

REVIEW ARTICLE

Avian influenza: recent developments

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This paper reviews the worldwide situation regarding avian influenza infections in poultry from 1997 to March 2004. The increase in the number of primary introductions and the scientific data available on the molecular basis of pathogenicity have generated concerns particularly for legislative purposes and for international trade. This has led to a new proposed definition of 'avian influenza' to extend all infections caused by H5 and H7 viruses regardless of their virulence as notifiable diseases, although this has encountered some difficulties in being approved.

The paper also reviews the major outbreaks caused by viruses of the H5 or H7 subtype and the control measures applied. The zoonotic aspects of avian influenza, which until 1997 were considered to be of limited relevance in human medicine, are also discussed. The human health implications have now gained importance, both for illness and fatalities that have occurred following natural infection with avian viruses, and for the potential of generating a reassortant virus that could give rise to the next human influenza pandemic.

Introduction

All avian influenza (AI) viruses belong to the *Influenzavirus A* genus of the *Orthomyxoviridae* family and are negative-strand, segmented RNA viruses. Influenza A viruses can be divided into subtypes on the basis of the possession of one of 15 antigenically distinct haemagglutinin (HA) antigens (H1 to H15) and one of nine neuraminidase (NA) antigens (N1 to N9). Virtually all HA and NA combinations have been isolated from birds. The genetic pool for all AI viruses is primarily in aquatic birds, which are responsible for the perpetuation of these viruses in nature (Alexander, 2000).

Influenza A viruses infecting poultry can be divided into two distinct groups on the basis of their ability to cause disease. The very virulent viruses cause highly pathogenic avian influenza (HPAI), which may result in flock mortality as high as 100%. These viruses have been restricted to subtypes H5 and H7, although not all viruses of these subtypes cause HPAI. All other viruses cause a much milder disease consisting primarily of mild respiratory disease, depression and egg production

problems in laying birds (low pathogenicity avian influenza [LPAI]). Sometimes infections with other organisms or environmental conditions may cause exacerbation of LPAI infections, leading to much more serious disease.

It has been demonstrated that the HA0 precursor of the main functional HA glycoprotein requires cleavage, to proteins HA1 and HA2, by host proteases before virus particles are infectious. HA0 proteins of AI viruses of low virulence for poultry are limited to cleavage by host proteases such as trypsin and 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). In contrast, virulent viruses appear to be cleavable by a ubiquitous protease(s), which remains to be fully identified, but appears to be one or more proprotein-processing subtilisin-related endoproteases of which furin is the leading candidate (Stienke-Grober *et al.*, 1992), and this enables these viruses to replicate throughout the animal, damaging vital organs and tissues, which brings about disease and death in the infected bird (Rott, 1992).

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Comparisons of the amino acid sequences at the HA0 cleavage site of AI viruses of high and low pathogenicity revealed that while viruses of low virulence have only two basic amino acids, at positions -1 and -4 from the cleavage site for H5 and at positions -1 and -3 for the H7 subtype, all HPAI viruses possessed multiple basic amino acids (arginine and lysine) adjacent to the cleavage site either as a result of apparent insertion or apparent substitution. The presence of the additional basic amino acids results in a motif recognized and cleavable by the putative ubiquitous protease(s) (Vey *et al.*, 1992; Wood *et al.*, 1993; Senne *et al.*, 1996).

Current evidence (Li *et al.*, 1990; Rohm *et al.*, 1995; Banks *et al.*, 2000a, 2001) strongly supports the hypothesis that HPAI viruses are not normally present in wild bird populations and only arise as a result of mutation after H5 or H7 LPAI viruses have been introduced to poultry from wild birds (Garcia *et al.*, 1996; Perdue, *et al.*, 1998).

Definition of Avian Influenza

Current European Union legislation for statutory control purposes (CEC, 1992) defines AI as 'an infection of poultry caused by any influenza A virus that has an intravenous pathogenicity index in 6-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'. However, on the basis of the evidence that HPAI viruses emerge in domestic poultry from LPAI progenitors of the H5 and H7 subtypes, there is a case that not only HPAI viruses but also their LPAI progenitors should be controlled in domestic poultry (Capua & Marangon, 2000; Alexander, 2003). As a result, the European Union Scientific Committee on Animal Health and Animal Welfare put forward a proposal for a new definition (EU SCAHAW, 2000), which is: 'an infection of poultry caused by either any influenza A virus that has an intravenous pathogenicity index in 6-week-old chickens greater than 1.2 or any influenza A virus of H5 or H7 subtype'. A very similar definition, for trade purposes, was also put forward in a draft OIE Code Chapter (OIE, 2002), but the relevant authorities have adopted neither of these proposals yet.

Recent Outbreaks due to H5 and H7 Viruses

Until recent times HPAI was considered a rare disease in domestic poultry with only 17 episodes being reported worldwide in the 40-year period 1959 to 1998 (Alexander, 2001). However, further outbreaks have occurred since 1999, resulting in eight episodes involving 12 countries in the 7 years

covering 1997 to March 2004. Recently, there also appears to have been a marked increase in the number of LPAI outbreaks caused by H5 and H7 viruses. The countries, subtypes and approximate number of birds involved in the outbreaks since 1997 are presented in Table 1. A brief summary of these outbreaks is now given.

