Vaccination as a tool to combat introductions of notifiable avian influenza viruses in Europe, 2000 to 2006

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Summary
In late 2000, Italy was the first country of the European Union (EU) to implement an emergency vaccination programme against notifiable avian influenza. Vaccination with a conventional vaccine containing a seed strain with a different neuraminidase subtype from that of the field virus was used to complement biosecurity and restriction measures as part of an overall eradication strategy. This vaccination technique, in line with the Differentiating Infected from Vaccinated Animals system (DIVA), was applied several times until March 2008. This strategy enabled the identification of field exposed flocks and ultimately the eradication of low pathogenic H7N1, H7N3 and H5N2 infections. Italy was also the first country to implement a bivalent H5/H7 prophylactic vaccination programme of defined poultry populations, which was discontinued in December 2006.

Following the incursion of highly pathogenic H5N1 into Europe, in 2005 and 2006, two other EU Member States, namely France and the Netherlands, implemented preventive vaccination programmes in 2006 but they targeted selected poultry populations different from those targeted in Italy and were implemented for short periods of time.

Data generated during six years of experience with vaccination against avian influenza in Italy indicate that it is a useful tool to limit secondary spread and possibly prevent the introduction of low pathogenic avian influenza viruses in a susceptible population. The experience of France and the Netherlands provides data on vaccination of ducks and hobby poultry respectively and monitoring programmes associated with vaccination and difficulties related to their application. The advantages and disadvantages of vaccination need to be considered in the decision-making process, including the financial aspects of vaccination.

Keywords
Avian influenza – Control – Epidemiology – Europe – Vaccination.

Introduction

Avian influenza (AI) is a World Organisation for Animal Health (OIE) listed disease and is of great importance both for animal and human health. Until 1999, highly pathogenic AI (HPAI) was a sporadic disease, with only 18 outbreaks reported in domestic poultry worldwide since 1959. The total number of birds involved in all outbreaks over this 40-year period was approximately 23 million (7). From 1999 onwards its epidemiology changed dramatically. Including estimations of the ongoing H5N1 epidemic, in the last five years HPAI has affected over 200 million birds. Several of these outbreaks (Italy in
1999 and 2000, the Netherlands in 2003, Canada in 2004 and throughout Eurasia and Africa in 2003 and 2006) have led to devastating financial consequences for the poultry industry. In some countries the effects have escalated to such a high level that the impact has been felt by the overall national economy (7). In addition, some AI infections have now been associated with significant adverse effects on human health. These include varying forms of human clinical disease ranging from mild disease complexes to fatalities and the risk of generating a novel pandemic virus for humans that possesses avian antigenicity combined with human host-adapted replicative ability.

The increased relevance of AI in the fields of animal and human health has highlighted the lack of scientific information available on several aspects of the disease. This lack of information has hampered the management of some of the recent crises and resulted in millions of dead animals, the loss of human lives and grave difficulty in managing the pandemic potential of the infection.

Several legislative and regulatory issues such as the inclusion of low pathogenicity AI (LPAI) viruses in the definition of AI have been addressed by the OIE and by the European Commission (EC), although not in a harmonised manner (8). Given the increase in the numbers of isolations of LPAI viruses of the H5 and H7 subtypes, this is of crucial importance in the management of future outbreaks.

Field experience with AI vaccination is very limited, in contrast to control strategies for other highly contagious diseases of animals such as foot and mouth disease and classical swine fever, where vaccination has been used extensively in different clinical situations (32).

Italy has been challenged with several successive epidemics of notifiable avian influenza (NAI) and has developed and applied compulsory vaccination programmes under official control. Recently, following the incursion of highly pathogenic H5N1 viruses into Europe, France and the Netherlands have also implemented vaccination programmes. A summary of the different experiences is reported below.

**Italy**

**Emergency vaccination programmes**

Between 1999 and 2001, Italy was affected by four successive epidemic waves of AI caused by viruses of the H7N1 subtype (9). The first epidemic wave was caused by an LPAI virus (LPAIV) of H7N1 subtype that subsequently mutated into an HPAI virus (HPAIV), having circulated in the industrial poultry population for approximately nine months. Following the emergence of the HPAIV, a complete depopulation of the infected area was carried out as a result of the implementation of the measures indicated in European legislation (Council Directive 92/40/CE [19]). The control measures resulted in the death or culling of about 16 million birds (13,733,000 in the affected farms and 2,072,000 in farms at risk of infection). 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.

From August to November 2000, the H7N1 LPAI strain infected 51 meat-type turkey farms (845,000 turkeys) and one quail farm (429,000 quails) located in the southern part of the province of Verona. Another three quail farms, with a total of 405,000 quails, located in a contiguous province were also affected. The latter were functionally linked to the farm situated in the province of Verona. All infected farms were depopulated. The origin of the outbreak was subsequently traced back. From the epidemiological investigation it appeared that the source of infection was the large Japanese quail (*Coturnix coturnix japonica*) farm located in the densely populated poultry area (DPPA). The absence of clinical signs in quail and lack of seroconversion misled the outcome of diagnostic efforts.

