

# Vaccination of birds other than chickens and turkeys against avian influenza

G. Koch<sup>(1)\*</sup>, M. Steensels<sup>(2)</sup> & T. van den Berg<sup>(2)</sup>

(1) Central Veterinary Institute Central Wageningen UR, Post Box 365, NL-8200 AB Lelystad, the Netherlands

(2) Veterinary and Agrochemical Research Centre (VAR), Groeselenberg 99, B-1180 Brussels, Belgium

\* Corresponding author: Guus.Koch@wur.nl

## Summary

Most avian influenza (AI) vaccination and field studies have focused on chickens and turkeys because of their high death rates and the large amounts of highly pathogenic avian influenza (HPAI) virus that they excrete into the environment when infected. Data on vaccination of other species against HPAI remain limited. An increasing number of studies have been conducted to test the efficacy of inactivated vaccines in ducks and geese since it became clear that these species are a source of HPAI H5N1. One problem is the varying susceptibility of waterfowl to H5N1 in general, and to different H5N1 clades in particular. This makes the extrapolation of protection results obtained for a particular waterfowl species against a particular viral strain very difficult.

At present, the vaccine industry only produces and licenses products for chickens and turkeys. Since the market for other birds is small, it does not invest heavily in testing products in other species. Most information on vaccination in other birds comes from zoo vaccination, and consists solely of serological data. Whenever experimental challenge was performed in birds other than chickens and turkeys, vaccination using inactivated vaccines always protected against disease and mortality, provided the vaccine was sufficiently matched antigenically with the challenge virus. Inactivated vaccines induce good antibody titres in most species when applied twice and when body weight is taken into account. Until the advent of more specific waterfowl vaccines that can be used in day-old chicks, inactivated vaccines can be applied to protect not only chickens and turkeys but also ducks and other valuable and/or endangered bird species.

## Keywords

Anseriformes – Avian influenza – Companion bird – Effectiveness – Efficacy – Endangered bird species – Game bird – H5N1 – Highly pathogenic avian influenza – Vaccination – Zoo bird.

## Introduction

Highly pathogenic avian influenza (AI) is a devastating disease for poultry. Mortality in an infected flock can be as high as 100%. Thus far, all viruses known to cause highly pathogenic AI (HPAI) belong to subtypes H5 and H7, but not all H5 and H7 viruses cause the disease. In the last decade, HPAI outbreaks have been reported in Italy in

1999 (H7N1), in Chile in 2001 and British Columbia (Canada) in 2004 (H7N3), and in the Netherlands in 2003 (H7N7). A major panzootic of HPAI H5N1 that most probably started in China extended over the whole of Southeast Asia in 2003, and spread, via Siberia, to Europe, the Middle East and Africa.

It has long been known that wild birds are a source for all influenza viruses. Surveillance programmes carried out in

wild birds showed that birds of the taxonomic orders of Anseriformes (ducks, swans, geese) and Charadriiformes (shorebirds, gulls, terns), in particular, carry low pathogenicity AI viruses (LPAIV), including precursor viruses of HPAI strains (25). These LPAIV can be carried over long distances by migratory birds and may be transmitted after direct or indirect contact with domestic poultry. Viruses of all subtypes can be introduced into domestic poultry, but LPAI H5 and H7 viruses mutate during replication in poultry into HPAI variants. The HPAI viruses (HPAIV) possibly use transmission routes in poultry that give them a selective advantage over LPAI precursors. In wild birds, these transmission routes probably do not exist or do not play a role (56).

Although wild birds are considered the natural reservoir of LPAIV, reports of epizootics of HPAI in wild birds are rare. In 1961, HPAIV H5N3 was reported to have caused the death of terns in South Africa (2). This HPAI H5N3 virus did not spread to domestic poultry and was not detected again in subsequent years, suggesting that it was not maintained in the wild bird population. During an outbreak of HPAIV H5N1 in Hong Kong in 2002, mortality was reported in migratory wild birds (resident mallards), as well as captive flamingos (Phoenicopteriformes) (10, 43). The H5N1 isolates of 2002 caused systemic infection and neurological signs after experimental infection of mallards (*Anas platyrhynchos*), confirming the high pathogenicity of this strain in wild ducks. At present, H5N1 infections resulting in neurological symptoms and mortality have been reported in a wide and still-growing range of avian species of different taxonomic orders:

- Anseriformes (7, 8, 10, 22, 30, 31, 38)
- Galliformes (30, 31)
- Charadriiformes (7, 8, 10, 22, 30, 31)
- Phoenicopteriformes (10, 39)
- Ciconiiformes (10, 37)
- Pelecaniformes (37)
- Falconiformes (13, 50)
- Strigiformes (13, 50)
- Struthioniformes (30)
- Columbiformes (10, 37)
- Passeriformes (10, 20, 31, 37)
- Psittaciformes (13).

