

## Should We Change the Definition of Avian Influenza for Eradication Purposes?

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**SUMMARY.** The current definitions of high-pathogenicity avian influenza (HPAI), formulated over 10 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 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.

The accumulating evidence is that HPAI viruses arise from low-pathogenicity avian influenza (LPAI) H5 or H7 viruses infecting chickens and turkeys 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, Mexico, and Italy 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 limit the probability of a mutation to HPAI occurring. This in turn may require redefining statutory AI. There appear to be three options: 1) retain the current definition with a recommendation that countries impose 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.

**RESUMEN.** Debemos cambiar la definición de influenza aviar para el propósito de erradicación?

Las definiciones actuales de la influenza aviar altamente patógena que fueron formuladas hace 10 años estaban enfocadas para incluir virus que fueran evidentemente virulentos en pruebas *in vivo* y aquellos que tuvieran el potencial de volverse virulentos. En esa época el único virus reconocido que había mutado a virulento fue el responsable de la epizootia en Pennsylvania en 1983 y 1984. El mecanismo involucrado no había sido visto en otros virus, pero la definición estableció un precedente para el control estatutario de virus potencialmente patógenos así como evidentemente virulentos.

La evidencia que se ha ido acumulando es la de que los virus altamente patógenos emergen de virus de influenza aviar de baja patogenicidad subtipos H5 ó H7 que tienen el potencial de infectar pollos y pavos después de diseminarse a partir de aves de vida libre. Actualmente, sólo se puede asumir que todos los virus de los subtipos H5 y H7 tienen este potencial y que la mutación a un virus virulento es un evento aleatorio. Por lo tanto, mientras más duradera sea la presencia del virus, mayor será la diseminación y mayor será la posibilidad de que aparezcan virus de alta patogenicidad. Los brotes en Pennsylvania, México e Italia son demostraciones de las consecuencias del fracaso para controlar la diseminación de los virus de influenza aviar de baja patogenicidad de los subtipos H5 y H7 dentro de la avicultura y limitar la probabilidad de que ocurra una mutación a alta virulencia. Esto, por lo tanto, puede requerir de una redefinición estatutaria de la influenza aviar. Parece que existen tres opciones: 1. Mantener la definición actual con la recomendación de que los países impongan limitaciones a la diseminación de los subtipos

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H5 y H7 de baja patogenicidad. 2. Definir estatutariamente a la influenza aviar como una infección de aves silvestres y domésticas con cualquier virus de los subtipos H5 ó H7. 3. Definir estatutariamente a la influenza aviar como cualquier infección con los subtipos H5 ó H7, pero modificar las medidas de control impuestas para diferentes categorías de virus y diferentes tipos de huéspedes.

Key words: avian influenza, cleavage site, definition, high pathogenicity, H5, H7, Office International Epizooties

Abbreviations: AI = avian influenza; EU = European Union; HPAI = high-pathogenicity avian influenza; LPAI = low-pathogenicity avian influenza [also termed moderately pathogenic avian influenza]; OIE = Office International des Epizooties; SCAHAW = Scientific Committee on Animal Health and Animal Welfare

Possibly the most important outcome of the First International Symposium on Avian Influenza held in Beltsville, MD, in 1981 was the recommendation for a definition of what should constitute avian influenza (AI) for which statutory control measures and trading restrictions should apply (2). Until that time definitions used in different countries of “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 hemagglutinin subtypes (H7 and H5) and that not all viruses of these subtypes were necessarily virulent for poultry. 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 a rational step forward, and with subsequent modifications to improve it, taking into account the greater understanding of the molecular basis of pathogenicity following the 1983–85 “Pennsylvania” high-pathogenicity avian influenza (HPAI) outbreak, it has evolved into the current Office International des Epizooties (OIE) definition quoted below.

At the First International Symposium it was also decided to abandon the term “fowl plague” for “highly pathogenic avian influenza.”

### MOLECULAR BASIS OF PATHOGENICITY

For all influenza A viruses the hemagglutinin glycoprotein is produced as a precursor, HA0, which requires posttranslational cleavage by host proteases before it is functional and virus particles are infectious (21). 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 (24,27,31) 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 (26). HPAI viruses are able to replicate throughout the bird, damaging vital organs and tissues, which results in disease and death (21).

### EMERGENCE OF HIGH-PATHOGENICITY 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 die-off of large numbers of terns in South Africa in 1961 (5), 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 nonpathogenic strains (3,20). Similarly, phylogenetic analyses of the preceding low-pathogenicity avian influenza (LPAI) H7 isolates and the subsequent HPAI H7 isolates in Italy in 1999–2000 indicated evolution from one to the other (4). These empirical findings are supported by the *in vitro* selection of mutants virulent for chickens from a LPAI H7 virus (17).

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.* (12) and Perdue *et al.* (19). 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 by the host polymerase complex. The assumption is that this transcription fault occurs more readily with chicken or turkey polymerase complex enzymes than those of free-living bird hosts. As pointed out by Perdue *et al.* (19), this may not be the only mechanism by which HPAI viruses arise, since 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.