To prevent a possible re-emergence of the H7N1 LPAIV, a coordinated set of measures, including strict biosecurity, a serological monitoring programme and a vaccination strategy based on differentiating infected from vaccinated animals (DIVA) were enforced (16). The DIVA strategy, which was implemented from December 2000, was based on the use of an inactivated oil emulsion vaccine containing the same haemagglutinin (H) subtype as the field virus, but a different neuraminidase (N) subtype, in this case an H7N3 strain (A/ck/Pakistan/95). To differentiate between vaccinated and naturally infected birds an ad hoc serological test based on the detection of specific anti-N1 antibodies was developed (10).

The control of the field situation was ensured through an intensive serosurveillance programme aimed at the detection of the LPAIV. This was achieved through the regular testing of sentinel birds in vaccinated flocks and 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. Quail farms have never been vaccinated.
Notwithstanding the depopulation of the infected premises, the serological monitoring programme revealed an additional incursion of the H7N1 LPAIV shortly after the start of the vaccination programme (December 2000 to March 2001). The H7N1 LPAIV infected three meat-type turkey farms in the vaccination area and 20 poultry holdings (19 turkey farms and one 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, with the last H7N1 LPAI-infected poultry flock stamped out on 26 March 2001. The results of the serological surveillance, carried out both within and outside the vaccinated area to assess the possible presence of Al infection, demonstrated that the H7N1 AI virus strain was no longer circulating (28). The application of the DIVA discriminatory test, with negative results, was considered an additional guarantee for Member States of the European Union (EU), and on 30 November 2001, Commission Decision 2001/847/EC (16) authorised the marketing of fresh poultry meat obtained from vaccinated birds for intra-Community trade. The emergency vaccination programme was discontinued in May 2002.

**Low pathogenicity H7N3 avian influenza epidemic, 2002 to 2003**

In 2002 and 2003 Italy once again experienced outbreaks of Al involving an H7N3 subtype influenza A virus of low pathogenicity. In the month of October 2002, an H7N3 LPAI strain was introduced from the wild reservoir into the domestic poultry population located in the DPPA which had previously been affected by the H7N1 epidemic in 1999 and 2001 (6). The new H7N3 virus was unrelated genetically to the H7N3 seed strain contained in the vaccine used in the 2000 and 2002 vaccination campaign (13).

Since the infection was spreading rapidly among poultry flocks, the use of vaccination was anticipated. The vaccination programme was based once again on a DIVA strategy and was carried out using an Al inactivated heterologous vaccine (strain A/ck/IT/1999-H7N1) in layers, capons and meat turkeys only. However, its implementation was delayed until 31 December 2002, due to the unavailability of the appropriate vaccine. Extensive spread of field virus therefore occurred initially within the affected area.

From 10 October 2002 to 10 October 2003, the H7N3 LPAIV was able to spread and infect a total of 388 poultry holdings, most of which were located in the southern part of two Italian regions (Veneto and Lombardy), as follows:

- 332 meat-type turkey farms
- 5 turkey breeder farms
- 12 broiler breeder farms
- 13 layer farms
- 6 guineafowl farms
- 4 broiler farms
- 3 quail farms
- 1 meat duck farm
- 12 backyard flocks.

A total of 7,660,005 birds were involved in the epidemic, and among these, 4,231,452 birds were stamped out in 163 affected flocks. The remaining 3,428,553 slaughtered birds were subjected to controlled marketing between winter/spring 2002 and winter/spring 2003. Of the 388 affected flocks, 88 were vaccinated. All of the latter were meat turkeys mainly located in a limited area of the southern part of Verona Province. It is interesting to note that despite the poultry density in the vaccination area only two unvaccinated poultry farms (one broiler breeder farm and one meat duck farm) were affected. These farms were located in close proximity to previously vaccinated meat turkey farms that had been exposed to field virus. Stamping out measures or controlled marketing were enforced in all vaccinated infected flocks, which housed a total of 1,523,320 birds.

The implementation of an emergency vaccination programme and the enforcement of strict restriction measures did not prevent the spread of the H7N3 LPAIV infection among meat turkey farms located in a DPPA. Nevertheless, massive spread of infection to unvaccinated poultry inside and outside the vaccination area did not occur. Only sporadic outbreaks of LPAI infection were detected in unvaccinated poultry farms, mainly located outside the vaccination area: three meat turkey, five dealer and two quail farms in Lombardy, one dealer farm in Piedmont, and two meat turkey farms and one back-yard flock in the Emilia-Romagna region. These flocks were identified promptly and stamped out. The last farm infected with H7N3 was cleared on 9 October 2003. On 13 January 2004, based on additional field data on the reliability of the DIVA strategy in detecting any LPAI-infected flock (12), Commission Decision 2004/159/EC authorised the marketing of fresh meat and of table eggs originating from vaccinated turkeys and layers for intra-Community trade (17).

During the 2003 to 2004 H7N3 LPAI outbreak, the authors estimated the impact of vaccination on the incidence of LPAI outbreaks by considering the poultry population before and after the implementation of the emergency vaccination programme. As an efficacy index, they calculated the reproduction rate (R) of the Al infection before and after the start of the vaccination campaign. The R parameter represents the average number of new cases generated in a susceptible population during the infectious
were proposed to the EC. These included:

- a complete reorganisation of the poultry sector in the area with the highest poultry population densities of Verona Province (long-term measure)
- a reduction of the turkey density in the same area through a temporary ban on the restocking of a certain percentage of turkey farms (short-term measure).

