

AVIAN INFLUENZA – CURRENT SITUATION AND FUTURE TRENDS

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In the last 10 years many aspects of the epidemiology of avian influenza (AI) infections in poultry and other birds appear to have changed dramatically from those established in the preceding century. The number of outbreaks of the highly pathogenic disease (HPAI) has increased alarmingly in the last 10 years and, even more noticeably the impact in terms of the number of birds involved and the costs of control disease have dramatically escalated. But what has been most marked is the apparently unprecedented emergence and spread of the HPAI H5N1 virus in SE Asia and beyond which, with the zoonotic infections, has resulted in AI being considered one of the most important animal diseases, if not the most important.

Influenza viruses have segmented, negative sense, single strand RNA genomes and are placed in the family *Orthomyxoviridae*. At present the *Orthomyxoviridae* family consists of five genera, only viruses of the *Influenzavirus A* genus are known to infect birds.

Influenza A viruses are further divided into subtypes based on the antigenic relationships in the surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). At present 16 HA subtypes have been recognised (H1-H16) and nine NA subtypes (N1-N9). Each virus has one HA and one NA antigen, apparently in any combination. All influenza A subtypes in the majority of possible combinations have been isolated from avian species. To date only viruses of H5 and H7 subtype have been shown to cause HPAI in susceptible species, but not all H5 and H7 viruses are virulent.

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 [1–3]. The HA0 precursor proteins of AI viruses of low virulence for poultry (LPAI viruses) have a single arginine at the cleavage site and another basic amino acid at position –3 or –4 from the cleavage site. 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 [2–4–6] and appear to be cleavable by an intracellular ubiquitous protease(s), probably one or more proprotein-processing subtilisin-related endoproteases of which furin is the leading candidate [5]. HPAI viruses are able to replicate throughout the bird, damaging vital organs and tissues, which results in disease and death.

The factors that bring about mutation from LPAI to HPAI are not known. In some instances mutation seems to have taken place rapidly (at the primary site) after introduction from wild birds, in others the LPAI virus has circulated in poultry for months before mutating [7–8]. Therefore, it is impossible to predict if and when this mutation will occur. However, it can be reasonably assumed that the wider the circulation of LPAI in poultry, the higher the chance that mutation to HPAI will occur. HPAI viruses are not necessarily virulent for all species of birds and the clinical severity seen in any host appears to vary with both bird species and virus strain [9, 11]. In particular ducks rarely show clinical signs as a result of HPAI infections although there are

reports that some of the Asian H5N1 viruses have caused disease [12] and the HPAI viruses A/duck/Italy/2000 (H7N1) and A/chicken/Germany/34 (H7N1) have been reported to cause disease and death in naturally and experimentally infected waterfowl [10].

Influenza viruses have been shown to infect a great variety of birds [for reviews see 13–17], including free-living birds, captive caged birds, domestic ducks, chickens, turkeys and other domestic poultry. It was not until the mid-1970s that any systematic investigations of influenza in feral birds were undertaken. These investigations revealed enormous pools of influenza viruses to be present in the wild bird population [17, 18–20] especially in waterfowl, Family Anatidae, Order Anseriformes. In the surveys listed by Stallknecht and Shane [19] a total of 21 318 samples from all species resulted in the isolation of 2317 (10.9%) viruses. However, 14 303 of these samples were from birds of the Order Anseriformes which yielded 2173 (15.2%) isolates. The next highest isolation rates were 2.9% and 2.2% from the Passeriformes and Charadriiformes, respectively; but these compare with an overall isolation rate of 2.1% from all birds other than ducks and geese. However, studies by Sharp et al., [21], suggest that waterfowl do not act as a reservoir for all avian influenza viruses. It seems likely that part of the influenza gene pool is maintained in shorebirds and gulls, from which the predominant number of isolated influenza viruses are of a different subtype to those isolated from ducks [22].

Until the spread of Asian HPAI H5N1 (see below), HPAI viruses had been isolated rarely from free-living birds and, apart from A/tern/S.Africa/61 [23], when they had been isolated it was usually in the vicinity of outbreaks of HPAI in poultry or geographically and chronologically close to known outbreaks in poultry. The different epidemiology of the Asian H5N1 HPAI has led to several groups re-examining the understanding of AI virus transmission. In particular the change in the primary route of transmission from faecal/oral to the respiratory route in land birds, especially minor poultry species such as quail and pheasants has been considered significant in the epidemiology of that virus, especially in its spread to mammals [24–26]

The emergence of HPAI H5N1 virus in SE Asia and its spread across Asia and into Europe and Africa is unprecedented in the virological era. The apparent progenitor virus for the subsequent outbreaks of HPNAI of H5N1 subtype was obtained from an infection of commercial geese in Guangdong province PR China in 1996 [27]. In some reports it has been considered that the virus continued to circulate in southern China primarily in domestic ducks and showing some genetic variation [28]. This apparent low-level, but probably endemic, situation changed dramatically in December 2003 to February 2004 when suddenly eight countries in E and SE Asia reported outbreaks of HPNAI due to H5N1 virus [28]. Although there seemed to be some success in controlling the outbreaks in some countries, it appeared to re-emerge in a second wave in July 2004 onwards. Malaysia reported an outbreak in poultry in August 2004 and became the ninth country in the region to be affected [29]. The virus appeared to affect all sectors of the poultry populations in most of these countries, but its presence in free range commercial ducks, village poultry, live bird markets and fighting cocks seemed especially significant in the spread of the virus [28, 27, 30].

