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Should there be a change in the definition of avian influenza for legislative control and trade purposes?

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Abstract

The current OIE and EU definitions of avian influenza (AI) to which control measures or trade restrictions should apply were both drafted over 10 years ago. These were aimed at including viruses that were overtly virulent in in-vivo tests and those that had the potential to become virulent. At that time the only virus known to have mutated to virulence was the one responsible for the 1983/84 Pennsylvania epizootic. The mechanism involved has not been seen in other viruses, but the definition set a precedent for statutory control of potentially pathogenic as well as overtly virulent viruses.

Evidence accumulated to date indicates that HPAI viruses arise from LPAI H5 or H7 viruses infecting chickens and turkeys sometime after spread from free-living birds. At present it can only be assumed that all H5 and H7 viruses have this potential and mutation to virulence is a random event. Therefore the longer the presence and greater the spread in poultry the more likely it is that HPAI virus will emerge. The outbreaks in Pennsylvania 1983, Mexico 1994 and Italy 1999 are demonstrations of the consequences of failing to control the spread of LPAI viruses of H5 and H7 subtypes. It therefore seems desirable to control LPAI viruses of H5 and H7 subtype in poultry to reduce the probability of a mutation to HPAI occurring. This in turn may require redefining statutory AI. There appears to be three options:

1. Retain the current definition with locally imposed restrictions to limit the spread of LPAI of H5 and H7 subtypes.
2. Define statutory AI as an infection of birds/poultry with any AI virus of H5 or H7 subtype.
3. Define statutory AI as any infection with AI virus of H5 or H7 subtype, but modify the control measures imposed for different categories of virus and/or different types of host.

Both the EU Scientific Committee on Animal Health and Animal Welfare in 2000 (Scientific Committee on Animal Health and Animal Welfare 2000) and the OIE *ad hoc* Committee on AI in 2002 (OIE 2002) recommended that relevant legislative processes concerned with control or trade should be extended to all infections of poultry with either H5 or H7 viruses.

Keywords: Avian influenza; definition; cleavage site; European Union; OIE

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Introduction

The first attempt at a universally acceptable definition of what should constitute avian influenza (AI) for which statutory control measures and trading restrictions should apply was agreed at the First International Symposium on Avian Influenza held in Beltsville, USA in 1981 (Bankowski 1982). Until that time definitions used in different countries for 'fowl plague' and 'fowl-plague virus' were extremely variable. It had been known since 1959 that highly virulent AI viruses for poultry could be of two different haemagglutinin subtypes (H7 and H5) and from the early 1970s that not all viruses of these subtypes were necessarily virulent for poultry (Beard and Easterday 1973). Nevertheless, many countries had historical definitions essentially based on identification of viruses as of H7 subtype or the presence of H7 antibodies. The 1981 definition was considered a rational step forward and with subsequent modifications, taking into account the greater understanding of the molecular basis of pathogenicity, it evolved into the current OIE (Office International des Epizooties) definition quoted below.

At the First International Symposium it was recommended that the term 'fowl plague' should be replaced by 'highly pathogenic avian influenza' (HPAI).

Molecular basis of pathogenicity

For all influenza-A viruses the haemagglutinin glycoprotein is produced as a precursor, HA0, which requires post-translational cleavage by host proteases before it is functional and virus particles are infectious (Rott 1992). The HA0 precursor proteins of avian influenza viruses of low virulence for poultry have a single arginine at the cleavage site and another basic amino acid at position -3 or -4. These viruses are limited to cleavage by extracellular host proteases such as trypsin-like enzymes and thus restricted to replication at sites in the host where such enzymes are found, i.e. the respiratory and intestinal tracts. HPAI viruses possess multiple basic amino acids (arginine and lysine) at their HA0 cleavage sites either as a result of apparent insertion or apparent substitution (Senne et al. 1996; Vey et al. 1992; Wood et al. 1993) and appear to be cleavable by (a) ubiquitous protease(s), probably one or more proprotein-processing subtilisin-related endoproteases of which furin is the leading candidate (Stieneke-Grober et al. 1992). HPAI viruses are able to replicate throughout a susceptible avian host, damaging vital organs and tissues, which results in disease and usually rapid death (Rott 1992).

