AGENDA

OFFLU Avian Influenza Virus Characterization meeting

Rome, Italy • Philippines Room (C-277)
29-30 March 2017

DAY 1

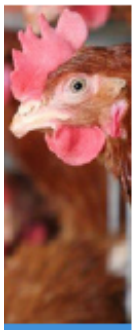
Wednesday 29 March

Chair: Ian Brown

09:00 – 09:15	**Opening and introductions**	
09:15 – 09:35	Overview of HPAI virus spread and evolution	*Ian Brown*
09:35 – 09:55	Challenges related to vaccines/diagnosis	*Timm Harder*
09:55 – 10:20	Current and future HPAI vaccine technologies – the US experience	*David Suarez*
10:20 – 10:45	**Coffee break and group photo**	
10:45 – 12:30	Country experience with HPAI virus evolution and vaccination	
(15 min each)	• Nigeria	*Clement Meseko*
	• Egypt	*Abdelsatar Arafa*
	• Viet Nam	*Tung Nguyen*
	• Indonesia	*Ernes Andesfha and Hendra Wibawa*
	• China	*Jiming Chen*
12.30 – 13.00	Questions and overall discussion	
13.00 – 14.00	**Lunch**	

Chair: David Swayne

14.00 – 14.40	WHO vaccine composition meeting (VCM) process – presentation and discussion	*Mia Torchetti and Lidewij Wiersma*
14.40 – 15.00	Options for a poultry vaccine selection process	*David Swayne*
15.00 – 15.30	**Working groups session 1** Application of the VCM process to poultry health	
15.30 – 15.50	**Coffee break**	
15:50 – 17.30	Discussion – the way forward	



Working groups session 1

3 groups will answer the same questions: Would the poultry industry benefit from an evidence based approach to strain selection? If so, by what process, e.g. like WHO VCM? What are the pros/cons/final decision on goals, structure, feasibility and pertinence of avian virus characterization/strain selection process?

Working group session 2

Group 1: Monitoring of virus genetic evolution: sharing data/barriers, protocols, practicalities (who, when), funding, full genome versus HA genome

Group 2: Antigenic characterization and reagent/antisera production: process for strain selection, protocols, which labs, production process

Working group session 3

All: Listing stakeholders

2 or 3 groups: Each group defines implications and messages for each category of stakeholders

DAY 2

Thursday 30 March

Chair: Gwenaelle Dauphin

08:45 – 09:15	Summarising challenges, options and way forward – key points	*Les Sims*
09:15 – 09:45	Discussion to define the process and needs	
09:45 – 10:00	Focus on H9N2	*David Suarez*
10:00 – 10:45	**Working groups session 2** Monitoring of virus evolution – poultry sera production	
10:45 – 11:00	**Coffee break**	
11:00 – 11:45	**Working groups session 2** Monitoring of virus evolution – poultry sera production	
11:45 – 12:45	Overall discussion	
12:45 – 13:45	**Lunch**	

Chair: Peter Daniels

13:45 – 14:15	OFFLU proficiency testing
14:15 – 15:30	**Working groups session 3** How to communicate with stakeholders
15:30 – 15:45	**Coffee break**
15:45 – 16:15	Overall discussions
16:15 – 17:00	Final recommendations
17:00 – 17.15	Meeting closure

MEETING GOAL

The objectives of this meeting are to engage participating laboratories, share virus characterization data, agree on a methodological approach for data compilation and analysis and to progress matters to ensure diagnostic test harmonization and to agree the delivery of proficiency testing (PT) among the Reference Centres designated by FAO and OIE. The meeting will be medium sized and interactive, with emphasis on brainstorming sessions and discussion (limited scientific presentations).

Minutes of the OFFLU Avian Influenza virus characterization meeting

FAO headquarters, Rome, Italy
Philippines room (C-277)
29-30 March 2017

List of acronyms and abbreviations

WHO	World Health Organization of the United Nations
FAO	Food and Agriculture Organization of the United Nations
OIE	World Organisation for Animal Health
AI	Avian Influenza
AIV	Avian Influenza virus
HPAI	Highly Pathogenic Avian Influenza
LPAI	Low Pathogenic Avian Influenza
RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Real time RT-PCR
VCM	WHO Influenza Vaccine Composition Meeting

Wednesday 29 March

Welcome and Introductions

The director of Animal Production and Health Division (AGA) of the Food and Agriculture Organization (FAO) Dr. Berhe Tekola officially opened the meeting on behalf of FAO. He stressed the importance of the work on avian influenza and OFFLU's role. Following this, the chair Dr. Ian Brown presented a brief overview of agenda and welcomed the participants, inviting them to introduce themselves in a *tour de table* (list of participants can be found on p. 16). The programme then proceeded as per agenda (see p. 2-3); the main points of each presentation are described in this document and the presentations are available upon request.

