

# Vaccination for notifiable avian influenza in poultry

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## Summary

Notifiable avian influenza (NAI) is a listed disease of the World Organisation for Animal Health (OIE) that has become a disease of great importance both for animal and human health. Prior to 2000, vaccination against NAI was discouraged and used to aid control of only a limited number of outbreaks, without reaching the goal of eradication. Pivotal work on the application of a vaccination programme aimed at, and resulting in, eradication was carried out in Italy, and was followed by other research, e.g. in Hong Kong and the United States of America. Given the spread of Asian lineage highly pathogenic avian influenza (HPAI) H5N1 to three continents, vaccination is now being used on a wide scale under different conditions, which in most cases are not ideal. Although in some countries, a lack of infrastructure and resources can greatly limit the overall success of control programmes that encompass vaccination, it is imperative that international organisations set guidelines to 'accredit' control strategies. These guidelines should include recommendations on seed strains to be used in vaccine preparations, the characteristics of the vaccine, the most appropriate field strategy to apply in the different phases of a control/eradication programme, and models of exit strategies. The availability of harmonised protocols would greatly facilitate the achievement of tangible results and would save time and avoid unnecessary wastage of resources.

## Keywords

Avian influenza – Biosecurity – Control – Epidemiology – Eradication – Monitoring – Stamping out – Vaccination.

## Introduction

Avian influenza (AI) is one of the greatest public health concerns to have emerged from the animal reservoir in recent times. Over the past five years there has been a sharp increase in the number of outbreaks of AI in poultry. It has been calculated that the impact of AI on the poultry industry has increased 100-fold, with 23 million birds affected in the forty year period between 1959 and 1998 and over 200 million from 1999 to 2006 (2). In fact, from the late 1990s, AI infections have assumed a completely different profile both in the veterinary and medical

scientific communities. In recent times some outbreaks have continued to be of only minor relevance while others, such as the ongoing Eurasian-African H5N1 epidemic and outbreaks that occurred in Italy (1999-2000), the Netherlands (2003) and Canada (2004) have led to devastating consequences for the poultry industry, negative repercussions on public opinion and in some cases have created significant human health issues, including the risk of generating a new pandemic virus for humans via the avian-human link.

The increased relevance of AI in the fields of animal and human health has highlighted the lack of scientific

information on several aspects of the disease. This has hampered the adequate management of some of the recent crises, thus resulting in millions of dead animals and concern over loss of human lives and over management of the pandemic potential.

Before 1999, AI did not have a high-profile, so the information and the specific tools necessary to manage AI epidemics adequately were not developed. Moreover, until recent times vaccination for avian influenza in its notifiable form had always been discouraged. As a result of this, studies and investments in the field of AI vaccinology have not been a priority in the past, and currently we are facing an emergency with only limited resources and experience.

## Definition of avian influenza

The revised chapter on AI in the World Organisation for Animal Health (OIE) *Terrestrial Animal Health Code (Terrestrial Code)*, which came into force in January 2006 (44), is the first document that approaches AI in a more modern manner, taking into account the new scientific data that is available on this disease, and making use of it for regulating trade. This revised definition logically follows the scientific evidence that low pathogenic avian influenza (LPAI) viruses of H5 and H7 subtypes are the progenitors of highly pathogenic avian influenza (HPAI) 1998 (19, 25, 29); recognising this is crucial for prevention and control of future outbreaks. In addition, the provisions in the revised chapter aim to limit the circulation of AI viruses, as this is one of the primary risk factors for the generation of reassortant viruses. The latter occurrence could represent the basis for the initiation of a human pandemic.

It is therefore imperative that official veterinary services identify surveillance and early detection measures for AI in poultry as a priority, and manage LPAI outbreaks caused by viruses of the H5 and H7 subtypes in an appropriate manner.

The definition adopted by the OIE during its 73rd General Session is as follows (44):

‘For the purposes of this *Terrestrial Code*, avian influenza in its notifiable form (NAI) is defined as an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described below. NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) and low pathogenicity notifiable avian influenza (LPNAI):

– HPNAI viruses have an IVPI in 6-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in 4- to 8-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the precursor haemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other HPNAI isolates, the isolate being tested should be considered as HPNAI;

– LPNAI are all influenza A viruses of H5 and H7 subtype that are not HPNAI viruses’.

The term low pathogenicity avian influenza (LPAI) is then used to define all infections caused by AI viruses that are not NAI viruses. Following the application of this revised definition there are significant changes in the notification obligations and in trading regulations with reference to AI. The main difference compared to the past is that in order to trade, countries/zones/compartments must demonstrate freedom from NAI infection. In the past, when only HPNAI was notifiable, freedom from infection relied primarily on the absence of clinical cases. With LPNAI being included in the definition, it follows that it is not possible to rely on clinical evidence only, but that freedom must be demonstrated through appropriate surveillance programmes.

The revision of the definition of AI has thus resulted in modified trade requirements, as these now also apply to LPAI of H5 and H7 subtypes (44).

Another major change in the OIE *Terrestrial Code* is that it introduces the concept of allowing the use of vaccination in avian populations whose commodities are destined for international trade. In fact, ‘infection’ is defined as:

‘The following defines the occurrence of infection with NAI virus:

a) HPNAI virus has been isolated and identified as such or viral RNA specific for HPNAI has been detected in poultry or a product derived from poultry; or

b) LPNAI virus has been isolated and identified as such or viral RNA specific for LPNAI has been detected in poultry or a product derived from poultry; or

c) antibodies to H5 or H7 subtype of NAI virus that **are not a consequence of vaccination have been detected in poultry** [author’s emphasis]. In the case of isolated serological positive results, NAI infection may be ruled out on the basis of a thorough epidemiological investigation

that does not demonstrate further evidence of NAI infection.'

