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Review article

The 1999–2000 avian influenza (H7N1) epidemic in Italy: Veterinary and human health implications

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Abstract

From the end of March to the beginning of December 1999, 199 outbreaks of low pathogenicity avian influenza (LPAI) were diagnosed in the Veneto and Lombardia regions, which are located in the northern part of Italy. The virus responsible for the epidemic was characterized as a type A influenza virus of the H7N1 subtype of low pathogenicity. On the 17th of December, highly pathogenic avian influenza (HPAI) was diagnosed in a meat turkey flock in which 100% mortality was observed in 72 h. The infection spread to the industrial poultry population of northern Italy including chickens, guinea-fowl, quail, pheasants, ducks and ostriches for a total of 413 outbreaks. Over 13 million birds were affected by the epidemic, which caused dramatic economic losses to the Italian poultry industry with severe social and economic implications. The possibility of H7 virus transmission to humans in close contact with the outbreaks was evaluated through a serological survey. Seven hundred and fifty nine sera were collected and tested for the detection of anti-H7 antibodies by means of the micro-neutralization (MN) and single radial haemolysis (SRH) tests. All samples resulted negative. A limited number of clinical samples were also collected for attempted virus isolation with negative results. Current European legislation considers LPAI and HPAI as two completely distinct diseases, not contemplating any compulsory eradication policy for LPAI and requiring eradication for HPAI. Evidence collected during the Italian 1999–2000 epidemic indicates that LPAI due to viruses of the H7 subtype may mutate to HPAI, and, therefore, LPAI caused by viruses of the H5 or H7 subtypes must be controlled to avoid the emergence of HPAI. A reconsideration of the current definition of avian influenza adopted by the EU, could possibly be an aid to avoiding devastating epidemics for the poultry industry in Member States.

Keywords: Avian influenza; Italy; Epidemic

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1. Introduction

Avian Influenza (AI) is a viral disease of poultry included in OIE List A. In the European Union (EU) its control is imposed by EU Directive 92/40/EEC (CEC, 1992). This disease may have a devastating effect on the poultry industry particularly following the high mortality rates in susceptible birds, but also its presence in a given territory results in restrictions on animal movements, marketing and trade of poultry and poultry products.

Since 1996, three accounts of AI viruses affecting humans, apparently as a result of spread directly from birds, have been reported, namely in the UK, Kurtz et al. (1996) and in Hong-Kong, Shortridge et al. (1998) and by Peiris et al. (1999).

Since the first Hong Kong outbreaks, AI infections have been seen in another perspective, thus, amplifying the issue of their control, not only for animal health but also for human health.

North-eastern Italy has been affected by a devastating epidemic of highly pathogenic avian influenza (HPAI), caused by a type A influenza virus of the H7N1 subtype that originated from the mutation of a low pathogenicity avian influenza (LPAI) virus of the same subtype (Capua and Marangon, 2000). The LPAI epidemic and the subsequent HPAI epidemic occurred in the Veneto and Lombardia regions, which raise 65% of Italy's commercial poultry. Furthermore, some areas affected by the epidemics (particularly south of Verona province), are so-called 'densely populated poultry areas' (DPPA), which may have (in some municipalities of Verona province) as many as 70 000 birds raised per km^2.

The HPAI epidemic resulted directly or indirectly in the death or culling of over 13 million birds, which inevitably resulted in the disruption of the marketing system and great economic losses to the poultry industry and social community. Following depopulation and restocking of the HPAI infected areas, LPAI re-emerged, thus, causing the poultry industry to demand and obtain the option to vaccinate against AI of the H7 subtype.

The awareness and concern of the possible zoonotic implications of this epidemic induced the Italian public health services to perform a survey of people exposed to the influenza virus. Descriptions of the Italian LPAI and HPAI epidemics from epidemiological and virological viewpoints, and the results of the survey in humans are reported below.