Overview of Highly Pathogenic Avian Influenza (HPAI) virus spread and evolution (Dr. Ian H. Brown)

The spread of the H5N8 HPAI virus has raised great concerns for animal health; it has been reported in Europe, Central Asia, Middle East and Africa. Germany, France and Hungary reported the highest numbers of outbreaks. In some countries, primary H5N8 outbreaks in poultry often were preceded by the detection of dead wild birds, whilst in others, secondary spread within domestic poultry has been reported to be contributed to the extent of notified poultry cases. To date, no H5N8 human cases have been reported. Important actions must be taken and these should be integrated into OFFLU activities: (i) study of genetic data (and antigenic characterization and ongoing evolution and drift); (ii) full genome sequencing to continue to track and define genotypic variation through mutation and reassortment; (iii) studies on host range; (iv) transmission related to infection kinetics and zoonotic risks of the different avian Influenza viruses.

Challenges related to vaccines/diagnosis (Dr. Timm Harder)

Generic detection and pathotyping of influenza viruses by RT-PCR is an established routine diagnostic method, and can be applied to all sub-types and various lineages of AIV. The use of pathotype-specific probes targeting the hemagglutinin cleavage site has been evaluated for the detection and differentiation between H5N1 HPAI and low pathogenicity avian influenza (LPAI) viruses with good results on field samples (H5 cleavage sites of viruses of the goose/Guandong lineage are fairly conserved). On the other hand, the H7 HP cleavage sites are not at all conserved, and degenerate primers to detect the different H7 viruses have been tested. The use of antigenic cartography, NGS sequencing from all isolated viruses, as well as the use of new approaches such as SISPA (Sequencing-independent sub- and pathotyping of avian influenza viruses) and RITA (Riems influenza typing assay) are also recommended.

Current and future HPAI vaccine technologies – the US experience (Dr. David L. Suarez)

Four H5 recombinant vaccines are now licensed in the US: herpesvirus turkey-(HVT)-AI and fowlpox-AI with heterologous H5 insert, and reverse genetic (RG) H5N1 avian influenza virus and RNA particle (RP) with clade 2.3.4.4 insert. The key determinants to measure vaccine success included protection from clinical disease (decreased morbidity and mortality), reduction in viral shedding and increased hemagglutination-inhibition (HI) serological response. The conclusions after initial vaccine trials performed in the US against A/gyrfalcon/Washington/40188-6/2014 (H5N8) and A/northern pintail/Washington/40964/2014 (H5N2) clade 2.3.4.4 field viruses were: i) homologous H5 killed vaccines provided the best protection, ii) vectored vaccines with partially matched HA displayed partial protection on their own when administrated as single dose, iii) Chinese RG viruses provided better protection (large reduction in viral shedding), and iv) older USA H5 vaccine strains gave the poorest protection across all three parameters[1]. Previous and subsequent studies have demonstrated that prime-boost regimes gave the best protection in experimental studies and some field trials and have been recommended for field application. Development of new vaccination strategies is an important area of current research, and potential users of vaccines are recommended to keep abreast of developments. The DNA vaccines provided only partial protection with two doses of vaccine. Regardless of the type of vaccine used, the standardization of tests able to differentiate vaccinated from vaccinated animals (DIVA) is strongly recommended.

Country experience with HPAI virus evolution and vaccination: In this section, the representatives of different countries presented their experience in a presentation of 15 min each. The principal points and discussions are reported below.

