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Currently available tools and strategies for emergency vaccination in case of avian influenza

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Abstract

Recent epidemics of highly contagious animal diseases included in the list A of the OIE such as foot-and-mouth disease, classical swine fever and avian influenza (AI) have led to the implementation of stamping-out policies resulting in the depopulation of millions of animals. The enforcement of a control strategy based on culling of animals that are infected, suspected of being infected or suspected of being contaminated, which is based only on the application of sanitary restrictions on farms, may not be sufficient to avoid the spread of infection, particularly in areas that have high animal densities, thus resulting in mass depopulation.

In the European Union, the directive that imposes the enforcement of a stamping-out policy (92/40/EC) for AI was adopted in 1992 but was drafted in the 1980s. The poultry industry has undergone substantial changes in the last twenty years, mainly resulting in shorter production cycles and greater animal densities per territorial unit. Due to these organizational changes, infectious diseases are significantly more difficult to control as a result of the greater number of susceptible animals reared per given unit of time and the difficulties in applying adequate biosecurity measures.

The slaughter and destruction of great numbers of animals is also questionable from an ethical point of view, particularly when human-health implications are negligible. For this reason, mass depopulation has raised serious concerns for the general public and has recently led to very high costs and economic losses for the national and federal governments, the stakeholders and ultimately for the consumers.

In the past, the use of vaccines in such emergencies has been limited by the impossibility of differentiating vaccinated/infected from vaccinated/non-infected animals. The major concern was that through trade or movement of apparently uninfected animals or products, the disease could spread further or might be exported to other countries. For this reason export bans have been imposed on countries enforcing a vaccination policy.

This paper takes into account the possible strategies for the control of avian influenza infections, bearing in mind the new proposed definition of AI. In detail, an overview of the advantages and disadvantages of using conventional inactivated (homologous and heterologous) vaccines and recombinant vaccines is presented and

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discussed. Reference is made to the different control strategies including the restriction measures to be applied in case of the enforcement of a vaccination policy. In addition, the implications of a vaccination policy on trade are discussed.

In conclusion, if vaccination is accepted as an option for the control of AI, vaccine banks including companion diagnostic tests must be established and made available for immediate use.
Keywords: avian influenza; vaccination; intervention strategies; poultry

Introduction

Recent epidemics of highly contagious animal diseases included in the list A of the Office International des Epizooties (OIE) such as foot-and-mouth disease, classical swine fever and avian influenza (AI) have led to the implementation of stamping-out policies resulting in a depopulation involving millions of animals. The implementation of a control strategy based on culling of animals that are infected, suspected of being infected or suspected of being contaminated, which is based only on the application of sanitary restrictions, may not be sufficient to avoid the spread of infection. This event is particularly foreseeable in areas that have high animal densities, and inevitably results in mass-depopulation policies. There is an increased risk of disease spread in these areas and the financial consequences of any occurring epidemic are severe (Capua and Marangon 2000; Dijkhuizen and Davies 1995; Gibbens et al. 2001; Meuwissen et al. 1999).

With reference to AI, the EU directive that imposes the enforcement of a stamping-out policy (92/40/EC) was adopted in 1992 but was drafted in the ’80’s (CEC 1992). The poultry industry has undergone substantial changes in the last twenty years, mainly resulting in shorter production cycles and in the development of densely populated poultry areas (DPPA). As a result of these organizational changes, infectious diseases are significantly more difficult to control due to the greater number of susceptible animals reared per given unit of time and to the difficulties in applying adequate biosecurity programmes. In order to avoid the destruction of great numbers of animals, the possibility of pursuing different control strategies should be considered.

Until recent times highly pathogenic avian influenza (HPAI) was considered a rare disease in domestic poultry with only 17 episodes being reported worldwide in the 40-year period 1959-1998. However, three further outbreaks have occurred since 1999, resulting in 11 outbreaks since 1991 and six in the six years covering 1997-2003. Recently, there also appears to have been a marked increase in the number of low-pathogenicity AI (LPAI) outbreaks caused by H5 and H7 viruses. The countries, subtypes and approximate number of birds involved are listed in Table 1. From 1997 to date 14 significant outbreaks due to H5 or H7 subtypes have been reported in poultry, three of which have had human-health implications. The approximate number of birds culled for AI in the past 6 years has been 63 million, with hundreds of millions of birds involved (Capua and Alexander 2004).

