# OFFLU

- Existing methodologies and protocols for identification of avian influenza virus infection in vaccinated and non-vaccinated populations

and

- Avian influenza matching (AIM) pilot project

Presented at Technical meeting on HPAI Vaccination: Approach, tools, knowledge, and experience for the Americas March 3rd 2023 by Gounalan Pavade – (OFFLU Secretariat)

1

1

# WOAH standards on vaccination

- The Terrestrial Animal Health Code (*Terrestrial Code*) recognises that vaccination can be used as an effective complementary control tool, part of a disease control programme
  - Chapter 10.4 https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_avian_influenza_viruses.htm
- Vaccination will not affect the high pathogenicity avian influenza status of a free country or zone **if surveillance supports the absence of infection.**
- In all vaccinated flocks tests should be performed to ensure the absence of virus circulation.
- Tests have to be repeated in accordance with the risk in the country.
- Evidence to show efffectiveness of the vaccination program should also be provided.
- Moreover, the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (*Terrestrial Manual*) provides standards on the requirements for vaccines
  - Chapter 3.3.4. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.03.04_AI.pdf

2

2

# Test methods available for the diagnosis of AI and their purpose

## B. DIAGNOSTIC TECHNIQUES

*Table 1. Test methods available for the diagnosis of avian influenza and their purpose*

| Method | Purpose | | | | | |
|---|---|---|---|---|---|---|
| | Population freedom from infection | Individual animal freedom from infection prior to movement | Contribute to eradication policies | Confirmation of clinical cases | Prevalence of infection – surveillance | Immune status in individual animals or populations post-vaccination |
| **Detection of the agent[1]** | | | | | | |
| **Virus isolation** | + | +++ | + | +++ | + | – |
| **Antigen detection** | + | + | + | + | + | – |
| **Real-time RT-PCR** | ++ | +++ | ++ | +++ | ++ | – |
| **Detection of immune response** | | | | | | |
| **AGID** | + (Influenza A) | + (Influenza A) | ++ (Influenza A) | + (convalescent) | ++ (Influenza A) | ++ (Influenza A) |
| **HI** | +++ (H5 or H7) | ++ (H5 or H7) | +++ (H5 or H7) | ++ (convalescent) | +++ (H5 or H7) | +++ (H5 or H7) |
| **ELISA** | + | + | ++ | + (convalescent) | ++ | ++ |

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
RT-PCR = reverse-transcription polymerase chain reaction; AGID = agar gel immunodiffusion;
HI = haemagglutination inhibition test; ELISA = enzyme-linked immunosorbent assay.

3

3

# Approaches to Differentiation of Infected from Vaccinated Animals (DIVA)- HPAI

- DIVA strategy has been put forward as a possible solution to the eventual eradication of HPAI and H5/H7 LPAI without involving mass culling of birds and the resulting economic damage, especially in developing countries.
- This strategy has the benefits of vaccination (less virus in the environment), but the ability to identify infected flocks, would still enable the implementation of additional control measures, including stamping out of infected flocks
- DIVA strategies use one of two broad detection schemes within the vaccinated population:
  - 1) detection of influenza A virus ('**virus DIVA**'), or
  - 2) detection of antibodies against influenza A field virus infection ('**serological DIVA**') - To use serological DIVA schemes, vaccination systems that enable the detection of field exposure in vaccinated populations should be used. Several systems have been used. These systems are yet to be validated in the field.

4

4

# Serological DIVA

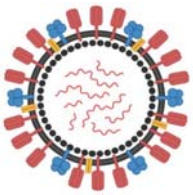
- To use serological DIVA schemes, vaccination systems that enable the detection of field exposure in vaccinated populations should be used. Several systems have been used.
- First, use of a vaccine containing a virus of the same haemagglutinin subtype but a different neuraminidase (N) from the field virus. Antibodies to the N of the field virus act as natural markers of infection.
- A second serological DIVA option is the use of vaccines that contain only HA, e.g. replicating or non-replicating recombinant vaccines, which allows validated, classical AGID and nucleoprotein (NP) or matrix protein-based ELISAs to be used to detect antibodies indicative of infection in vaccinated birds.
- Finally, for inactivated vaccines, a test that detects antibodies to the nonstructural viral or M2e proteins have been developed
- These systems are yet to be validated in the field.

