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REVIEW ARTICLE

The avian influenza epidemic in Italy, 1999–2000: a review

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During 1999, northern Italy has been affected by an epidemic of low pathogenicity avian influenza (LPAI) caused by a virus of the H7N1 subtype. Due to the characteristics of the poultry industry in the area and to the absence of specific legislative tools to eradicate infection, the virus continued to circulate for several months until a highly pathogenic virus of the same subtype emerged. The highly pathogenic virus had caused death, at the time of writing, of over 13 million birds in 3 months. The consequences of the highly pathogenic avian influenza (HPAI) epidemic appear to be devastating for the poultry industry and the social community.

Several conditions generated the current situation, including the high density of susceptible animals and the structure of the poultry industry in the infected area. In addition, the circulation of LPAI virus for a number of months inevitably delayed the prompt identification of HPAI and complicated the interpretation of diagnostic results. A reconsideration of current European legislation and a reorganization of the poultry industry are suggested to prevent the occurrence of similar situations in countries of the European Union.

Introduction

Avian influenza viruses are known to cause two different diseases on the basis of the severity of clinical signs they induce in susceptible species. Highly pathogenic avian influenza (HPAI) is a devastating disease of poultry caused by some viruses of the H5 and H7 subtypes, in which the deduced amino acid sequence of the region coding for the cleavage site of the precursor haemagglutinin molecule, HA0, contains multiple basic amino acids (Wood *et al.*, 1993). The presence of multiple basic amino acids at this site enables HPAI viruses to replicate throughout the body, damage vital organs and tissues, and thus bring about the death of the bird (Rott, 1992). In contrast, low pathogenicity avian influenza (LPAI) viruses have only two basic amino acids in the cleavage site motif, and are capable of replicating only in limited tissues and organs, mainly the respiratory and digestive tracts, and do not invade the rest of the body. The mutation of LPAI viruses of the H5 and H7 subtypes to HPAI has occurred in the field in the Pennsylvania and in the Mexico epidemics (Kawaoka *et al.*, 1987; Eckroade &

Silverman-Bachin, 1987; Campos-Lopez *et al.*, 1996; Senne *et al.*, 1996; Villarreal & Flores, 1997) and has been reported in the laboratory (Swayne *et al.*, 1998).

In Italy, HPAI was probably enzootic at the end of the nineteenth and beginning of the twentieth centuries, and Petek (1982) considered outbreaks of HPAI in Italy to have occurred during 1935 to 1937. Although, up to 1997, no HPAI infections had been recorded since that time, there have from time to time been isolations of influenza viruses of low pathogenicity and various subtypes from poultry, especially turkeys, and feral waterfowl (Franciosi *et al.*, 1981; Petek, 1982; Papparella *et al.*, 1994, 1995). Similar isolations have been reported in most countries producing poultry and have often been linked with the introduction of the viruses by migratory waterfowl (Alexander, 1995). Between October 1997 and January 1998, eight outbreaks of highly pathogenic avian influenza were diagnosed in backyard and semi-industrial flocks of the Veneto and Friuli-Venezia Giulia regions in northeastern Italy, and for each of the eight outbreaks, HPAI viruses of the H5N2 subtype were isolated (Capua *et al.*, 1999b).

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Highly pathogenic avian influenza appeared again in Italy in December 1999. It affected the industrial poultry population in northern Italy with the devastating strength of a viral disease that is capable of causing 100% mortality in less than 3 days.

The disease has been seen in the Veneto and Lombardia regions, which account for approximately 65% of the Italian industrially reared poultry, particularly in the provinces of Verona, Vicenza, Mantova, and Brescia. To date, HPAI has affected virtually all intensively reared avian species regardless of age or housing system.

The Poultry Industry in the Affected Region

The poultry industry has grown substantially and developed in the past 20 years in this particular area of Italy, which is a densely populated poultry area (up to 70 000 birds/km² in certain municipalities of Verona province). The increase in the number of poultry houses and other connected establishments has often occurred irrationally and without planning, to the point that this area may be considered one, large, epidemiological unit. This is particularly emphasized by a series of "local factors" such as the absence of physical barriers between establishments and the common practice of sharing staff and equipment among farms. Furthermore, the development of a semi-vertical integration system (i.e. house owned by the farmer, and 1-day-old chicks and feed supplied by a private company) frequently means that feed trucks and other vehicles (e.g. abattoir delivery) visit a number of farms daily, regardless of the species reared and of the type of production. Added to this, even basic biosecurity measures are rarely practised.

Moreover, the local poultry industry has developed and grown in intensively rearing a number of different avian species such as chickens, turkeys, guinea fowl, quail and ostriches. Production circuits of these different birds often overlap, since the feed mills and slaughtering plants are owned by single companies that serve a specific number of farms. It is therefore easy to see how a highly diffusive infectious disease may spread rapidly in this epidemiological situation.