Pakistan 1995 to 2003 H7N3

An outbreak of HPAI caused by a virus of H7N3 subtype affected northern Pakistan in 1995, causing the death of 3.2 million birds, primarily broiler breeders and broilers. A vaccination campaign with a homologous vaccine was implemented, coupled to an upgrading of biosecurity measures, and this led to the eradication of HPAI (Naeem, 1998). During this outbreak HPAI viruses with three different HA0 cleavage site amino acid sequences were detected: PETPKRKRKR*GLF, PETPKRKR*GLF and PETPKRRNR*GLF (Banks *et al.*, 2000a).

In March 2001, AI viruses were identified in chickens from an isolated and relatively new poultry region of Pakistan, 200 km southwest of Islamabad. The affected population was layers, broiler breeders and broilers with approximately 75% of the flocks experiencing mortality ranging between 20 and 85%. H9N2 and H7N3 AI and Newcastle disease viruses were isolated in various combinations. Both the HPAI and LPAI forms of the H7N3 virus were identified. Vaccination is being practiced using a bivalent H7 + H9 oil-based inactivated vaccine. The 2001 H7N3 viruses are very similar to the 1995/96 H7N3 HPAI virus (Swayne & Suarez, 2001). Interestingly, an H7N3 HPAI virus showing close genetic similarity to the Pakistan viruses was isolated from a peregrine falcon (*Falco peregrinus*) dying in the United Arab Emirates (Manvell *et al.*, 2000; Banks *et al.*, 2000a). There was no evidence of links to Pakistan. Further isolations of HPAI virus of H7N3 subtype were reported in Pakistan in 2003 and 2004.

Mexico 1994 to 2003 H5N2

The LPAI H5N2 virus, which mutated to a HPAI virus with the HA0 cleavage site sequence RKRKTR*GLF (Senne, 1998) and caused an outbreak of HPAI in Mexico in 1994/95, continued to circulate in chickens, and the virus has been isolated each year from 1997. Since 1997, Mexico has used more than 1.423 billion doses of inactivated H5N2 vaccine and an additional 459 million doses of a recombinant Pox-H5 vaccine in their H5N2 control programme (Villareal-Chavez & Rivera-Cruz, 2003).

Central America 2000 to 2001 H5N2

H5N2 viruses, genetically similar to the virus circulating in Mexico, were isolated in the Central

Table 1. Outbreaks of LPAI and HPAI caused by H5 and H7 viruses in recent years

Country	Year(s)	Subtype	Virulence	Approximate number of birds infected or culled	Control measure
Mexico	1994–2003	H5N2	LPAI/HPAI	>1 000 000 000	Vaccination
Guatemala,	2000				
El Salvador	2001				
Pennsylvania	1996–1998	H7N2	LPAI	2 623 116	Depopulation
Australia	1997	H7N4	HPAI	310 565	Stamping out
Hong Kong	1997–2003	H5N1	HPAI	~3 000 000	Stamping out, vaccination
Italy	1997	H5N2	HPAI	7741	Stamping out
Ireland	1998	H7N7	LPAI	320 000	Depopulation
N. Ireland	1998	H7N7	LPAI	?	Depopulation
Italy	1998	H5N9	LPAI	2000	Stamping out
Belgium	1999	H5N2	LPAI	100	Stamping out
			LPAI		Stamping out
Italy	1999–2001	H7N1	HPAI	17 000 000	Vaccination + stamping out
			LPAI		Stamping out
Canada (Ont.)	2000	H7N1	LPAI	?	None
Germany	2001	H7N7	LPAI	145	Stamping out
Pakistan	2001–2004	H7N3	HPAI/LPAI	>10 000 000?	Vaccination
USA (NC/VA)	2002	H7N2	LPAI	~5 000 000	Stamping out
Chile	2002	H7N3	LPAI/HPAI	~1 000 000	Stamping out
Italy	2002–2003	H7N3	LPAI	>6 000 000	Vaccination + stamping out
					Stamping out
The Netherlands	2003	H7N7	HPAI	30 283 000	Stamping out
Belgium				2 700 000	
Germany				419 000	
USA (Connecticut)	2003	H7N2	LPAI	2 900 000	Vaccination
Denmark	2003	H5N7	LPAI	12 000	Stamping out
USA (Delaware and Maryland)	2004	H7N2	LPAI	425 000	Stamping out
South-East Asia ^a	2003–2004	H5N1	HPAI	>100 000 000	Variable
Taiwan	2004	H5N2	LPAI	66 600	Stamping out
Canada (BC)	2004	H7N3	LPAI/HPAI	>16 000	Stamping out
USA (TX)	2004	H5N2	HPAI	6608	Stamping out

^a Cambodia, China, Indonesia, Japan, Lao PDR, Republic of Korea, Thailand, and Viet Nam.