If HPAI virus becomes widespread in poultry, especially in domestic ducks that are reared on free range, spill-over into wild bird populations is inevitable. In the past such infections have been restricted to wild birds found dead in the vicinity of infected poultry, but there has always been concern that infections of wild birds in which HPAI virus caused minimal or no clinical signs (i.e. ducks) could result in spread of the virus over large areas and long distances. Outbreaks affecting many wild bird species at two waterfowl parks in Hong Kong were recorded in 2002 [31] and further, possibly more significant, outbreaks in wild migratory birds were reported in China and Mongolia in 2005. In particular it was suggested that presence of virus in migratory birds at Lake

Qinghai in Western China could be the means by which the H5N1 [32, 33] virus could spread West and South.

There is no certain evidence that wild birds were responsible for the introduction into Russia but HPAI H5N1 virus, genetically closely related to isolates obtained at Lake Qinghai, reached poultry there in the summer of 2005. Whether spread from there to other Western Asian and some Eastern European countries occurred or virus was introduced independently is not clear, nor is whether spread was associated with movements of poultry or wild birds, probably both were involved, but during 2005 to the beginning of 2006 genetically closely related H5N1 viruses appeared in a number of countries in the region.

Reports of HPAI H5N1 virus infections continued in Europe and in Africa during the first six months of 2006 and by the end of 2006 56 countries in Asia, Europe and Africa had reported HPAI caused by H5N1 virus to the World Organisation for Animal Health (OIE) since the end of 2003 [29].

The epidemiology of AI has changed in the last 10 years, not only because of the failure to control and eradicate infections in poultry due to HPAI H5N1 viruses, but also because the continued development and industrialisation of the poultry industries throughout the World has meant that AI infections, especially HPAI outbreaks have had a far greater impact in terms of spread and loss of birds than in earlier years. In addition, in the past the spread of HPAI virus to wild birds has not been recorded on the scale reached by the Asian HPAI H5N1 virus. Whether the virus is likely to become or remain endemic in some species of wild birds or would gradually die out if there was no further spread from infected poultry is not clear.

This change in the ecology and epidemiology of AI infections requires the urgent generation of new knowledge on issues related to epidemiology, pathogenesis and control. The Asian HPAI H5N1 viruses have spread to three continents, with completely different agricultural, ecological social and economic backgrounds. This in turn is likely to result in the establishment of different mechanisms by which the virus may be perpetuated in a given area. The generation of such cycles will be influenced by the diversity and availability of hosts in that area. As the virus encounters new hosts – within and outside the Class *Aves*, it may well acquire mutations that may reflect replication advantages in one or more species, but affect the pathogenicity and transmissibility in those and other species.

In view of the zoonotic potential it would appear important that the Asian HPAI H5N1 virus is eliminated from poultry at least and not just contained by the use of vaccination, as has been the strategy with other poultry viruses, especially Newcastle disease virus [34], which remains endemic in many parts of the world. Additionally, the application of control programmes encompassing vaccination may result in the generation of strains that have progressively drifted away from the original antigenic profile [35]. To date it is unclear how the immunological pressure generated by the variety of seed strains contained in available and planned veterinary vaccines will affect the antigenic properties of isolates.

The results of these two driving forces in the genetic and antigenic profile require careful monitoring of viral strains and a close collaboration between the parties involved in the crisis management. The monitoring effort should aim at the collection and characterisation of strains in order to identify genetic mutations and antigenic properties. Information should be collated and made available to the international scientific community, so that those involved in both animal and human health are fully informed of the current situation.

Efforts to bring about control and eradication internationally will have to take into account the extremely complex situation especially in any given geographical location the characteristics of the poultry producing sector in its entirety, the eco-epidemiological situation, the response

capacity of the veterinary infrastructure and the availability of adequate resources. These features must be integrated with the social environment, including those linked to the rearing of birds for recreational and farming purposes. It is possible that in some areas control and eradication will never be achieved and great changes in the way poultry are reared and they and their products marketed will be necessary.

For this reason, international organisations that govern trade regulations and animal disease control should establish a set of guidelines so that control programmes may be “accredited” and consequently internationally recognized. Such a policy would appear to have several practical advantages, ultimately resulting in an improved crisis management. These include rapid approval of established control programmes, constant update on the field situation, feedback of information on successes and failures, harmonisation of protocols and systems and public availability of control and eradication programmes. In this way, even inexperienced countries can maximise the outcome of other experiences to combat this infection in an educated manner – thus avoiding wastage of resources and time.

At least two AI subtypes, H5N1 and H9N2, both of which have zoonotic implications are currently endemic in vast areas of the world. It is impossible to predict whether either of them will represent the progenitor of the next human pandemic virus. Certainly, both of them are causing losses to the poultry industry and H5N1 is also causing the loss of human lives and the reduction of the livelihood of rural establishments. The extensive and uncontrolled circulation of these strains could result in catastrophic consequences for both human and animal health and therefore requires an extraordinary and coordinated international effort so that control and eradication can be successfully managed and achieved.

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