Low Pathogenicity Avian Influenza

On 29 March 1999, the first isolation of a type A, H7 avian influenza virus was officially notified. Within one week, the virus was further characterized, in accordance with European Union (EU) Directive 92/40/EEC (CEC, 1992), by the EU Reference Laboratory for Avian Influenza and Newcastle Disease, Weybridge, UK, as an H7N1 virus with an intravenous pathogenicity index of 0.0 in 6-week-old specific pathogen free (SPF) chickens and a deduced amino acid sequence for the cleavage site of the haemagglutinin molecule of . . . PEIPKGR*GLF. . ., which is a typical motif of

LPAI viruses since it does not contain multiple basic amino acids (Wood *et al.*, 1994).

During the next 2 weeks, 63 outbreaks were notified in meat turkey flocks mainly located south of the city of Verona, which incidentally contains approximately 50% of the national meat turkey flocks and one of the largest turkey hatcheries in Europe (450 000 poult hatched per week), and in a limited number of farms located in Vicenza province. Infection was also detected in the Lombardia region, mainly in Mantova and Brescia provinces. The main problem at the time was that the infection was causing severe losses to the industry, particularly when it affected young turkeys (Capua *et al.*, 1999a). In fact, in a number of flocks, mortality reached 90%, and farmers and private company veterinarians insistently asked the National Reference Laboratory to inquire with the Commission on the possibility of using a vaccine to control the disease and allow the local economy to survive.

The Commission and Member States were informed of the field and economic situation, and were concerned and supportive with the problems that the Italian veterinary authorities and poultry industries were facing. Unfortunately, the main problem encountered was that with current legislation there are no tools to support Member States in eradicating this infection. The current definition of avian influenza specified in Directive 92/40/EEC is: "an infection of poultry caused by an 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". The Italian LPAI H7N1 strains did not meet either of these criteria. Therefore, the eradication of the infection could not be aided financially by the EU, but had to be achieved by the Italian veterinary authorities, without having the possibility of compensating farmers. It was considered not possible to implement a voluntary stamping out policy, and all that could be done was to monitor the situation and attempt to reduce the number of new outbreaks by imposing biosecurity measures on the companies and farmers.

Another issue that was discussed was the possibility of controlling the field situation with a homologous vaccine. An EU expert committee recommended not to use this tool to control the disease, since it is well known that although vaccination protects the birds from clinical signs and death, it does not prevent the virus from replicating (Swayne *et al.*, 1999). Therefore, from a biological point of view, it would not prevent virus circulation or mutation. In addition, it was possible that masking the clinical condition would lead to greater perpetuation of infection. Another point stressed by the expert committee was that vaccination with an H7 vaccine would mask clinical signs

caused by an HPAI virus that might arise by mutation.

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 (e.g. to the abattoir) or to other establishments (e.g. young breeders), and to avoid movement of dead birds and infected litter, which were identified as being among the primary sources of infection.

These restriction policies, aided by the oncoming warm season, resulted in a decrease in the number of outbreaks, which reached the minimum of six during the month of August.

It is well known that influenza viruses are not very resistant to warm temperatures, while they can remain vital for greater periods of time in cold and humid environments. With the arrival of the winter, the number of outbreaks increased again, reaching 199 notified outbreaks at the end of November.

Emergence of Highly Pathogenic Avian Influenza

On 13 December 1999, a private practitioner submitted samples from a suspected influenza outbreak in a meat-turkey flock exhibiting high mortality. HPAI was diagnosed on 17 December, with the characterization of an H7N1 isolate with an intravenous pathogenicity index of 3.0 and a deduced haemagglutinin cleavage site amino acid sequence of PEIPKGSRRVRR*GLF, i.e. containing multiple basic amino acids, typical of highly pathogenic viruses.

Eradication procedures are likely to be successful if two main conditions are fulfilled: first, an early identification of the primary outbreaks and, second, the prompt implementation of measures as required by EU Directive 92/40/EEC. Since the notification of an outbreak with high mortality in a meat-turkey flock, associated with the isolation of an H7 virus, was not unusual at the time, the presence of HPAI virus was not suspected immediately when it first appeared. As a result, eradication measures were not implemented at an early stage and this resulted in a spread of infection. In fact, from the epidemiological follow-up of subsequent outbreaks, it appeared that at least 16 flocks were already infected on 17 December 1999. This evidence suggested that infection spread significantly before the implementation of restriction measures, possibly related to the increased numbers of slaughtered birds prior to the holiday season. The resulting loss of control of the infection has culminated in 378 outbreaks notified to date.