When these two articles are combined, it appears that although infection with any virus of the H5 and H7 subtype, or any other virus fulfilling the virulence criteria must be notified, the presence of antibodies as a result of vaccination does not limit international trade in the commodities originating from those birds. With this in mind, the use of vaccination for NAI viruses can be considered in a wider range of circumstances.

In this paper, vaccination for NAI viruses of the H5 and H7 subtypes will be addressed with particular reference to the field experiences gathered to date.

## The use of vaccination

### Currently available vaccines

At the time of writing there are two different types of vaccines available on the market: inactivated vaccines based on adjuvanted whole virions and live recombinant vaccines. The former can be prepared using master seed viruses isolated from natural outbreaks or generated by reverse genetics. Recombinant vector-based vaccines are engineered products, based on a vector virus expressing the haemagglutinin (H) protein of avian influenza. Only a fowlpox vectored vaccine and a Newcastle disease virus (NDV) vaccine are commercially available.

### Rationale behind the use of vaccination for control and eradication purposes

It is proven that any product containing an antigenic fraction derived from the haemagglutinin of AI viruses is cross-protective against challenge, provided the H component of the vaccine is of the same subtype as the H component of the challenge virus, e.g. any H5 haemagglutinin will protect against any H5 challenge virus, even if they belong to different lineages (30).

In order to fully understand the potential use of vaccination to combat AI infections, the term cross-protection should be explained. Vaccines defined as cross-protective are those that prevent the onset of clinical signs. In order to achieve a reduction or prevention of clinical symptoms, viral replication in the host must be reduced. However, in most cases, a certain degree of viral replication occurs even in the vaccinated host, and the extent of this replication is assessed and measured by evaluating shedding (42). The latter is achieved in the laboratory by collecting tracheal and cloacal swabs at fixed day intervals and the amount of viable virus eliminated by natural routes

into the environment is assessed by means of appropriate tests such as the inoculation of specific-pathogen-free embryos. Most vaccines prevent clinical signs and mortality and significantly reduce, but do not completely suppress, shedding. In experimental trials only a limited number of vaccine candidates have completely suppressed shedding (34). Another effect of vaccination is that it increases the resistance to challenge with infectious virus. For example, in one experiment, naïve turkeys were susceptible to an infectious dose containing  $10^4$  EID<sub>50</sub> (50% embryo infective dose) of virus, while vaccinated birds were resistant to this infectious dose and susceptible to a challenge suspension containing  $10^6$  EID<sub>50</sub> (6).

It appears, therefore, that if vaccinated birds are placed in the field and they are subsequently challenged by a field virus of the same subtype, they will be more resistant to infection and shed less infectious virus. If the farms at risk of exposure are also vaccinated, it is possible that the infectious cycle may be blocked because not enough virus is shed in the environment to infect vaccinated birds, as the latter are more resistant to challenge. This has been shown experimentally (37) and in the field (10).

However, in order to exploit the advantages of vaccination, it is imperative that vaccination is carried out in an adequate manner (healthy population, full dose, and appropriately stored and administered product) in the framework of a control programme. Such a programme must be based on the application of biosecurity and restriction measures developed to prevent spread from a vaccinated/exposed to an unexposed flock. The failure to implement such a system may result in the circulation of virus in a vaccinated population, with the subsequent undesirable consequences. The most worrisome is probably the emergence of antigenic drift within the viral population. Antigenic drift is a well-known effect of extensive immunological pressure on antigenic properties of influenza viruses. It has been reported for mammalian (human, equine and swine) influenza viruses, but to date, there has only been one instance for avian influenza viruses (21). The result of such occurrence is an antigenic modification of the H protein, which 'escapes' the immune response generated by the vaccine strain. For this reason, influenza vaccines designed for mammalian viruses need to be frequently updated to contain a new H component, more similar to the drifted field virus.

There is no logical reason to exclude the possibility that antigenic drift for avian influenza viruses could occur under the above-mentioned circumstances. The consequences of the emergence of multiple antigenic variants from extensive immunological pressure, generated by a variety of different vaccines are unpredictable, but certainly inauspicious.

## Emergency vaccination

### Examples of emergency vaccination for low pathogenicity notifiable avian influenza viruses of the H5 and H7 subtypes

#### Mexico

An LPNAI virus of the H5N2 subtype was first diagnosed in Mexican poultry in May 1994. This virus mutated to a highly pathogenic variant in December of the same year in two different states, Puebla and Querétaro. Both HPNAI outbreaks were extinguished by stamping out (40). Despite the application of this control policy the LP virus continued to circulate in the country and a vaccination programme under official control was implemented. The programme consisted of improved biosecurity, monitoring and vaccination. Two types of vaccines, an oil emulsion inactivated vaccine and a fowlpox recombinant vaccine were licensed for use (40). Monitoring of field exposure within the vaccinated population was not a part of the strategy. Most probably as a result of this, infection is still present eleven years after the implementation of the vaccination campaign (40). The extensive use of vaccination has generated – for the first time with reference to avian influenza – an antigenic drift. A study by Lee *et al.*, 2004 (21) indicated a reduced antigenic cross-reactivity between the vaccine seed and the currently circulating field viruses.

#### Italy

Between 1999 and 2001 Italy was affected by four successive epidemic waves of AI caused by viruses of the H7N1 subtype. The first epidemic wave was caused by an LPAI virus of H7N1 subtype that subsequently mutated into an HPAI virus after having circulated in the industrial poultry population for approximately nine months. Following the emergence of the HPAI virus, a complete depopulation of the infected area was carried out in accordance with the measures indicated in Council Directive 92/40/CE (12). Approximately 16 million birds either died or were culled. Emergency vaccination was not applied during this epidemic. Four months later, in late summer, the H7N1 LPAI virus re-emerged following the repopulation of poultry farms (4).