American countries of Guatemala in 2000 and El Salvador in 2001. These were the first reports of AI virus in Central America. Movement of contaminated poultry products and equipment between Mexico and Guatemala were the most probable means of virus introduction (Senne, 2003). Both countries are using inactivated and recombinant fowlpox H5 vaccine to control the disease.

Australia 1997 H7N4

Australia had experienced outbreaks of HPAI in 1976 (H7N7), 1985 (H7N7), 1992 (H7N3) and 1994 (H7N3), which have been reviewed by Westbury (1998). In November 1997 a further outbreak of HPAI occurred near Tamworth, New South Wales (Selleck *et al.*, 2003). The viruses isolated from chickens on two commercial farms were identified as HPAI viruses of H7N4 subtype, with HA0 cleavage site amino acid sequences of RKRKR*G and intravenous pathogenicity index (IVPI) values of 2.52 and 2.90. The outbreak resulted in the destruction of a total of 310 565 chickens and 1 232 074 eggs, at a cost of A\$4 445 000.

Italy 1997 to 1998 H5N2

Between October 1997 and January 1998 eight outbreaks of HPAI due to infections with virus of H5N2 subtype were recorded in the Veneto and Friuli-Venezia Giulia regions of NE Italy (Capua *et al.*, 1999). IVPI values of 2.98 to 3.00 were recorded for the viruses isolated and the HA0 cleavage site was PQR RRK KR*GLF. The eight farms were essentially backyard or small dealer farms, often with mixed poultry. The number of birds dying or culled as part of the stamping out procedures was 7741.

Italy 1998 H5N9

A LPAI H5N9 virus was isolated from a flock of 2000 chickens in Emilia Romagna Italy in 1998. The IVPI was 0.00 and the cleavage site motif P QKETR*GLF (Fioretti *et al.*, 1999). The flock was stamped out.

Ireland 1998 H7N7

Between 18 March and 17 April 1998, 29 outbreaks of LPAI, due to a virus of H7N7 subtype, were recorded in the Republic of Ireland; 27 commercial

turkey, one turkey breeder and one broiler breeder farm were affected (Campbell & De Geus, 1999). All viruses tested had an IVPI value of 0.00 and a HA0 cleavage site of PEIPKGR*GLF. Control was by voluntary slaughter, cleaning and disinfection. In total, 320 000 birds were affected. Serological surveillance of 229 farms in the area during and after the outbreaks was carried out; 12 920 blood samples were tested, with negative results.

Northern Ireland 1998 H7N7

Three outbreaks, one in meat turkeys, one in broiler breeders and one in turkey breeders, of H7N7 LPAI occurred in Northern Ireland in March to April 1998, apparently as a result of spread from the Republic of Ireland (Graham *et al.*, 1999). Each of the three viruses produced an IVPI value of 0.00 and a HA0 cleavage site motif of PEIPKGR*GLF.

Belgium 1999 H5N2

LPAI virus of H5N2 subtype was isolated in Belgium in 1999 from a backyard flock of 100 chickens with 10% mortality and diarrhoea and respiratory signs. The IVPI value obtained for the virus was 0.00 and the cleavage site amino acid motif PPKETR*GLF. Despite the LPAI nature of the virus, a stamping-out policy was employed (Meulemans *et al.*, 2000).

Italy 1999 to 2001 H7N1

LPAI epidemic. In March 1999 the first isolation of a LPAI virus of H7N1 subtype was reported. The isolate had an IVPI value of 0.00 and HA0 cleavage site PEIPKGR*GLF. The affected farms were located in a densely populated poultry area (DPPA) and, from March to December 1999, 199 farms were shown to be infected with the virus. Since the virus did not have the characteristics defined in EU Directive 92/40/EEC, no compulsory stamping-out policy was implemented. It was not possible at the time to stamp out such a number of flocks on a voluntary basis, and therefore infection spread further.

HPAI epidemic. On the 17 December 1999 HPAI was confirmed in a meat turkey flock showing high mortality, with the characterization of an H7N1 isolate with an IVPI value of 3.0 and a deduced HA0 cleavage site amino acid sequence of PEIPKGSRRR*GLF, (Capua *et al.*, 2000). In all, 413 HPAI outbreaks were diagnosed. Over 13 000 000 birds died or were slaughtered as part of the stamping-out policy (Capua & Mutinelli, 2001). The last outbreak was notified on 5 April 2000. Following the implementation of Directive 92/40/EEC (CEC, 1992) infected flocks were stamped out, and cleaning and disinfection of infected premises was carried out. To improve eradication

procedures, the depopulation of intensive and semi-intensive chicken and turkey farms was imposed in an area of 5500 km².

Re-emergence of LPAI. In August 2000, clinical suspicion of LPAI in a turkey flock located in the DPPA was confirmed by the laboratory. The Italian Ministry of Health ordered the eradication of infection with a stamping-out policy imposed by an extraordinary Act. Fifty-two LPAI outbreaks 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 programme was drawn up and approved by the European Commission.

