

Guidelines for the Administration of Nobilis Influenza H5 Vaccine as Part of an Avian Influenza Control Strategy

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1. Introduction

The current epizootic of highly pathogenic avian influenza (HPAI), subtype H5N1, which started in Asia end 2003 and has since spread towards the west into Russia, Turkey and Romania has raised international concern. The ever increasing number of cases reported of people infected by this avian influenza strain (Vietnam, Thailand and Indonesia), often with fatal consequences, has raised concerns that this could trigger the start of a human influenza pandemic, thus calling for prompt control measures.

Traditional control measures for HPAI have centred on stamping out, which entails the large scale culling of infected flocks and contact flocks. This policy has in the past proven effective however the high concentration of poultry found in certain areas leads to the culling of millions of animals at great expense.

Vaccination against AI has proven to be a successful additional control measure implemented alongside controlled culling (Italy (H7N1 & H7N3), Mexico (H5N2), Pakistan (H7N3), Hong Kong (H5N1), Vietnam (H5N1) and Indonesia (H5N1)). In 2004 the FAO, OIE and WHO issued a joint statement that called for a targeted strategy, including poultry vaccination, to help curb AI in Asia.

The expected advantages of a vaccination policy are two fold. Firstly vaccination reduces susceptibility to infection, a higher dose of virus is necessary for establishing an infection in vaccinated birds. Secondly there is a significant reduction in the amount of virus shed by infected birds, thus less virus to contaminate the environment reducing the risk of spread to other avian species and reducing the occupational risk faced by poultry workers. (Reducing the incidence of human infections reduces the chance of the AI strain mutating and the possible emergence of a new human influenza strain.) Reducing the amount of avian virus allowed to multiply in poultry also reduces the likelihood of mutations caused by random errors in transcription.

However, we hasten to caution that vaccination is not intended to replace stamping out policies. The primary goal remains eradication of AI. Vaccination, by increasing the infection threshold and decreasing virus shedding, is a valuable aid in the eradication of AI infection when combined with appropriate control measures.

The following document provides a guideline to the effective implementation of an AI eradication policy using an inactivated influenza vaccine, Nobilis Influenza H5.

2. Inactivated Avian Influenza Vaccines

Influenza virus is grown in embryonated eggs and is chemically inactivated. Subsequently the inactivated viral antigen is formulated with an oil emulsion adjuvant to enhance the immune response. Vaccine efficacy is dependant on the vaccine antigen and field virus being of the same H type (homologous haemagglutinin).

Two options exist when selecting an inactivated vaccine:

- a. *Inactivated homologous vaccines:* These are generally autogenous vaccines prepared from the field strain. Efficacy of homologous vaccines has been proven, however the disadvantage is that no serological distinction can be made between vaccinated and field exposed birds.
- b. *Inactivated heterologous vaccines:* These vaccines are prepared from a virus with the same H type as the field strain but a different N type (heterologous neuramidase). The immune response to the homologous H type ensures protection, while antibodies against the neuramidase of the field virus can be used as a marker.

Selecting an inactivated heterologous vaccine enables the application of a 'DIVA' (Differentiating Infected from Vaccinated Animals) strategy to demonstrate that the field virus is no longer circulating in a vaccinated poultry population, a prerequisite for the lifting of trade bans.

(The current highly pathogenic avian influenza epizootic affecting a number of Asian countries is caused by a subtype H5N1 avian influenza strain. Intervet's Nobilis Influenza H5 vaccine has as its active component the inactivated low pathogenic Mexican avian influenza strain, subtype H5N2 (A/chicken/Mexico/232/94/CPA). Nobilis Influenza H5 is thus a suitable candidate heterologous vaccine. It has been shown to afford protection against H5N1 viruses in the region)

3. Vaccination Schedule for Nobilis Influenza H5*

- a. *Dosage:* 0.5 ml per dose in birds older than 3 weeks of age, 0.25 ml per dose in younger birds.
- b. *Administration:* subcutaneously in the lower back of the neck or intramuscularly in older birds.
- c. *Emergency Vaccination Schedule:*
Primary vaccination administered to all poultry irrespective of age.
Booster vaccination administered 4 – 6 weeks later.
(If the primary vaccination was administered to birds younger than 3 weeks of age a third vaccination is recommended at 16 – 18 weeks of age)
- d. *Vaccination of Replacement Flocks:*
Vaccination schedule is dependant on perceived risk of infection.
In high risk areas (active infection) primary vaccination (0.25 ml) is recommended at day old to establish immunity as early as possible.
Two booster vaccinations (0.5ml) are recommended at 4 – 6 and 16 – 18 weeks of age.
In areas with high infection pressure revaccination at midlay may be indicated.