From August to November 2000, the H7N1 LPAI strain infected 51 meat-type turkey farms, and 4 quail farms. All birds were stamped out. In order to intervene against a possible re-emergence of the H7N1 LPAI virus, a coordinated set of measures, including strict biosecurity, a serological monitoring programme and a DIVA (Differentiating Infected from Vaccinated Animals) vaccination strategy were enforced. The DIVA strategy, which was implemented as from December 2000, was

based on the use of an inactivated oil emulsion vaccine containing the same H subtype as the field virus, but a different N subtype, in this case an H7N3 strain. A serological test based on the detection of specific anti-N1 antibodies was developed as a way of using the diverse N group to differentiate between vaccinated and naturally infected birds (5).

The control of the field situation was ensured through an intensive serosurveillance programme aimed at the detection of the LPAI virus, through the regular testing of sentinel birds in vaccinated flocks and through the application of the anti-N1 antibody detection test. Serological monitoring was also enforced in unvaccinated flocks, located both inside and outside the vaccination area. In addition, the efficacy of the vaccination schemes was evaluated in the field through regular testing of selected flocks. The vaccination programme did not include broilers, but only longer-living birds such as meat turkeys and layers and a very limited number of chicken and turkey breeders.

Notwithstanding the depopulation of the infected premises, the serological monitoring programme revealed an additional incursion of the LPAI H7N1 virus shortly after the beginning of the vaccination programme (December 2000 to March 2001). The H7N1 LPAI virus infected 3 meat-type turkey farms in the vaccination area and 20 poultry holdings (19 turkey farms and 1 layer farm) located in a contiguous unvaccinated area. Only one vaccinated flock was affected and the virus did not spread from this to other vaccinated farms. All infected flocks were culled; the last H7N1 LPAI infected poultry flock was stamped out on the 26 March 2001. The results of the serological surveillance carried out both within and outside the vaccinated area to assess the possible presence of AI infection, demonstrated that the H7N1 AI virus strain was not circulating any longer. In addition, the DIVA discriminatory test was performed and the results were negative; consequently, on 30 November 2001, Commission Decision 2001/847/EC (13) authorised the marketing of fresh poultry meat obtained from vaccinated birds for intra-community trade. The emergency vaccination programme was discontinued in May 2002.

In 2002 and 2003 Italy once again experienced outbreaks of AI involving an H7N3 subtype influenza A virus of low pathogenicity. In October 2002, an H7N3 LPAI strain was introduced from the wild reservoir into the domestic poultry population located in the densely populated poultry area which had previously been affected by the H7N1 epidemic in 1999-2001 (2).

Since the infection rapidly spread among poultry flocks, it was decided to implement a vaccination campaign. The vaccination programme designed was based once again on a DIVA strategy and was carried out using an AI inactivated

heterologous vaccine (strain A/ck/IT/1999-H7N1), which was administered only to layers, capons and meat turkeys. The implementation of the DIVA vaccination campaign was delayed until 31 December 2002, due to unavailability of an appropriate vaccine. From 10 October 2002 to 10 October 2003, the H7N3 LPAI virus was able to spread and infect a total of 388 poultry holdings. Stamping out measures or controlled marketing were enforced in all infected flocks.

On the 13th January 2004, based on the field data generated on the reliability of the DIVA strategy in detecting any LPAI virus infected flock (7), Commission Decision 2004/159/EC (14) authorised the marketing of fresh meat and of table eggs originating from vaccinated turkeys and layers for intra-community trade (13, 14).

### United States of America

Although vaccination has been used for many years to combat LPAI viruses (20), only very recently was it applied to combat LPNAI – and it has never been used against HPAI. In 2003, a large layer operation, consisting of three farms all owned by the same company, was infected with an H7N2 virus. This virus was phylogenetically related to the H7N2 virus which had been circulating in the live bird market system in the United States of America (USA) and that had spilled over to the industrial poultry rearing systems on more than one occasion. Given the size of the outbreak, it was decided to vaccinate the animals, rather than apply a stamping out policy (28).

Both naïve and infected birds were vaccinated with two doses of a conventional inactivated vaccine containing a seed virus of the same subtype as the field virus. The reason for this choice was the degree of homology with the field virus and the immediate availability of 3.2 million doses. Subsequently, a vaccine containing an H7N3 strain was used. In order to complement vaccination efforts biosecurity was upgraded and the farmer agreed to comply with certain rules laid down by the health authorities. In order to monitor the evolution of infection, individually identified sentinels were introduced on to the premises and tested serologically every two weeks, and virus isolation or reverse transcription polymerase chain reaction (RT-PCR) was performed daily on the animals that died. Once the vaccination process began, there was no evidence of further viral circulation in the flock. H7N2 was eradicated and the effort was considered a success (28).

### Examples of emergency vaccination for highly pathogenic avian influenza

#### Pakistan

Since 1995 Pakistan has suffered several incursions of H7N3 viruses, and recently of H5N1. In 1995 an HPAI

virus was introduced in the northern part of the country, primarily affecting the broiler industry, and an LPNAI virus of the same subtype affected the same area in 2000. Both infections were controlled, although not eradicated, through the application of biosecurity measures and the implementation of a vaccination programme based on an inactivated preparation containing a field isolate (24).

