5

The control of avian influenza in areas at risk: the Italian experience 1997-2003

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Abstract

From 1997 to 2003, Italy has been affected by two epidemics of highly pathogenic avian influenza and by several outbreaks of low-pathogenic avian influenza (LPAI). In 1999-2000 a severe avian influenza (AI) epidemic affected the country. The epidemic was caused by a type-A influenza virus of the H7N1 subtype, originated from a low-pathogenic (LP) AI virus, which spread in 1999 among poultry farms in Northeastern Italy and eventually became virulent by mutation with the emergence of a highly pathogenic (HP) strain. From 17 December 1999 to 5 April 2000, a total of 413 outbreaks (178 meat-type turkey, 5 turkey breeder, 29 broiler breeder, 119 layer, 37 broiler, 9 guinea fowl, 11 game farm and 25 back-yard flocks) were identified and the last affected flock was stamped out on 5 April 2000. A total of about 16 million birds died or were stamped out on affected and at-risk premises.

From August 2000 to March 2001 in two epidemic waves, the H7N1 LPAI strain infected 73 turkey farms, which housed 1 million turkeys, 4 quail farms, with about 800,000 quails, and 1 layer farm (40,000 layers) located in the southwestern part of the Veneto Region (Verona and Padua provinces). To supplement disease-control measures already in force an emergency vaccination programme was implemented based on the 'DIVA' (differentiating infected from vaccinated animals) strategy. After the implementation of the vaccination programme, only 3 meat-type turkey farms were infected inside the vaccination area and among these, only one vaccinated flock was affected. The last affected flock was stamped out on 26 March 2001.

In October 2002, another LPAI virus of the H7N3 subtype emerged in the northern part of the country. The H7N3 LPAI strain rapidly spread among poultry flocks located in the densely populated poultry area (DPPA) which had been affected by the H7N1 epidemic in 1999-2001. Eradication measures were based on stamping out or controlled marketing of slaughterbirds on infected farms and on the prohibition of restocking of poultry farms. Restriction measures on the movement of live poultry, vehicles and staff were also imposed in the areas at risk. To supplement disease-control measures already in force, an emergency vaccination programme, based once again on the 'DIVA' strategy was drawn, approved by the EU Commission and

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implemented in the area. From 10 October 2002 to 10 October 2003, the H7N3 LPAI virus was able to spread and infect a total of 387 poultry holdings, mainly meat-type turkey farms; of these 88 were vaccinated. The implementation of a vaccination programme and the enforcement of strict restriction measures did not avoid the spread of the H7N3 LPAI virus infection among meat-turkey farms located in a DPPA. Nevertheless, it was possible to prevent the massive spread of infection to poultry farms other than turkey and to neighbouring vaccinated areas.
Keywords: avian influenza; LPAI and HPAI; outbreak; Italy

Introduction

Since 1997 Italy has been affected by six epidemics of avian influenza caused by viruses of the H5 or H7 subtypes. These epidemics were caused by H5N2 HPAI, H7N1 (HPAI and LPAI) and H7N3 (LPAI) (Capua and Alexander 2004). The characteristics of these epidemics and their different impact on the poultry industry and possible control strategies are presented.

The most severe episode was observed during 1999-2001, in which Italy was affected by four subsequent epidemic waves of avian influenza caused by viruses of the H7N1 subtype. The first epidemic wave was caused by a low-pathogenicity avian influenza virus of the H7N1 subtype that subsequently mutated into a highly pathogenic avian influenza virus, after circulating in the industrial poultry population for approximately nine months. Following the emergence of the HPAI virus, which caused death or culling of about 16,000,000 birds on infected and at-risk farms, and the implementation of the measures indicated in Council Directive 92/40/CE, the LPAI virus re-emerged twice.

Following the eradication of the H7N1 virus an H7N3 virus was introduced in the industrial poultry population of Northern Italy. A vaccination programme based on the 'DIVA' strategy was implemented and is currently being used.

The present paper reports of the strengths and weaknesses of the control strategies implemented to deal with AI epidemics occurring in diverse conditions.