## Vaccination

Most field use and most avian influenza vaccine studies have focused on chickens and turkeys because of their

high death rates and the significant amounts of HPAIV excreted into the environment by these species, when infected. However, the epidemiology of AI has changed with the spread of H5N1 HPAIV in Asia, indicating extended susceptibility of wild and exotic birds. The infection of domestic ducks and geese has become a very important contributor to the maintenance and spread of H5N1 HPAIV (16). In reaction to the altered epidemiology and the increased risk of H5N1 infections, many zoos decided to vaccinate their birds to protect them from H5N1. The results of several vaccination campaigns against H5N1 were reported in the literature. However, different vaccination schedules, doses and routes; various methodologies and antigens used in the haemagglutination inhibition (HI) tests in different laboratories, as well as the use of different vaccines for different taxonomic orders, make it difficult, if not impossible, to directly compare results of all reports. Field validation of vaccine effectiveness is often species-specific, i.e. most of the AI vaccines have been validated for chickens and turkeys, but little is known about their efficacy in other species, such as ducks and geese. Field experience with vaccination against HPAI, especially in exotic bird species, is scarce (28). Research has mainly been performed on poultry and, most recently, ducks (24, 42, 44, 52). Data on the vaccination of other species remain limited (32, 34).

At present, two different types of vaccine are commercially available for use in poultry: inactivated vaccines, based on adjuvanted whole viruses, and live recombinant vaccines.

### Inactivated vaccines

Inactivated vaccines are based on viruses from natural outbreaks or generated by reverse genetics (46, 51). In most countries, only inactivated whole-virus vaccines, produced using LPAI H5 and H7 viruses, are in use. The efficacy of inactivated vaccines is determined mainly by the vaccine dose, the adjuvant and the antigenic homology between the vaccine and challenge virus. In vaccination challenge experiments, it was demonstrated that vaccines based on seed viruses from the American lineage provided protection against challenge viruses of Eurasian lineages and vice versa. Swayne *et al.* demonstrated that a fowlpox virus with a haemagglutinin (HA) gene insert of A/turkey/Northern Ireland/87 H5N8 protected against challenge viruses whose HA gene showed only 86% homology (47). Swayne and colleagues also showed that a higher homology of the HA between vaccine and challenge virus reduced virus shedding by a greater degree. Avian influenza vaccines are developed mainly for chickens but have also been used in the field for multiple avian species, ranging from domestic poultry (turkeys, ducks, geese, quail) to exotic or endangered species. The efficacy of inactivated vaccines depends on the specific characteristics of the vaccine, but also on the targeted host. The immune

status and age of the host are important factors influencing the immunogenicity of influenza vaccines in all species.

More recently, inactivated vaccines have been produced using reverse genetics. The advantage of reverse genetics is that seed viruses can be produced that have HA and neuraminidase (NA) genes matching those of circulating wild-type strains. In most cases, the genes encoding the envelope glycoproteins are combined with the six remaining segments of viruses that are well adapted to growth in embryonated eggs, e.g. the originally human PR8 isolate. For safe production, the HA gene is modified to remove basic amino acids at the cleavage site to reduce the pathogenicity of the virus and increase the safety of vaccine production, thus enabling production at a lower biosafety level.

Experimental vaccination of chickens and turkeys reduced their susceptibility to infection and decreased the level and duration of virus shedding (6). Moreover, increased resistance and reduced shedding also resulted in the reduction or inhibition of transmission in groups of chickens and turkeys (4, 53). In chickens, only one vaccination was sufficient to block transmission, whereas, for turkeys, vaccination must be repeated, as recommended by most manufacturers. However, in the field a single vaccination was insufficient, and at least one repeat (in chickens) or two repeats (in turkeys) were necessary to obtain the desired immunity (23), demonstrating that results obtained experimentally cannot easily be extrapolated to the field situation.