In 2003 and 2004 the southern part of the country, which specialises in the layer industry, was affected by a novel incursion of an LPNAI H7N3 that subsequently mutated to HPNAI H7N3. It appears that an LPAI virus of the H9N2 subtype was also co-circulating at the time. Infection rapidly spread throughout the area causing severe economic damage to the poultry industry.

A control policy, based on voluntary depopulation (without compensation), establishment of a vaccination policy and active surveillance, was implemented by the veterinary authorities. Two months after the peak mortality in the field, the severity and incidence of the disease started to decline. The vaccination campaign covered 80% of the commercial layers and was extended to the breeding stock in the north of the country.

The surveillance efforts showed a reduced circulation of the H7N3 HPAI virus following the implementation of the national control programme (24).

#### Hong Kong

In 1997 in Hong Kong, a severe outbreak of HPAI H5N1 in poultry spilled over to human beings, infecting 18 people and causing the death of 6 of them. This outbreak was controlled by stamping out approximately 1.3 million poultry. Despite the implementation of restriction measures on the introduction of waterfowl into the territories, further H5N1 outbreaks occurred in chickens and other birds between 2001 and 2003 (11).

Between February 2002 and March 2003, a field trial including vaccination, biosecurity and monitoring was implemented in the Pak Sha district of the Northern Territories, considered at high risk of exposure. The trial was carried out using a heterologous H5N2 virus, included 22 chicken farms and was based on a double vaccine administration (with 4 weeks between the first and second shot) and restocking with vaccinated birds. Thirty unvaccinated birds were left in each farm as sentinels. Surveillance was based on serology and on virus detection in sentinels or dead birds using RT-PCR.

The control programme was extended to 53 farms in December 2002 and during an outbreak between December 2002 and January 2003 vaccination was used to interrupt transmission. In June 2003, the Administration

approved a universal vaccination programme of chickens entering the live-bird market system.

Despite the high infectious environmental pressure in Southeast Asia, and the significant numbers of introductions of live poultry from the mainland, since the implementation of the first vaccination trial no H5N1 virus has been detected in the vaccinated populations. Similarly, no active circulation of H5N1 was detected following the extension of the vaccination programme, and the attempt to interrupt transmission was successful. The vaccination programme will continue to be implemented as long as the high-risk of introduction of H5N1 remains.

### Other Asian countries

Several Asian countries are combating avian influenza infection with the aid of vaccination. These include China, Indonesia and Vietnam – which have implemented official programmes encompassing vaccination. Although there is some information on the products that are being used in these campaigns details of the precise conditions under which these products are used are currently unavailable (27).

Certainly the infrastructure and resources necessary to manage a vaccination campaign, following guidelines which have been successfully used in developed countries, are mostly unavailable in these countries. In addition, the logistics of implementing a harmonised programme in a big and populated country such as China, which rears over five billion poultry (27), or in Indonesia where the cultural diversity and various different languages represent an additional problem to the logistics of organising a campaign across approximately 17,000 islands, cannot even be compared to the difficulties that may be encountered in the European Union (EU) or in the USA.

However, one substantiated piece of encouraging information concerning public health has emerged following the implementation of a 'blanket' vaccination campaign in Vietnam: as a result of mass vaccination in poultry, no human cases have been detected since December 2005 (43).

## Prophylactic vaccination for notifiable avian influenza

Prophylactic vaccination for viruses of the H5 and H7 subtypes is a completely innovative concept. This is primarily due to the fact that it is only recently that situations for which this policy may be cost-effective have been pinpointed and identified.

The rationale behind the use of prophylactic vaccination is that it should be able to generate a level of protective immunity in the target population. The immune response may be boosted if there is evidence of the introduction of a field virus.

Prophylactic vaccination should increase the resistance of birds and, in case of virus introduction, reduce levels of viral shedding. It should be perceived as an additional tool to maximise biosecurity measures when a high risk of exposure exists. Ultimately, it should result in preventing the index case, or alternatively in reducing the number of secondary outbreaks, thus minimising the negative aspects of animal welfare and the potential economic losses in areas where the density of the poultry population would otherwise result in uncontrollable spread unless there was pre-emptive culling.

Prophylactic vaccination should only be considered when there is circumstantial evidence that a country/area/compartment is at risk of infection. Risk of infection may be subdivided into two categories:

- a) risk of infection with an unknown subtype, either H5 or H7, (e.g. from migratory birds)
- b) risk of infection with a known subtype (e.g. H5N1, which is present in Asian and African countries, or subtypes that are known to circulate in live bird markets in the USA).

In the first case, a bivalent (H5 and H7) vaccination programme could be implemented. In the second case, a monovalent (either H5 or H7) programme would be sufficient.

The choice of the vaccine is crucial to the outcome of prophylactic vaccination campaigns. Ideally, vaccines that do not interfere with diagnosis in the case of field exposure with any AI virus should be used and vaccination should be carried out as long as the risk of infection exists; some countries are using it in a targeted manner in the face of an increased risk of H5N1 introduction. Appropriate and detailed exit strategies should be formulated before prophylactic vaccination is undertaken.

What appear to be lacking in some situations are guidelines that define an appropriate approach to field surveillance. These guidelines may be derived from general guidelines on surveillance for epizootic diseases, but must be adapted to the local situations and must be targeted towards a well-defined and pursuable objective. In addition, due to recent exposure of a vast variety of avian species to HPAI, it is imperative that specific research programmes are developed to evaluate the efficacy of vaccination in these species and to develop and validate novel vaccination concepts that enable the DIVA system.