The slaughter and destruction of great numbers of animals is also questionable from an ethical point of view. For this reason, mass depopulation has raised serious concerns from the general public. The policy has also led to very high costs and economical losses for the Community budget, the Member States, the stakeholders and ultimately for the consumers.

Table 1. Outbreaks of LPAI and HPAI caused by H5 and H7 viruses in recent years

Country	Year[s]	Subtype	Virulence	Approximate no. of birds infected/culled	Control
Mexico Guatemala, El Salvador	1994-2003 2000 2001	H5N2	LPAI/HPAI	>1.000,000,000	Vaccination
Pennsylvania	1996-1998	H7N2	LPAI	2,623,116	Depopulation
Australia	1997	H7N4	HPAI	310,565	Stamping out
Hong Kong	1997-2003	H5N1	HPAI	~3,000,000	Stamping out Vaccination
Italy	1997	H5N2	HPAI	7741	Stamping out
Ireland	1998	H7N7	LPAI	320,000	Depopulation
N. Ireland	1998	H7N7	LPAI	?	Depopulation
Italy	1998	H5N9	LPAI	2,000	Stamping out
Belgium	1999	H5N2	LPAI	100	Stamping out
Italy	1999-2001	H7N1	LPAI HPAI LPAI	17,000,000	Stamping out Vaccination + stamping out
Germany	2001	H7N7	LPAI	145	Stamping out
Pakistan	2001	H7N3	HPAI/LPAI	>10,000,000?	Vaccination
USA (NC/VA)	2002	H7N2	LPAI	~5,000,000	Stamping out
Chile	2002	H7N3	LPAI/HPAI	~1,000,000	Stamping out
Italy	2002-2003	H7N3	LPAI	>6,000,000	Vaccination + stamping out
The Netherlands Belgium Germany	2003	H7N7	HPAI	30,283,000 2,700,000 419,000	Stamping out
USA (CT)	2003	H7N2	LPAI	2,900,000	Vaccination

In the EU, the possibility of vaccinating to aid control policies in such emergencies has been limited by the inability to differentiate vaccinated–infected from vaccinated–non-infected animals. The major concern was that through trade or movement of vaccinated animals or their products, the disease could spread further or might be exported to other countries, primarily because it was not possible to establish whether the vaccinated animals had been field-exposed.

The following paper takes into account the recent developments in vaccinology, which may represent valid tools for the control of avian-influenza infections, bearing in mind the new definition of AI proposed by the EU (Document Sanco/B3/AH/R17/2000) and by the OIE (*ad hoc* expert group on Avian Influenza, Animal Health Code Commission meeting of 29-30 October 2002) and the possibility of enforcing an emergency vaccination programme with the products currently available. Reference will be made to the type of vaccines available, the efficacy of these vaccines, their limitations and the possibility of identifying infected animals in a vaccinated population.

Definition of avian influenza

Avian influenza viruses all belong to the *Influenzavirus A* genus of the *Orthomyxoviridae* family and are negative-stranded, segmented RNA viruses. The influenza-A viruses can be divided into 15 subtypes on the basis of the haemagglutinin (H) antigens. In addition to the H antigen, influenza viruses possess one of nine neuraminidase (N) antigens. Virtually all H and N combinations have been isolated from birds, thus indicating the extreme antigenic variability that is a hallmark of these viruses. Changes in the H and N composition of a virus may be brought about by genetic reassortment in host cells. One of the consequences of genomic segmentation is that if co-infection by different viruses occurs in the same cell, progeny viruses may originate from the reassortment of parental genes originating from different viruses. Thus, since the influenza-A virus genome consists of 8 segments, from two parental viruses 256 different combinations of progeny viruses may arise theoretically.