### Live recombinant vaccines

Live recombinant vaccines are based on a vector virus expressing the HA protein of AI (5, 51). Examples of recombinant vaccines that are currently used in the field are fowlpox virus (48), and Newcastle disease virus expressing the H5 of AI (26). Although these vaccines are claimed to be effective in the field, particularly in broilers, they have not been tested in other birds under field conditions.

## Vaccination of ducks and geese

Vaccination experiments in ducks received more attention when it became clear that ducks, in particular, free-range ducks, and geese are a source of HPAI H5N1 (15). An increasing number of studies have therefore been conducted to test the efficacy of vaccines in these birds.

One important problem resides in the variable susceptibility of waterfowl to H5N1, in general, and to

different H5N1 clades, in particular (24, 29, 41). It is important to bear in mind that Muscovy ducks (*Cairina moschata*) and Pekin ducks (*A. platyrhynchos*) belong not only to different genera, but are also members of different tribes (Cairinini and Anatini, respectively) of the subfamily Anatinae. Therefore, although they are all called ducks, their sensitivity to virus infections may differ widely. In this context, geese seem closer to Muscovy than to Pekin ducks. Therefore, the results of vaccine efficacy obtained with one waterfowl species cannot be extrapolated to another species. In addition, although HI-specific antibody titres correlate with clinical protection in chickens, where an HI titre of  $\geq 5 \log_2$  was associated with good protection (19), a similar correlation has not been made in other avian species.

### Inactivated vaccines

Most published studies show that inactivated whole-virus vaccines are also effective in ducks (1, 18, 41, 42, 44, 49, 52, 54, 55) and geese (49) but species react differently (40). However, the current inactivated AI vaccines have a limited efficacy in Anseriformes and therefore require twice the antigenic load used for chickens and/or the addition of a strong stimulator for the immune response to be effective (41, 42). Recently, it was shown that a double dose of a bivalent vaccine protected ducks against disease and mortality, while only a low antibody response was induced (4 to 8  $\log_2$ ), and virus could be re-isolated from some (13%) of the vaccinated ducks (100% for non-vaccinated ducks) (24, 42). The failure of the challenge H5N1 virus to stimulate a secondary antibody response in ducks vaccinated with the closely related H5 of the monovalent vaccine is very strong evidence that there was minimal to no replication of the challenge virus in those vaccinated ducks (24). Another study showed that, after vaccination with a reverse genetics-derived H5N1 inactivated vaccine (49), chickens, geese and ducks were protected from clinical disease and death, and viral shedding was reduced, upon challenge with HPAI H5N1.

Van der Goot *et al.* (55) quantified transmission by estimating the reproduction ratio (R0) as being the average number of susceptible contact birds that become infected by, on average, one infectious bird. After one vaccination of Pekin ducks with a commercially available inactivated vaccine, A/chicken/Mexico/232/94/CPA H5N2, birds were fully protected against disease and mortality upon challenge with a clade 2 HPAI A/China/1204/04 H5N1 strain. After two vaccinations, the R0 was reduced from  $>1.5$  in unvaccinated ducks to 0.2 (95% confidence limits: 0.005-1.5) in vaccinated ducks. Thus, as in chickens and turkeys, these vaccines increase resistance and reduce shedding in ducks but seem to be less effective in reducing disease transmission within the flock (55).

In a recent study (18), the dominant strains of HPAI H5N1 circulating in Asian poultry were identified. Although four of them caused symptomatic illness in domestic ducks, not all were lethal. In addition, the researchers reversed the genetics of the viruses in domestic ducks to develop three different inactivated, oil emulsion, whole-virus H5 influenza vaccines. Following one inoculation with these vaccines, ducks were completely protected when challenged with a lethal dose of the H5N1 virus. Experimentally, the vaccines provided complete protection against lethal challenge with a homologous or heterologous H5N1 HPAIV, with no evidence of morbidity, mortality or shedding of the challenge virus. The effect of these vaccines on reducing viral excretion of H5N1 HPAIV among vaccinated agricultural avian populations remains to be established.

The duration of immunity after vaccination in these species is mostly unknown. In ducks, antibodies could be detected up to 38 weeks after two vaccinations, ten weeks apart, and so these birds were protected from infection during that period of time, but the longevity of antibodies in geese proved to be much shorter (49).