[1] N.B. A subsequent publication [Bertran et. al, Vaccine 2017 Nov 1; 35(46):6336-6344] showed better protection from mortality and shedding of the rHVT-H5 vaccine alone against challenge by A/turkey/Minnesota/12582/2015 (H5N2) clade 2.3.4.4.

a) **Nigeria (Dr. Clement Meseko).** Several factors such as wildlife diversity, extensive wetlands, backyard poultry, free range birds, live bird trade, and the movement of poultry and poultry products across the country, have created the optimal conditions for the introduction, spread and persistence of avian influenza in Nigeria. After the first outbreak of HPAI H5N1 occurred in 2006, different H5 clades (2.2.1, 2.3.2.1c, 2.3.4.4b) have circulated in the country. Moreover, H5N2 (HPAI & LPAI) and H5N8 HPAI strains have been reported. The AI control policy in Nigeria is based on surveillance, early detection, depopulation, compensation and biosecurity which have been successful. No vaccines are used for the control of AI since 2006 (although unregulated vaccines have been used), thus the application of DIVA strategy may be necessary to avoid the abuse of vaccines especially after outbreaks of a new clade (H5N1 2.3.2.1c) started in 2015.

b) **Egypt (Dr. Abdelsatar Arafa).** H5N1 HPAI outbreaks have been reported in backyard poultry, commercial farms and live bird markets (LBM), and H5N1 human cases continue to be detected. The H9N2 LPAI and H5N8 HPAI viruses circulating in Egypt are closely related to those circulating in Middle East and Europe (H5N8), respectively. Imported (China, Europe, Mexico) and locally produced vaccines are used in the country (inactivated and/or recombinant). To be licensed for use, vaccines must be tested through challenge studies, as well as through the evaluation of the vaccine matching with circulating strains (cross HI test and HA sequence comparison). The control strategy in Egypt has been based on: update of surveillance plans, continuous monitoring and characterization of circulating strains, vaccine selection, sharing information with stakeholders, and monitoring of matching between vaccine and circulating strains (cartographic analysis and genetic markers).

c) **Viet Nam (Dr. Tung Nguyen).** In 2016, H5N6 and H5N1 HPAI viruses circulated and co-circulated in different regions of the country. The strains circulating in the northern part of the country are closed related to those circulating in China, whilst those circulating in the south are more closely related to those from Cambodia. Several vaccines have been used against the different H5N1 clades circulating in the country. For licensing the poultry H5 vaccines, quality control tests and challenge studies using newly viral strains are performed. The vaccination strategy has been carried out in two phases: (i) 2005-2012: mass vaccination (chickens and ducks) in high risk zones; and (ii) vaccination at province level (2013-2017). Challenge strains change every 2-3 years, and the selected H5 vaccines are tested for their antigenic matching with circulating viruses, using HI tests of vaccine sera with field viruses of different clades, and challenge experiments (annually performed or upon the emergence of new strains).

d) **Indonesia (Dr. Ernes Andesfha and Dr. Hendra Wibawa).** H5N1 HPAI remains endemic in some areas, particularly in Java. Some reasssortant events have also been observed among H5 clades, particularly between H5N1 clade 2.1.3.2 and clade 2.3.2.1c viruses. In a period of 9 years (2007-2016), the number of HPAI H5N1 outbreaks in poultry has decreased considerably, as well as the number of confirmed H5N1 human cases. The vaccination strategy started with mass poultry vaccination (including backyard and small farmers) with a local inactivated H5N1 isolate. The government continued the vaccination on targeted poultry sectors, particularly small holder commercial farms of Sector 3. Sector 1, Sector 2 and intensive Sector 3 did vaccination at their own cost. OFFLU Projects started for the selection of master seed vaccines and challenge antigens based on genetic, antigenic cartography and challenge studies. From 2011 to now, vaccine companies are allowed to make monovalent or bivalent vaccines (combination of strain/clades as recommended by the government), or use their own seed strains as long as originated from local isolates that have been analyzed using an antigenic

cartography testing. The Influenza Virus Monitoring (IVM) network has played an important role on the monitoring and communication of AIVs circulating in animals.

e) **Bangladesh (Dr. Muzaffar Goni Osmani (Jewel)).** Currently, only the clade 2.3.2.1 of H5 HPAI is circulating in Bangladesh. The vaccines that are used in the country are imported from the U.S (vector-vaccines) and from China (inactivated vaccines). Vaccination is applied throughout the country. For the use of vaccines, the permission from the Drug Administration of Bangladesh to import vaccines is mandatory. According to the current strategy for the vaccine selection, the first step is the characterization and sequencing of the HPAI circulating strains, followed by evaluation of antigenic matching between the vaccines and the field strains. The real-time monitoring of the vaccine is performed through the evaluation of the post-vaccination titers in vaccinated animals, and the protection conferred by the vaccines against strains circulating in the country (challenge studies) performed on vaccinated birds in the field.