1.1. Low pathogenicity avian influenza (LPAI) epidemic

On the 29th of March 1999 the first isolation of a type A, H7 AI virus was officially notified. The virus was further characterized, in accordance to EU Directive 92/40/EEC (CEC, 1992), by the EU Reference Laboratory for AI and Newcastle Disease, Weybridge UK, as a type A AI virus of the H7N1 subtype. The intravenous pathogenicity index (IVPI) of the isolate, in 6-week-old SPF chickens was of 0.0, and the deduced amino acid sequence of the genome segment which encodes for the cleavage site of the precursor of the haemagglutinin molecule was typical of LPAI viruses since it did not contain multiple basic amino acids (Wood et al., 1993, 1997).

Following the first official notification, a total of 199 infected flocks was diagnosed and notified. The highest number of outbreaks affected meat turkeys (164), and only a limited number of turkey breeder flocks were affected (six). Infection also affected chickens (12 outbreaks in layers, 11 in broiler breeders and four in broilers) and two guinea-fowl flocks. From the epidemiological inquiry it appeared that at the time of the first submission approximately 60–70 turkey farms had already been infected. Infection was particularly severe in the turkey industry, causing severe losses to farmers (Capua et al., 1999).

Nevertheless, this virus did not have the characteristics listed in EU Directive 92/40/EEC, therefore, no compulsory stamping out policy could be implemented, and it was not possible at the time to stamp out such a significant number of flocks on a voluntary basis. Moreover, since LPAI is not considered a disease that requires statutory intervention in Italian veterinary legislation, there were no legislative tools to prevent its spread. However, the regional authorities of the two affected regions, implemented restriction orders with the

aim of reducing the number of new outbreaks. The main strategies of these orders were to avoid movement of viraemic birds, and to avoid movement of dead birds and infected litter, which were identified as being among the primary sources of infection. These policies, aided by the oncoming warm season, resulted in a decrease in the number of outbreaks during the summer, which, inevitably, increased from the month of September.

1.2. Highly pathogenic avian influenza (HPAI) epidemic

On the 13th December 1999 a private practitioner submitted pathological samples from a meat turkey flock exhibiting high mortality rates. The outbreak was confirmed as HPAI on the 17th December with the characterization of an H7N1 isolate with an IVPI index of 3.0 and a deduced amino acid sequence at the H cleavage site containing multiple basic amino acids, typical of highly pathogenic viruses (Capua et al., 2000).

Due to the complex field situation (isolation of an H7 virus was not unusual at the time) HPAI virus was not suspected immediately and there was some delay in the application of eradication measures. This resulted in the spread of the virus. Furthermore, the holiday season was approaching and high slaughter levels resulted in a further spread of the virus with complete loss of control of infection. Four hundred and thirteen outbreaks were diagnosed involving 177 meat turkey flocks, 121 table-egg layer flocks, 39 broiler flocks, 29 broiler breeder flocks, 25 backyard flocks, nine guinea fowl flocks, six turkey breeder flocks, three ostrich farms, two pheasant flocks, one Pekin duck flock and one quail flock and death of over 13 000 000 birds. The last outbreak was notified on 5th April 2000.

As a result of mass mortality, (stamping out policy and pre-emptive slaughter), several establishments such as hatcheries, feed mills, abattoirs, processing plants and other connected activities were forced to interrupt their activity, causing unemployment and heavy economic losses to the poultry industry and to the social community, due to the disruption of the marketing system. Further economic losses were also caused by the export bans imposed on the infected regions and by the depopulation of the infected area.

1.3. Eradication of HPAI

Following the implementation of Directive 92/40/EEC (CEC, 1992) infected flocks were stamped out, and cleaning and disinfection of infected premises were carried out. To improve eradication procedures, a complete depopulation of the infected area was imposed. An area of 5500 km^2 was depopulated of poultry, including intensive, semi-intensive and backyard flocks, and left empty for a minimum period of 60 days.

1.4. Re-emergence of LPAI

On the 14th August 2000 samples were submitted from a turkey flock located in the DPPA on clinical suspicion of LPAI. This was confirmed by the laboratory on 20th August 2000. The Italian Ministry of Health ordered the eradication of infection with a stamping out policy imposed by an extraordinary act. Fifty-two outbreaks of LPAI were diagnosed and stamped out.