### 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 (18):

- 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 hemagglutinin 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 (10). 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<sup>1</sup> 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 hemagglutinin.

The differences between the two definitions are slight in terms of assessing virus virulence. The election 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 both definitions or neither.

These definitions, formulated over 10 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 (15,16). 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.

### REASONS FOR REVIEWING THE DEFINITION

The current theories and the accumulating evidence suggest that HPAI viruses arise from LPAI

<sup>1</sup>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.

H5 or H7 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, when and if this will occur remains unpredictable. Presumably in outbreaks of HPAI such as that occurring in England in 1991 (1), in which only a single house of turkeys was affected, the mutation happens very quickly after introduction. In Australia in 1976 there was evidence of limited spread before mutation took place (19,30). Whereas in Pennsylvania in 1983 (29), Mexico in 1993–94 (7,28), and Italy in 1999–2000 (9) 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 the longer the presence and greater the spread in poultry the more likely it is that HPAI virus will emerge. It would therefore seem reasonable to limit the spread and presence of LPAI viruses of H5 and H7 subtype in poultry to limit the probability of a mutational event occurring.

In some instances practices aimed at restricting the spread of LPAI viruses of H5 or H7 subtype have been implemented voluntarily. For example, 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 Northern Ireland (three 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 program of biosecurity measures, voluntary slaughter, early marketing, cleansing and disinfection, and extensive surveillance (6,13). Similarly, outbreaks of H5 or H7 LPAI in the US have often been controlled by strict biosecurity measures and voluntary depopulation (11,23). In Utah in 1995, strict biosecurity measures were combined with vaccination (14). In effect in these instances, additional control measures were imposed locally.

In contrast, in Italy in 1999 LPAI H7 virus continued to spread despite the recommendation of strict biosecurity regimens, with the inevitable emergence of HPAI virus in December 1999 after 199 confirmed LPAI outbreaks (8). 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 1993–94 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 influenza viruses is necessary to avoid probable emergence of HPAI viruses when the options are relatively limited, then 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 since 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. There may well be a 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 than 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 some countries (25).

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. 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. Infections of commercial ducks could be controlled differently, but the need to prevent spread to other poultry would be paramount.

### CONCLUSIONS

The object of the present paper is to encourage thought and discussion of the definition of avian influenza virus infections that require eradication by stamping out policies and other statutory controls, and, as such, conclusions are inappropriate. However, 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 (22).

### REFERENCES

1. Alexander, D. J., S. A. Lister, M. J. Johnston, C. J. Randall, and P. J. Thomas. An outbreak of highly pathogenic avian influenza in turkeys in Great Britain in 1991. *Vet. Rec.* 132:535–536. 1993.
2. Bankowski, R. A. Proc. First International Symposium on Avian Influenza, 1981. U.S. Animal Health Association, Richmond, VA. pp. viii. 1992.
3. Banks, J., E. C. Speidel, J. W. McCauley, and D. J. Alexander. Phylogenetic analysis of H7 hemagglutinin subtype influenza A viruses. *Arch. Virol.* 145:1047–1058. 2000.
4. Banks, J., E. S. Speidel, E. Moore, L. Plowright, A. Piccirillo, I. Capua, P. Cordioli, A. Fioretti, and D. J. Alexander. Changes in the hemagglutinin and the neuraminidase genes prior to the emergence of highly pathogenic H7N1 avian influenza viruses in Italy. *Arch. Virol.* 146:963–973. 2001.
5. Becker, W. B. The isolation and classification of tern virus: influenza virus A/tern/South Africa/1961. *J. Hygiene* 64:309–320. 1966.
6. Campbell, G., and H. De Geus. Non-pathogenic avian influenza in Ireland in 1998. In: Proc. Joint Fifth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, Vienna, Austria. pp. 13–15. 1999.
7. Campos-Lopez, H., E. Rivera-Cruz, and M. Irastorza-Enrich. Situacion y perspectivas del programa de erradicacion de la influenza aviar en Mexico. In: Proc. 45th Western Poultry Disease Conference, Cancun, Mexico. pp. 13–16. 1996.
8. Capua, I., and S. Marangon. The avian influenza epidemic in Italy 1999–2000: a review. *Avian Pathol.* 29:289–294. 2000.
9. Capua, I., F. Mutinelli, S. Marangon, and D. J. Alexander. H7N1 Avian influenza in Italy (1999–2000) in intensively reared chickens and turkeys. *Avian Pathol.* 29:537–543. 2000.
10. Council Directive 92/40/EEC of 19th May 1992 introducing Community measures for the control of avian influenza. *Off. J. European Communities* L167:1–15. 1992.
11. Eckroade, R. J. Comment. In: Proc. Fourth International Symposium on Avian Influenza. D. E. Swayne and R. D. Slemons, eds. U.S. Animal Health Association, Richmond, VA. p. 55. 1998.
12. Garcia, M., J. M. Crawford, J. W. Latimer, E. Rivera-Cruz, and M. L. Perdue. Heterogeneity in the hemagglutinin gene and emergence of the highly pathogenic phenotype among recent H5N2 avian influenza viruses from Mexico. *J. Gen. Virol.* 77:1493–1504. 1996.
13. Graham, D., S. McCullough, and T. Connor. Avian influenzas in Northern Ireland: Current situation. In: Proc. Joint Fifth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union. Vienna, Austria. pp. 18–19. 1999.
14. Halvorson, D. A., D. D. Frame, A. J. Friendshuh, and D. P. Shaw. Outbreaks of low pathogenicity avian influenza in USA. In: Proc. Fourth International Symposium on Avian Influenza. D. E. Swayne and R. D. Slemons, eds. U.S. Animal Health Association, Richmond, VA. pp. 36–46. 1998.
15. Kawaoka, Y., C. W. Naeve, and R. G. Webster. Is virulence of H5N2 influenza viruses in chickens associated with loss of carbohydrate from the hemagglutinin? *Virology* 139:303–316. 1984.
16. Kawaoka, Y., A. Nestorowica, D. J. Alexander, and R. G. Webster. Molecular analyses of the hemagglutinin genes of H5 influenza viruses: origin of a virulent turkey strain. *Virology* 158:218–227. 1987.