Current EU legislation (CEC 1992) defines avian influenza as "an infection of poultry caused by any influenza-A virus which has an intravenous pathogenicity index in six-week-old chickens greater than 1.2 or any infection with influenza-A viruses of H5 or H7 subtype for which nucleotide sequencing has demonstrated the presence of multiple basic amino acids at the cleavage site of the haemagglutinin". However it has been proved that highly pathogenic avian influenza (HPAI) viruses emerge in domestic poultry from low-pathogenicity (LPAI) progenitors of the H5 and H7 subtypes. It therefore seems logical that HPAI viruses and their LPAI progenitors must be controlled, when they are introduced in domestic poultry populations (Scientific Committee on Animal Health and Animal Welfare 2000). The new proposed definition of AI for the OIE and the EU (Scientific Committee on Animal Health and Animal Welfare 2000) is "an infection of poultry caused by either any influenza A virus which has an intravenous pathogenicity index in 6-week-old chickens greater than 1.2 or any influenza A virus of H5 or H7 subtype". With reference to the present paper, the term avian influenza applies to all avian influenza viruses of the H5 and H7 subtype, regardless of their virulence and of their pathogenicity for domestic poultry.

Rationale behind the use of vaccines

When an outbreak of avian influenza occurs in an area with a high population density, the application of rigorous biosecurity measures might not be possible. In this case the disease may spread very rapidly, and the tracing and culling capacities might not be adequate. This results in enormous efforts being spent on chasing the disease rather than on actively imposing a barrier to its spread. The only additional measure that can be taken to attempt to reduce disease spread is vaccination. The expected results of the implementation of a vaccination policy on the dynamics of infection are primarily those of reducing the susceptibility to infection (i.e. a higher dose of virus is necessary for establishing productive infection) and reducing the amount of virus shed into the environment. The association between a higher infective dose necessary to establish infection and less virus contaminating the environment represents a valuable support to the eradication of infection.

The efficacy of an emergency vaccination programme is inversely correlated with the time span between the diagnosis in the index case and the implementation of mass vaccination. For this reason, it is imperative that if emergency vaccination is

considered as a possible option in a given country, vaccine banks must be available in the framework of national contingency plans.

It should be clear that vaccination can be used for a variety of different scopes. It can be used to protect birds and reduce spread in case an HPAI epidemic is out of control and there is the risk of spread to other DPPAs. When there is no more evidence of virus circulation the vaccinated birds can be culled or dealt with appropriately. It can also be used to support eradication measures for LPAI in a DPPA. This is a longer-term strategy, which may also imply trade of commodities, provided they come from vaccinated animals that have not been field-exposed.

Currently available vaccines

Conventional vaccines

Inactivated homologous vaccines

These vaccines were originally prepared as 'autogenous' vaccines, i.e., vaccines that contain the same avian-influenza strain as the one causing the problems in the field. They have been used extensively in Mexico and Pakistan during the AI epidemics (Swayne and Suarez 2000).

The efficacy of these vaccines in preventing clinical disease and in reducing the amount of virus shed in the environment has been proven through field evidence and experimental trials (Swayne and Suarez 2000). The disadvantage of this system is the impossibility of differentiating vaccinated from field-exposed birds unless unvaccinated sentinels are kept in the shed. However, the management (identification, bleeding and swabbing) of sentinel birds during a vaccination campaign is time-consuming and rather complicated since they are difficult to identify, and they may be substituted with seronegative birds in the attempt to escape restrictions imposed by public health officials.

Inactivated heterologous vaccines

These vaccines are manufactured in a similar way to the previous ones. They differ in the fact that the virus strain used in the vaccine is of the same H type as the field virus but has a heterologous neuraminidase. Following field exposure, clinical protection and reduction of viral shedding are ensured by the immune reaction induced by the homologous H group while antibodies against the neuraminidase induced by the field virus can be used as a marker of natural infection (Capua and Marangon 2000).

For both homologous and heterologous vaccines, the degree of clinical protection and the reduction of shedding are improved by a higher antigen mass in the vaccine (Swayne et al. 1999). For heterologous vaccines the degree of protection is not strictly correlated to the degree of homology between the haemagglutinin genes of the vaccine and challenge strains (Swayne and Suarez 2000). This is definitely a great advantage because it enables the establishment of vaccine banks since the master seed does not contain the virus that is present in the field and may contain an isolate (preferably of the same lineage) available before the epidemic.