Emergence of highly pathogenic avian influenza

Avian influenza viruses, including those of H5 or H7 subtype, isolated from free-living birds are invariably of low virulence for poultry. Apart from the deaths of large numbers of terns in South Africa in 1961 (Becker 1966), from which A/tern/South Africa/61 (H5N3) was isolated, isolations of HPAI viruses from free-living birds have been associated with contact with infected poultry, usually as a result of surveillance of birds trapped or found dead on or near infected premises. In addition, results of phylogenetic studies of H7 subtype viruses indicate that HPAI viruses do not constitute a separate phylogenetic lineage or lineages, but appear to arise from non-pathogenic strains (Banks et al. 2000; Rohm et al. 1995). Similarly phylogenetic analyses of the preceding LPAI H7N1 isolates and the subsequent HPAI H7N1 isolates in Italy in 1999-2000 indicated evolution from one to the other (Banks et al.

2001). These empirical findings are supported by the in-vitro selection of mutants virulent for chickens from a LPAI H7 virus (Li, Orlich and Rott 1990).

Theories of the molecular basis for the mutation of avian influenza subtype H5 and H7 viruses from low to high virulence in poultry have been put forward by Garcia et al. (1996) and Perdue et al. (1997). Essentially it is proposed that spontaneous duplication of purine triplets results in the insertion of basic amino acids at the HA0 cleavage site and that this occurs due to a transcription fault. The assumption is that this transcription fault occurs more readily in chickens or turkeys than in free-living bird hosts. As pointed out by Perdue et al. (1997) this may not be the only mechanism by which HPAI viruses arise, as some appear to result from nucleotide substitution rather than insertion while others (including the 1999-2000 Italian H7N1 HPAI virus) have insertions without repeating nucleotides. In addition, the H7N3 HPAI virus responsible for the outbreak in Chile in 2002 appears to be somewhat unique. The extremely virulent virus was reported to have a 10-amino-acid insert at the cleavage site giving the unusual motif PEKPKTCSPLSRCRETR*GLF, which does not seem wholly compatible with the need for multiple basic amino acids. The virus is also unique in that the insert appears to have arisen by intergenic recombination between the HA gene and the nucleocapsid gene of the progenitor LPAI virus that had also been isolated (Suarez et al. 2003).

Current definitions

Office International des Epizooties

The following definition for viruses that cause HPAI is taken from the Manual of Standards for Diagnostic Tests and Vaccines 2000 (Alexander 2000)

"*a) Any influenza virus that is lethal for six, seven or eight of eight 4- to 8-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid.*

b) The following additional test is required if the isolate kills from one to five chickens but is not of the H5 or H7 subtype: growth of the virus in cell culture with cytopathogenic effect or plaque formation in the absence of trypsin. If no growth is observed, the isolate is considered not to be a HPAI isolate.

c) For all H5 and H7 viruses of low pathogenicity and for other viruses, if growth is observed in cell culture without trypsin, the amino acid sequence of the connecting peptide of the haemagglutinin must be determined. If the sequence is similar to that observed for other HPAI isolates, the isolate being tested will be considered to be highly pathogenic."

European Union

European Union (EU) legislation on avian influenza is contained in Council Directive 92/40/EEC (CEC 1992). The disease is defined as follows in Annex III of the directive;

"*For the purpose of the diagnostic procedures for the confirmation and differential diagnosis of avian influenza the following definition shall apply.*

'Avian influenza' means an infection of poultry caused by any influenza A virus which has an intravenous pathogenicity index[1] *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 differences between the two definitions are slight in terms of assessing virus virulence. The decision by the EU to use the Intravenous Pathogenicity Index (IVPI) test means that disease as well as death is assessed, but this involves some subjectivity in reading the test. In practice viruses have qualified by either definitions or neither.