### Recombinant vaccines

The efficacy of an inactivated vaccine, from a Eurasian isolate (A/chicken/Italy/22A/98 H5N9), was compared with that of a commercially available recombinant AI H5 fowlpox virus (recFPV-H5) vaccine in five-week-old Muscovy ducks. The ducks were vaccinated with either the H5N9 vaccine (0.5 ml) or Trovac™ (5 log<sub>10</sub> 50% tissue culture infective dose [TCID<sub>50</sub>]/dose) and boosted two weeks later with the same vaccine (1.0 ml of H5N9 or 5 log<sub>10</sub> TCID<sub>50</sub>/dose Trovac™). The ducks were challenged at nine weeks with 10<sup>7</sup> 50% egg infective dose (EID<sub>50</sub>) of A/crested eagle/Belgium/01/2004 (H5N1). All unvaccinated challenged birds showed severe nervous signs (loss of balance, torticollis), starting at seven days post infection, while all vaccinated ducks were protected against nervous signs. Both vaccines protected the ducks against clinical signs and mortality and both vaccines were effective in reducing viral excretion through the oropharynx and cloaca. However, the classical inactivated H5N9-It vaccine proved to be significantly more effective than the recombinant vaccine, Trovac™ (42).

The efficacy of different vaccination schedules was also evaluated in 17-day-old Pekin ducks, using the same experimental inactivated H5N9 vaccine and/or a fowlpox recFPV-H5 expressing a synthetic HA gene from an Asian H5N1 isolate (A/chicken/Indonesia/7/2003). Full protection against clinical signs and shedding was induced by the different vaccination schemes. However, the broadest antibody response and the lowest antibody increase after challenge were observed in the group of

ducks whose immune system was primed with the fowlpox-vectored vaccine and boosted with the inactivated vaccine, suggesting that this prime-boost strategy induced optimal immunity against H5N1 and minimal viral replication after challenge. In addition, this prime-boost vaccination scheme was shown to be effective in one-day-old ducklings (41).

Although not commercialised so far, other recombinant approaches have been considered for the vaccination of ducks. Virus-like particles proved to be immunogenic in different species. A lysate of recombinant baculovirus-infected cells, containing H5, N3 and M1, was tested on Muscovy ducks for protection against a homologous LPAI H5N3 challenge. A double vaccination with this experimental vaccine was compared with vaccination with a commercial H5N9 inactivated vaccine. The commercial vaccine only reduced tracheal excretion while the recombinant baculovirus reduced both cloacal and tracheal shedding (35, 36).

## Vaccination of other bird species

### Ornamental ducks and game birds

Transmission experiments were also conducted in two commonly kept bird species, ringed teal (*Callonetta leucophrys*) and golden pheasants (*Chrysolophus pictus*). Vaccination with H7 vaccines protected both these species against challenge with HPAIV H7N7. These transmission experiments demonstrated that, depending on the host species, the virus can spread in unvaccinated birds with or without disease symptoms but also that vaccination of both bird species does not always reduce virus transmission (54).

### Raptors

Collections of birds of prey have a high commercial and species conservation value; therefore, protection from HPAI is important. Lierz *et al.* (21) used hybrid breeds of falcons (*Falco rusticolus* × *F. cherrug*) to determine the susceptibility of this species to H5N1 infection. An inactivated influenza virus (H5N2 vaccine) was used in an attempt to protect birds by vaccination. Vaccinated falcons seroconverted after two rounds of vaccination, with titres against the challenge virus that were four-fold lower than those against the vaccine virus. Only vaccinated falcons were protected and survived infection with HPAI A/*Cygnus cygnus*/Germany/R65/2006. Non-vaccinated birds shed high levels of infectious virus from the oropharynx and cloaca, while the vaccinated birds shed virus at much lower titres and only from the oropharynx.

## Pigeons

Pigeons are abundant throughout the world and live in close proximity to humans. Moreover, pigeons are used for racing across borders, raising questions about their possible role in transmitting influenza viruses from infected regions. Domestic rock pigeons (*Columba livia*) (33), vaccinated with a 0.5 chicken dose of commercially available vaccine (A/duck/Potsdam/2243/84 H5N6), were protected against disease and mortality when challenged with a clade 2.1.1 Indonesian or clade 2.2 Turkey virus. The vaccinated birds did not shed detectable amounts of virus through the trachea or faeces, and vaccination did not cause adverse effects in the pigeons.