f) **China (Dr. Gwenaelle Dauphin, on behalf of Prof. Jiming Chen).** Different H5, H7 and H9 subtypes (H5N1, H5N2, H5N6, H5N8 subtypes) and clades have been circulating in the country. The LPAI H7N9 mutated into HPAI H7N9 in Guangdong province in late 2016 and spread to Hunan province in early 2017. Several factors such as large scale poultry production, high poultry density, and low poultry biosecurity have played an important role in the H5, H7 and H9 circulation. Mass vaccination has been applied in the country (and maintained for a dozen years). Six inactivated RG PR8-based vaccine strains have been used, matching with different H5 clades. For the update or vaccine selection, epidemiological surveys (prevalence), serological assays (antigenic changes) and challenge studies (protection potency) are conducted. Although H5 mass vaccination has shown to be effective to decrease the prevalence of some terrestrial clades (e.g. 7.2), mass vaccination likely accelerated the mutation of the virus to escape from the vaccination, and more vaccines strains are needed to curb the viral infections (bivalent or trivalent vaccines used). The H5 and H7 crises have spurred modernization of the poultry, development of veterinary technologies, and optimization of animal disease control in China.

World Health Organisation (WHO) vaccine composition meeting (VCM) process – presentation and discussion (Dr. Mia Torchetti and Dr. Lidewij Wiersma)

To ensure that existing human candidate vaccine viruses protect against circulating strains of human seasonal and zoonotic avian (and porcine) viruses, WHO organizes consultations with an advisory group of experts (including OFFLU) twice annually, with the aim of analysing data generated from the influenza virus surveillance. The report of the recommendations is available online after each meeting, and summarizes the analysis and selection (if applicable) of new vaccine candidates for the following influenza season. OFFLU has played an important role in this process, identifying and sharing information on animal influenza viruses with zoonotic potential. OFFLU provides a report with the antigenic and genetic characteristics of recent zoonotic influenza viruses circulating in birds, thereby contributing to pandemic preparedness through the selection of new human candidate vaccine viruses (CVVs). OFFLU is also responsible for compiling the data generated by different Labs (OIE/FAO Reference Centres, Regional laboratories, National Reference laboratories), and offering assistance for virus characterization and epidemiological data collection. Given the success of the contribution, OFFLU and WHO extended their agreement until 2018 and remain committed to further close collaboration on influenza risks at the human-animal interface.

Some of the other points that were discussed following the presentation:

- Data sharing (e.g. sequences), sharing of biological material, and diagnostic results among laboratories should be improved. The biggest concern is international sharing of biological material between laboratories (Nagoya protocol, MTAs, shipping process etc.). However regardless of these difficulties, the evaluation of antigenic matching with human/animal sera panels is very important and efforts should therefore be made to overcome these difficulties.
- Sharing of viruses is insufficient (sequences, RNA, biological material), as well as the generation of timely HI (antigenic) data. This remains problematic and disproportionate to genetic data (due to the lack of sharing of viruses to reference Labs as well as difficulties in obtaining well-standardized antigenic reference panels).
- Financial solutions must be found to facilitate the sharing of material (like the WHO Shipping Fund).
- The production, standardization of protocols and reagents and sharing of serum panels is crucial.
- Data collection and correct linking of this field data to samples is essential
- OFFLU should focus on improving communication with the countries
- Assist the laboratory focal points in obtaining permission from competent authority for sharing the data to the VCM, a copy of the OIE resolution is sent along with the OFFLU VCM letter; laboratory focal points should be reminded of this resolution and their role in its implementation.
- Prepare a *letter of acknowledgement* to national authorities on behalf of OFFLU every time data is shared. In addition, a full report and analysis of the data shared can be enclosed to each letter; this can be useful to the national authorities and may incentivize continued sharing.

Options for a poultry vaccine selection process (Dr. David Swayne)

Annual or biannual meeting can be held with the different members (OFFLU – WHO VCM and OFFLU contributors) with the aim to define and optimize the data gathering process, define AIV subtypes to be explored, update the inventory and phylogeny of HA accessible sequences, review and analyze antigenic data, conduct serological tests and antigenic analysis on circulating viruses using poultry and ferret antisera, review the vaccination/challenge studies, to update previous inventories of available vaccine products in different countries, among others in relation to AIV vaccines for use in poultry. The communication of these results with national authorities and private industry to facilitate the open sharing of data with international and national vaccine manufacturers is the main goal. This process will make recommendations for poultry AI vaccine seed and challenge strains, as well as regularly update of the strain inventory and availability. FAO and OIE could explore funding (including from external sources) to pay for a meeting and consultancy to help for the coordination or data analysis.