These definitions, formulated over ten years ago, were aimed at including viruses that were overtly virulent in in-vivo tests *and* those that had the potential to become virulent. At that time the only virus known to have mutated to virulence was the one responsible for the 1983/84 Pennsylvania panzootic. In this epizootic viruses isolated at the beginning of AI infections of poultry in Pennsylvania were of low virulence for chickens although possessing multiple basic amino acids at the cleavage site (Kawaoka, Naeve and Webster 1984; Kawaoka et al. 1987). These early viruses possessed a carbohydrate chain close to the cleavage site in the three-dimensional structure of the HA molecule that was absent in the later HPAI isolates. The inference is that the presence of this carbohydrate chain prevented access of the ubiquitous host protease(s), but not trypsin-like enzymes, to the cleavage site and when lost the potential virulence of the virus was realized. This mechanism has not been seen in other viruses. However, the inclusion in these internationally accepted definitions of *potentially* virulent viruses does set a precedent for future definitions.

It should also be noted that both definitions allow the confirmation of HPAI by sequencing the amino acids at the HA0 cleavage site, but in-vivo tests are still required to confirm a virus is LPAI. This is important as RT-PCR primers may well identify only the consensus population or show a better 'fit' with LPAI virus and not detect the presence of HPAI virus in a mixed population of LPAI and HPAI viruses.

Reasons for reviewing the definition

The current theories and the accumulating evidence suggest that HPAI viruses arise from H5 or H7 LPAI viruses infecting chickens and turkeys and that when viruses of these subtypes spread from free-living birds there is always a potential that they may become virulent. However, at present we are unable to predict when and if this will occur. Presumably in outbreaks of HPAI such as that occurring in England in 1991 (Alexander et al. 1993), in which only a single house of turkeys was affected,

[1] The intravenous pathogenicity index (IVPI) is the mean score per bird per daily observation over 10 days of 10 six-week-old chickens inoculated intravenously with the virus under test when birds are scored: Score 0 = normal, Score 1 = sick, Score 2 = very sick or paralysed, Score 3 = dead. An IVPI = 0 means that no signs were seen in the 10-day observation period. An IVPI = 3 means that all birds died within 24 hours.

the mutation happens very quickly after introduction. In Australia in 1976 there was evidence of limited spread before mutation took place (Westbury 1997). Whereas in Pennsylvania in 1983 (Webster and Kawaoka 1988), Mexico in 1993/94 (Campos-Lopez, Rivera-Cruz and Irastorza-Enrich 1996; Villarreal and Flores 1997) and Italy 1999/2000 (Capua et al. 2000) there had been extensive outbreaks of LPAI for a considerable period of time before the emergence of HPAI. If it is assumed that mutation to virulence is a random event, then it seems logical that the longer the presence and greater the spread in poultry the more likely it is that HPAI virus will emerge. It would therefore seem a reasonable policy to reduce the spread and presence of LPAI viruses of H5 and H7 subtype in poultry to limit the probability of a mutational event occurring.

Policies pursued, either locally or nationally for different LPAI outbreaks in recent years have varied enormously; ranging from none, through reliance on biosecurity with or without vaccination, voluntary depopulation/slaughter, to a full stamping-out policy, or combinations of these strategies.

In 1998 outbreaks of LPAI caused by virus of H7N7 subtype occurred on the island of Ireland in the Republic of Ireland (29 outbreaks) and in Northern Ireland (3 outbreaks). In both countries the potential to mutate to HPAI viruses and the potential public-health risks were considered serious threats by regulatory authorities and industry. The spread of virus was successfully eliminated by a programme of biosecurity measures, voluntary slaughter, early marketing, cleaning and disinfection and extensive surveillance (Campbell and De Geus 1999; Graham, McCullough and Connor 1999). Similarly, outbreaks of H5 or H7 LPAI in the US have often been controlled by strict biosecurity measures and voluntary depopulation (Eckroade 1997; Senne, Swayne and Suarez 2003). In Utah in 1995 strict biosecurity measures were combined with vaccination (Halvorson et al. 1997). Straightforward stamping out was applied to H5 or H7 LPAI outbreaks in Belgium in 1999 (H5N2), Germany 2001 and Virginia 2002 (H7N2) (review Capua and Alexander 2004) and more recently in Denmark 2003 (H5N7 in ducks). In Italy a 'DIVA' (differentiating infected from vaccinated animals) vaccination strategy was employed with the re-emergence of H7N1 LPAI in 2000 and the H7N3 outbreaks in 2003 (Capua and Alexander 2004), but it should be emphasized that this strategy involves stamping out vaccinated flocks shown to have been infected with the field LPAI virus. All these strategies appear to have been successful in controlling the spread of LPAI.