Vaccination policy. The vaccination programme in Italy began on 15 November 2000 and lasted until May 2002. The vaccination programme was restricted to meat-type birds and table-egg layers that applied an all-in, all-out system, but involved 15 000 000 birds raised in the 1155 km² restriction zone south of Verona. The vaccine used did not contain an homologous H7N1 virus, but was prepared from an inactivated H7N3 virus (A/ck/Pakistan/95/H7N3). The reason for this was the possibility of using it as a natural 'marker' vaccine, or, more correctly, a 'DIVA' (differentiating infected from vaccinated animals) vaccine (Capua *et al.*, 2003). 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 that 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 induces specific antibodies (against N3 rather than N1), meant that, with the aid of an *ad hoc* diagnostic kit, it was possible to discriminate between infected and vaccinated flocks, and to monitor and follow the evolution of the situation. This system, coupled to an intensive monitoring system, with restriction and biosecurity measures, enabled the eradication of the LPAI H7N1 virus.

Hong Kong and China 1997 to 2002

HPAI due to H5N1 virus, with a HA0 cleavage site amino acid sequence of PQRERRRKKR*GLF, first occurred in poultry in Hong Kong on three chickens farms in March to May 1997 and then re-emerged in November (Shortridge, 1999). Surveillance of Hong Kong poultry markets in December 1997 (Shortridge, 1999) indicated that H5N1 infections were widespread, especially in chickens (19.5% isolation rate) but also in ducks (2.4%) and geese (2.5%). Control was established by slaughter of all poultry in Hong Kong, which was carried out between 29 December 1997 and 2 January 1998. HPAI H5N1 virus re-emerged in poultry in Hong Kong in 2001 and 2002, but the virus was

genetically distinguishable from the 1997 virus (Sims, 2003). The 2002 outbreaks resulted in the infection of 22 farms between January and March 2002 and the slaughter of 950 000 birds (Sims *et al.*, 2003). In April 2002 a vaccination policy was adopted in Hong Kong, using a heterologous H5N2 strain.

There has been little published information on the situation of HPAI H5N1 in mainland China during 1997 to 2002, although the absence of HPAI in China was reported to the Office International des Epizooties for the years 1999 to 2001. However, in 2001 an HPAI isolate of subtype H5N1 was isolated in Korea from duck meat imported from China (Tumpey *et al.*, 2002).

USA 1997 to 2004 H7N2

Pennsylvania 1996 to 1998. Between December 1996 and April 1998, over 2.5 million layer chickens on 24 premises and 47 flocks in Pennsylvania were infected with a LPAI H7N2 virus with HA0 cleavage site sequence PENPKTR*GLF. Approximately 25% of birds exhibited clinical signs of respiratory disease and a temporary decrease in egg production (Davison *et al.*, 2003; Henzler *et al.*, 2003; Senne, 2003). The outbreaks were believed to have originated from two separate introductions as a result of contacts with live-bird markets in metropolitan New York (Kleven, 1998). Control was by strict biosecurity measures and depopulation of infected flocks.

Virginia 2002. LPAI virus of H7N2 subtype continued to circulate in the live bird market system in the US, and the virus again spilled over into the industrial poultry population of the Shenandoah valley in Virginia in 2002. Some farms in North Carolina and West Virginia were also affected. In all, 197 outbreaks were diagnosed (primarily in turkeys) and a total of about 5 million birds was stamped out at a cost of about \$149 000 000. The virus did not mutate to HPAI, although there had been the acquisition of some additional basic amino acids at the cleavage site of the HA (Spackman & Suarez, 2003), indicating a progression towards virulence.

Connecticut 2003. In March 2003, two outbreaks of LPAI H7N2 virus were confirmed in New London County, Connecticut, affecting 2 900 000 table-egg layer hens in two commercial operations managed by the same enterprise. Vaccination of recovered hens and replacement pullets with a homologous vaccine was implemented. Again it appeared that the outbreaks had been the result of spread from live bird markets.

Delaware and Maryland 2004. In February 2004 two farms in Delaware were confirmed as infected with LPAI of H7N2 subtype. One was a small-scale

quasi-backyard type farm with ~11 000 chickens of various types, which supplied live-bird markets. The other was a commercial broiler operation with 85 800 birds. At the beginning of March 2004 a flock in Maryland was shown to be infected with H7N2 virus as a result of surveillance in the Delmarva Peninsula following the Delaware outbreaks. The infected farm containing 118 000 6-week-old broilers and a farm close to the infected premises belonging to the same owner with 210 000 2-week-old birds were depopulated.

Canada 2000 H7N1

In 2000 a flock of turkeys in Ontario, with slightly elevated mortality and egg production problems, was shown to be infected with a LPAI virus of H7N1 subtype, which had an IVPI of 0 and a HA cleavage site sequence of PENPKTR*GLF (Pasick *et al.*, 2003). The flock fully recovered.

Germany 2001 H7N7

Werner *et al.* (2003) reported the isolation of a LPAI virus of H7N7 from a small mixed free-range flock consisting of 60 layer-type chickens, 10 adult broilers, two bronze turkeys, 30 geese and 25 ducks in Southern Germany. The virus had an IVPI of 0.03 and a HA0 cleavage site motif of PEIPKGR*GLF. The flock was stamped out, as was a contact flock of 18 hens that was serologically positive for H7.