Application of the VCM process to poultry health (working group session 1)

1) Would the poultry industry benefit from an evidence-based approach to vaccine strain selection?

The overall conclusion was that the poultry industry would indeed benefit from such a process. The following points were raised:

- Not just the poultry industry but also the national licensing agencies would greatly benefit from the process in licensing only efficacious vaccines. However, AIV vaccine selection will be more complex as national needs vary and there is significantly more diversity of subtypes and strains in poultry than in humans. Therefore direct extrapolation of the human (or equine) process will likely not be valid. The process is also different from human strain selection because of cross-reactivity due to adjuvants in inactivated vaccines, or recombinant vectored vaccines.

- There is lack of transparency of some countries and the difficulties encountered with sharing of information (confidentiality? lack of communication?). This problem will have to be addressed and (standardized and accurate) information will need to be made available to compare virus circulation at local, regional and national level.

- For the production of human influenza vaccines, the process is already globalized whereas for animal health, AI vaccines are frequently locally produced for national use. On the animal health side, there needs to be a shift in perception; due to the transboundary nature and the rapid spread of influenza viruses it must be made clear to decision makers that such approaches on a country-by-country basis are no longer appropriate.

2) If so, by what process, e.g. like WHO VCM?

- Because of the complexity of the animal health situation (no one-fits-all approach possible), it may be more realistic to provide criteria for antigen selection rather than recommending the exact antigen to use. Such guidance should include standardized methods for preparing antigens, antisera and protocols to be used. All protocols should be revised and updated on a regular (yearly) basis to ensure they are still fit-for-purpose.

- A system that detects mismatch or impending mismatch is required. This system should include high quality data collected through a well-defined surveillance programme available in real-time and adequate downstream analysis of the match (e.g. using antigenic cartography). Data sharing and an up to date and complete database of circulating viruses is crucial. In addition, labs with more expertise (country or regional level) that may act as collection points of samples and information can be identified to help this process.

- In order to identify mismatches, clear criteria for vaccine efficacy must be formulated: How do we know vaccine efficacy in the field? How do we know if there is a breakdown in vaccine efficacy? Laboratory testing of field strains with available vaccines is ones way, but should also include field trials. The process used in Indonesia worked well in ascertaining what worked and what did not for seed selection and challenge virus selection; this could be used as starting point. Other ways include the use of a zonal/local approach in each country (e.g. Egypt) which may be more feasible to begin with. Whatever the exact approach, local capacity must be built (funding required) for labs to perform vaccine trials and challenge studies.

3) What are the final decision on goals, structure, feasibility and pertinence of avian virus characterization/strain selection process?

- Develop criteria for a good vaccine match;
- Develop criteria for strain selection;
- Develop standardized protocols that are revised and updated regularly (including field surveillance guidelines);
- Monitor whether standardized approaches have been adopted;
- Advocate for, and facilitate, real-time data sharing and increase the communication between countries and laboratories. For this, updating of the different websites is required (including OFFLU).
- Initially, aim at identifying antigenic mismatch rather than strain selection as this is likely more manageable;

- Capacity building needed to carry out well designed surveillance, perform vaccine trials and challenge studies and improve genotyping and monitoring of antigenic changes through sequencing. Training and funding will be the essential.
- Some countries are vaccinating and have an existing vaccine selection process (e.g. China, Egypt, Indonesia, Viet Nam) but others have nothing – thus country specific needs will require different approaches which would make a global process like the WHO VCM more difficult to implement. There is a need cooperation of multiple countries for data/material sharing and assist in field trials.
- Collect existing information at regional and local level in each country regarding vaccination trials and challenge studies that are not publically shared or published. Even if this information cannot be shared by OFFLU, it can help ensure better recommendations are made (same as for the WHO VCM which relies heavily on non-public data to inform its decision).
- Increase sharing of results of any vaccine used in the field (including results of poor vaccine efficacy in the field).

Questions raised during the discussion:

- Overcoming regulatory inertia for change – would more data on strain selection overcome or make this process better or worse?
- What happens with subtypes other than H5/H7? Could H9N2, which is less politically sensitive present an opportunity to trial the process? Once this is shown to be effective it could be extended to other subtypes.
- There is a gap regarding the evaluation of the vaccines when other secondary diseases are present (e.g. E. coli, Mycoplasma, co-infection with other AI viruses, etc.), how can this be addressed?
- What exactly is OFFLU's role in this process?