In July 2004, the EC authorised Italy to implement a prophylactic vaccination programme in the high-risk areas of Veneto and Lombardy regions (29). Italy was authorised to enforce the programme from 1 October 2004 onwards based on the availability of a bivalent (H5/H7) inactivated vaccine containing seed strains of the H5N9 and H7N1 or H7N4 subtypes supplied by the Italian National Reference Laboratory. From 7 October 2004, the vaccination programme was implemented only in categories of poultry considered at high risk of exposure, namely meat turkeys and layers. The bivalent vaccination programme replaced the monovalent (H7) vaccination programme from 31 August 2004.

Re-occurrence of the low pathogenicity H7N3 avian influenza virus and introduction of low pathogenicity H5N2 avian influenza

In September 2004, about one year after the depopulation of the last LPAI-affected flock, the AI virus of the H7N3 subtype re-emerged in the southern part of Verona province. Affected farms were mostly in close proximity to each other and functionally connected. This occurrence required the application of strict control measures in association with the rapid implementation of a booster immunisation of the susceptible population.

From 15 September to 1 December 2004, the H7N3 LPAIV infected 27 meat turkey farms and 1 quail farm, housing a total of 744,000 birds, located in a cluster of 9 municipalities with one of the highest turkey population densities in Europe. All the affected meat turkey flocks had been vaccinated with the monovalent (H7N1) inactivated vaccine, but the majority of these flocks had been vaccinated only once or twice. The recommendation for this species is three administrations. The increased resistance to challenge of the vaccinated birds and the reduction of the shedding levels combined with restriction measures, biosecurity and appropriate surveillance resulted in a significant reduction of the infectious pressure in the environment, and aided the rapid eradication of the infection. Only 28 farms were affected, the same virus having caused 388 outbreaks the previous year.

Successful virus isolation proved that the outbreak once again originated from a quail farm. This flock had displayed inconclusive serological results, with haemagglutination inhibition (HI) titres of 1:4
to 1.8 detected during the monitoring programme. In retrospect it appeared that the virus had circulated undetected in this reservoir for one year. Infection was eradicated in December 2004, and as a result of this epidemic, quail farming rules in the area were revised, requiring separate locations for breeder and meat quails.

A similar situation was also observed in 2005, when a novel introduction of an H5N2 LPAI occurred in vaccinated turkey flocks in Lombardy. Infection spread to 15 turkey farms. Two of these farms were unvaccinated and 13 were vaccinated. The latter consisted of adult turkeys close to slaughter, with post-vaccinal HI titres at that age ranging between 1:4 and 1:16. These findings demonstrated the difficulty in obtaining an adequate duration of immunity in the turkeys (compared with previous experiences in chickens) and may explain the ability of the virus to enter the vaccinated population following a field challenge towards the end of the birds’ production period. Field evidence thus indicated that vaccinated layers were resistant to field challenge, as no vaccinated holding developed infection. In addition, although it was not possible to prevent the introduction of AI viruses into vaccinated turkey flocks, the spread of the infection was limited and the containment of the outbreaks was successful in a shorter time compared to previous epidemics, with a marked reduction in economic losses.

Since H5N2 LPAI has been eradicated there have been no additional introductions of AI into the vaccinated poultry population of the DPPA in northern Italy. Thus, no virus of the H5 or H7 subtype was introduced into the vaccinated population between April 2005 and October 2006, notwithstanding ten viral isolations of H5/H7 LPAI strains in wild birds and the 16 swans and one mallard found positive to the Asian H5N1 HPAI virus (33).

Outbreak of low pathogenicity H7N3 avian influenza, 2007

From 24 May to 30 October 2007, 16 H7N3 LPAI outbreaks were identified in north-eastern Italy. Ten outbreaks were detected in rural or hobby flocks during the implementation of the national AI monitoring plan and at the time of sampling none of the birds showed any clinical signs. From 22 August to 2 October 2007, a total of six infected meat turkey farms were identified in the Lombardy region. The introduction of the H7N3 LPAIV into the industrial turkey sector could be related to the previous circulation of the same virus in rural and hobby poultry.

Phylogenetic analysis performed on the haemagglutinin gene indicated that all the viruses from 2007 were unrelated (haemagglutinin and neuraminidase sequences with respective homologies of 94.5% and 93.5%) to the H7N3 virus that caused the 2002/2004 LPAI epidemic. All the H7N3 viruses obtained from the 2007 outbreaks showed a high level of homology between each other (98.7% to 99.8%) with the exception of one isolate.

Eradication measures were implemented as specified by EU Directive 94/2005/EC (20). All the birds (126,705 in total) on the infected poultry farms were humanely killed and the carcasses disposed of. In three outbreaks, a total of 244 birds of endangered species were not killed.

From 9 October 2007 to 31 March 2008 an emergency DIVA vaccination programme with an inactivated H7N1 vaccine was implemented in areas with the highest turkey densities in the Lombardy and Veneto regions. No additional viral incursion or circulation was detected.

France

In France AI vaccination of poultry had not been implemented in the field prior to 2006. When outbreaks of H5N1 HPAIV were detected in western Siberia during summer 2005, the French Veterinary Authority (DGAL, part of the Ministry of Agriculture and Fishing) asked the French Food Safety Agency (AFSSA) for an opinion on the possibility of implementing an AI vaccination campaign. In late August 2005, AFSSA considered that, in principle, vaccination of force-fed free-range ducks was acceptable provided that:

- satisfactory experimental results were obtained with a reverse genetics-derived inactivated vaccine
- an appropriate adjustment of vaccine needs, in accordance with the availability of commercial vaccines, was provided
- an appropriate balance between surveillance requirements and laboratory network capacity could be secured (4).