A vaccination policy against AI was, at this point, strongly requested by the farmers and by the poultry industry, and a vaccination program was drawn up and approved by the European Commission.

1.5. Vaccination policy

The vaccination programme began on 15th November 2000 and will last until May 2002. About 15 000 000 birds [only meat type birds and table-egg layers (that apply the all-in all-out system)] raised in a restricted zone (1155 km^2) south at Verona will be vaccinated. Live birds or poultry products that originate from the vaccination zones will be subjected to trade restrictions.

The vaccine that is being used does not contain a homologous H7N1 virus, but has been prepared from an inactivated H7N3 virus (A/CK/Pakistan/95/H7N3). The reason for this is to allow the possibility of using it as a natural

'marker' vaccine, or more correctly a DIVA [Differentiating Infected from Vaccinated Animals] vaccine. In fact, the presence in the vaccine of an H7 antigen ensures protection against clinical signs and the reduction of virus shedding, since it is well known the neutralizing antibodies to influenza A viruses are induced primarily by the haemagglutinin molecule (Swayne et al., 1999). The presence of a different neuraminidase (N) subtype, which will induce specific antibodies against N3 rather than N1, will enable us, with the aid of an 'ad hoc' diagnostic test, to discriminate between flocks infected with the H7N1 virus and those receiving the H7N3 vaccine, and to monitor and follow the evolution of the situation.

1.6. Survey in humans

Between February and June 2000, 765 biological samples were collected from staff (veterinarians, technicians, abattoir employees and farmers) that had been in contact with infected birds. The greatest number of samples was collected from farmers. The total number of establishments monitored was 249.

Serum samples were processed by the cell-culture (MDCK) microneutralisation test, as described by Rowe et al. (1999); and by single radial haemolysis test (Mancini et al., 1984). Both tests were performed using strain A/Ck/It/2676/99 as the reference virus.

Virological investigations were performed on six pharyngeal swabs, which were processed by RT-PCR as described by Campitelli et al. (1997).

All the serum samples tested yielded negative results with both tests. Similarly, the pharyngeal swabs also yielded negative results.

2. Discussion

A few considerations can be made from this experience. Firstly, 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 EU in case of LPAI. Therefore, on one hand permanent surveillance programmes should be implemented in order to allow the prompt diagnosis of infection by H5 and H7 LPAI viruses, to allow the stamping out of infected flocks while this is economically feasible. In the spring of 1999 we were faced with 60–70 outbreaks and it was not possible to stamp out infected flocks without compensation.

The spread of infection was also a result of the structure and organization of the local poultry industry. In several areas worldwide, the poultry industry has grown substantially in an often irrational way, particularly where the system has developed as a semi vertical integration. The latter (i.e. house owned by the farmer and day-old chicks and feed supplied by private company) has the disadvantage that there is no planning behind the spatial distribution of the units that are involved in the system. Furthermore, the number of contacts between establishments is much higher than is desirable for good biosecurity. In fact, feed trucks and other vehicles (e.g. abattoir delivery), frequently visit a number of farms daily, regardless of the species reared or of the type of production, and basic biosecurity measures are rarely respected. The concentration of poultry houses, hatcheries, abattoirs, litter processing plants and other establishments in a restricted area is definitely convenient from an organizational point of view, but has a series of drawbacks from the sanitary point of view, which emerge dramatically when an epidemic of a highly contagious disease is faced.

As a result of this epidemic, in which a LPAI virus of the H7 subtype apparently mutated to a HPAI virus, with the devastating consequences mentioned above, the control policies for AI in the EU should be reviewed. It would seem logical that control measures should be implemented aimed a eradicating all infections of poultry caused by viruses of H5 and H7 subtypes, regardless of their virulence. In addition to the potential of LPAI viruses to mutate to HPAI, the combined presence of HPAI and LPAI of the same subtype in a given poultry population, complicates the interpretation of diagnostic results, thus, delaying eradication procedures.

In conclusion, the Italian experience with AI shows that it is extremely difficult to control

HPAI in densely populated areas, especially if infection with LPAI is already widespread in the area. Therefore, in order to avoid similar situations, prevention systems should be implemented either on a voluntary or on a compulsory basis.