Materials and methods

HPAI eradication measures

The measures required under the European Union (EU) legislation (CEC 1992) to control the disease were enforced. The identification of the AI infected farms was based on the notification of suspected cases by farmers and on official inspections of flocks at risk of infection. Epidemiological investigations were carried out on the affected farms by means of a standardized questionnaire. HPAI-virus-infected and at-risk flocks were stamped out. In the areas at risk, the prohibition of restocking of poultry farms associated with the enforcement of restriction measures on the movement of live poultry, vehicles and staff was imposed.

LPAI (H7N1-H7N3) control and eradication measures

No compulsory eradication measures to control LPAI-virus infections are provided for in the current EU legislation. In order to avoid the spread of the LPAI virus and the possible re-emergence of a HPAI-virus strain, the Italian authorities put in force a series of control measures in affected and at-risk areas. These included: monitoring of flocks at risk of infection, stamping out or controlled marketing of slaughterbirds on infected farms, restriction policies to restocking and movement of live birds, vehicles

and staff. Furthermore, in order to supplement these control measures, a 'DIVA' vaccination programme against the disease was implemented. This vaccination strategy was based on heterologous vaccination. Briefly, since the antigen that induces the production of neutralizing antibodies is represented by the haemagglutinin, a vaccine containing an isolate with a homologous H group and heterologous N, from the field strain, was used as a 'natural marker' vaccine. Vaccination was carried out on meat-type turkey farms, layer flocks, capon and cockerel farms. Other species and production types were not vaccinated. Serological testing of sentinel birds and a discriminatory test able to distinguish the different type of anti-neuraminidase antibodies (anti-N1 and anti-N3) were applied in vaccinated flocks to monitor the epidemiological situation.

Laboratory diagnosis

During the LPAI and HPAI epidemics the diagnostic guidelines reported in the EU legislation on AI were followed (CEC 1992). With reference to the LPAI epidemic, in case of HI-positive results in vaccinated flocks, a discriminatory test (Capua et al. 2003) was used to differentiate between vaccinated/non-exposed and vaccinated/field-exposed birds/farms.

Data analysis

Epidemiological data collected during the HPAI epidemic on each affected flock were used to trace back the origin of the infection. The characteristics of HPAI outbreaks were analysed using χ^2 test and Mann-Whitney U test for categorical and ordinal variables, respectively. The risk of neighbourhood spread, and the association between inter-event distance and time were evaluated by multivariate logistic regression model (PROC LOGISTIC, SAS system 8.2). Inter-outbreak distance ≤500 metres was used as outcome, and quartiles of inter-outbreak times were used as predictors. Another predictor was included in the model, taking value equal to 1 when outbreak pairs belonged to the same avian species, 0 otherwise.

Results

1997-1998 H5N2 HPAI epidemic

The epidemic consisted of a total of 8 outbreaks (Capua et al. 1999), in backyard or semi-intensive flocks, located in the Veneto and Friuli Venezia Giulia regions. Although the origin of the epidemic was not established, the epidemiological investigation allowed identifying risk factors in the affected farms, primarily the marketing of infected birds, presence of mixed species and rearing in the open of birds. The disease was eradicated by the prompt implementation of Directive 92/40/EEC. A total of 7,731 birds were depopulated and no further isolations of the H5N2 virus have been made to date.

1999-2000 H7N1 HPAI epidemic

From 17 December 1999 to 5 April 2000 413 HPAI-infected poultry farms, mainly located in the Po Valley, were detected. A total of 13,731,253 birds were culled or died on the affected farms, and more than 2 million animals were pre-emptively slaughtered on 80 premises at risk of infection. The infection was detected more frequently in turkey ($\chi2$= 118.37, P<0.0001) and layer farms ($\chi2$= 373.04, P<0.0001), which accounted for 73% of the outbreaks, in larger flocks (z= 5.895, P<0.0001), in poultry farms located on the plain (altitude ≤300 m) ($\chi2$=37.27, P<0.0001). Tracing