## Zoo birds

During the outbreak of H7N7 in the Netherlands in 2003, zoos were in danger of being enclosed in the protection zone, which meant that they could be included in the culling. As a result, zoos in Belgium, Germany and the Netherlands proposed vaccination as an alternative to pre-emptive culling. At that time, vaccinating commercial poultry against HPAI was not permitted, under European Union (EU) Directive 92/40/EEC (12). Vaccinating poultry, however, had been approved in the past for LPAI H7N1 by the EU Commission (11) because LPAI viruses were not yet included in the list of notifiable diseases, among other reasons. Vaccination is allowed for certain species under controlled conditions because of the educational role of zoos and their important breeding programmes for maintaining endangered avian species (the International Union for Conservation of Nature and Natural Resources red list: [www.iucnredlist.org](http://www.iucnredlist.org)). This replaced the need for culling and confinement, which was considered unacceptable, particularly since the period for which this would be necessary was unknown. Vaccination was only allowed for species listed as being susceptible to avian influenza, i.e.:

- Galliformes (fowl, quail, pheasants)
- Anseriformes
- Struthioniformes (emus, nandus and ostriches)
- Columbiformes (rock doves), which were not kept for consumption.

Moreover, extensive additional biosecurity requirements had to be met.

Since commercially available vaccines have only been developed for chickens and turkeys, and since the market for other birds is small, the vaccination industry does not invest heavily in testing products in other bird species. Moreover, vaccination challenge experiments cannot easily

be performed in species of birds that are kept as zoo or companion birds. Thus, most of the available information on the effect of vaccination in other bird species stems from zoo vaccination, and consists solely of serological data. Direct comparison of such data is virtually impossible, for the reasons given above (see 'Vaccination').

Inactivated H5 or H7 vaccines produced for application in chickens are used for zoo vaccination, which creates difficulties when body weight has to be taken into account. Oh *et al.* (27) reported on the vaccination of birds against H5N1 in the Singapore Zoological Gardens. Birds of the orders of Galliformes and Anseriformes, as well as birds in open exhibits, were vaccinated with an inactivated H5N2 vaccine produced for poultry, using an A/chicken/Mexico/232/94/CPA as seed virus. Seroconversion of 100% of the birds occurred after the booster vaccination of the following Anseriformes:

- a) Egyptian geese (*Alopochen aegyptiacus*)
- b) bar-headed geese (*Anser indicus*)
- c) Radjah shelducks (*Tadorna radjah*)
- d) black swans (*Cygnus atratus*)
- e) domestic ducks (*A. platyrhynchos*)
- f) ringed teal (*Callonetta leucophrys*)
- g) Canadian geese (*Branta canadensis*)

Similarly, seroconversion of 100% of the following Galliformes occurred after booster vaccination:

- a) peafowl (*Pavo spp.*)
- b) golden pheasants (*Chrysolophus pictus*)
- c) crested fireback pheasants (*Lophura ignita*)
- d) silver pheasants (*L. nycthemera*).

In contrast, a partial response was recorded for:

- spur-winged geese (*Plectropterus gambensis*), white-faced whistling ducks (*Dendrocygna viduata*), and spotted whistling ducks (*D. guttata*) (Anseriformes)
- guineafowl (*Numida meleagris*) (Galliformes)
- cormorants (*Phalacrocorax carbo*) (Pelecaniformes).

Some species, including the pelicans (Pelecaniformes) and owls (Strigiformes), failed to respond to vaccination. Titres measured with A/duck/Singapore-Q/F119-3/97 H5N3 varied between 1:16 and 1:2.048 for those birds that responded.