HPAI has affected a greater number of establishments than LPAI, and has been often characterized by 100% morbidity and 100% mortality in the affected flocks. All intensively reared species have been affected; turkeys, chickens, guinea-fowl and

quail often exhibited 100% mortality rates in a few days. Apart from waterfowl, in which the resistance to HPAI is well known, the only birds that appeared to show some resistance were ostriches, in which clinical signs and mortality were limited to young birds (Capua *et al.*, 2000).

Consequences of the HPAI Outbreaks

The HPAI epidemic started south of Verona and Mantova provinces (similar to what had occurred with the LPAI outbreaks) and spread rapidly, affecting the neighbouring provinces of Vicenza, Padova, Brescia and Bergamo, causing death of over 13 million birds at the time of writing this editorial (March 2000). As a result of mass mortality (due to the 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. The resulting disruption of the marketing system caused unemployment and heavy economic losses to the poultry industry and to the social community. Further economic losses also occurred due to the export bans imposed on the infected regions.

A delay to restocking cleaned and disinfected farms, and therefore to normalization of commercial activities, was caused by new outbreaks, which resulted in overlapping of the protection and surveillance zones imposed by Directive 92/40/EEC. Because of the high population density in the area, the only possibility of ensuring that susceptible restocking birds may not contract the disease has been to impose depopulation of some infected areas, which has inevitably caused further economic losses.

At present, we have no certainty that after depopulation the disease will not reappear, bearing in mind the enormous infective pressure in the environment, and the possible presence of virus reservoirs such as wild birds and waterfowl.

Conclusions

A few conclusions can be drawn from this experience. Most importantly, farmers and private companies should understand that within current legislation there is no provision for financial aid from local or national governments, or from the European Union in cases of LPAI. There would therefore appear to be a need to diagnose such infections promptly so that appropriate control measures, including voluntary stamping-out policies, can be implemented while they are still economically viable. One way to achieve this would be to put in place permanent surveillance programmes, which may result in the identification of possible risk factors and critical points in the area and in the production system. In Italy, in 1999, by the time LPAI was recognized and diagnosed, it was already

affecting 60 to 70 flocks in the area, and voluntary slaughter did not seem to be a feasible option. In several geographical areas throughout the world, poultry industries have developed without any thought being given to the possibility of having to isolate an infected unit from the system. Concentrating 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 in the face of an epidemic of a highly contagious disease. Without planning, it is inevitable that semi-vertical integration systems similar to those in the affected area of Italy will develop in this way. Considering the animal density and the geographical limits in the existing situation, it is imperative that all efforts to obtain early diagnosis and control LPAI must be made.

One further point should be emphasized. If HPAI represents a "model" of how viruses may spread within the poultry industry, other viral infections (such as infectious bursal disease, infectious bronchitis and avian pneumoviruses) may spread among the different establishments with a similar pattern, resulting in an increase in drug consumption and vaccine administration. There seems little doubt, particularly in densely populated livestock areas, that the implementation and application of basic biosecurity measures would reduce the diffusion of infectious diseases throughout the poultry industry, thus reducing drug administration and improving the quality of poultry products. It therefore appears that the basic concepts developed for densely populated livestock areas of cattle and pigs with regard to the eradication of Office International des Epizooties (OIE) list A diseases should be also applied to poultry (Dijkhuizen & Davies, 1995).

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 already mentioned, the control policies for avian influenza in the EU should be reviewed. It would seem logical that the control measures should be implemented aimed at 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 in a given poultry population complicates the interpretation of diagnostic results, thus delaying eradication procedures.

Another aspect that should be emphasized is the cost of eradication in such a complex situation. At present, the estimated costs for compensation of farmers are approximately 100 million Euros. But this does not take into account indirect costs to the poultry industries and other companies (e.g. vaccine producers and feedmills) and the additional costs linked to dealing with such an emergency situation (e.g. prices for rendering and disposal of carcasses

become significantly higher than normal). These costs could have been greatly reduced if the LPAI epidemic was eradicated at the time.

One of the problems that was faced and which inevitably caused an increase in the number of outbreaks was that, at present, there is no official test for detecting an infected flock, apart from virus isolation in SPF eggs, which is an expensive and time-consuming test. In a dense livestock area, after diagnosing the first outbreak and identifying the flocks placed in the restriction zones, it is important to have a priority list among the possibly contaminated flocks to establish which flocks are truly infected. The priority list may be decided on the basis of data that originate from the epidemiological inquiry or from laboratory testing. The possibility of using an EU-validated antigen detection system or reverse transcription-polymerase chain reaction test would be extremely useful in emergency situations to avoid delays in eliminating infected flocks, and would definitely be convenient from the laboratory point of view.