Chile 2002 H7N3

Up to 2002 there had been no reports that HPAI had ever occurred in South America. Between the end of April and the first week of May 2002, a clinical condition characterized by low mortality, a slight drop in egg-production and egg-peritonitis appeared in some sheds of a broiler breeder farm located in the Vth region in Chile. Samples were collected from the premises and processed in the laboratory, resulting in the isolation of a LPAI virus of H7N3 subtype. On 23 May notification of a disease causing high mortality in the same farm was forwarded to the Servicio Agrícola y Ganadero, with the suspicion of poisoning. However, following an official inspection the next day, HPAI was suspected. The laboratory confirmed HPAI with the isolation of a virus, of H7N3 subtype, which proved to be highly virulent for chickens (Rojas *et al.*, 2002). The HPAI virus was reported to have a 10-amino acid insert at the cleavage site giving the unusual motif PEKPKTCSPLSR-CRETR*GLF, which furthermore appears to have arisen by intergenic recombination between the HA gene and the nucleocapsid gene of the LPAI virus (Suarez *et al.*, 2003). All the birds (617 800 broiler breeders) on the premise and 116 000 hatching eggs were destroyed. The following week HPAI was diagnosed in two sheds

housing breeder poults of a turkey breeder farm, located 4 km from the index case but functionally connected to it. Since the turkey farm had good biosecurity measures on site and the remaining units on the farm were isolated from the affected houses, there was no spread to the other sheds. All infected birds were slaughtered.

Italy 2002 to 2003 H7N3

During August 2002, serological testing at the abattoir detected antibodies to H7 virus in three meat-type turkey flocks. Intensive surveillance in the whole area did not reveal any additional outbreaks. In October 2002, haemagglutination inhibition tests on serum samples from meat turkeys in the Brescia province were again found to be positive for antibodies to the H7 subtype of AI. Virus isolation yielded a LPAI virus of H7N3 subtype and HA0 cleavage site sequence PEIPKGR*GLF. Laboratory investigations indicate that isolate A/ty/Italy/2002/H7N3 appears to be a new introduction of H7 virus into the domestic poultry population of Northern Italy. Soon after the first outbreaks had been diagnosed, a vaccination programme based on the 'DIVA' strategy was drawn up and approved by the European Union Commission. However, vaccination began 2.5 months after the index case since there was no vaccine commercially available. The delay in the implementation of the vaccination programme resulted in significant spread of the H7N3 virus and further outbreaks continued, including detection of challenge in vaccinated turkeys. The total number of outbreaks caused by this virus was 388; the infection has been eradicated, the last outbreak being recorded on 30 September 2003.

The Netherlands, Belgium and Germany 2003 H7N7

At the end of February 2003, HPAI was suspected in layer farms located in the Gelder Valley province in The Netherlands. The outbreak was confirmed with the isolation of a HPAI virus of H7N7 subtype with an IVPI of 2.98, HA0 cleavage site sequence PEIPKRRRR*GLF. The

infection spread, causing 241 confirmed outbreaks and death or culling of over 30 million birds. In the middle of April the infection spread to Belgium, causing eight outbreaks and death or culling of 2.3 million birds. A single outbreak also occurred in Germany close to the border with The Netherlands, with the death or culling of 419 000 birds.

South-East Asia H5N1 2003 to 2004

Between 12 December 2003 and 27 January 2004 eight countries in South-East Asia first reported the presence of HPAI, virus of H5N1 subtype in poultry. The countries, the number of birds involved and control measures implemented are presented in Table 2. The spread of this HPAI virus appears to be particularly severe in China, with 49 outbreaks in 16 of the country's 31 provinces, Indonesia with 127 outbreaks in 11 provinces, Thailand with outbreaks in 61 of 76 provinces and Viet Nam with virus affecting poultry in 57 of 64 provinces declared by 8 March 2004. All isolates that have been tested and reported have an IVPI approaching 3 and a cleavage site amino acid motif of PQRERRRKKR*GLF, which is identical to that of the Hong Kong 1997 H5N1 virus. In Japan the disease was thought to have been stamped out following a single outbreak in January, but two further outbreaks were reported at the end of February and beginning of March 2004. There was a single isolation of the H5N1 HPAI virus in Hong Kong in January 2004 from a peregrine falcon (*Falco peregrinus*) that was found dead.

Taiwan 2004 H5N2

On 20 January 2004 Taiwan confirmed the infection of two farms, one of table-egg layers, the other native birds, with a LPAI virus of H5N2 subtype. The flocks were slaughtered (54 600 birds). A further outbreak in a flock of 12 000 birds was reported at the beginning of March.