At the beginning of November, despite the occurrence of outbreaks in Romania, Turkey and Croatia, AFSSA did not recommend implementing vaccination but confirmed that the preventive vaccination of ducks was conceivable in three locations in the West of France, namely, the wetland areas of Loire-Atlantique, Vendée and Landes (from north to south), since these would have been at higher risk if there was evidence of virus entry via the East Atlantic flyway. In addition, AFSSA recommended considering the use of emergency ring vaccination of long-living poultry and possibly meat turkeys if outbreaks occurred in high density poultry areas and/or biosecurity measures alone were insufficient. Also, AFSSA proposed guidelines for the choice of the vaccine (either a fowlpox virus recombinant vaccine for chickens or an inactivated vaccine for poultry.
and zoo birds) that would induce the best protection against H5N1 HPAIV in terms of decreasing virus shedding by the respiratory and digestive tract following infection (3).

The DGAL took this opinion into consideration and asked the French Agency for Veterinary Medicinal Products (also included in AFSSA) to examine vaccine dossiers that could meet appropriate requirements for a provisional marketing and use authorisation. Two inactivated vaccines obtained these authorisations: including one inactivated vaccine comprising seed virus that was obtained by reverse genetics. In addition, a genetically modified vector vaccine (a recombinant fowlpox virus encoding the H5 gene of H5N8 A/turkey/Ireland/1378/83) was also authorised after the favourable additional opinion given by the Commission of Biomolecular Engineering (a group of experts and public representatives who evaluate the risks to public health and the environment of all genetically modified organisms). A vaccine bank (with 20 million doses) was then established at the beginning of 2006, for preventive (e.g. in zoo birds) and/or emergency vaccination.

Following the occurrence of H5N1 HPAIV outbreaks in Greece at the beginning of February, AFSSA recommended preventive vaccination of zoo birds and free-range ducks and geese that could not be maintained indoors, in order to allow the development of a sufficient level of immunity (5). The recommendation was influenced by several factors:

- the high susceptibility of free-range ducks to avian influenza infection as confirmed by the observations of the authors (15, 24)
- the unprecedented speed with which the H5N1 HPAI panzootic spread in European countries, due most probably to unusual wild bird movements resulting from very hard winter conditions in Eastern Europe
- the confused situation (in mid-February 2006) regarding the origin of the first outbreaks in Africa (probably Nigeria)
- the period of wild bird migration from Africa to northern areas through or above France.

Finally, preventive vaccination of zoo birds and free-range ducks and geese was implemented after the authorisation of the EC (18).

**Preventive vaccination of free-range ducks and geese in winter/spring 2006**

Between the end of February 2006 and the end of March vaccination was implemented for free-range flocks of ducks and geese with more than 100 birds.

Nearly 500,000 free-range domestic waterfowl (98% ducks reared for force feeding) belonging to 143 holdings (96.5% in Landes, corresponding to 218 flocks) were vaccinated using an H5N2 (A/duck/Potsdam/1402/86) inactivated vaccine, which was available in sufficient amounts out of two inactivated authorised vaccines. The schedule was two shots, three to five weeks apart, and the full operation was implemented under the responsibility and supervision of the DGAL through delegation to appointed veterinarians.

In every vaccinated flock unvaccinated sentinel birds were maintained to detect a possible viral incursion. Their number depended on the size of the flock (10% for flock sizes of 10 to 50, 50 sentinel for flocks of 100 to 500, 100 sentinel for flocks of 500 and over).

Early warning surveillance systems with predetermined criteria were implemented on farms with vaccinated flocks (≥ 2% mortality in one day or ≥ 0.25% mortality for two successive days; drop of food and/or water intake ≥ 50% in one day or ≥ 25% for three successive days) and the birds were clinically inspected on a monthly basis by a veterinarian. In addition, sentinels from every vaccinated flock were checked for the absence of H5N1 HPAIV excretion via respiratory and digestive routes, within seven days of loading, using M- and H5-based real-time reverse transcription polymerase chain reaction (RT-PCR) (15 cloacal swabs and 15 tracheal swabs from 30 ducks taken at random). No systematic serological surveillance was implemented because of the high natural prevalence of NP antibodies (24) and the lack of validation, at that time, of N1-based enzyme-linked immunosorbent assay (ELISA) in ducks and geese. However, since HI titres following vaccination were assessed by sampling 5% of the vaccinated flocks (using both the vaccine seed and a French H5N1 highly pathogenic 2006 isolate [24] as antigens), a serological survey of the corresponding sentinels was implemented at the same time.

Following the analysis of 6,500 cloacal and oropharyngeal swabs, no H5N1 HPAIV was detected. However, H5 LPAI viruses were found by real-time RT-PCR in sentinels of 7% of flocks and one H5N3 LPAIV was detected in one flock. Out of 2,640 HI tests, it was observed that geometric mean HI titres at the onset of the vaccine-induced immune responses were around 6 and in some cases hardly 4, using the vaccinal and H5N1 antigens, respectively. There also appeared to be significant variability (from 0 to 9) and poor responses in ducks primed early, at three weeks old (1, 25).