Thursday 30 March

Summarizing Day 1: challenges, options, and way forward – key points (Dr. Les Sims)

Vaccination is still needed to assist in control and prevention of (potential) zoonotic avian influenza infection. Two principal pillars are necessary to implement a vaccination program: evidence of antigenic variation that has the capacity to affect vaccine efficacy/effectiveness, and the standardization of protocols for challenge studies to measure vaccine efficacy. In this regard, a more nuanced approach than just percentage of survival is needed (e.g. failure to prevent systemic infection in the face of antibody), also taking into consideration the percentage reduction in shedding. All countries that use vaccines need up-to-date information on antigenic characteristics of circulating viruses, and some of them already have a system in place that results in vaccine updates (e.g. China, Indonesia). Unfortunately, the proper selection of antigens and serological DIVA tests may be complicated in areas with multiple subtypes circulating.

Actions (proposed by experts):

- Obtaining access to the vaccine information is a challenge, because vaccine producers may not share information about the composition of the vaccines. For this reason, the creation of a central dataset – independent of the pharmaceutical sector – can be created, containing minimum information about the available vaccines used.
- It could be also important to create and test vaccines that induce immunity in ducks in order to reduce the multiplication of the viruses.

Following the overview provided by Dr. Les Sims, Dr. Suarez presented a focus on H9N2, followed by presentation by Dr. Nicola Lewis on H5 characterization. Between working group discussions, Dr. Frank Wong provided a presentation on the current OFFLU proficiency test.

Focus on H9N2 (Dr. David Suarez)

H9N2 avian influenza is widely endemic in Asia, the Middle East and North Africa, causing important clinical disease in poultry and sporadic zoonotic infections in humans. Some transmission studies have demonstrated that H7 transmission is reduced compared to H9 (100%) from infected to in-contact birds. Although 3 poultry adapted lineages have been classified, the establishment of one online source to predict lineage for easy analysis may be useful. Regarding the diagnosis, it is necessary to provide validated H9 real-time RT-PCR tests to identify H9 influenza viruses, and to provide serologic reagents to assure accurate HI testing with the aim to provide understanding of cross-reactivity between different sublineages.

H5- enhanced characterization (Dr. Nicola Lewis)

H5 characterization is important to understand the antigenic and genetic evolution of currently circulating H5 clades of public and animal health importance. Key to improving H5 characterization are: real-time sharing of genetic data and isolates among laboratories, generation of a standardized chicken serum reference panel (using at least 4 sera per clade), antigenic characterization of different clades using full serum panels, antigenic cartography of chicken HI assay data, generation of standardized ferret serum reference panels, and performing HI assays and antigenic cartography of currently circulating H5 strains using ferret antisera, to allow integration into the WHO VCM analyses . The principal outputs of this process are the real-time assessment of evolution (natural host and ferret sera), the standardization and generation of high quality paired chicken/ferret sera, and the evaluation of the antigenic drift relative to vaccine strains in both poultry and pandemic preparedness human vaccine.

Discussion:

- Should not be prohibitively expensive to perform (it is relatively economical to generate standardized chicken serum panels).
- It is recommended to work as a large network with the aim of sharing serum samples among laboratories (this would require funding).

OFFLU proficiency testing (Dr. Frank Wong)

Proficiency testing (PT) is an important tool that facilitates laboratory networking at national and regional level, thus assuring the quality of laboratory services. Moreover, it is essential to maintain relevant diagnostic capability and the international reference designation. The molecular AIV PT tailored for the OFFLU Reference Labs is formed using a panel for the identification of the type A influenza viruses (different H and N types), and a panel to assess H5 virus detection (different subtypes and clades, including H5Nx). The PT should be performed every year and includes a network of 11 OIE/FAO Reference Centres. AAHL, Australia has agreed to lead the PT exercise in 2017 for coordination, dispatch of panels and analysis of results. Points that were raised included:

- The results presented displayed a wide range of variability among the laboratories (Ct values of real time RT-PCR), thus there is a necessity to harmonize the methods.
- It could be important to include AIVs pathotyping (discriminate HPAI and LPAI) in the PT. This will give an idea of the capacity of the lab.
- To evaluate the speed of detection: from receiving samples to analysis.