Vaccination campaigns against H7N7 were conducted in zoo birds in the Netherlands and Germany. In the Netherlands, birds were given two doses of an inactivated

vaccine, using A/ckicken/Italy/473/99 H7N1 as the seed virus. The vaccine had a nucleotide homology of 97.4% and 98.7% homology on the basis of amino acids. Birds were vaccinated twice, six weeks apart. To compensate for body weight, birds under 1.5 kg were given two doses of 0.25 ml and those over 1.5 kg were inoculated with two doses of 0.5 ml, using average published body weights for each species. The antibody response was evaluated by determining HI titres using turkey erythrocytes, whereas vaccine immunity was evaluated by determining the antibody titre in the virus neutralisation test, using post-vaccination sera of selected birds with different HI titres. After booster vaccination, 81.5% of the birds developed a titre of 1:40 or higher, while the geometric mean titre was 190 (95% confidence interval [CI]: 144-251). No significant difference in titre was found between birds of the orders Galliformes, Anseriformes and Phoenicopteriformes. However, mean titres were higher in birds from these orders, and a higher percentage of these birds had titres  $\geq$ 1:40 than did birds from other orders. The route of vaccination had no significant effect on titre development but the mean titre was inversely related to body weight in birds over 1.5 kg.

To protect Dutch zoo birds against H5N1, a commercially available vaccine was employed, which used A/duck/Potsdam/1402/86 H5N2 as a seed virus. The vaccine virus HA had 90% nucleotide homology and 92.4% amino acid homology with A/turkey/Turkey/1/05. After booster vaccination, the overall geometric mean titre, using the homologous vaccine strain as the antigen in an HI test of 334 birds, was 1:190 (95% CI: 152-236), and 80.5% of vaccinated birds developed a titre of  $\geq$ 1:40. When HPAI A/turkey/Turkey/1/05 H5N1 was used as the antigen, the geometric titre was lower (geometric mean titre: 1:61 [95% CI: 49-76]; 61% = 40). The cross-reactivity of the response was further demonstrated by measuring antibody titres against prototype strains from four antigenic clades of currently circulating H5N1 viruses (32).

Furger *et al.* (14) reported on the results of four major Swiss zoos in which selected birds were vaccinated with an adjuvanted inactivated vaccine based on A/chicken/Mexico/232/95/CPA H5N2. The geometric mean HI titre was 187 (n = 139) after booster vaccination, using the vaccine virus as antigen in the test. Within the non-Galliformes, significant differences in geometric mean titres were found among different species. In general, flamingos (Phoenicopteriformes) showed a strong response to vaccination, reaching a geometric mean titre of 659 at week 10, while Sphenisciformes did not show high antibody titres, even after booster vaccination, reaching a maximum geometric mean titre of only 65.

Similar results were found in a vaccination programme of Danish zoo birds (3). Five hundred and forty birds in three

zoos were vaccinated twice against avian influenza, at a six-week interval, using an inactivated H5N9 vaccine. The serological response was evaluated by HI test, four to six weeks after the second administration of vaccine. In all, 84% of the birds seroconverted and 76% developed a titre  $\geq$ 32. The geometric mean titre after vaccination was 137. As in other studies, a significant variation in response was noted among different species. Those that showed a very poor response to vaccination included:

- penguins (Sphenisciformes)
- pelicans (Pelecaniformes)
- ducks and geese (Anseriformes)
- herons (Ciconiiformes)
- guineafowl (Galliformes)
- cranes (Gruiformes)
- cockatiels and lovebirds (Psittaciformes)
- barbets (Piciformes).

On the other hand, very high titres and seroconversion rates were seen in:

- flamingos (Phoenicopteriformes)
- ibis (Ciconiiformes), with the exception of herons, which demonstrated poor responsiveness
- rheas (Rheiformes)
- Congo peafowl (Galliformes)
- black-winged stilts (Charadriiformes)
- Amazon parrots (Psittaciformes)
- kookaburras (Coraciiformes).

## Field situation

The results of vaccine efficacy studies cannot easily be extrapolated to the field situation, and so effectiveness studies are essential to evaluate the value of vaccines, while taking into consideration the many factors that have an effect on acquired immunity (24, 45). As with chickens, only inactivated vaccines that must be administered individually by the parenteral route have been used in the field for vaccinating ducks and geese. This is inconvenient since individual administration is time-consuming, expensive and increases the risk of wild-type virus being spread within and between farms by the vaccination teams. This is particularly true for waterfowl, which are more difficult to catch and handle than chickens, especially for booster vaccinations.