Table 2. Outbreaks of HPAI caused by subtype H5N1 viruses in South-East Asia 2003 to 2004

Country	Date reported (to OIE)	Type of birds	Approximate number of birds infected or culled ^a	Control measures
Republic of Korea	17 December 2003	Layers, ducks	350 000	Stamping out
Viet Nam	08 January 2004	Broilers	36 000 000	Modified stamping out
Japan	12 January 2004	Layers	222 000	Stamping out
Thailand	23 January 2004	Layers	36 000 000	Stamping out
Cambodia	24 January 2004	Layers, ducks	7 500	Stamping out
Lao PDR	27 January 2004	Layers	3 000	Stamping out
Indonesia	06 February 2004	Chickens, ducks, quail	15 000 000	Modified stamping out, vaccination
China	06 February 2004	Ducks, chickens, geese	8 000 000	Stamping out, vaccination

^a FAO estimates.

Canada (BC) H7N3 2004

LPAI of H7N3 subtype was detected on a broiler breeder farm in British Columbia Canada in February 2004 as a result of a routine monitoring programme. The flock of 16 000 birds was depopulated. Further work showed that viruses obtained on the premises proved to be both LPAI (IVPI = 0.0) and HPAI (IVPI = 3.0). A second farm 3 km from the first outbreak was confirmed as infected with HPAI on 12 March 2004.

USA (Texas) 2004 H5N2

In February 2004 HPAI caused by a virus of H5N2 subtype was confirmed in a broiler flock of 6608 birds in Gonzales County, Texas. The farm was depopulated on 21 February. The source of the virus and its relationship to the Mexican H5N2 virus is unknown at the time of writing.

LPAI due to H9N2 Subtype Viruses

Special mention should be made of infections of poultry, mainly chickens, with H9N2 LPAI viruses, which, in the 1990s, reached panzootic proportions. Outbreaks due to H9N2 LPAI occurred in domestic ducks, chickens and turkeys in Germany during 1995 to 1998 (Werner, 1998, 1999), in chickens in Italy in 1994 and 1996 (Fioretti *et al.*, 1998), pheasants in Ireland in 1997 (Campbell, 1998), ostriches in South Africa in 1995 (Banks *et al.*, 2000b), and turkeys in the US in 1995 and 1996 (Halvorson *et al.*, 1998). Infections of chickens with H9N2 viruses in Korea were first detected in 1996 and reported to be widespread (Mo *et al.*, 1998; Lee *et al.*, 2000). Outbreaks due to H9N3 subtype virus were reported to have occurred in China in 1994 (Yingjie, 1998). There have been later reports of widespread infections in China, including Hong Kong, due to H9N2 viruses (Guan *et al.*, 2000; Liu *et al.*, 2003a,b,c). LPAI H9N2 infections have been reported in the Middle East since 1998 and have also caused widespread outbreaks in commercial chickens in Iran (Nili & Asasi, 2002, 2003) and Pakistan (Naeem *et al.*, 1999, 2003; Bano *et al.*, 2003), often associated with serious disease problems.

Phylogenetic analysis of isolates (Banks *et al.*, 2000b) suggests that reports of H9 AI viruses from different parts of the world represent both spread among poultry and separate introductions from wild birds. Vaccination against H9N2 infections has been used in Pakistan (Naeem *et al.*, 1999), Iran (Vasfi Marandi *et al.*, 2002) and China (Liu *et al.*, 2002).

Zoonotic Aspects

Although it has been known for sometime that the human pandemic viruses of 1957 and 1968 appeared to arise by reassortment between viruses present in the human population and AI viruses (Schlotissek *et al.*, 1978; Gething *et al.*, 1980; Kawaoka *et al.*, 1989), until recently direct infection of humans with AI viruses had not been considered an important zoonosis. Up to 1996 there were three instances on record of an isolation of AI viruses from humans. The first was an HPAI virus of H7N7 subtype obtained from a patient with hepatitis in 1959 (Campbell *et al.*, 1970). The second related to a laboratory worker in Australia who developed conjunctivitis after accidental exposure directly in the eye with a HPAI virus of H7N7 subtype (Taylor & Turner, 1977). The third was of conjunctivitis associated with an avian LPAI virus, again of H7N7 subtype, which spread to an animal handler from an infected seal (Webster *et al.*, 1981). In this case four other people handling the infected seals also developed conjunctivitis, but the cause was not confirmed by virus isolation. Despite these cases, human infections with AI viruses were considered rare events of little consequence. This view was largely supported by volunteer experiments, which had shown that only transitory infections resulted when humans were infected with some viruses of avian origin (Beare & Webster, 1991). However, in the period under review in the present paper, a series of events has dramatically raised the profile of human infections with AI viruses. In the past 7 years AI virus infections of humans have been detected on at least seven occasions, with four different subtypes.

In 1996, a LPAI H7N7 virus was isolated in England from the eye of a woman with conjunctivitis who kept ducks. This virus was shown to be genetically closest in all eight genes to viruses of avian origin and to have >98% nucleotide homology in the HA gene with a virus of H7N7 subtype isolated from turkeys in Ireland in 1995 (Kurtz *et al.*, 1996; Banks *et al.*, 2000a).