* The vaccination schedule for Nobilis Influenza H5, which is mentioned by Intervet in product related information, is based on existing registrations. However in the light of recent experience with vaccination, adaptation seems advisable from an international point of view. Under all circumstances the vaccination schedule implemented has to comply with the vaccination program of the national regulatory authorities

In low risk areas, or where poultry flocks are reared in an AI free zone with the intention of future transfer into an AI positive zone primary vaccination can be delayed to a later age.

Primary vaccination (0.5ml) administered at 4 weeks of age and booster vaccination (0.5 ml) administered at 16 – 18 weeks of age.

e. *Vaccination of Broilers:*

Vaccination of broiler type chickens that are slaughtered within 7 – 8 weeks is in principle discouraged, as there is not sufficient time to develop adequate immunity following a primer and booster vaccination. However in situations where live bird trade predominates and meat chickens are raised for longer periods this may be reconsidered. (In Hong Kong local meat type chickens are vaccinated at 8 and 36 days of age.)

4. Vaccination of Poultry in a Defined Zone

To successfully eradicate AI, vaccination must be implemented together with strict surveillance and biosecurity measures. This is best achieved if well defined manageable zones are identified. A zone should as far as possible be self contained limiting the need to transfer poultry or poultry related products (including feed and manure) across the zone's borders.

Stamping out is the preferred control option for an outbreak of HPAI and should be used on all flocks exhibiting clinical disease. The decision to create a vaccination zone should be driven by the following key priorities.

- a. Vaccination to create a buffer zone between infected and non infected areas.
- b. Vaccination of areas free of AI but at a high risk of infection (e.g. functional connection with infected areas)
- c. Vaccination of flocks in the initial restocking of affected areas that were depopulated and disinfected.

All poultry, including backyard chickens, within a vaccination zone must be vaccinated with an approved AI vaccine. In commercial flocks 30 to 60 birds must be left unvaccinated to act as sentinels. The measures applied in the vaccination zone should where feasible include:

- a. The identification of all holdings having poultry within the zone.
- b. Clinical and serological examination of flocks prior to vaccination to verify AI free status.
- c. For at least a three week period following primary vaccination (time required to establish immunity) strict control of the movements of persons handling poultry and eggs as well as vehicles transporting eggs or poultry feed.
- d. The transport of poultry and poultry manure should be prohibited during this three week period.
- e. For detailed guidelines on the movement of poultry in a vaccination zone refer to Table 2 from the article "The Use of Vaccination as an Option for the Control of Avian Influenza" (Capua and Marangon, 2003 – Refer to [OIE website](#)).
- f. Regular serological monitoring of the sentinels. In case of HPAI infection the sentinels will most likely die within 2-3 days of infection. Thus it is important that pathology/virus testing is done on all dead sentinels.

5. Vaccination of at Risk High Value (Pedigree) Poultry Flocks

It may be deemed necessary in certain circumstances to vaccinate high value poultry flocks located outside of a vaccination zone. In such cases it is recommended that all poultry in a 3 – 5 km radius of the concerned poultry flock be vaccinated, or culled. If surrounding poultry is culled restocking should be discouraged until at least 4 weeks after the second vaccination. The same guidelines as discussed in point 4 above apply to this ‘mini’ zone.

6. Serological Monitoring

a. Tests

ELISA: Detects antibodies to all AI viruses irrespective of subtype (antibodies to nucleocapsid antigens). The ELISA is only suitable for testing turkey and chicken sera. Within one week of infection antibodies are detected in more than half of tested specimens. ELISA is of limited value when multiple serotypes of virus are circulating (e.g. H9 in Asia)

AGID: The agar gel immunodiffusion test too makes no distinction between AI subtypes. Like ELISA antibodies are detected in more than half of the specimens within one week of infection and the test is of limited value when multiple serotypes of virus are circulating (e.g. H9 in Asia).