Recombinant vaccines

Several recombinant fowlpox virus expressing the H5 antigen have been developed (Beard, Schnitzlein and Tripathy 1992; Beard, Schnitzlein and Tripathy 1991; Swayne et al. 2000; Swayne, Beck and Mickle 1997; Webster et al. 1996) and one has

been licensed and is currently being used in Mexico (Swayne and Suarez 2000). Experimental data have also been obtained for fowlpox-virus recombinants expressing the H7 antigen (Boyle, Selleck and Heine 2000). Other vectors have been used to deliver successfully the H5 or H7 antigens such as constructs using infectious laryngotracheitis virus (ILTV) (Lüschow et al. 2001).

The only field experience with a recombinant virus to control AI has been obtained in Mexico (Villareal-Chavez and Rivera Cruz 2003), where it has been used in the vaccination campaign against an LPAI H5N2 virus.

No such product has been licensed in the EU to date.

Trade implications

Until recent times, vaccination against avian influenza viruses of the H5 and H7 subtypes, was not considered or practised in developed countries since it implied export bans on live poultry and on poultry products (CEC 1994). In case of an infection with an H5 or H7 virus, regardless of the virulence of the isolate, export bans have also been imposed. Export bans frequently represent the major cause of economic loss due to OIE List A diseases.

Whilst the severe clinical signs caused by HPAI ensure a prompt diagnosis and facilitate the implementation of a stamping-out policy, the inconspicuous nature of the disease caused by viruses of low pathogenicity make this infection difficult to diagnose. Detection of infection is only possible with the implementation of appropriate surveillance programmes. Bearing in mind the new proposed definition of AI, and the potential mutation of LPAI of the H5 and H7 subtypes to HPAI it is easy to understand why these bans have been imposed. For the sake of trade, freedom from AI should be demonstrated in a given country or compartment by ongoing surveillance programmes. This approach is supported by the fact that in several recent outbreaks, infection with a virus of low pathogenicity was only detected once infection was widespread, and often out of control.

In absence of vaccination, trade bans imposed on a given area last until freedom from infection can be demonstrated in the affected population. In the case of the adoption of a vaccination policy that does not enable the application of a ‘DIVA’ (differentiating infected from vaccinated animals) strategy (either for the type of vaccine used or because the monitoring system in place does not guarantee that infection is no longer circulating) this also results in prolonged trade bans. On the contrary, if it is possible to demonstrate that the infection is not circulating in the vaccinated population trade bans may be lifted.

Such ‘marker’ vaccination strategies offer attractive control options for OIE List-A diseases. In case of an outbreak of avian influenza in a DPPA the option of vaccinating should be pursued. To safeguard international trade a control strategy that enables the differentiation between vaccinated–infected and vaccinated–non-infected animals should be implemented. The possibility of using vaccines would support restriction-based control measures, thus reducing the risk of a major epidemic and the subsequent mass stamping-out policy.

Options for control

It is extremely difficult to establish fixed rules for the control of infectious diseases in animal populations, due to the unpredictable number of variables involved.

However, with reference to AI, some basic scenarios may be hypothesized, and on the basis of the considerations made above some guidelines may be drawn, which are reported in Table 2.

Table 2. Guidelines for the application of control policies for AI

H5/H7 virus pathogenicity	Index case flock	Evidence of spread to industrial circuit	Population density in area	Policy
HPAI/LPAI	Backyard	No	High/Low	Stamping out
HPAI/LPAI	Backyard	Yes	Low	Stamping out
			High	Vaccination
HPAI/LPAI	Industrial	No	High/Low	Stamping out
HPAI/LPAI	Industrial	Yes	Low	Stamping out
			High	Vaccination

There are several crucial steps that must be planned for if avian influenza represents a risk. Firstly the index case must be promptly identified. This should not represent a problem if the virus is of high pathogenicity, but it can be a serious concern if the virus if of low pathogenicity. For this reason countries or areas at risk of infection should implement specific surveillance systems to detect infection with LPAI as soon as it appears.