Besides a structural change in the industrial system, which must inevitably take place in order to reorganize production circuits, veterinary surveillance, quarantine and controlled marketing, particularly in restocking procedures, are also essential to prevent sanitary emergencies. In addition to this, education of farmers and staff to the basic concepts of biosecurity is a critical point in the eradication of AI and fundamental to the management of intensively reared poultry.

Although no evidence of transmission to humans was detected during the 1999–2000 Italian AI epidemic, this risk should not be underestimated, and, therefore, specific surveillance systems in human beings should be implemented during AI epidemics.

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References

Campitelli, L., Donatelli, I., Foni, E., Castrucci, M.R., Fabiani, C., Kawaoka, Y., Krauss, S., Webster, R.G., 1997. Continued evolution of H1N1 and H3N2 influenza viruses in pigs in Italy. Virology 232, 310–318.

Capua, I., Marangon, S., 2000. Avian influenza in Italy (1999–2000): a review. Avian Pathol. 29, 289–294.

Capua, I., Marangon, S., Dalla Pozza, M., Mutinelli, F., Vincenzi, G., Santucci, U., 1999. The low pathogenicity avian influenza (H7N1) epidemic in the Veneto region, Italy. Proceedings of the Sixth Joint Annual Meeting of the EU Reference Laboratories for Newcastle Disease and Avian Influenza, Brussels 29–30 November 1999, pp. 66–71.

Capua, I., Mutinelli, F., Marangon, S., Alexander, D.J., 2000. H7N1 Avian Influenza in Italy (1999–2000) in intensively reared chickens and turkeys. Avian Pathol. 29, 537–543.

CEC, 1992. Council Directive 92/40/EEC of 19 May 1992 introducing Community measures for the control of avian influenza. O. J. of the European Commission L167, 1–15.

Kurtz, J., Manvell, R.J., Banks, J., 1996. Avian influenza virus isolated from a woman with conjunctivitis. Lancet 348, 901–902.

Mancini, G., Donatelli, I., Arangio Ruiz, G., Rozera, C., 1984. Detection of antibodies to strain-specific and cross-reactive antigenic determinants on the haemagglutinin of influenza viruses A/Texas/1/77 and A/Bangkok/1/79 in human sera. Arch. Virol. 79, 117–121.

Peiris, M., Yam, Y.C., Chan, K.H., Ghose, P., Shortridge, K.F., 1999. Influenza A H9N2: aspects of laboratory diagnosis. J. Clin. Microbiol. 37, 3426–3427.

Rowe, T., Abernathy, R.A., Hu-Primmer, J., Thompson, W.W., Lu, X., Lim, W., Fukuda, K., Cox, N.J., Katz, J.M., 1999. Detection of antibody to avian influenza (H5N1) virus in human serum by using a combination of serologic assays. J. Microbiol. 37, 937–943.

Shortridge, K.F., Zhou, N.N., Guan, Y., Gao, P., Ito, T., Kawaoka, Y., Kodihalli, S., Krauss, S., Markwell, D., Murti, K.G., Norwood, M., Senne, D.A., Sims, L., Takada, A., Webster, R.G., 1998. Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. Virology 252, 331–342.

Swayne, D.E., Beck, J.R., Garcia, M., Stone, H.D., 1999. Influence of virus strain and antigen mass on the efficacy of H5 avian influenza inactivated vaccines. Avian Pathol. 28, 245–255.

Wood, G.W., Banks, J., McCauley, J.W., Alexander, D.J., 1993. Deduced amino acid sequences of the haemagglutinin of H5N1 avian influenza virus isolates from an outbreak in turkeys in Norfolk, England. Arch. Virol. 134, 185–194.

Wood, G.W., Banks, J., Brown, I.H., Strong, I., Alexander, D.J., 1997. The nucleotide sequence of the HA1 of the haemagglutinin of an H1 avian influenza isolate from turkeys in Germany provides additional evidence suggesting recent transmission from pigs. Avian Pathol. 26, 347–355.