5

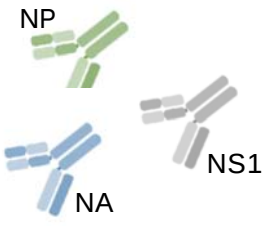
5

# Serological DIVA compatible vaccines

Immunization against the hemagglutinin



Infection




Detection of antibodies against other proteins

6

6

# Serological DIVA compatible vaccines

Vectored HVT vaccines
(live cell-associated)

Vectored ND vaccines
(killed + adjuvant

DNA vaccines

Subunit vaccines
(recombinant HA)

Self-amplifying RNA vaccines

**Multiple DIVA tools**

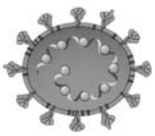
- Anti-NA ELISA matching the circulating Nx
- Anti NP/M antibodies

7

7

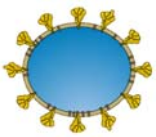
# Serological DIVA compatible vaccines

Reverse Engineered (rg)
Whole Inactivated Virus
(WIV) Vaccines



Some limitations, but still compatible
(need for heterologous NA in the vaccine to differentiate; anti-NS1 antibodies although lower sensitivity)

Virus-like particles



(need for heterologous NA in the vaccine to differentiate; anti NP and NS1 ELISA)

8

8

# "DIVA-like" strategy: unvaccinated sentinels

**Sentinel surveillance**

- ❑ The use of sentinel birds and the close monitoring of flock status do not closely fit the definition of a DIVA strategy, but these methods can be used to monitor vaccinated flocks
- ❑ If sentinel surveillance is to be used, a certain number of unvaccinated sentinel birds (typically 1%) should be kept and appropriately scattered in the vaccinated flock and tested by **virological and serological methods**

There are practical issues…

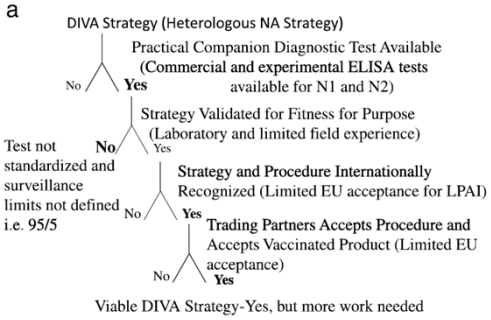
- ❑ Without good compliance of farmers and a proper management system, the presence of sentinel birds in the flock may facilitate introduction and transmission of HPAIV in this group of birds
- ❑ Logistically challenging to identify animals in big flocks.
- ❑ "False positive" serological results are not uncommon → interpretation difficulty and unnecessary disruptions of trade
- ❑ Cross reactivity of antibodies against LPAIV

9

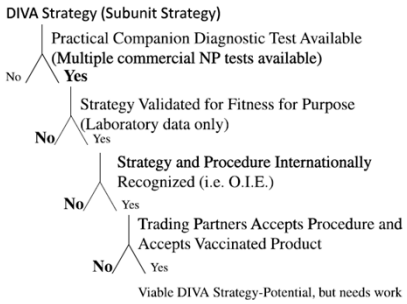
9

# A viable DIVA strategy

a




b




Source: David Suarez (2012)

10

10

# DIVA strategies

TABLE 1. LIST OF AVAILABLE STRATEGIES FOR DIFFERENTIATING INFECTED ANIMALS FROM VACCINATED ANIMALS, WITH SOME OF THEIR ADVANTAGES AND LIMITATIONS IN GENERAL

| *Strategy* | *Sentinel bird* | *Recombinant subunit vaccines* | *Heterologous NA* | *Differential immune response against protein (NS1, M2 and HA2 gp)* |
|---|---|---|---|---|
| Procedure and vaccine used | Naïve unvaccinated birds are marked and randomly spread in a vaccinated flock<br>Sentinel birds are routinely tested for influenza virus exposure | Vaccine using a vector expressing HA and NA proteins<br>Example: Fowlpox-vectored recombinant vaccine for the H5 subtype | Vaccines containing the same HA subtype as the field strain, but a different NA subtype.<br>Example: If the field virus is H7N2, the vaccine is H7N3 | Vaccination using whole-killed virus<br>Observation of the differential immune responses to the targeted protein (NS1, M2, or HA2) |
| Available companion diagnostic test | Hemagglutinin Inhibition (HI) test<br>Agar gel immunodiffusion<br>Type A-specific ELISA (detect anti-NP antibodies) | Agar gel precipitin<br>ELISA targeting the antibodies to matrix (M) protein or the nucleoprotein (NP)<br>Fluorescence microsphere immunoassay (FMIA) | Neuraminidase Inhibition (NI) test<br>Indirect immunofluorescence assay (iIFAT)<br>FMIA<br>Modified NI test | ELISA-based targeting the antibodies to specified proteins |
| Advantages | Low cost<br>Readily applicable<br>Sensitive procedure for monitoring in vaccinated flock | Efficacious in providing protection<br>Commercially available<br>Mass administration<br>The standard diagnostic tests are applicable | Efficacious in providing protection<br>Rapidly available through reverse genetics technology | Conventional inactivated virus can be used for vaccination<br>Only a single diagnostic test needed |
| Limitations | Labor intensive<br>Time consuming<br>Naïve birds can potentially act as virus amplifiers and be the source of infection | Test sensitivity is yet to be determined | Prior knowledge on circulating strain<br>Possible introduction of the same NA subtype field strain with the NA subtype used for vaccination<br>Undetermined sensitivity of serologic testing<br>Low-throughput screening capacity<br>iIFAT–time consuming, laborious and the result interpretation is subjective | Risk of false-positive due to the presence of protein contaminant from nonpurified vaccine i.e NS1 protein<br>Risk of false-negative in surinfected host due to the inability of host to seroconvert<br>HA2 gp approach–need more studies |