Secondly, a timely assessment must be performed of whether there has been spread to the industrial poultry population of that area. This is a crucial evaluation, which must be made available for decision-makers.

Once an AI outbreak has been identified eradication measures based on the stamping out or controlled marketing of slaughter birds on infected farms must be enforced. The choice between these two options must be taken bearing in mind the pathogenicity and transmissibility of the virus, the density of poultry farms around the affected premises, the economical value of the affected birds, the logistics for slaughter/stamping out and the collaborative approach of farmers/producers. With reference to the Italian experience a stamping-out policy was generally applied to LPAI-infected young meat birds, breeders and layers, while controlled marketing was applied for older meat birds approaching slaughter age. This strategy enables the reduction of the restriction periods (i.e. if infected young turkeys, breeders or layers were kept on the farms the restriction period could last several months) and hence facilitates faster restocking.

In addition, restriction measures on the movement of live poultry, vehicles and staff must be imposed in the areas at risk.

Finally, if vaccination is the proposed strategy, vaccine banks should be available for immediate use and a contingency plan must be enforced. A territorial strategy must be implemented. It must include restriction measures (Tables 3 and 4) and an ongoing set of adequate controls (Figure 1) that enable public authorities to establish whether the virus is circulating or not in the vaccinated population and assess the efficacy of the vaccination programme.

Table 3. Basic restriction and monitoring measures to be enforced on the movements of live poultry and poultry products originating from and/or destined for farms or plants located in the vaccination area (VA)

Commodity	Restrictions to movements towards the VA	Restrictions to movements inside the VA	Restrictions to movements outside the VA
Hatching eggs	- shall be transported directly to the hatchery of destination - (and their packaging) must be disinfected before dispatch - tracing-back of egg lots in the hatchery shall be guaranteed	- must originate from a vaccinated or unvaccinated breeding flock that has been tested, with negative results, according to Table 4 - shall be transported directly to the hatchery of destination - (and their packaging) must be disinfected before dispatch - tracing-back of egg lots in the hatchery shall be guaranteed	- must originate from a vaccinated or unvaccinated breeding flock that has been tested, with negative results, according to Table 4 - shall be transported directly to the hatchery of destination - (and their packaging) must be disinfected before dispatch - tracing-back of egg lots in the hatchery shall be guaranteed
Day-old chicks	must be destined for a poultry-house where: - no poultry is kept - cleansing and disinfection operations have been carried out	- must originate from hatching eggs satisfying the conditions mentioned above - must be destined for a poultry house where no poultry is kept and where cleansing and disinfection operations have been carried out	- must originate from hatching eggs satisfying the conditions mentioned above - must be destined for a poultry house where no poultry is kept and where cleansing and disinfection operations have been carried out
Ready-to-lay pullets	must be: - housed in a poultry house where no poultry has been kept for at least 3 weeks, and cleansing/ disinfection operations have been carried out - vaccinated at the farm of destination	must: - have been vaccinated regularly against avian influenza - have been tested, with negative results, according to Table 4 - be destined for a farm located in the VA and housed in a poultry house where no poultry has been kept for at least 3 weeks, and cleansing/disinfection operations have been carried out - be officially inspected within 24 hours before loading - be virologically and serologically tested with negative results before loading (sentinel birds)	must: - not have been vaccinated - have been tested, with negative results, according to Table 4 - be destined for a poultry house where no poultry has been kept for at least 3 weeks, and cleansing/ disinfection operations have been carried out - be officially inspected within 24 hours before loading - be virologically and serologically tested with negative results before loading