Field and laboratory evaluation of a vaccination programme against H5N1 in waterfowl in Vietnam indicated that, in addition to the species of the bird, other factors, such as maternally derived antibody and proper use of vaccines, may also significantly influence the outcome of a vaccination programme (9). In an experimental field trial conducted in Germany, vaccination of geese and ducks with an H5N2 inactivated vaccine was successful in protecting against illness and death. However, geese vaccinated under field conditions became infected with, and transmitted the challenge virus to, non-vaccinated geese. These contact animals became ill, shed virus at higher titres than the vaccinated animals, and served as amplification hosts for the virus. But, when vaccinated in-contact geese were used, transmission was strongly reduced. In conclusion, this indicates that, even under field conditions, vaccination is one means to limit and, ideally, abolish virus transmission if a high enough level of immunity can be achieved within the population. In the same trial, it was demonstrated that vaccinated Pekin ducks were able to resist infection with HPAIV H5N1. Moreover, virus excretion was found to be intermittent and at low titres (40).

## Surveillance and testing to differentiate infected animals from vaccinated animals

Control measures for H5N1 avian influenza involve increased biosecurity, monitoring, surveillance and vaccination. Subclinical infection in farmed ducks is important for virus persistence. Ducks, especially adults, do not always show clear clinical symptoms, hence syndrome surveillance is not effective as an early warning system in ducks. Differentiating infected from vaccinated animals (DIVA) tests, using the detection of heterologous NA antibodies, have not been validated in ducks. In addition, in major duck-rearing countries, homologous H5N1 vaccines are being used, making serosurveillance using H5- or N1-specific antibodies insufficient to identify infected flocks. Since wild birds are a natural reservoir for LPAI strains, the other existing DIVA tests, based on antibodies against the non-structural protein 1, M2 or viral nucleoprotein, are of limited use when using recombinant vector vaccines. An alternative has been recently proposed: including tetanus toxoid (TT) as a positive marker for vaccination (17). High levels of TT-specific antibodies, produced in twice-TT-vaccinated Muscovy ducks,

persisted for up to 19 weeks. There was no interference, by the inclusion of TT in an inactivated H6N2 vaccine, in H6- or TT-seroconversion. Although such a system allows the identification of vaccinated birds, it cannot be used to detect whether vaccinated birds are subsequently infected.

## Conclusions

Wherever experimental challenge was performed in birds other than chickens and turkeys, vaccinations using inactivated vaccines always protected against morbidity and mortality, provided that the vaccine sufficiently matched the challenge virus antigenically. Moreover, inactivated vaccines induced good antibody titres in most species when they were applied twice, and body weight was taken into account. Therefore, vaccination can, and will, be used to protect not only chickens and turkeys but also ducks and valuable and/or endangered bird species in zoos. However, since vaccinated birds may still transmit virus, strict biosecurity measures and virus surveillance are needed to prevent silent virus spread in vaccinated populations.

In the field, the application of inactivated vaccines is more difficult, as there are many practical limitations; for instance, the difficulty in reliably assuring double vaccinations.

In conclusion, there is a clear need for a new generation of cost-effective and efficient vaccines for other birds, including ducks and geese, that can be applied by mass immunisation methods (by spray, in drinking water) and induce strong local immunity to control the shedding of the virus. Several vectored vaccines show promise as this next generation of AI vaccines. Until the advent of more specific waterfowl vaccines that can be used in day-old birds, fowlpox vectors could help to achieve a good prime-boost regimen. Field studies are also necessary to evaluate vaccine efficacy under real conditions. Control or eradication can only be achieved if all aspects of the intervention strategy operate efficiently. A major point of concern is the selection of antigenic variants which may occur in the field. Thus, vaccination should always be accompanied by good control and prevention strategies, including an effective surveillance plan for vaccinated birds, using DIVA tools. ■

## La vaccination d'espèces aviaires autres que les poulets et les dindes contre l'influenza aviaire

G. Koch, M. Steensels & T. van den Berg

### Résumé

La plupart des études consacrées à la vaccination contre l'influenza aviaire et à la situation de la maladie sur le terrain traitent principalement des poulets et des dindes, en raison des taux de mortalité très élevés dans ces espèces et des quantités importantes de virus de l'influenza aviaire hautement pathogène (IAHP) qu'elles excrètent dans l'environnement. Les informations publiées sur la vaccination d'autres espèces contre l'IAHP sont plus rares.