## European Union

Prophylactic vaccination has been recently approved in several countries in the EU, and its application is now foreseen in EU Directive 2005/94/EC (18). Italy started a prophylactic vaccination programme in October 2004 following the EU Commission Decision on introducing vaccination to supplement control measures to control LPAI in Italy (15). At risk categories in an area at high risk of exposure were vaccinated with a bivalent (H5/H7) inactivated vaccine containing seed strains supplied by the Italian National Reference Laboratory. The vaccination programme was part of a wider control strategy encompassing monitoring, restriction and biosecurity.

In 2006, the Netherlands and France were also authorised to implement a prophylactic vaccination programme to combat the possible introduction of HPAI H5N1 from the wild bird reservoir (16, 17). The Dutch programme was developed as a voluntary effort concerning hobby flocks and free-range farms. Vaccinated farms or flocks were monitored for field exposure under official control. The French programme was implemented in free-range duck and geese farms in defined areas at risk and was coupled with a monitoring effort. At the time of writing, monitoring efforts in all three countries have been able to exclude the circulation of any HPNAI.

## Conclusions

The epidemiology of AI infections has changed over the last decade due to the persistence and the continuous spread of HPAI H5N1 in domestic poultry populations in vast geographical areas of three continents, and to the marked increase of AI outbreaks worldwide. Furthermore, H5N1 has been able to spread to previously unaffected areas and its recent isolation from apparently healthy migratory birds in southern China suggests that they can contribute to virus transmission over long distances during migration (8). This unprecedented situation could be the result of reintroductions from the wild or of a re-emergence in domestic poultry populations worldwide, with inauspicious and unpredictable consequences.

To combat this global threat, the international veterinary community has designed a strategy and identified a set of coordinated measures that can be enforced to prevent, control and eventually eradicate AI infections (3, 45, 46).

The application of the different control options, which may include vaccination, should be used in different ways on the basis of the characteristics of the poultry producing sector in its entirety, the eco-epidemiological situation, the response capacity of the veterinary infrastructure and the

availability of adequate resources. Previous experiences have indicated that in order to succeed in controlling and ultimately eradicating the infection, vaccination programmes must be part of a wider field strategy that includes ongoing monitoring and feedback inputs as the situation evolves. Essentially, this strategy must include monitoring the evolution of infection (DIVA approach), early detection of any possible outbreaks, and enforcement of adequate biosecurity, restriction and eradication measures. Whenever such a strategy cannot be implemented, the establishment of an endemic status due to sub-clinical virus circulation in the vaccinated poultry population cannot be ruled out.

Research in the field of AI vaccinology has greatly increased in the last few years with a variety of novel preparations ranging from recombinants (e.g. vaccines for fowlpox virus, NDV and infectious laryngotracheitis virus) (1, 9, 23, 26, 31, 32, 33, 36, 38, 39, 41) to products based on reverse genetics (22, 35) that are being developed to complement existing conventional inactivated products. Most of these have undergone experimental evaluation and some of them appear to perform very well, and will certainly be valid options in the future. However, the concerns regarding vaccinating against AI should not be limited to the vaccine itself, as there are other aspects, which must be taken into account and that are crucial to the success of a vaccination programme both in the short and in the long term.

One issue which will certainly be a concern in the coming years will be the occurrence and extent of antigenic drift in avian influenza viruses. At the time of writing this occurrence has been identified with certainty only in the Mexican lineage of H5N2 viruses, following a 10-year vaccination programme with two different vaccines containing diverse haemagglutinin proteins (21). The antibody response to the plethora of seed viruses that are currently being proposed in vaccines will certainly impact the antigenicity of circulating viruses and should be closely monitored. Under these conditions it would seem appropriate that only a very limited number of the most cross-protective AI strains be selected as vaccine seed strains.

As no country can consider itself not at risk for AI, it is imperative that contingency plans are prepared and discussed in advance. These contingency plans should also contain control strategies that can be applied on a country-by-country basis, bearing in mind the realistic possibilities of each country. If vaccination is considered a possible control option, vaccination programmes should be laid down in the framework of national AI contingency plans. In fact, the probability of implementing an efficacious emergency vaccination programme is mainly related to the level of preparedness and the capacity of the Veterinary Services in the affected countries or in those at risk.

Vaccination enabling the DIVA system in conjunction with a strategy that includes monitoring and appropriate management of field exposed flocks can be successful in controlling and eradicating AI infections in poultry and is compatible with the continuation of international trade. However, it is complicated to manage and may include failures such as the spread of infection within the vaccinated population. It is therefore essential that the vaccination programme be implemented using a suitable product that enables the detection of field exposed flocks and that the monitoring system in place is developed to guarantee early detection of and rapid response to AI introduction. For this reason, international organisations that govern trade regulations and animal disease control should establish a set of guidelines so that control programmes may be 'accredited' and consequently internationally recognised. Such a policy would appear to have several practical advantages which would result in improved crisis management. These include harmonisation of seed viruses to be used in vaccines, rapid approval of established control programmes, constant updating on the field situation, feedback on successes and failures, harmonisation of protocols and systems and increased public awareness of control and eradication programmes. In this way, even developing countries that are experiencing notifiable avian influenza infections – and

that have no experience with AI management – can maximise the outcome of other experiences to combat this infection in an educated manner, thus avoiding wastage of resources and time.

The harmonisation of programmes and the sharing of information would also improve our knowledge on the epidemiology of NAI infections and this would benefit our management skills, including the identification of reservoir species.

Vaccination is now being used extensively to aid the prevention of introduction or to control widespread HPAI H5N1. Overall, the veterinary community has limited first-hand experience with managing this disease aided by vaccination. It is most likely that if managed appropriately positive results will be achieved. However, errors will be made, and it is imperative that control and eradication processes can benefit from the learning process generated by such errors. In addition, control programmes need to be managed in a flexible and transparent manner to respond to the continuous challenges that AI infections pose.