Commodity	Restrictions to movements towards the VA	Restrictions to movements inside the VA	Restrictions to movements outwards the VA
Poultry for slaughter	- must be sent directly to the abattoir for immediate slaughter - must be transported by lorries that operate, on the same day, only on farms located outside the VA - lorries must be washed and disinfected under official control before and after each transport	- shall undergo a clinical inspection within 48 hours before loading - must be directly sent to the abattoir for immediate slaughter - must be serologically tested before loading - the abattoir must guarantee that accurate washing and disinfection operations are carried out under official supervision - shall be transported by lorries that operate, on the same day, only on farms located inside the VA - lorries must be washed and disinfected before and after each transport	- shall undergo a clinical inspection within 48 hours before loading - must be sent directly to an abattoir designated by the competent veterinary authority for immediate slaughter - must be serologically tested before loading - the abattoir must guarantee that accurate washing and disinfection operations are carried out under official supervision - shall be transported by lorries that operate, on the same day, only on farms located inside the VA - lorries must be washed and disinfected before and after each transport
Table eggs	must be: - sent directly to a packaging centre or a thermal-treatment plant designated by the competent authority - transported using disposable packaging materials that can be effectively washed and disinfected	must: - originate from a flock that has been tested, with negative results, as laid down in Table 4 - be sent directly to a packaging centre or a thermal-treatment plant designated by the competent authority - be transported using disposable packaging material or packaging material that can be effectively washed and disinfected	must: - originate from a flock that has been tested, with negative results, as laid down in Table 4 - be sent directly to a packaging centre or a thermal-treatment plant designated by the competent authorities - be transported using disposable packaging material or packaging material that can be effectively washed and disinfected

Table 4. Basic restrictions to be applied to the trade of fresh meat produced from poultry originating from the vaccination area (VA)

Commodity	Unrestricted to international trade	Restricted to national trade
Fresh poultry meat	- originating from birds vaccinated against avian influenza with a heterologous subtype vaccine can be dispatched to other countries, provided that the meat comes from slaughter-turkey flocks that: (i) have been regularly inspected and tested with negative results for avian influenza as follows. For the testing of: — vaccinated animals, the anti-N discriminatory test shall be used — sentinel animals, either the haemagglutination-inhibition test (HI), the AGID test or the ELISA test shall be used. However, anti-N discriminatory test shall also be used if necessary (ii) have been clinically inspected by an official veterinarian within 48 hours before loading. Sentinel animals shall be inspected with particular attention (iii) have been serologically tested with negative results with the iIFA test (iv) must be sent directly to a slaughterhouse designated by the competent authority and be slaughtered immediately on arrival - produced from poultry not vaccinated against avian influenza and originating from the VA	originating from holdings located in the VA cannot be dispatched to other countries, if produced from poultry: (i) vaccinated against avian influenza with a homologous subtype vaccine (ii) vaccinated against avian influenza with a heterologous subtype vaccine and not tested, with negative results, using the anti-N discriminatory test (iii) originating from seropositive poultry flocks subjected to controlled marketing (iv) coming from poultry holdings located in the restriction zone (minimum 3 km radius) that must be established around any LPAI-infected farms for at least two weeks