The titre of vaccinated ducks varied between 2log 0 and 9. The geometric mean titre of 2,640 serum samples measured early after vaccination was 6 when using the vaccine virus and 4 when using H5N1 as antigen in the HI test. Ducks responded poorly when vaccinated at an early age of three weeks.
The Netherlands

In the Netherlands, before 2003, vaccination against HPAI was not considered an option because of the potential repercussions on the export market. In 2003, the Netherlands suffered a devastating outbreak of HPAI of the H7N7 subtype causing the destruction of 30 million birds and losses of 300 million euros in direct costs. About 15,000 hobby owners were confronted with the culling of their birds due to the implementation of rigorous stamping out policies. Most of these birds were healthy, which caused discomfort among owners. Moreover, analyses performed after the epidemic indicated that most of the culled hobby birds were not infected and that hobby flocks only played a minor role (if any) in the spread and perpetuation of the epidemic (34).

Aim of the Dutch vaccination programme

The Netherlands are frequented by migratory birds: waterfowl, in particular, are attracted by the many foraging areas and open waters. Free-range and organic farms as well as hobby flocks are considered risk factors for influenza virus introduction from wild birds. To reduce the risk of introduction these categories of birds had to be moved into confinement during the migration season of 2005 and 2006. For free-range farming this measure caused major problems because, according to European legislation, free-range birds are not allowed to be kept in confinement for periods longer than 12 weeks. If this period is exceeded eggs cannot be labelled ‘free-range’ or ‘organic’. Several groups within Dutch society protested against confinement out of concerns about the welfare of confined birds. Vaccination was suggested as an alternative measure. Moreover, associations of hobby-bird owners lobbied for vaccination as an alternative to other control measures and for welfare reasons. Preventive vaccination was offered as an alternative to temporary confinement, following approval of a vaccination plan by the EC in February 2006 (31). A total of eight commercial flocks (19,687 birds) and 1,613 hobby flocks (22,300 birds) were vaccinated. Post-vaccination surveillance by anti-N1 ELISA and the indirect immunofluorescence antibody test indicated that no flock had been infected.

Vaccination programme

Organisation

Three organisations, the Ministry of Agriculture (LNV), the Dutch Food and Consumer Product Safety Authority (VWA) and the Dutch Animal Health Service (GD Deventer) worked closely together for the proper implementation of the vaccination programme. Vaccination was performed by veterinarians. Whereas LNV carried overall political responsibility it made the VWA responsible for the practical execution. The VWA relied on the GD Deventer for the implementation because the GD is better equipped to organise a vaccination campaign. The GD Deventer is the organisation farmers appeal to either directly or via their veterinarian whenever confronted with health or management issues. Thus, whereas the VWA supervised and eventually collected all data, the GD Deventer acted as the central organisation where vaccination could be requested, vaccines obtained, and blood samples submitted. A summary of the organisation and responsibilities of the different parties involved is shown in Figure 1.

Vaccination

According to the vaccination programme approved by the EC, only veterinary practitioners were authorised to administer vaccine. Therefore, poultry owners had to inform the local veterinary practitioner of their intention to vaccinate their birds. The practitioner subsequently completed a vaccination form issued by the GD Deventer containing the information on the location of the owner, species and/or type of birds, number of birds, etc. A unique ‘farm’ number was given to each owner. Unique farm numbers were already available for all commercial farms. Birds that came under the plan were species belonging to the order Galliformes (including chickens and turkeys), and ducks, geese and other species belonging to the Anseriformes, provided they were not kept for commercial purposes. Hobby keepers who had birds of these species were strongly advised to vaccinate them. Based on the number of birds to be vaccinated, GD Deventer supplied the practitioner with appropriate doses of vaccine via a supplier, a vaccination declaration and vaccination leg rings.

Ministry of Agriculture, Nature and Food Quality: overall responsibility

Food and Consumer Product Safety Authority: responsible for central administration, implementation of the plan and supervision

Dutch Animal Health Service: responsible for administration, issues vaccination declarations, and supplies leg rings and vaccines

Veterinarian: submits registration and carries out vaccination

Owner: participates voluntarily and registers via veterinarian

Fig. 1
Organisation of the highly pathogenic avian influenza vaccination programme in the Netherlands: summary of responsibilities
The vaccination declaration stated the address and registration details of the practitioner, the name and address of the owner, the location where the animals were housed and vaccinated, and the number of birds of each species vaccinated with the number of vaccine doses used. The declaration was signed by both practitioner and owner.

By signing the vaccination declaration the owner of vaccinated birds agreed that:

- all vaccinated animals would again be presented for vaccination at least three weeks after the initial vaccination; a new date was set immediately for this next vaccination
- animals that had been vaccinated only once and were awaiting their second vaccination would not be removed from the holding in the intervening period
- the animals and products would not enter the food chain
- he/she would not transfer the animals to another holding at a later date without communicating this to the competent authority. A copy of the vaccination declaration would accompany the animals if they were transferred.
- Permanent transfers were included in an official transfer register
- he/she would not remove the leg ring (or other means of identification) attached by the veterinarian
- he/she would comply with any monitoring or checks required by the competent authority
- he/she would keep a register containing all relevant information concerning the vaccinated poultry.