- It would be important to include some of the WHO labs in the PT, especially those labs that are involved in the H5 and H7 viruses detection in humans.

Monitoring of virus evolution and Poultry sera production (working group session 2)

Monitoring of virus genetic evolution: *sharing data/barriers, protocols, practicalities (who, when), funding, full genome versus HA genome.*

- **Data generation**. HA/NA sequences, AIV whole genome and antigen characterization of viruses should be included. The principal concern is the accuracy of the data generated, for example there is a necessity to define and to develop a quality assurance system for the NGS and sequencing methods. Moreover, the process of data collection must be improved.
- **Barriers**. Some barriers that pose problems to free data sharing were identified as:
  (i) political: complex process;
  (ii) economical: Sanger sequencing or NGS may be expensive for some laboratories, fear to notify the disease (trade barriers) when sharing data;
  (iii) confidentiality and publications: in several cases, the data is not shared until articles are published (and if not published, the data is never shared);
  (iv) technical capacity: problems in shipping material (permits) or the shipping process is complex, costly and long.
- **Some of solutions to these barriers** could be :
  (i) political: avoid the legalization of the process; standardize data sharing process case by case; to carry out trainings for officials that provide permissions (often non-technical personnel) to allow them to understand the ramifications of their decisions;
  (ii) economical: increase the budget and the funding resources (to different countries) to incentivize the sharing of data and material;
  (iii) confidentiality of publications: to create a central sequencing service (to assure the harmonization of methods) and to generate automatically the data;
  (iv) technical capacity: to find solutions for shipping material a regional, national an country level and to make easier the process.

- The two different groups that discussed this topic showed some differences in opinion regarding sharing of sequences (mandatory vs. voluntary). One group felt that not creating a legal framework for the process would be a more sustainable solution, whereas the other group felt this was unrealistic. Both groups may have valid points and the timeline may the deciding difference (i.e. in the short term we cannot count on a legal framework but over time this should be built). The groups also varied in their opinion on the need for the creation of a new database with confidential information (controlled vs. free access). Some believed a private data sharing space would be beneficial, others argued that many platforms already exist and these should be used. Again time may be a factor; creating a private space may address data sharing issues in the short term, but in the long term, real-time sharing in open access databases should be advocated for.

Antigenic characterization and reagent/antisera production*: process for strain selection, protocols, which labs, production process*

- Antigenic characterization can be performed at zonal, regional and national level (example of Indonesia).
- Reagent/antisera production:

(i) Standardize protocols and produce guidelines about the antigen selection;
(ii) Perform and to share results of the HI data and antigenic cartography among laboratories;
(iii) Standardize and generate protocols to raise serum panels. The group recommends identifying and giving responsibility to raise poultry sera to 1 or 2 partners (centralized production). This will harmonize/standardize the quality of sera produced. The objective is to share (for free) the sera panels produced by these partners to the different reference laboratories and different countries, with the aim of performing cross HI tests with the new AIV strains isolated;
(iv) Standardize the use of adjuvants and vectors.

- Standardize and define the criteria to assess vaccine protection: percentage of mortality, reduction of shedding and clinical signs. The level of protection can be very subjective; thus, the establishment of quantitative criteria to decide the correlates of protection is important. It could be important to harmonize the time points when samples can be taken for the evaluation of viral shedding (days post challenge).
- Define criteria to assess vaccine protection for LPAI viruses. It is important to understand how to evaluate the protection conferred for LPAI viruses (vaccines can block viremia in challenge animals with HPAI viruses, but it is more difficult to understand the protection conferred for LPAI viruses).
- Harmonize methods to evaluate post-vaccination monitoring on the field studies.
- Standardize the challenge models: to evaluate the use of different adjuvants, age and breed of experimental animals the use of prime/boost vaccines, challenge doses, and time of challenge. The evaluation of the level of maternal antibodies can also be important.
- Standardize the challenge studies and link the results with antigenic cartography (mismatches)
- Besides virus shedding, the transmission from vaccinated and infected to in contact birds may be evaluated for each vaccine. This could be helpful to understand the transmission kinetics from vaccinated to naïve animals, and to evaluate whether the vaccine is able to reduce the transmission. However, this parameter was considered as impractical by some participants of the second group, since the lab condition is different than those observed in the field, as well as the different coefficient of transmission of the AIVs.
- Ask collaboration of vaccine producers to provide more information on the composition of vaccines and the results of challenge studies performed.