## La vaccination des volailles contre l'influenza aviaire à déclaration obligatoire

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### Résumé

L'influenza aviaire à déclaration obligatoire figure sur la liste de l'Organisation mondiale de la santé animale (OIE) et revêt une importance désormais capitale tant pour la santé animale que pour la santé publique. Jusqu'en 2000, la vaccination contre l'influenza aviaire à déclaration obligatoire était déconseillée et n'a été appliquée que dans de très rares foyers, en appui aux mesures de lutte et sans objectif d'éradication. Des études décisives portant sur des programmes de vaccination visant l'éradication ont été conduites en Italie, puis dans d'autres pays tels que Hong Kong et les États-Unis d'Amérique. Compte tenu de la propagation sur trois continents de la souche asiatique H5N1 du virus de l'influenza aviaire hautement pathogène, la vaccination est maintenant appliquée à grande échelle, dans des conditions qui ne sont généralement pas idéales. En dépit de l'obstacle que constitue, dans certains pays, le manque de ressources et d'infrastructures pour la réussite d'ensemble des programmes de contrôle appliquant la vaccination, il est impératif que les

organisations internationales fixent des lignes directrices pour « accréditer » les stratégies de lutte, en prévoyant des recommandations sur les souches à utiliser pour la préparation de vaccin, sur les caractéristiques de ces vaccins, sur les meilleures stratégies à appliquer sur le terrain à chaque étape des programmes de lutte et d'éradication, et sur le modèle à suivre pour sortir de l'application des mesures. L'application concertée de protocoles harmonisés permettrait d'obtenir des résultats concrets, de gagner du temps et de rationaliser l'utilisation des ressources.

**Mots-clés**

Abattage sanitaire – Biosécurité – Épidémiologie – Éradication – Influenza aviaire – Lutte contre les maladies – Suivi – Vaccination.



## Vacunación de aves de corral contra la influenza aviar de notificación obligatoria

I. Capua

**Resumen**

La influenza aviar de notificación obligatoria es una enfermedad inscrita en la lista de la Organización Mundial de Sanidad Animal (OIE), que últimamente ha cobrado gran relieve desde el punto de vista de la salud tanto humana como animal. Antes de 2000 se desaconsejaba la vacunación, utilizada sólo esporádicamente como instrumento auxiliar de lucha contra algún brote, pero no como medio de erradicación. En Italia se llevó a cabo un trabajo de gran trascendencia sobre la aplicación de un programa de vacunaciones que perseguía el objetivo, a la postre cumplido, de erradicar la enfermedad. Después siguieron otras investigaciones en países como Hong Kong y los Estados Unidos de América. Ante la extensión a tres continentes del linaje asiático H5N1 de la influenza aviar altamente patógena, la vacunación se está utilizando ahora a gran escala en diversas circunstancias, que a menudo distan de ser idóneas. Aunque en algunos países la falta de infraestructuras y recursos pueda reducir considerablemente el éxito global de programas de lucha que incluyen la vacunación, resulta imperativo que las organizaciones internacionales establezcan pautas para 'homologar' las estrategias de control. Tales pautas deberían incluir recomendaciones sobre las cepas originales que deben usarse para preparar vacunas, las características de éstas y la estrategia que conviene aplicar sobre el terreno en las distintas fases de un programa de control o erradicación, junto con modelos para finalizar la aplicación de las medidas. La existencia de protocolos armonizados facilitaría considerablemente la obtención de resultados tangibles, ahorraría tiempo y evitaría un inútil despilfarro de recursos.

**Palabras clave**

Bioseguridad – Control – Epidemiología – Erradicación – Influenza aviar – Sacrificio sanitario – Vacunación – Vigilancia.