In May 1997, a virus of H5N1 subtype was isolated from a young child who died in Hong Kong, and by December 1997 the same virus was confirmed, by isolation, to have infected 18 people, six of whom died (Shortridge *et al.*, 1998). There was some evidence of very limited human-to-human spread of this virus, but clearly the efficiency of transmission was extremely low (Buxton Bridges *et al.*, 2000). The viruses isolated from the human cases appeared to be identical to viruses first isolated from chickens in Hong Kong in March 1997 following an outbreak of HPAI. Both human and avian isolates possess multiple basic amino acids at the HA0 cleavage site. In 2003 an H5N1 virus was isolated from a father and son in Hong Kong who presented with respiratory illness after returning from the Chinese mainland;

the father died. A daughter and mother had become ill and the daughter died while visiting the Chinese mainland. It is not known if she was infected with H5N1 virus. There were reported to be some genetic differences between the 1997 and the 2003 H5N1 viruses (World Health Organization website: <http://www.who.int/mediacentre/releases/2003/pr17/en/>).

In March 1999, two independent isolations of AI subtype H9N2 were made from girls aged 1 and 4 years who recovered from influenza-like illnesses in Hong Kong (Peiris *et al.*, 1999). Subsequently, five isolations of H9N2 virus from humans on mainland China in 1998 were reported.

During the 2003 HPAI H7N7 outbreaks in The Netherlands, of 260 people involved in some aspect of the outbreak and presenting with conjunctivitis and/or influenza-like illness, 82 were confirmed as infected with H7 virus (Koopmans *et al.*, 2003). There was also evidence of three cases of human-to-human transmission within families. Six people tested proved positive for H3N2 influenza, but none were also positive for H7N7. Following these cases, all staff involved in the outbreaks were treated prophylactically with antiviral drugs and subjected to vaccination against human influenza (to reduce the chance of reassortment between human and avian viruses). During this outbreak a human fatality also occurred. The victim was a 57-year-old veterinarian who had not received prophylactic antiviral drugs and had contact with infected birds during outbreak management. He was admitted to hospital with severe headache and fever. Subsequently he developed a severe respiratory condition, kidney failure and died. H7 virus was recovered from a broncho-alveolar lavage collected 9 days after the onset of illness (Koopmans *et al.*, 2003).

To date, in 2004 in South-East Asia, there have been 33 confirmed human infections and 23 people have died; 11 infections with eight deaths in Thailand, and 22 infections with 15 deaths in Vietnam. No other country involved in the 2004 H5N1 outbreaks has so far reported human infections. The number of deaths is worrying, but this extremely high infection to mortality rate may be misleading as there is no information available on the number of people infected with mild or no symptoms.

Discussion

The H5 and H7 outbreaks in domestic poultry since 1997 listed in the present paper apparently represent a significant increase over recent previous similar periods. The reasons for this are not altogether clear. Possibly it may be that in the past decade there has been increased awareness and better diagnostic tools, but this would seem unlikely in the case of HPAI, due to the severe clinical

disease. Climate change and consequent variations in wild bird migratory patterns or populations may also have some impact on the incidence of AI introductions to domestic poultry. There have also been relevant changes in poultry production in recent years with an increase in DPPAs coupled with a move towards rearing on open range. If there truly has been an increase in infections of poultry with H5 and H7, resulting in the consequent high incidence of HPAI infections, it is likely to be a complex interaction of many factors, including those suggested earlier.

The 2003 HPAI epizootic in The Netherlands, with limited spread to Belgium and Germany, resulted in the death or slaughter of over 30 000 000 birds, which was an unprecedented number in the history of the control of HPAI. The reason for this was the extremely high density and close proximity of poultry farms (up to 25 farms/km² in the Gelder Valley), which resulted in difficulties in containment, and the large numbers of birds in the buffer zones when it was decided to depopulate those. The extensive Italian problems since 1997 have also occurred in the DPPAs in NE Italy. It may well be that to reduce the risk of future introduction and spread, to facilitate rapid effective control once disease has occurred and to minimize the economic and sociological impact there is a need for limitations to be placed on the concentrations of poultry farms and poultry in specified geographical areas.

The situation in South-East Asia appears to even over-shadow the outbreaks in DPPAs in Europe, with FAO estimates of over 100 million birds affected by 24 February 2004. As in The Netherlands, because of the concentration of poultry, many of the birds killed have been healthy birds situated close to HPAI outbreaks. For example, in China by 1 March only 144 800 birds had been reported to be infected with H5N1, but to implement the stamping-out policy of culling all birds in a 3 km radius, 7 961 000 had been slaughtered and a further 10 211 800 in a 5 km radius vaccinated.

Control measures for LPAI viruses especially those of H5 and H7 subtype, which do not currently fall within virus definitions for statutory control, cause a dilemma, for both farmers and national authorities, which was highlighted by the problems in Italy during 1999 and 2000 (Capua & Marangon, 2000). In addition, despite falling outside the current agreed definitions, trading partners are most reluctant to receive poultry and poultry products from countries or areas where LPAI H5 or H7 viruses are known to be present. Equally, the consequences of the LPAI mutating to HPAI after circulating for some time, as occurred in Pennsylvania in 1983, Mexico in 1994 and Italy 1999 are recognized as extremely serious. As a result most countries faced with outbreaks of H5 or H7 LPAI in recent years have implemented either a stamp-

ing-out policy or one of early depopulation and marketing (Table 1).