exercises carried out on affected premises allowed the identification of the possible origin of the infection in 66.3% of the outbreaks. In particular, the origin of infection could be attributed to: movement of animals (1.0%), indirect contacts at the time of loading for slaughter of female turkeys (8.5%), neighbourhood spread (within 1 km radius) (26.2%), lorries for the transport of feedstuff, litter and carcasses (21.3%), and other indirect contacts (e.g. exchange of manpower, machinery, equipment) (9.4%). The logistic regression model for space–time association was significant (likelihood ratio $\chi 2 = 53.5$, 4 df, $P<0.001$) and showed no evidence of poor fit (Hosmer and Lemeshow test: $\chi 2 = 0.6$, 3 df, $P = 0.90$). Inter-event distance ≤500 m and inter-event time ≤10 days (first quartile) were strongly associated, indicating rapid viral transmission among contiguous farms (Table 1). Outbreak pairs involving the same avian species were more likely to be within 500 m from one another than outbreaks in different species.

Table 1. Results of the logistic regression model for space–time association

Inter-event time	OR estimate	95% Wald confidence limits	
quartile 1 vs 4	13.2	3.2	55.1
quartile 2 vs 4	3.6	0.75	17.3
quartile 3 vs 4	2.3	0.45	11.9
same species	1.3	1.2	1.7

2000-2001 H7N1 LPAI epidemic

From August to November 2000, the H7N1 LPAI strain infected 51 meat-type turkey farms, which housed 845,000 turkeys, and 1 quail farm, with a total of 429,000 quails, located in the southern part of the province of Verona. Another 3 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. To supplement disease-control measures already in force, an emergency vaccination programme against the disease was implemented in this area. Vaccination was implemented according to the 'DIVA' strategy based on the use of an inactivated heterologous vaccine (A/ck/PK/95-H7N3). Shortly after the beginning of the vaccination programme (December 2000 to March 2001), the H7N1 LPAI virus infected 3 meat-type turkey farms in the vaccination area and 20 poultry holdings (19 turkey farms and 1 layer farm) located in a contiguous unvaccinated area. Only one vaccinated flock was affected, and the virus did not spread from this to other vaccinated farms. The last H7N1 LPAI infected poultry flock was stamped out on 26 March 2001, and the results of the serological surveillance carried out both within and outside the vaccinated area to assess the possible presence of AI infection, demonstrated that the H7N1 AI virus strain was not circulating anymore. The application with negative results, of the 'DIVA' discriminatory test, was considered an additional guarantee for Member States, and on 30 November 2001, Commission Decision 2001/847/EC, authorized the marketing of fresh poultry meat obtained from vaccinated birds for intra-community trade.

2002-2003 H7N3 LPAI epidemic

In October 2002, another LPAI virus of the H7N3 subtype was introduced in the northern part of the country. The H7N3 LPAI strain rapidly spread among poultry flocks located in the densely populated poultry area (DPPA) that had been affected by

the H7N1 epidemic in 1999-2001. The vaccination programme was based once again on a 'DIVA' strategy and was carried out using an AI inactivated heterologous vaccine (strain A/ck/IT/1999-H7N1). The beginning of the DIVA vaccination campaign was delayed up to 31 December 2002, due to unavailability of an appropriate vaccine. From 10 October 2002 to 10 October 2003, the H7N3 LPAI virus was able to spread and infect a total of 387 poultry holdings: 332 meat-type turkey, 5 turkey breeder, 12 broiler breeder, 13 layer, 6 guinea fowl, 4 broiler, 3 quail, 1 meat-duck farms and 11 back-yard flocks mainly located in the southern part of the two Italian regions. A total of 7,659,303 birds were involved in the epidemic, and among these 4,230,750 animals were stamped out in 163 affected flocks. The remaining 3,428,553 slaughterbirds were subjected to controlled marketing. Of the affected farms, 88 were vaccinated flocks. The first outbreak in a vaccinated flock occurred on 18 April. All the infected vaccinated flocks were meat turkeys mainly located in a limited area of the southern part of Verona province. It is interesting to point out that despite the poultry density in the latter area only 2 unvaccinated poultry farms (1 broiler breeder and 1 meat-duck farms) were affected. These farms were located in close proximity to previously vaccinated meat-turkey farms which had been field-exposed. Stamping-out measures or controlled marketing were enforced in all infected flocks, which housed a total of 1,523,320 birds.