HI: The haemagglutination inhibition test is serotype specific. Each H-subtype has an individual HI test. Positive HI titres (> 1:8) develop a few days later than seen in ELISA or AGID tests; and titres persist till long after the infection. The HI test is the standard test for all avian species.

IFT: An ‘*ad hoc*’ immunofluorescence test has been developed and used to detect antibodies to a specific N-subtype. This technique was employed in Italy as part of the ‘DIVA’ strategy. An IFT was developed to detect antibodies to the specific N-subtype (neuramidase) of the circulating field virus. An AI virus with a different N-subtype was used in the vaccine, thus enabling differentiation between vaccinated and infected flocks.

b. Monitoring Efficacy of Vaccination

- Assessment of vaccination should be done by HI test one month after the second vaccination.
- Test 10 - 20 serum samples per flock.
- Require an HI titre greater than 1:16 in more than 70% of tested samples (Guidelines set by Agriculture, Fisheries and Conservation Department, Hong Kong).

c. Monitoring for Virus Circulation in Vaccinated Flocks

- Thirty to sixty clearly identified sentinels (chickens left unvaccinated) must be placed in each house.
- Ten to twenty serum samples collected from sentinels should be tested every 30 – 45 days (ELISA or HI).
- If the sentinels seroconvert the flock is considered AI positive. However, in case of HPAI infection the sentinels will most likely die within 2-3 days of infection. Thus it is important that pathology/virus testing is done on all dead sentinels.
- Alternatively, if suitable testing/laboratory facilities are available and a heterologous AI vaccine has been used serological testing can be performed in accordance with the 'DIVA' strategy.
- A definite diagnosis requires virus isolation and identification by standard virus culture techniques on embryonated eggs or alternatively using RT-PCR techniques. If HPAI is suspected virus isolation work should preferably be done in a suitably equipped laboratory and all necessary precautions must be taken to prevent exposure of laboratory personal to the virus.

7. Recommendations in Case of Confirmed AI Outbreak within a Vaccination Zone

Confirmation of an AI infection in a vaccinated flock (mortality or seroconversion of sentinels) should be dealt with as per standard stamping out procedures. Such procedures should include:

- Immediate quarantine of infected site.
- Depopulation of flock, preferably with disposal of the birds on site (burial).
- Correct disposal of litter, preferably on site to limit the risk of spreading virus. Options include burial or piled in heaps to ensure maturation. The heap must be covered with a resistant sheet of plastic (Virus can persist in wet manure for up to 4 months).
- Where applicable products such as eggs, egg-trays, animal feed, etc. must be suitably destroyed.
- A zone with immediate increased surveillance for AI (radius of 3 – 5 km) should be established around the infected site. Sudden mortality of sentinels should be considered highly suspicious and thoroughly investigated.
- The movement of poultry and poultry related products should be prohibited in this zone for at least 21 days after carrying out the depopulation preliminary cleaning and disinfection of the infected site.

8. Useful references

For more detailed information on measures for the control of AI we refer to the following websites:

OIE – Avian influenza: methods for the disease control

http://www.oie.int/eng/info/en_avinf.htm

This is the portal to OIE information on AI and control measures. Information includes links to relevant current documents categorised according to among others the following headings:

- a. Background information: Up to date statements regarding the current AI pandemic in Asia. Included is a link to:
 - The conclusions and recommendations of the FAO/OIE/WHO expert meeting held in Rome from the 3 – 4 February 2004.
 - The use of vaccination as an option for the control of avian influenza (Ilaria Capua & Stefano Marangon).
- b. Safety of International Trade: Links to the actual and proposed OIE Chapters on Avian Influenza
- c. Vaccines and Diagnostic Methods: Links to documents discussing sampling and diagnostic techniques.
- d. Methods of Humane Killing and Carcass Disposal: Links to documents with guidelines on the topic.
- e. Guidelines for Control of the Disease: Link to the IZSV (Italian) Contingency Manual for Avian Influenza.
- f. Food Safety: Risks for consumers

European Union – Control measures: avian influenza

<http://europa.eu.int/scadplus/leg/en/lvb/l12020.htm>

This is the portal to EU regulations on Avian Influenza with a link to Council Directive 92/40/EEC on measures for the control of AI.

Avian Influenza.com

<http://www.avian-influenza.com>

AI information website hosted and maintained by Intervet International.