In the past 10 years, the severity of clinical signs induced by both LPAI and HPAI strains, and the massive and uncontrolled spread of the virus in some of the outbreaks, have resulted in the adoption of vaccination policies in some countries. Vaccination has been and continues to be used against H5N2 virus in Mexico, H7N3 virus in Pakistan and against H5N1 virus in at least China and Indonesia in the recent outbreaks in South-East Asia. However, the usefulness of conventional vaccination as an eradication tool is unclear, particularly because there is a probable decline in biosecurity measures once vaccination has been undertaken. In addition, the constant monitoring of the epidemiological situation is of vital importance for eradication purposes, and this requires a tremendous amount of effort from the public veterinary services and laboratories that is not always available.

Vaccination against LPAI was reported to have been a success in Utah in 1995 where inactivated vaccine was prepared from the LPAI virus of H7N3 responsible for a series of outbreaks in turkeys (Halvorson *et al.*, 1998; Halvorson, 2002). However, strict biosecurity measures, including controlled slaughter, were kept in place and enforced. The use of vaccination plus a 'DIVA' strategy for the LPAI H7N1 re-emergence in Italy in 2000 to 2002 was successful in the eradication of the virus. A similar system was implemented in 2002 in Italy for the H7N3 LPAI, and was also successful. It should be emphasized that these control measures are in effect 'stamping out with vaccination' as vaccinated birds found to be infected subsequently with the field virus are stamped out or depopulated by controlled marketing (Capua & Marangon, 2003).

At the FAO summit meeting in Rome (3 to 4 February 2004), vaccination using the 'DIVA' strategy was recommended for South-East Asian countries where there is risk that stamping out of infected animals may result in the removal of a major source of food for rural communities and damage the commercial viability of the local poultry industry (FAO, 2004). However, as in Mexico and Pakistan, attempts at controlling HPAI infections of poultry by vaccination without a well-defined strategy, including monitoring of field exposure and the application of stringent control measures on field exposed farms (regardless of the state of vaccination), may not result in eradication. From experimental data it is known, for both LPAI and HPAI, that vaccination protects against clinical signs and mortality, reduces virus shedding and increases resistance to infection (EU SCAHAW, 2003; Capua *et al.*, 2004). However, the virus is still able to replicate in clinically healthy vaccinated birds and this is probably why vaccina-

tion alone has been unsuccessful in achieving eradication. Without the application of monitoring systems, strict biosecurity and depopulation in the face of infection, there is the possibility that HPAI could become endemic in vaccinated poultry populations and the concomitant public health threat will not be removed. In addition, long-term circulation of the virus in a vaccinated population may result in antigenic drift. This has never been described for AI viruses, but could occur if the circumstances allowed it. The outcome of such a situation could invalidate the use of vaccination to support eradication.

One of the most alarming aspects of AI infections during the period under discussion has been the human infections. The reported incidents in the past 7 years raise several important issues. The fact that AI viruses now appear to be able to infect humans fairly regularly, especially via the conjunctival route, and cause disease signs is likely to have public health repercussions on the handling of all AI outbreaks.

The H5N1 HPAI infections of humans in Hong Kong in 1997 and 2003 and in South-East Asia in 2004 have been of particular concern because of the apparent high mortality rates. The particular concern for HPAI viruses is that mammals including humans also possess furin, the putative ubiquitous protease that cleaves HPAI virus HA0 cleavage sites with multiple basic amino acid motifs, leading to systemic infections in birds. Linked to this is possibly the most important consideration in AI virus infections of humans: that while infections to date have been extremely limited in their human-to-human spread, it is quite feasible that AI and human influenza viruses could infect the same individual. This could result in reassortment between the two viruses with the consequence that a virus emerged with the internal genes from the human virus, allowing easy transmission in humans, but with the HA from the AI virus, which, inevitably, would lead to a new pandemic. At the time of writing all human isolates tested in South-East Asia have had eight avian genes, indicating that, so far, no reassortment had taken place. The public health implications of the co-circulation of human and avian viruses are severe. For this reason, it is important that AI should be eradicated from poultry rather than controlled by vaccination and that vaccination should be used only as a tool in delivering eradication.

Finally, humans infected with AI viruses could represent a mechanism for the dissemination and spread of the virus, not just locally, but over long distances; this could lead to further infections of poultry or other susceptible animals such as pigs.

It is important that, regardless of the species or size of the flock, all rearers of poultry and other birds treat the possibility of infections of

their birds with AI viruses extremely seriously, both from animal and public health viewpoints, and take all practicable measures to minimize the risk of their birds becoming infected with any AI virus.

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In addition to the references cited in the text, the authors have obtained information from the following European Union, OIE and FAO websites: http://europa.eu.int/comm/health/ph_threats/com/Influenza/avian_influenza
<http://www.fao.org/newsroom/en/news/2004>
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