Source: Hasan et al (2016)

11

11

# Serological DIVA complications

- Serological sampling is more time consuming than virological sampling
- Ducks are particularly susceptible to other subtypes of AI

- Organic farming, free range and backyard birds at higher risk of exposure to other non-H5Nx AI viruses.

- Heterologous boosting strategies with different vaccine technologies might limit the number of assay options

12

12

# Conclusion DIVA

- Many different DIVA strategies have been proposed for AIV, but they all rely upon several key elements and needs field validation
- DIVA serological testing might be one component but suffers from being relatively insensitive and subject to false positives and provides virtually no information on the status of infection in the last two weeks of the life of a vaccinated flock.
- Negative DIVA serology on a flock basis does provide some information but it is likely there will be many false alarms when using it because the tests available rely on detection of antibody to NP which is common to all Influenza A viruses.
- All DIVA vaccines require a sensitive, specific, cost-effective companion diagnostic antibody test that can be run on large numbers of samples.
- Sufficient scientific research must be performed to show that the test has a ‘‘fitness for purpose.’’

13

13

# Surveillance for detecting infection

- It should be multilayered and risk based/targeted rather than relying on random sample to detect infection at a certain level
- Routine dead bird testing but it is also important to establish appropriate sample numbers for flocks of different sizes and existing background mortality rates
- Surveillance systems that demonstrate infection in flocks is not being transmitted (R is <1). Experts discussing ways to do this at present

14

14

# Burden of testing when vaccination is applied (Europe)

REGULATIONS

COMMISSION DELEGATED REGULATION (EU) 2023/361
of 28 November 2022
supplementing Regulation (EU) 2016/429 of the European Parliament and the Council as regards rules for the use of certain veterinary medicinal products for the purpose of prevention and control of certain listed diseases
(Text with EEA relevance)

THE EUROPEAN COMMISSION,

PART 2

**SPECIFIC CONDITIONS FOR THE REINFORCED LABORATORY SURVEILLANCE TO BE IMPLEMENTED IN THE VACCINATION AND PERI-VACCINATION ZONES DURING EMERGENCY PROTECTIVE VACCINATION FOR PREVENTION AND CONTROL OF HPAI**

**Emergency vaccination**

Laboratory surveillance by collection of samples for virological testing shall be implemented every two weeks in the establishments where emergency protective vaccination has been carried out to detect occurrence of infection with HPAI field virus. The surveillance has to enable detection of a prevalence of infection with the HPAI virus in the vaccinated establishment of 5 % or less with a confidence level of 95 %.

15

15

# Burden of testing when vaccination is applied (Europe)

REGULATIONS

COMMISSION DELEGATED REGULATION (EU) 2023/361
of 28 November 2022
supplementing Regulation (EU) 2016/429 of the European Parliament and the Council as regards rules for the use of certain veterinary medicinal products for the purpose of prevention and control of certain listed diseases
(Text with EEA relevance)

THE EUROPEAN COMMISSION,

PART 5

**SPECIFIC CONDITIONS FOR PREVENTIVE VACCINATION OF HPAI**

**Preventive vaccination**

2. Reinforced surveillance to be implemented in case of preventive vaccination:

2.1. enhanced passive surveillance shall be implemented in the vaccinated establishments by weekly virological testing of a representative sample of dead birds collected within one week;

16

16

# Burden of testing when vaccination is applied (Europe)

REGULATIONS

COMMISSION DELEGATED REGULATION (EU) 2023/361

of 28 November 2022

supplementing Regulation (EU) 2016/429 of the European Parliament and the Council as regards rules for the use of certain veterinary medicinal products for the purpose of prevention and control of certain listed diseases

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

PART 5

SPECIFIC CONDITIONS FOR PREVENTIVE VACCINATION OF HPAI

**Preventive vaccination**

2.2. after the start of vaccination, the following active surveillance has to be carried out by an official veterinarian in vaccinated establishments at least every 30 days to detect occurrence of infection with HPAI field virus:

(b) a collection of representative samples for laboratory surveillance by serological or virological testing to enable detection of a prevalence of HPAI virus infection in the epidemiological unit of 5 % with a confidence level of 95 %, using appropriate methods and protocols that allow early detection of the virus and taking into account the specific characteristics of the vaccine used;

17

17

# Example of preventive vaccination (Europe)

**Densely Populated Poultry Areas**

3 provinces in Italy

PART 5

SPECIFIC CONDITIONS FOR PREVENTIVE VACCINATION OF HPAI

2.2. after the start of vaccination, the following active surveillance has to be carried out by an official veterinarian in vaccinated establishments at least every 30 days to detect occurrence of infection with HPAI field virus:

(b) a collection of representative samples for laboratory surveillance by serological or virological testing to enable detection of a prevalence of HPAI virus infection in the epidemiological unit of 5 % with a confidence level of 95 %, using appropriate methods and protocols that allow early detection of the virus and taking into account the specific characteristics of the vaccine used;

| Regione | Province | Production type | Number of Holdings | Number of animals |
|---|---|---|---|---|
| A | 1 | Layer breeders | 10 | 227.620 |
| | | Breeder pullets | 4 | 158.000 |
| | | Laying pullets | 14 | 631.100 |
| | | Meat turkeys | 45 | 988.483 |
| | 2 | Layer breeders | 1 | 39.500 |
| | | Laying pullets | 6 | 739.086 |
| | | Meat turkeys | 30 | 528.312 |
| B | 3 | Layer breeders | 3 | 70.000 |
| | | Breeder pullets | 3 | 300.600 |
| | | Laying pullets | 30 | 1.862.605 |
| | | Meat turkeys | 199 | 4.174.615 |
| **Cumulative number** | | | **345** | **9.719.921** |

5% prevalence (95% CI)

**20,000 samples to be tested on a monthly basis**

**(plus weekly testing of carcasses)**

**Challenges:**

- Sampling capacity
- Diagnostic capacity

18

18

# Avian Influenza Matching (AIM) Pilot

## Scope and objectives

- Vaccination against high pathogenicity avian influenza is already being used in countries where viruses are endemic.
- Inactivated vaccines are economically cost effective so expected to be widely used in the region.
- Antigenic heterogeneity and drift can potentially reduce the effectiveness of vaccines in protecting against disease and shedding of virus from infected birds.
- Providing information to stakeholders on the antigenic characteristics of currently circulating avian influenza viruses can be used to facilitate the selection of appropriate vaccines for poultry and used in conjunction with other information.
- Need for real-time monitoring early warning updates to vaccine seed strains

19

19

# Avian Influenza Matching (AIM) Pilot

**Sera**
- Generation of a panel of standardised poultry sera - **December 2022**
- Isolates *similar* to vaccine seed strains and H5 clade 2.3.4.4b

**HI**
- Selection of representative contemporary viruses – **February 2023**
- Harmonised antigenic characterisation

**Cartography**
- Antigenic characterisation mapped using cartography **– March 2023**

**Report**
- Summary report presenting the antigenic diversity of currently circulating H5 viruses
- Shared with stakeholders **- April 2023**

20

20

# Avian Influenza Matching (AIM) Pilot

**Barriers**

- Timely reporting of outbreak events
- Surveillance
- Sequencing information
- Sharing of viral isolates

**Solutions**

- Strengthening networks such as OFFLU
- Improving surveillance and genomic sequencing capacity

21

21

## Avian Influenza Matching (AIM) Pilot

The Animal and Plant Health Agency (APHA, UK) and Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe, Italy) are generating a panel of poultry sera and homologous antigens. This will serve as an OFFLU standard for the antigenic characterization of currently circulating avian influenza viruses. This will allow for the assessment of avian influenza virus evolution, between OFFLU laboratories in a harmonised way. Outputs will be antigenically mapped by the Royal Veterinary College (RVC, London) using cartography. Reference sera panels will be shared with partners to expand geographical representation. Results will allow for continuous monitoring of antigenic changes in currently circulating viruses and inform the continuous expansion and updating of sera panels.

Food and Agriculture Organization of the United Nations

World Organisation for Animal Health
Founded as OIE

22

22

# Thank you for your attention

With thanks to Francesco Bonefante, David Suarez, Amelia Coggon, Guillermo Zavila and Les Sims for contributions to the presentation

The OFFLU website has regular updates on OFFLU and parent organisations' publications, technical advice, protocols and many other useful links. Please visit: www.offlu.org for more information

For any questions please contact: secretariat@offlu.org