## References

1. Boyle D.B., Selleck P. & Heine H.G. (2000). – Vaccinating chickens against avian influenza with fowlpox recombinants expressing the H7 haemagglutinin. *Aust. vet. J.*, **78** (1), 44-48.
2. Capua I. & Alexander D.J. (2004). – Avian influenza: recent developments. *Avian Pathol.*, **33** (4), 393-404.
3. Capua I. & Alexander D.J. (2006). – The challenge of avian influenza to the veterinary community. *Avian Pathol.*, **35** (3), 189-205.
4. Capua I. & Marangon S. (2000). – The avian influenza epidemic in Italy, 1999-2000: a review. *Avian Pathol.*, **29**, 289-294.
5. Capua I., Terregino C., Cattoli G., Mutinelli F. & Rodriguez J.F. (2003). – Development of a DIVA (Differentiating Infected from Vaccinated Animals) strategy using a vaccine containing a heterologous neuraminidase for the control of avian influenza. *Avian Pathol.*, **32** (1), 47-55.
6. Capua I., Terregino C., Cattoli G. & Toffan A. (2004). – Increased resistance of vaccinated turkeys to experimental infection with an H7N3 low-pathogenicity avian influenza virus. *Avian Pathol.*, **33** (2), 158-163.
7. Cattoli G., Milani A., Bettini F., Beato M.S., Mancin M., Terregino C. & Capua I. (2006). – Development and validation of an anti-N3 indirect immunofluorescent antibody test to be used as a companion diagnostic test in the framework of a "DIVA" vaccination strategy for avian influenza infections in poultry. *Avian Pathol.*, **35** (2), 154-159.
8. Chen H., Smith G.J., Zhang S.Y., Qin K., Wang J., Li K.S., Webster R.G., Peiris J.S. & Guan Y. (2005). – Avian flu: H5N1 virus outbreak in migratory waterfowl. *Nature*, **436** (7048), 191-192.
9. De B.K., Shaw M.W., Rota P.A., Harmon M.W., Esposito J.J., Rott R., Cox N.J. & Kendal A.P. (1988). – Protection against virulent H5 avian influenza virus infection in chickens by an inactivated vaccine produced with recombinant vaccinia virus. *Vaccine*, **6** (3), 257-261.
10. Ellis T.M., Bousfield R.B., Bissett L.A., Dyrting K.C., Luk G.S., Tsim S.T., Sturm-Ramirez K., Webster R.G., Guan Y. & Malik Peiris J.S. (2004). – Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian Pathol.*, **33** (5), 492-505.
11. Ellis T.M., Sims L.D., Wong H.K., Wong C.W., Dyrting K.C., Chow K.W., Leung C. & Peiris J.S. (2006). – Use of avian influenza vaccination in Hong Kong. *In Proc. OIE/FAO International Scientific Conference on Avian Influenza. Dev. Biol. (Basel)*, **124**, 133-143.
12. European Union (1992). – Council Directive 92/40/EEC of 19 May 1992 introducing Community measures for the control of avian influenza. *Off. J. Eur. Communities*, **L167**, 1-15.
13. European Union (2001). – Commission Decision 2001/847/CE of 30 November amending for the third time Decision 2000/721/EC to modify the Italian avian influenza vaccination programme and current trade restrictions for fresh meat originating from vaccinated turkeys. *Off. J. Eur. Communities*, **L315**, 61-63.
14. European Union (2004). – Commission Decision 2004/159/EC of 16 February 2004. *Off. J. Eur. Communities*, **L50**, 63-64.
15. European Union (2004). – Commission Decision 2004/666/EC on introducing vaccination to supplement the measures to control infections with low pathogenic avian influenza in Italy and on specific movement control measures and repealing Decision 2002/975/EC. *Off. J. Eur. Communities*, **L303**, 35-44.
16. European Union (2006). – Commission Decision 2006/147/EC on introducing preventive vaccination against highly pathogenic avian influenza H5N1 and related provisions for movements in the Netherlands. *Off. J. Eur. Communities*, **L55**, 47-50.
17. European Union (2006). – Commission Decision 2006/148/EC introducing preventive vaccination against highly pathogenic avian influenza H5N1 and related provisions for movements in France. *Off. J. Eur. Communities*, **L55**, 51-54.
18. European Union (2006). – Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing 92/40/EEC. *Off. J. Eur. Communities*, **L10**, 16-65.
19. Garcia M., Crawford J.M., Latimer J.W., Rivera-Cruz E. & Perdue M.L. (1996). – Heterogeneity in the haemagglutinin gene and emergence of the highly pathogenic phenotype among recent H5N2 avian influenza viruses from Mexico. *J. gen. Virol.*, **77** (Pt 7), 1493-1504.
20. Halvorson D.A. (2002). – The control of H5 or H7 mildly pathogenic avian influenza: a role for inactivated vaccine. *Avian Pathol.*, **31** (1), 5-12.
21. Lee C.W., Senne D.A. & Suarez D.L. (2004). – Effect of vaccine use in the evolution of Mexican lineage H5N2 avian influenza virus. *J. Virol.*, **78** (15), 8372-8381.
22. Lee C.W., Senne D.A. & Suarez D.L. (2004). – Generation of reassortant influenza vaccines by reverse genetics that allows utilization of a DIVA (Differentiating Infected from Vaccinated Animals) strategy for the control of avian influenza. *Vaccine*, **22** (23-24), 3175-3181.
23. Luschow D., Werner O., Mettenleiter T.C. & Fuchs W. (2001). – Protection of chickens from lethal avian influenza A virus infection by live-virus vaccination with infectious laryngotracheitis virus recombinants expressing the hemagglutinin (H5) gene. *Vaccine*, **19** (30), 4249-4259.