In case of free-range farming, the owner had to inform their veterinary practitioner that they wanted to vaccinate their flock. Prior to vaccination the veterinarian was required to check the health status of the flock and determine the number of non-vaccinated sentinel animals. In contrast to the hobby birds the sentinel birds were identified by a non-removable leg ring. Vaccinated animals are registered at flock level using the Flock Information System (KIP). The KIP system is the official database in the Dutch poultry sector, which is also used, for example, within the Salmonella monitoring programme. In KIP, both farms and flocks are registered, which offers the opportunity to monitor the location and movement of (vaccinated) flocks.

**Supervision and security**

A copy of the vaccination declaration was submitted to GD Deventer to prepare a database of the records.

Whether all of the vaccine doses were administered was checked by comparing the vaccination application forms with the completed vaccination declaration. This check was performed by the GD Deventer under the supervision of the VWA. Both the GD Deventer and the General Inspection Service of the LNV (AID) carried out random checks on the implementation of the programme with regard to legitimate use. Together with the VWA, the GD Deventer organised the training of the veterinarians, checked the administration of the veterinary practitioners, monitored the delivery of vaccines and coordinated the serological sampling of the flocks. Vaccination of commercial flocks was reported regularly by the GD Deventer to both the VWA and the production boards (PVE) so that they could be registered in the KIP system.

The VWA approved and supervised the relevant protocols and checked before and afterwards whether activities were performed in accordance with these protocols. In addition, both the AHS (under responsibility of the competent authority) and the AID carried out random checks on the implementation of the programme with regard to the legitimate use of the vaccines. Moreover, the VWA together with the GD Deventer verified the application of ordered doses afterwards by means of the provided vaccination declarations.

**Movement restrictions**

Vaccinated birds could not be moved outside the Netherlands unless such movement was approved by the competent authorities of the importing EU Member State. Eggs of vaccinated free-range animals could be handled only by designated packing stations which, when located outside the Netherlands, had to be approved by the competent authorities of the Member State. Meat of vaccinated birds could be exported only with the consent of the recipient. Finally, manure of vaccinated animals had to remain in the Netherlands and slaughterhouses were not allowed to slaughter vaccinated hobby poultry.

**Results**

Vaccination started in March 2006 and ended in August 2006. During this period only eight commercial farms and 1,613 owners of hobby birds made use of the opportunity to vaccinate. In total 19,687 free-range chickens were vaccinated twice with commercial H5N9 vaccine. Of the 22,330 hobby birds that were vaccinated, 14,207 were chickens, 372 turkeys, 3,395 ducks and 3,467 geese; 710 belonged to other species within the order Galliformes and 194 in the order Anseriformes. These numbers were disappointingly low.

The high costs that had to be paid to the veterinarians may have kept a lot of hobby owners from vaccinating. Costs, and trade restrictions, also had a negative impact on the number of participating free-range holdings.
Post-vaccination monitoring

Before the vaccination period that ran from March to July 2006 blood samples were collected from 2,380 hobby birds. Samples originating from 1,133 hobby flocks were tested using generic influenza A antibody ELISA. Samples that were positive in the ELISA were confirmed by HI test using two H5 and H7 antigens. In total, 39 samples obtained from 31 different flocks were confirmed to be positive for H5 or H7 in the HI test. All of these flocks were inspected by specialist teams of the VWA and a maximum of 20 tracheal and cloacal swabs were collected to exclude a prevalence of more than 15%. Swabs were tested using real-time RT-PCR targeted at the M gene. All samples tested negative in the PCR and thus no evidence for ongoing AIV infections was obtained. Since no sentinel birds were present in vaccinated hobby flocks, the birds were serologically monitored for infection with H5N1 HPAIV using a commercial competitive N1 antibody ELISA to detect antibodies against neuraminidase type 1. Serum samples were collected at a maximum of 20 per flock. By prior studies using a panel of 75 sera of experimentally infected birds and 427 negative field sera of various species, the sensitivity of the competitive N1 antibody ELISA had been estimated to be 90.1% (95% confidence limits, 91.8% to 100%) and the specificity 91.1% (98.2% to 100%). In total, 2,545 sera were collected, of which 2,500 were tested in the N1 competitive ELISA. Most of the sera originated from chickens. Of the 1,862 chicken sera collected, 1,829 were tested and only 40 were positive. These 40 samples were tested in the immunofluorescence test (IFT) on insect cells infected with N1 recombinant baculovirus (14). None of the sera were confirmed positive in the N1 IFT; 30 sera were negative, 9 sera showed a non-specific reaction and 1 sample could not be tested because of lack of volume. One hundred and sixty-four duck sera were tested in the N1 ELISA, 30 of which were positive and 134 negative. Only 29 of the positive duck sera could be tested in N1 IFT but none was confirmed. Of the 379 goose sera tested, 52 were positive in the N1 ELISA. Eight of 109 sera obtained from other bird species were positive in the N1 ELISA. The positive sera of the last two bird categories (goose and other birds) could not be tested for confirmation in the IFT because the fluorescein conjugated secondary antibody needed for the IFT was not available.

Discussion

The epidemiology of AI infections has changed over the last decade, due to the persistence and the continuous spread of H5N1 HPAIV in domestic poultry populations in vast geographical areas of three continents, and the marked increase of AI outbreaks worldwide. 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 (22).

The application of the different control options, which may include vaccination, depends on 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 to succeed in controlling and ultimately eradicating the infection, vaccination programmes must be part of a wider territorial strategy. 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 as a whole cannot be implemented, the establishment of an endemic status due to sub-clinical virus circulation in the vaccinated poultry population cannot be ruled out.