The implementation of a vaccination programme and the enforcement of strict restriction measures did not avoid the spread of LPAI-virus infection among meat-turkey farms located in a DPPA. Nevertheless, it was possible to prevent the massive spread of infection to poultry farms not rearing turkeys and to neighbouring vaccinated areas. Only sporadic outbreaks of LPAI infection were detected in unvaccinated poultry farms, mainly located outside the vaccination area: 3 meat-turkey, 5 dealer and 2 quail farms in Lombardia, 1 dealer farm in Piemonte, and 2 meat-turkey farms in the Emilia-Romagna region. These flocks were promptly identified and stamped out.

Discussion

A few considerations can be made from retrospectively analysing the experience gained in the past 6 years with avian influenza in Italy. Firstly, Northeastern Italy can definitely be considered an area 'at risk' for avian influenza infections. This is also supported by AI epidemics which have occurred in the past (Franciosi et al. 1981; Petek 1982; Meulemans 1986; Papparella, Fioretti and Menna 1994; 1995) caused by viruses of the H6 and H9 subtypes. This could probably be linked both to the great numbers of wild birds which fly over the area during their migration and to the great numbers of imports of live birds into the area. For this reason, and considering the poultry density in the area, it is imperative that surveillance programmes are implemented to diagnose AI infections promptly.

The comparison between the 1997-1998 and 1999-2000 epidemics points out that if HPAI is diagnosed promptly and is not preceded by extensive circulation of the LPAI progenitor, the application of the measures imposed by Directive 92/40/EEC are efficacious in disease eradication. The devastating impact of the HPAI H7N1 epidemic in 1999-2000 was linked to loss of control of infection, primarily due to the previous circulation of the LPAI virus, which caused difficulties in identifying infected flocks promptly. Clearly, eradication efforts are more successful if there is no massive spread into the industrial circuits of intensively reared poultry.

The Italian 1999-2000 AI epidemic also emphasized that farmers and private companies should bear well in mind that within the current European legislation there is no financial aid from local or national governments or from the European Union in case of LPAI. Therefore, voluntary and permanent surveillance programmes should be implemented in order to allow the prompt diagnosis of infection by H5 and H7 LPAI viruses, to allow the enforcement of restriction and eradication policies until this is economically feasible.

The control of LPAI infections in DPPA is a challenging experience. A co-ordinated set of control measures including the application of adequate biosecurity measures, the enforcement of restriction policies to restocking and movement of live birds, vehicles and staff, and the implementation of a vaccination programme and of intensive monitoring measures in the areas at risk of infection, may have different outcomes on the basis of a series of variables. These include primarily the biological characteristics of the strain, the animal species and density at the moment of AI introduction and the functional organization of both the poultry industry and the veterinary services in the area. However, the availability of a well-structured legal basis for LPAI control, the prompt availability of vaccine, the general economic situation and the motivation of farmers and companies to eradicate the infection also play a major role in the eradication of avian influenza infections.

The experience gathered during the Italian 1997-2003 AI epidemics suggests that countries at risk of infection should have contingency plans and a general preparedness in order to deal appropriately with such infections. Outbreaks caused by avian influenza viruses of the H5 and H7 subtypes can no longer be considered rare events and therefore alternative strategies to a stamping-out policy should be considered, particularly for outbreaks occurring in densely populated poultry areas.

In our opinion it is imperative that this disease is dealt with as a problem of the industry and of veterinary public-health services. The different sets of data that are generated from surveillance and control programmes at the industry level must be made available to support decision-making; this can only be achieved if there is extensive collaboration between farmers, official and field veterinarians, poultry industry, the diagnostic laboratories, the epidemiology units and the central and local governments. Only in this way it will be possible to establish a network of collaboration able to make the best of the data and tools available in the effort to control avian influenza infections in poultry.

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