24. Naeem K. & Siddique N. (2006). – Use of strategic vaccination for the control of avian influenza in Pakistan. In Proc. OIE/FAO International Scientific Conference on Avian Influenza. *Dev. Biol. (Basel)*, **124**, 145-150.
25. Perdue M.L., Crawford J., Garcia M., Latimer J. & Swayne D.E. (1998). – Occurrence and possible mechanism of cleavage site insertions in the avian influenza hemagglutinin gene. Proceedings of the 4th International Symposium on avian influenza, Athens, Georgia, USA (D.E. Swayne & R.D. Slemons, eds). US Animal Health Association, 182-193.
26. Qiao C.L., Yu K.Z., Jiang Y.P., Jia Y.Q., Tian G.B., Liu M., Deng G.H., Wang X.R., Meng Q.W. & Tang X.Y. (2003). – Protection of chickens against highly lethal H5N1 and H7N1 avian influenza viruses with a recombinant fowlpox virus co-expressing H5 haemagglutinin and N1 neuraminidase genes. *Avian Pathol.*, **32** (1), 25-32.
27. Sims L.D. (2006). – Lessons learned from Asian H5N1 outbreak control. *Avian Dis.* (in press).
28. Suarez D.L., Lee C.W. & Swayne D.E. (2006). – Avian influenza vaccination in North America: strategies and difficulties. In Proc. OIE/FAO International Scientific Conference on Avian Influenza. *Dev. Biol. (Basel)*, **124**, 117-124.
29. Suarez D.L., Senne D.A., Banks J., Brown I.H., Essen S.C., Lee C.W., Manvell R.J., Mathieu-Benson C., Moreno V., Pedersen J.C., Panigrahy B., Rojas H., Spackman E. & Alexander D.J. (2004). – Recombination resulting in virulence shift in avian influenza outbreak, Chile. *Emerg. infect. Dis.*, **10** (4), 693-699.
30. Swayne D.E., Beck J.R., Garcia M. & Stone H. (1999). – Influence of virus strain and antigen mass on efficacy of H5 avian influenza inactivated vaccines. *Avian Pathol.*, **28**, 245-255.
31. Swayne D.E., Beck J.R. & Mickle T.R. (1997). – Efficacy of recombinant fowl poxvirus vaccine in protecting chickens against a highly pathogenic Mexican-origin H5N2 avian influenza virus. *Avian Dis.*, **41** (4), 910-922.
32. Swayne D.E., Suarez D.L., Schultz-Cherry S., Tumpey T.M., King D.J., Nakaya T., Palese P. & Garcia-Sastre A. (2003). – Recombinant paramyxovirus type 1-avian influenza-H7 virus as a vaccine for protection of chickens against influenza and Newcastle disease. *Avian Dis.*, **47** (3 Suppl.), 1047-1050.
33. Taylor J., Weinberg R., Kawaoka Y., Webster R.G. & Paoletti E. (1988). – Protective immunity against avian influenza induced by a fowlpox virus recombinant. *Vaccine*, **6** (6), 504-508.
34. Terregino C., Toffan A., Beato M.S., De Nardi R., Drago A. & Capua I. (2006). – Conventional H5N9 vaccine suppresses shedding in SPF birds challenged with HPAI H5N1 A/ck/Yamaguchi/7/2004. *Avian Dis.* (in press).
35. Tian G., Zhang S., Li Y., Bu Z., Liu P., Zhou J., Li C., Shi J., Yu K. & Chen H. (2005). – Protective efficacy in chickens, geese and ducks of an H5N1-inactivated vaccine developed by reverse genetics. *Virology*, **341** (1), 153-162.
36. Tripathy D.N. & Schnitzlein W.M. (1991). – Expression of avian influenza virus hemagglutinin by recombinant fowlpox virus. *Avian Dis.*, **35** (1), 186-191.
37. Van Der Goot J.A., Koch G., De Jong M.C. & Van Boven M., (2005). – Quantification of the effect of vaccination on transmission of avian influenza (H7N7) in chickens. *Proc. natl Acad. Sci. USA*, **102** (50), 18141-18146.
38. Veits J., Luschow D., Kindermann K., Werner O., Teifke J.P., Mettenleiter T.C. & Fuchs W. (2003). – Deletion of the non-essential ULO gene of infectious laryngotracheitis (ILT) virus leads to attenuation in chickens, and ULO mutants expressing influenza virus haemagglutinin (H7) protect against ILT and fowl plague. *J. gen. Virol.*, **84** (Pt 12), 3343-3352.
39. Veits J., Wiesner D., Fuchs W., Hoffmann B., Granzow H., Starick E., Mundt E., Schirrmeier H., Mebatsion T., Mettenleiter T.C. & Romer-Oberdorfer A. (2006). – Newcastle disease virus expressing H5 hemagglutinin gene protects chickens against Newcastle disease and avian influenza. *Proc. natl Acad. Sci. USA*, **103** (21), 8197-8202.
40. Villarreal C.L. (2006). – Control and eradication strategies of avian influenza in Mexico. In Proc. OIE/FAO International Scientific Conference on Avian Influenza. *Dev. Biol. (Basel)*, **124**, 125-126.
41. Webster R.G., Kawaoka Y., Taylor J., Weinberg R. & Paoletti E. (1991). – Efficacy of nucleoprotein and haemagglutinin antigens expressed in fowlpox virus as vaccine for influenza in chickens. *Vaccine*, **9** (5), 303-308.
42. Webster R.G., Webby R.J., Hoffmann E., Rodenberg J., Kumar M., Seiler P., Krauss S. & Songserm T. (2006). – The immunogenicity and efficacy against H5N1 challenge of reverse genetics-derived H5N3 influenza vaccine in ducks and chickens. *Virology*, **351** (2), 303-311.
43. World Health Organization (2006). – Epidemic and Pandemic Alert and Response: Avian Influenza. Available at: [www.who.int/csr/disease/avian\\_influenza/en/](http://www.who.int/csr/disease/avian_influenza/en/) (accessed on 20 February 2007).
44. World Organisation for Animal Health (OIE) (2005). – Terrestrial Animal Health Code, 14th Ed. Chapter 2.7.12.1: avian influenza. Also available at: [www.oie.int/eng/normes/mcode/en\\_chapitre\\_2.7.12.htm](http://www.oie.int/eng/normes/mcode/en_chapitre_2.7.12.htm) (accessed on 20 February 2007).
45. World Organisation for Animal Health/Food and Agriculture Organization (OIE)/(FAO) (2004). – Recommendations of the FAO/OIE emergency regional meeting on avian influenza control in Asia, Rome, 3-4 February 2004, and Bangkok, 26-28 February 2004. Available at: [http://www.fao.org/AG/AGAInfo/subjects/en/health/diseasescards/HPAI\\_Bangkok.pdf](http://www.fao.org/AG/AGAInfo/subjects/en/health/diseasescards/HPAI_Bangkok.pdf) (accessed on 20 February 2007).
46. World Organisation for Animal Health/Food and Agriculture Organization (OIE)/(FAO) (2005). – Recommendations of the second FAO/OIE regional meeting on avian influenza control in Asia, Ho Chi Minh City 23-25 February 2005. Available at: [http://www.fao.org/AG/AGAInfo/subjects/documents/ai/AI\\_2nd\\_RegMtg\\_HoChiMinhCity\\_Rep.pdf](http://www.fao.org/AG/AGAInfo/subjects/documents/ai/AI_2nd_RegMtg_HoChiMinhCity_Rep.pdf) (accessed on 20 February 2007).