In contrast, in Italy in 1999 LPAI H7 virus continued to spread despite the recommendation of strict biosecurity regimens, with the emergence of HPAI virus in December 1999 after 199 confirmed LPAI outbreaks (Capua and Marangon 2000).

Many factors appear to influence the ability to control LPAI solely by the application of biosecurity measures including: the degree of spread prior to notification, the population density of poultry farms, the degree of integration and the economic pressures on poultry farmers. The situations in Italy in 1999 and Mexico in 1993/4 are lessons that failure to control LPAI virus spread *will* result in the emergence of HPAI and further complicate the control of the more pathogenic disease. Attempts to control LPAI infections with H5 or H7 viruses without any statutory instrument in place or the ability to pay compensation for birds slaughtered voluntarily may not prove successful.

Controlling H5 and H7 virus infections

If it is accepted that greater statutory control of H5 and H7 LPAI viruses is necessary to avoid probable emergence of HPAI viruses then the options are relatively limited. The apparent choices are:

1. Retain the current definition with a recommendation that countries impose restrictions to limit the spread of LPAI of H5 and H7 subtypes.

This option essentially maintains the status quo, in that in recent years most countries/states have reacted to try and limit infections of LPAI H5 and H7 viruses when they have occurred in poultry. It has proved successful in some countries and unsuccessful in others.

2. Define statutory AI as an infection of birds/poultry with any AI virus of H5 or H7 subtype.

This option follows the precedent in present definitions of slaughter of birds infected with potentially HPAI viruses (see above), since it is currently thought that all H5 or H7 LPAI viruses may mutate to virulence. The added advantages of this option are that diagnosis of both LPAI and HPAI is greatly simplified and would result in quicker implementation than the current definition as it requires neither in-vivo testing or sequencing of the amino acids at the HA cleavage site.

There are however several disadvantages. There is currently lack of knowledge of the prevalence of H5 and H7 virus infections of poultry, especially species other than turkeys and chickens. In the EU during 2003 member states have been carrying out point prevalence surveillance studies in poultry in an attempt to address this lack of knowledge. There may well be reluctance among farmers to consider slaughter of birds showing few, if any, signs and this could lead to failure to investigate mild respiratory disease or even to covering up infections with LPAI. Some decision would have to be made on whether to treat species such as commercial ducks differently to turkeys and chickens. There is no evidence that H5 and H7 LPAI viruses are likely to mutate while infecting ducks and the prevalence of LPAI viruses of these subtypes could be high in commercial ducks in some countries (Shortridge 1999).

3. Define statutory AI as any infection with AI virus of H5 or H7 subtype, but modify the control measures imposed for different categories of virus and/or different types of host.

This option is intermediate to options 1 and 2. It is envisaged that there would be a legal requirement for the notification of all H5 and H7 infections to the regulatory authorities and there would be statutory imposition of control measures. However, although the presence of HPAI virus would require stamping out, lesser measure could be imposed for LPAI virus infections. Such measures would need to be carefully considered and specified, but could include: voluntary slaughter or early marketing, stringent defined biosecurity measures, epizootiological tracing and surveillance. Possibly infections of commercial ducks could be controlled differently, but the need to prevent spread to other poultry would be paramount.

Conclusions

The EU Scientific Committee on Animal Health and Animal Welfare was asked by the EU Commission to reconsider the definition of AI requiring statutory control and recommended that the current control measures laid down in Council Directive 92/40/EEC should be extended to all infections with either H5 or H7 viruses (Scientific Committee on Animal Health and Animal Welfare 2000). A very similar definition was put forward in an OIE draft chapter for the OIE International Animal Health Code (OIE 2002).

To date there has been considerable debate on the desirability of making this change for control or trade reasons, which is continuing. It was not in the terms of reference of this paper to review the emerging public-health implications of AI infections of poultry, but this may well have a future impact, and any decision based on scientific or poultry-industry criteria may be completely nullified by public-health concerns and public opinion.

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