Les études visant à tester l'efficacité des vaccins à virus inactivé chez les canards et les oies sont plus nombreuses depuis que l'on sait que ces espèces sont une source avérée du virus H5N1 de l'IAHP. Les variations de susceptibilité que présentent les oiseaux aquatiques aux virus H5N1 en général, et plus particulièrement aux différents clades des virus H5N1, posent un véritable problème. Il en ressort que les résultats sur la protection obtenue chez une espèce particulière contre une souche virale donnée ne peuvent être extrapolés à d'autres espèces ni à d'autres souches.

À l'heure actuelle, les seuls vaccins autorisés produits par l'industrie pharmaceutique sont destinés aux poulets et aux dindes. Le marché des vaccins destinés aux autres espèces aviaires étant limité, les investissements pour tester des produits dans ces espèces sont insuffisants. La plupart des informations disponibles sur la vaccination de ces espèces provient des parcs zoologiques et se limite à des données sérologiques.

Toutes les inoculations d'épreuve pratiquées sur des espèces aviaires autres que les poulets et les dindes ont montré que les oiseaux vaccinés avec un vaccin à virus inactivé acquéraient systématiquement une immunité protectrice contre la maladie et contre la mortalité qui lui est associée, à condition d'utiliser un vaccin suffisamment proche, au plan antigénique, du virus inoculé. Les vaccins à virus inactivé, s'ils sont administrés en deux fois et si le poids corporel des oiseaux est pris en compte, induisent une production d'anticorps suffisamment protectrice chez la plupart des espèces. En attendant que des vaccins plus spécifiques utilisables sur des poussins d'un jour soient mis au point pour les oiseaux aquatiques, il est possible d'utiliser les vaccins à virus inactivé pour protéger les poulets et les dindes ainsi que les canards et d'autres espèces aviaires de grande valeur ou menacées.

### Mots-clés

Ansériforme – Efficacité vaccinale – Espèce aviaire menacée – Gibier à plumes – Influenza aviaire – Influenza aviaire hautement pathogène – Oiseau de compagnie – Oiseau de parc zoologique – Sous-type H5N1 – Vaccination.



## Vacunación contra la influenza aviar de aves distintas del pollo y el pavo

G. Koch, M. Steensels & T. van den Berg

### Resumen

La mayoría de los estudios sobre el terreno y de las campañas de vacunación contra la influenza aviar (IA) se han centrado en el pollo y el pavo porque éstos presentan elevadas tasas de mortalidad y excretan al medio una gran cantidad de virus de la influenza aviar altamente patógena (IAAP) cuando resultan infectados. No existen datos suficientes sobre la vacunación de otras especies contra la IAAP.

Cada vez se vienen realizando más estudios para determinar la eficacia en patos y ocas de vacunas inactivadas, desde que se pudo comprobar que ambas especies son fuente de virus de la cepa H5N1 causante de IAAP. Uno de los problemas que se plantean es la variación de la susceptibilidad de las aves acuáticas a los virus H5N1 en general y a distintos clados de esa cepa en particular. Ello hace muy difícil extrapolar los resultados obtenidos en una determinada especie acuática respecto al grado de protección contra una cepa vírica concreta.

En la actualidad, la industria farmacéutica sólo fabrica y registra vacunas destinadas al pollo y el pavo. Dado el reducido tamaño del mercado para otras aves, el sector privado no invierte grandes sumas en ensayos de productos en otras especies. La mayoría de los datos existentes sobre el uso de vacunas en otras aves provienen de vacunaciones practicadas en parques zoológicos y aportan información exclusivamente serológica.

En todos los casos de infección experimental de aves distintas del pollo y el pavo se ha observado que la administración de vacunas inactivadas confiere invariablemente protección contra la enfermedad y su desenlace mortal, siempre y cuando la vacuna guarde la suficiente correspondencia antigénica con el virus utilizado. En la mayoría de las especies, las vacunas inactivadas inducen títulos elevados de anticuerpos si se administran dos veces y si se tiene en cuenta el peso corporal. Hasta que existan vacunas destinadas más específicamente a las aves acuáticas que puedan administrarse a pollitos de un día de edad, será posible utilizar las vacunas inactivadas para proteger no sólo a pollos y pavos, sino también a patos y otras especies avícolas que estén en peligro y/o revistan interés económico.

### Palabras clave

Anseriformes – Aves de compañía – Aves de recreo – Aves de zoológico – Efectividad – Eficacia – Especies avícolas en peligro de extinción – H5N1 – Influenza aviar – Influenza aviar altamente patógena – Vacunación.



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