Communicating with stakeholders (working group session 3)

The stakeholders were defined as: vaccine manufacturers, the biotechnology industry, Journals, regulatory agencies, sequence databases, poultry industry, consumers, governments, public health sector and donors.

- **Governments:** communicate the need and benefits of doing surveillance and sharing data/virus, communicate the economic benefits of improved vaccines for AI control and the animal and public health impacts (using e.g. examples of success stories), this could be achieved through advocacy by e.g. FAO and OIE, interaction with AI taskforce/experts and communication from other stakeholders.
- **Regulatory agencies:** similar to government (see above), changing regulations is an internal issue, regulations will have an impact on trade; effects of use or non-use of vaccination must be communicated well.
- **Donors:** identify possible donors (e.g. USAID, USDA, Wellcome Trust, EC, ECOWAS, Bill and Melinda Gates, etc) and start outcome oriented discussions, create proposals with clear objectives and outcomes to benefit animal and public health, invite donors to OFFLU meetings.

- **Vaccine manufacturers/ Biotechnology industry**: Communicate on the added value of having a neutral coordinated scientific platform to provide regular updates on influenza viruses of interest for vaccine development and challenge efficacy trials.
- **Poultry industry**: Communicate why well matched vaccines are important for their business and how they can contribute to improving this process (by e.g. making data from their businesses available).
- **Consumers**: Communicate the importance of healthy livestock for protection of their own health.
- **Public health sector**: through WHO contact person, use public health meetings such as IMED or organizations as IFPMA as a platform, encourage within country sharing of information for public health, explain the objectives and differences with the public health sector, stress that animal vaccine selection procedures may generate interesting data for public health use.
- **Journal**s: Depositing viral sequences in the public domain before publishing affects the novelty factor of a paper (perceived and/or real concern), an open letter to the editor of some top journals could bring attention to this issue. In instructions to authors it should be encouraged to share data as early as possible and that this will not (or even positively) affect the chances of getting a paper accepted.
- **Sequence databases**: an ‘‘agnostic’’ stakeholder, communication with sequence databases not necessary but communication about sequence databases and their importance to the other stakeholders (e.g. government) is crucial.

Final recommendations

From the meeting the following actions were recommended:

- Draft guidelines for proposed antigen selection process
    - OFFLU to draft in a smaller group and circulate widely
    - OFFLU to advocate for implementation the guidelines

- Draft criteria for testing of vaccine efficacy both in the laboratory *and* in the field and circulate with wide audience for validation, including vaccine manufacturers
    - OFFLU to draft in a smaller group and circulate widely
    - OFFLU to advocate for implementation the guidelines

- Collect field data on the impact of HPAI vaccination on the larger scale to conduct studies on vaccine efficacy, including developing guidelines and identifying potential sources of funding to conduct such studies (possibilities for public-private partnerships).

- Standardization of protocols for antisera generation
    - OFFLU to draft in a smaller group and circulate widely
    - Shared on OFFLU website

- Standardize use of adjuvants and vectors

- Monitor whether standardized approaches have been adopted and are revised regularly (yearly)

- Create an inventory of vaccine seed strains and hemagglutinin inserts as well as commercial vaccines in use (for more detail, see page …)
    - To be created with the help of industry
    - Posted on the OFFLU websites and kept up-to-date

- Capacity building needed to carry out well designed surveillance (including for post-vaccination monitoring), perform vaccine trials and challenge studies and improve genotyping and monitoring of antigenic changes through sequencing. Training and funding will be the essential.

- Advocacy to address need for quality assurance in sequence databases (e.g. IRD pipeline)

- Advocate for, and facilitate, real-time data sharing;
    - OFFLU to advocate for open data and biological material sharing, through e.g. an open letter to journals to encourage data sharing before publication;
    - Explore opportunities to facilitate sharing of materials (model on WHO shipping fund?);
    - Continue to explore solutions for better linking of field and laboratory data;
    - Send letters of acknowledgement for all data shared and other incentives for data sharing;
    - Assist OIE laboratory focal points in obtaining permission from competent authority, including training/information for competent authorities;
    - Monitor and participate in the discussions on the implementation of the Nagoya protocol;

Meeting closure

The meeting was closed by Dr Ian Brown thanking OIE and FAO for organizing this meeting and the experts for their contribution to the meeting.

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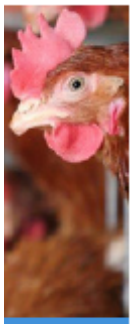

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