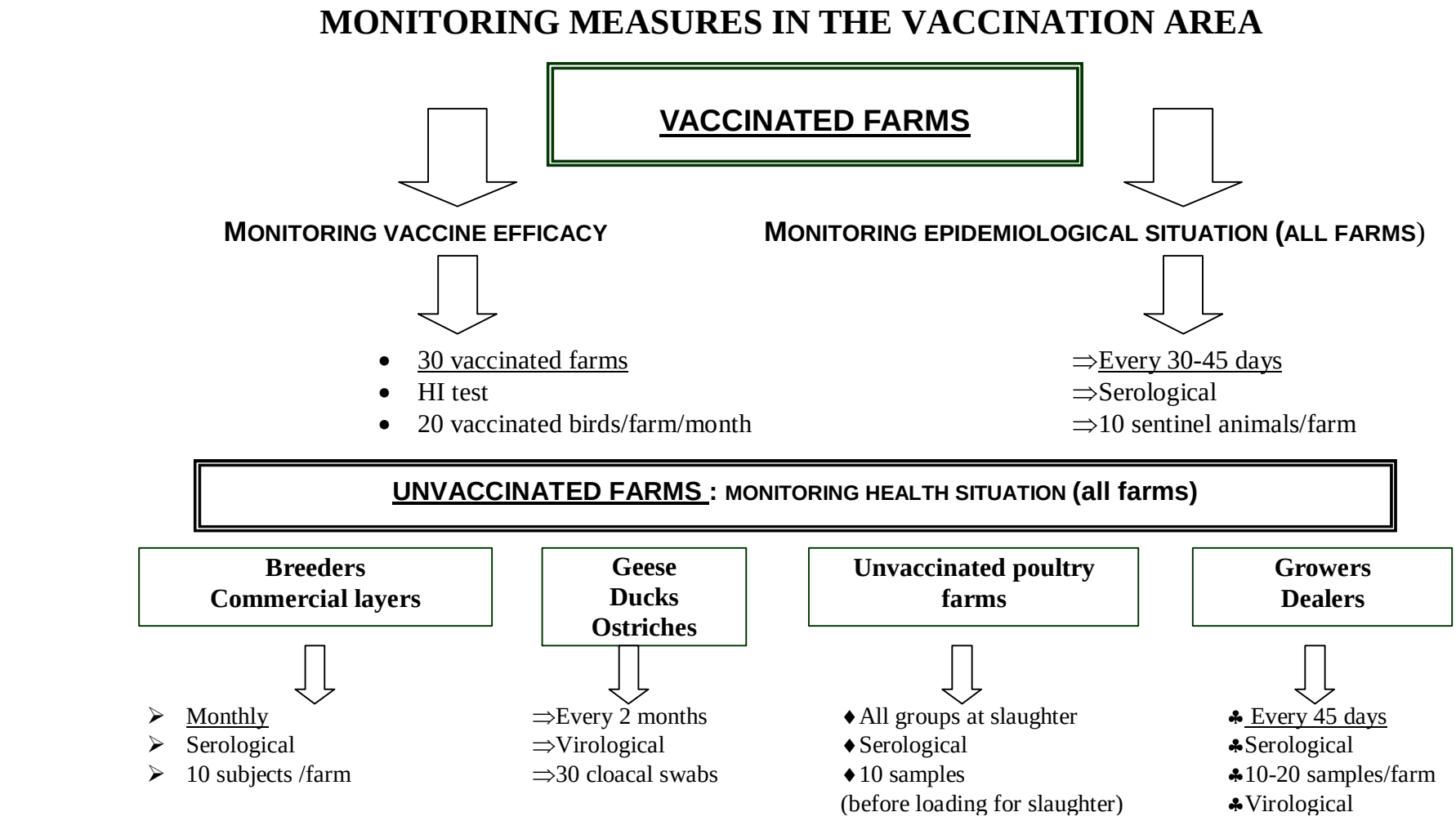

Figure 1. Monitoring measures to be applied in the vaccination area

Applications in the field

Conventional vaccines

Inactivated homologous vaccines

These products have recently been used in the attempt to control avian-influenza infections in Pakistan and Mexico (Swayne and Suarez 2000), but under those specific conditions they have not been successful in eradicating the infection. Conversely, in one instance, in Utah (Frame et al. 1996), the use of this vaccination strategy has been successful. The reason for the discrepancy of the results probably lies in the efficacy of the direct control measures, which must be implemented to support a vaccination campaign.

Inactivated heterologous vaccines

This vaccination strategy has been used successfully over the years in Minnesota (Halvorson 2002); however, in these instances vaccination was never implemented to control infections caused by viruses of the H5 or H7 subtypes. In addition the heterologous neuraminidase was not used as a marker of infection.

Conversely, in Italy during 2000-2002 this strategy was used to supplement control measures for the eradication of the H7N1 LPAI virus (CEC 2000). In order to control the re-emergence of LPAI virus and to develop a novel control strategy, a co-ordinated set of measures, including strict biosecurity, a serologic monitoring programme and a 'DIVA' strategy were enforced (Commission Decision 2001/721/CE as amended). The 'DIVA' strategy was based on the use of an oil-emulsion-based inactivated vaccine with the same haemagglutinin and a heterologous neuraminidase (N) subtype from the field virus, in this case an H7N3 strain.

The possibility of using the diverse N groups to differentiate between vaccinated and naturally infected birds, was achieved through the development of an *ad hoc* serological test to detect the specific anti-N1 antibodies (Capua et al. 2003).