The Italian vaccination programme was initiated when the concept of large-scale immunisation against AIV of the H5/H7 subtypes was virtually unheard of, and had only been attempted, without achieving eradication, in a few countries (27, 30). The choice of the vaccine was strongly influenced by what was available at the time and could be licensed provisionally in a timely manner. The only products that complied with these requirements were inactivated conventional vaccines, and the DIVA discriminatory test was developed on the basis of available options.

The DIVA system using heterologous neuraminidase has some limitations in its application in epidemiological situations where there is the risk of introduction of multiple AI subtypes. The main limitation is that under certain circumstances, for example, if there is a risk of multiple introductions, it is impossible to predict the neuraminidase of the forthcoming virus. This can cause problems in the interpretation of the DIVA test. An approach to resolving this difficulty is to use seed vaccine strains of the H5 and H7 subtypes exhibiting rare neuraminidase subtypes such as N5 or N8. These criteria for the selection of vaccine strains will greatly reduce the chance that an AIV of the same N subtype is introduced. However, in any case, unvaccinated sentinels should always be present in vaccinated flocks for surveillance purposes.

Research in the field of AI vaccinology has increased in the last few years with a variety of novel preparations being produced, ranging from recombinant-based products (using vectors such as fowlpox virus, Newcastle disease virus and infectious laryngotracheitis virus) (2) to those based on reverse genetics (35) (see paper by Fuchs et al. in
Vaccination enabling the DIVA system in conjunction with a territorial strategy can be successful in controlling and eradicating AI infections in poultry, and is compatible with the continuation of international trade. However, the management of such a programme is complex and may include failures such as the spread of infection within the vaccinated population. It is therefore essential that the vaccination programme uses a suitable vaccine that enables the detection of field-virus-exposed flocks and that the monitoring system in place is developed to guarantee early detection of, and rapid response to, AI introductions. 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 that would result in improved crisis management. These include rapid approval of established control programmes, constant update on the field situation, feedback on successes and failures, harmonisation of protocols and systems and public availability of control and eradication programmes. In this way, even developing countries that are experiencing NAI infections, and that have no experience with AI management, can learn from other experiences and avoid any waste of resources and time.

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

The Italian experience with vaccination against NAI, gathered between 1999 and 2006, is one example of the potential use of vaccination and of the evolution of a programme on the basis of the learning process in itinere. Extensive monitoring has allowed the identification of infection in the vaccinated and unvaccinated avian population, which in turn has resulted in the eradication of infection. In addition, field evidence has generated important data such as those concerning the higher susceptibility of turkeys to LPAI infection, which has been subsequently demonstrated in experimental studies (37), and the role that quail may play as reservoirs of H7 LPAI viruses has been identified. This evidence has resulted in an improvement of control programmes, including structural changes such as the re-organisation of the quail farming system.

Vaccination of commercial ducks and geese was implemented for the first time in Europe in France. Apart from HI responses, levels of protection achieved were not assessed following vaccination since no H5N1 HPAI outbreaks occurred in the areas where ducks and geese had been vaccinated and, unfortunately, no experimental H5N1 HPAIV challenge could be implemented. The vaccination campaign was terminated and the last vaccinated birds were slaughtered in June 2006. In fact, the epidemiological situation evolved favourably and the risk of exposure was reduced. In addition, the direct and indirect costs of vaccination were very high, and France wanted to recover its HPAI-free status as soon as possible.

Although the implementation of such a vaccination programme has been criticised, it should be remembered that there was no way to maintain these poultry birds indoors, and nobody could predict the geographical extent of outbreaks in wild birds or the consecutive exposure of free-range ducks and geese. Thus, the goal was to avoid any introduction of virus into French poultry. If nothing had been done, the situation in France might have evolved as it did in Hungary, with 29 H5N1 HPAIV outbreaks in free-range ducks and geese (1). Vaccination of commercial poultry has not been recommended or implemented since that time and the vaccine bank no longer exists.

The Dutch vaccination programme revealed valuable information on various aspects of the implementation of such a campaign, such as central coordination, vaccine selection, registration of owners, logistics of materials, movement control of vaccinated poultry, and monitoring of vaccinated holdings.

Unfortunately, participation in the vaccination programme, which was entirely voluntary, was disappointingly low. The limited participation was an unexpected finding as the vaccination programme had been requested and implemented as a result of specific requests from the owners and farmers. No investigation was conducted to investigate factors that determined participation, but the high costs of vaccination to be paid by the owner and the bureaucracy linked to the implementation of the programme certainly did not promote participation. For the commercial sector, restrictions imposed upon the marketing of eggs and movement of spent hens played a role in discouraging participation. In fact, farmers who were in favour of participating in the programme were put under pressure by the rest of the poultry industry, who feared losing markets, to withdraw their support for vaccination.

As for the vaccination itself, no adverse effects were reported in vaccinated birds and vaccination technically went rather smoothly.
The absence of H5N1 HPAIV introduction into vaccinated holdings of hobby birds was checked serologically by using a competitive N1 ELISA that had been marketed only recently and had not yet been used in the field. Using this ELISA the authors detected 130 positive serum samples mainly from ducks and geese. However, none could be confirmed by other methods. Moreover, extensive epidemiological investigation did not indicate that virus circulated in those flocks. Therefore the authors conclude that the sera either reacted non-specifically, or that the positive reactions were the result of previous exposure to non-pathogenic N1.