17. Li, S., M. A. Orlich, and R. Rott, Generation of seal influenza virus variants pathogenic for chickens, because of hemagglutinin cleavage site changes. *J. Virol.* 64:3297–3303. 1990.
18. Office International Epizooties. Highly pathogenic avian influenza. In: *Manual of standards for diagnostic tests and vaccines*, chapter 2.1.14, pp. 212–220. Office International des Epizooties, Paris, France. 2001.
19. Perdue, M., J. Crawford, M. Garcia, J. Latimer, and D. Swayne. Occurrence and possible mechanisms of cleavage site insertions in the avian influenza hemagglutinin gene. In: *Proc. Fourth International Symposium on Avian Influenza*. D. E. Swayne and R. D. Slemons, eds. U.S. Animal Health Association, Richmond, VA. pp. 182–193. 1998.
20. Rohm, C., T. Horimoto, Y. Kawaoka, J. Suss, and R. G. Webster. Do hemagglutinin genes of highly pathogenic avian influenza viruses constitute unique phylogenetic lineages? *Virology* 209:664–670. 1995.
21. Rott, R. The pathogenic determinant of influenza virus. *Vet. Microbiol.* 33:303–310. 1992.
22. Scientific Committee on Animal Health and Animal Welfare (SCAHAW). The definition of avian influenza: the use of vaccination against avian influenza. Report 17 of the Scientific Committee on Animal Health and Animal Welfare adopted 27.06.00, Sanco/B3/AH/R17/2000. [http://europa.eu.int/comm/food/fs/sc/scah/outcome\\_en.html](http://europa.eu.int/comm/food/fs/sc/scah/outcome_en.html). pp. 38. 2000.
23. Senne, D. A. Avian influenza in the Western Hemisphere including the Pacific islands and Australia. *Avian Dis.* 47:798–805. 2003.
24. Senne, D. A., B. Panigrahy, Y. Kawaoka, J. E. Pearson, J. Suss, M. Lipkind, H. Kida, and R. G. Webster. Survey of the hemagglutinin (HA) cleavage site sequence of H5 and H7 avian influenza viruses: amino acid sequence at the HA cleavage site as a marker of pathogenicity potential. *Avian Dis.* 40:425–437. 1996.
25. Shortridge, K. F. Poultry and the influenza H5N1 outbreak in Hong Kong, 1997: Abridged chronology and virus isolation. *Vaccine* 17:S26–S29. 1999.
26. Stieneke-Grober, A., M. Vey, H. Angliker, E. Shaw, G. Thomas, C. Roberts, H.-D. Klenk, and W. Garten. Influenza virus hemagglutinin with multibasic cleavage site is activated by furin, a subtilisin endoprotease. *EMBO J.* 11:2407–2414. 1992.
27. Vey, M., M. Orlich, S. Adler, H.-D. Klenk, R. Rott, and W. Garten. Hemagglutinin activation of pathogenic avian influenza viruses of serotype H7 requires the recognition motif R-X-R/K-R. *Virology* 188:408–413. 1992.
28. Villarreal, C. L., and A. O. Flores. The Mexican avian influenza H5N2 outbreak. In: *Proc. Fourth International Symposium on Avian Influenza*. D. E. Swayne and R. D. Slemons, eds. U.S. Animal Health Association, Richmond, VA. pp. 18–22. 1997.
29. Webster, R. G., and Y. Kawaoka. Avian influenza. *Critical Rev. Poul. Biol.* 1:211–246. 1988.
30. Westbury, H. A. History of high pathogenic avian influenza in Australia and the H7N3 outbreak 1995. In: *Proc. Fourth International Symposium on Avian Influenza*. D. E. Swayne and R. D. Slemons, eds. U.S. Animal Health Association, Richmond, VA. pp. 23–30. 1998.
31. Wood, G. W., J. W. McCauley, J. B. Bashiruddin, and D. J. Alexander. Deduced amino acid sequences at the hemagglutinin cleavage site of avian influenza A viruses of H5 and H7 subtypes. *Arch. Virol.* 130:209–217. 1993.