Finally, the Italian experience with HPAI shows that it is extremely difficult to control this disease in densely populated areas, especially if infection with LPAI is already widespread in the area, and, therefore, in order to avoid similar situations, prevention systems should be implemented. Besides a structural change in the industrial system that must inevitably take place in order to reorganize production circuits, veterinary surveillance, quarantine and controlled marketing are also essential to prevent sanitary emergencies. In addition, education of farmers and staff to the basic concepts of biosecurity is possibly the most critical point in the eradication of avian influenza, and fundamental to the management of poultry that are reared intensively.

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RÉSUMÉ

L'épizootie Italienne d'Influenza aviaire en 1999–2000

Durant l'année 1999, le nord de l'Italie a été affecté par une épizootie d'influenza aviaire de faible pathogénicité (LPAI) due à un virus de sous-type H7N1. Du fait des caractéristiques de l'élevage industriel dans cette région et de l'absence de mesures législatives spécifiques pour éradiquer l'infection, le virus a continué à circuler pendant plusieurs mois jusqu'à l'émergence d'un virus hautement pathogène appartenant au même sous-type. Le virus hautement pathogène a entraîné, jusqu'à la rédaction de cet article, la mort de plus de 13 millions d'oiseaux en trois mois. Les conséquences de cette épizootie d'influenza aviaire hautement pathogène sont l'anéantissement de l'aviculture industrielle et l'apparition de problèmes sociaux.

Plusieurs conditions ont généré la situation présente parmi lesquelles la forte densité d'animaux sensibles et la structure de l'élevage industriel dans la région infectée. De plus, la circulation de virus LPAI durant plusieurs mois a inévitablement retardé l'identification rapide de HPAI et a compliqué l'interprétation des résultats des diagnostics. Une reconsidération de l'actuelle législation européenne et une réorganisation de l'élevage industriel sont suggérées pour prévenir l'apparition de situations similaires dans les pays de l'Union Européenne.

ZUSAMMENFASSUNG

Eine Influenza-Epidemie bei Geflügel in Norditalien, 1999–2000

Im Jahr 1999 kam es in Norditalien zu einer Epidemie von schwach pathogener aviärer Influenza (LPAI), die durch ein Virus des Subtyps H7N1 verursacht wurde. Wegen der Eigenart der Geflügelindustrie in dem Gebiet und wegen des Fehlens spezifischer legislativer Instrumente zur Tilgung der Infektion, zirkulierte das Virus mehrere Monate lang weiter, bis ein hoch pathogenes Virus desselben Serotyps zum Vorschein kam. Das hoch pathogene Virus verursachte innerhalb von drei Monaten, bis zu der Zeit als diese Arbeit geschrieben wurde, den Tod von über 13 Millionen Vögeln. Die Folgen der hoch pathogenen aviären Influenza (HPAI)-Epidemie scheinen für die Geflügelindustrie und die Sozialgemeinschaft verheerend zu sein.

Die gegenwärtige Situation wurde durch mehrere Umstände bewirkt, darunter die hohe Dichte empfindlicher Tiere und die Struktur der Geflügelindustrie in dem infizierten Gebiet. Und außerdem verzögerte die mehrere Monate lange Zirkulation des LPAI-Virus zwangsläufig die prompte Feststellung der HPAI und komplizierte die Interpretation der diagnostischen Befunde. Eine Revision der gegenwärtigen europäischen Gesetzgebung und eine Reorganisation der Geflügelindustrie werden vorgeschlagen, um das Auftreten einer ähnlichen Situation in Ländern der Europäischen Union zu verhüten.

RESUMEN

Epidemia de gripe en aves en Italia 1999–2000

Durante el año 1999, el norte de Italia se vio afectado por una epidemia de influenza aviar de baja patogenicidad (LPAI) causada por un virus del subtipo H7N1. Debido a las características de la industria avícola en esta área y a la ausencia de legislación específica para erradicar la infección, el virus continuó circulando durante varios meses hasta que aparecieron virus altamente patogénicos del mismo subtipo. Los virus

altamente patogénicos causaron la muerte, en el momento de escribir este artículo, de unos 13 millones de aves en tres meses. Las consecuencias de esta epidemia fueron devastadoras para la industria avícola y para la comunidad.

Varias condiciones generaron la actual situación, entre las cuales destacan la alta densidad de población de animales susceptibles y la organización de la industria avícola en el área infectada. Además, la

circulación de virus LPAI durante algunos meses retrasó inevitablemente la rápida identificación de los virus altamente patogénicos y complicó la interpretación de los resultados diagnósticos. En el presente artículo se sugiere una reconsideración de la legislación europea y una reorganización de la industria avícola para prevenir la aparición de situaciones similares en otros países de la Unión Europea.