Non-vaccinated sentinel birds were used in free-range farms. There were no reports of death or disease in the sentinels. The sera of the sentinels were tested in the regular serological monitoring programme that is obligatory in the Netherlands. Sera collected within the framework of this programme were tested using a commercially available indirect antibody ELISA. In the programme free-range farms were checked every three months. None of the sera of sentinel birds were positive during the vaccination campaign, whereas flocks of two conventional non-vaccinated farms were serologically positive and H7N7 LPAIV was recovered from one of them in August 2006. At the time of writing H5N1 HPAIV has not been detected in the Netherlands.

Vaccination is now being used extensively to help control widespread H5N1 HPAI or prevent its introduction. Overall, the veterinary community does not have first-hand experience with managing this disease using vaccination. It is most likely that, if managed appropriately, positive results will be achieved. However, errors will be made, and unexpected events will occur and it is imperative that control and eradication processes can benefit from the information generated by such situations. In addition, control programmes need to be managed in a flexible and transparent manner to respond to the continuous challenges that AI infections pose.

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La vaccination comme moyen de lutte contre l’introduction des virus de l’influenza aviaire à déclaration obligatoire en Europe, 2000-2006

I. Capua, A. Schmitz, V. Jestin, G. Koch & S. Marangon

Résumé
À la fin de l’année 2000, l’Italie a été le premier pays de l’Union européenne (UE) à mettre en place un programme de vaccination d’urgence contre l’influenza aviaire à déclaration obligatoire. Le vaccin utilisé était un vaccin classique contenant une neuraminidase hétérologue par rapport à celle de la souche virale circulant dans le terrain ; la vaccination a été appliquée en complément des restrictions et des mesures de biosécurité imposées dans le cadre d’une stratégie intégrée d’éradication. La vaccination, réalisée suivant le système DIVA (pour différencier les animaux infectés des animaux vaccinés) a été pratiquée à plusieurs reprises jusqu’en mars 2008. Elle a permis d’identifier les troupeaux exposés aux virus de terrain et d’obtenir à terme l’éradication des infections dues aux virus faiblement pathogènes H7N1, H7N3 et H5N2. L’Italie a été également le premier pays à appliquer un programme de vaccination préventive avec un vaccin bivalent H5/H7 destiné à certaines populations de volailles ; ce programme a été interrompu en décembre 2006.


Les observations recueillies en Italie pendant les six années aux cours desquelles la vaccination a été pratiquée contre l’influenza aviaire montrent que la vaccination est un outil efficace pour limiter le nombre de foyers secondaires et probablement pour éviter l’introduction des virus de l’influenza aviaire faiblement pathogène dans les populations susceptibles. L’expérience de la France et des Pays-Bas a permis de réunir des informations sur la vaccination des canards et des volailles de loisir, ainsi que sur les programmes de suivi de la vaccination et sur les difficultés de mise en œuvre rencontrées. L’analyse des avantages et des inconvénients de la vaccination, y compris ses aspects financiers, doit faire partie intégrante du processus de décision.

Mots-clés
La vacunación como instrumento para combatir la penetración de virus de la influenza aviar de notificación obligatoria en Europa, 2000-2006

I. Capua, A. Schmitz, V. Jestin, G. Koch & S. Marangon

Resumen
Italia fue, a finales del año 2000, el primer país de la Unión Europea (UE) que aplicó un programa de vacunación de emergencia contra la influenza aviar de notificación obligatoria. Para complementar las medidas de seguridad biológica y de restricción adoptadas como parte de la estrategia general de erradicación, se administró una vacuna convencional constituida por una cepa original con un subtipo de neuraminidasa distinto del subtipo salvaje. Esta técnica de vacunación, acorde con el sistema para distinguir entre animales infectados y vacunados (DIVA), fue utilizada repetidas veces hasta marzo de 2008. Ello permitió detectar bandadas salvajes expuestas y, a la postre, erradicar las infecciones causadas por las cepas de influenza leve H7N1, H7N3 y H5N2. Italia también fue el primer país en aplicar a determinadas poblaciones avícolas un programa de vacunación profiláctica bivalente H5/H7, que fue interrumpido en diciembre de 2006. Tras las incursiones de la cepa altamente patógena H5N1 registradas en Europa en 2005 y 2006, otros dos Estados de la Unión Europea, Francia y los Países Bajos, instauraron sendos programas de vacunación preventiva en 2006, aunque no iban dirigidos a las mismas poblaciones avícolas que en el caso de Italia y duraron poco tiempo. Los datos obtenidos gracias a los seis años de experiencia de vacunación contra la influenza aviar en Italia indican que se trata de un instrumento útil para limitar la propagación secundaria y, posiblemente, impedir la penetración de virus de la influenza aviar levemente patógena en poblaciones susceptibles. La experiencia de Francia y los Países Bajos ofrece datos sobre la vacunación de patos y de aves de corral domésticas, respectivamente, y sobre los programas de seguimiento asociados a la vacunación y las dificultades que se plantean al aplicarlos. A la hora de adoptar decisiones conviene sopesar las ventajas e inconvenientes de la vacunación, comprendidos los aspectos económicos.

Palabras clave
References