Control of the field situation was achieved through an intensive sero-surveillance 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 serological testing of selected flocks.

After the first year of vaccination, the epidemiological data collected indicated that the H7N1 virus was not circulating. This was considered sufficient by the EU Commission to lift the marketing restrictions on fresh meat obtained from vaccinated poultry provided that animals had been tested with negative results using the discriminatory test (Commission Decision 2001/847/CE) (CEC 2001).

It is clear that due to the unpredictable nature of the epidemiology of this disease, which could result in the introduction of other avian-influenza subtypes, this solution is to be considered 'tailored' for a given epidemic.

Recombinant vaccines

The only field experience with these vaccines has been obtained in Mexico, where it has been used in the vaccination campaign against the H5N2 virus. Avian influenza has not been eradicated in Mexico, probably because an eradication programme based on a territorial strategy and including monitoring and restriction was not established.

Recombinant live vectored vaccines also enable the differentiation between infected and vaccinated birds, since they do not induce the production of antibodies against the nucleoprotein antigen, which is common to all AI viruses. Therefore, only field-infected birds will exhibit antibodies to the AGP or ELISA test directed towards the detection of group A (nucleoprotein) antibodies.

Since these vaccines have encountered some difficulties in licensing, their use is restricted to countries in which they are legally available. In addition, these vaccines will not replicate, and induce protective immunity, in birds that have had field exposure to the vector (i.e. fowlpox or infectious laryngotracheitis viruses) (Lüschow et al. 2001; Swayne, Beck and Kinney 2000). Since serological positivity to these viruses is widespread (due to field exposure and vaccination) in the poultry population, and can be in some instances unpredictable, the use of these vaccines is limited to a population which is seronegative to the vector virus. In addition, the use of these vaccines is restricted to species in which the vector virus will replicate. For example, ILTV will not replicate in turkeys, and since these birds are particularly important in the epidemiology of AI, the use of this vaccine is limited to areas in which turkeys are not present.

Discussion

From the data presented it appears that emergency vaccination is a sensible option if there is evidence of the introduction of a highly transmissible AI virus in a densely populated poultry area, or whenever the epidemiological situation indicates that there could be massive and rapid spread of infection. In addition, emergency vaccination should be considered where applicable, when birds of high economic value (e.g. pedigree flocks) or rare (endangered) birds are at risk of infection. It is clear that vaccination represents a tool to support eradication, and will be a successful tool only if coupled with restriction and increased biosecurity.

Considering the advantages and disadvantages of the products and diagnostic tools taht are currently available, if no recombinant products are licensed in that country, heterologous vaccination rather than homologous vaccination should be practiced in case of an emergency. The main reason for this would be that it would enable the differentiation between vaccinated and naturally exposed birds, through the development/application of an appropriate test. At present only the anti-neuraminidase-based test is available and has been validated. In our opinion however, this test represents a starting point on which future developments of the ‘DIVA’ strategy can be based. The development of novel candidate vaccines and of additional tests that enable the detection of field infection in vaccinated populations should be a priority for pharmaceutical industries and for research institutions, since for all the reasons listed above vaccination is already an option for the control of avian influenza.

If the country has access to licensed recombinant products, the use of these vaccines is acceptable taking into consideration the immune status of the population against the vector, since seropositivity impedes the replication of the vector virus and therefore the establishment of immunity. The issue of the replicating capacity of the vector in different species must also be addressed.

In conclusion, recent events including devastating epidemics in densely populated poultry areas, public-health concerns on animal-welfare issues and the introduction of novel technology into vaccinology have encouraged consideration of alternative

control strategies for OIE List-A diseases which were unthinkable of only a few years ago. This has also been supported by the development of reliable, sensitive and specific diagnostic companion tests. Countries, areas and enterprises at risk of infection should imperatively enforce surveillance programmes and have contingency plans in case of a disease outbreak, which may include vaccination. If the latter is considered an option, among other issues, the contingency plan must foresee the establishment of licensed vaccine banks that enable the 'DIVA' strategy, thus safeguarding animal health, animal welfare and international trade.

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