# 2

# Avian influenza viruses in Hong Kong: zoonotic considerations

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## Abstract

Since the mid-1970s, Hong Kong has functioned as an influenza sentinel post for southern China, a region identified as a hypothetical epicentre for the emergence of pandemic influenza viruses. Nineteen ninety-seven marked the coming-of-age of animal-influenza studies with the recognition in Hong Kong of an incipient pandemic situation brought about by the infection of chicken and humans with an avian influenza H5N1 (H5N1/97) virus. Slaughter of all poultry across the Hong Kong SAR possibly averted a pandemic. Tracking down the source of the H5N1/97 virus to geese and quail and precursor avian H5N1, H9N2 and H6N1 viruses revealed that it was a triple reassortant. This provided a framework for influenza-pandemic preparedness at the baseline avian level, H5N1-like viruses being recognized in chicken in 2001 and twice in 2002; at the human level, H9N2 and H5N1-like isolations were made in 1999 and 2003, respectively.

In contrast to Europe and elsewhere, where outbreaks of disease in chicken (by H5 and H7 subtypes) often follow migratory bird activity in an area and the subsequent detection of low-pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) virus forms, in southern China, chicken and other poultry are raised in a permanent, year-round avian-influenza milieu as a consequence of duck-raising practices. Given this long-established milieu, the question is raised whether East-Asian avian influenza viruses comprise a group with a greater propensity for interspecies transmission and potential for pandemicity. The intensification of the poultry industry worldwide in recent years may influence the behaviour of these viruses in the milieu and elsewhere. Clearly, there are scientific and veterinary health needs to redefine the terms LPAI and HPAI. The extent of their applicability in southern China where there is now evidence of H5 and H9 subtype viruses exhibiting swings in avian host range perhaps with ‘smouldering virulence’ remains to be seen.

With animal influenza now part of the World Health Organization’s “Global Agenda on Influenza Surveillance and Control” the time is now opportune for it and its sister organization dealing with animal health, Office International des Epizooties, to strengthen their links to combat collaboratively the serious threat of avian influenza viruses for humans and animals.

**Keywords**: influenza; H5N1; H9N2; zoonosis; China; Hong Kong; pandemic

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## Hong Kong situation

The import of the term 'influenza' in the public's perception was ratcheted up a number of notches in 1997 when a novel, highly pathogenic influenza-A virus of avian origin, H5N1 (H5N1/97), killed chicken on farms and in live-poultry markets in the Hong Kong SAR as well as six of 18 people known to have been infected (Claas et al. 1998; Subbarao et al. 1997; Yuen et al. 1998). Ominously, a purely avian influenza virus was causing respiratory disease and death in humans. An important source of food was also the source of a zoonotic infectious disease that stood to become a global health threat. Moreover, the viability of this food source globally stood to be affected (Shortridge, Peiris and Guan 2003).

Termed locally as 'bird flu', this avian influenza (AI) outbreak put Hong Kong into an incipient influenza pandemic situation, and a pandemic was probably averted by the slaughter of all chicken and other poultry across the SAR (Shortridge et al. 2000). By building a profile of influenza ecology over the years particularly as it applied to domestic animals in southern China, Hong Kong essentially functioned as an influenza sentinel post for it and the wider region. This led to an element of influenza-pandemic preparedness in animals and humans (Shortridge 1988; 1992). The H5N1 or 'bird flu' incident upheld the hypotheses that southern China is an epicentre for the emergence of pandemic influenza viruses (Shortridge and Stuart-Harris 1982) and that pandemic influenza is a zoonosis (Shortridge 1992; Webster et al. 1992). The latter hypothesis was engendered from the observation that the surface haemagglutinins (HAs) of the 1968 H3N2 (Hong Kong) pandemic influenza virus and an avian influenza virus were antigenically related (Webster and Laver 1975).

The worth of this understanding in the post-1997 period might be measured by the detection of genotypes of H5N1-like viruses in asymptomatically infected chicken in markets in 2001 before they showed signs of disease. Poultry were killed market-by-market as signs became evident, leading to the pre-emptive slaughter of all poultry to prevent human infection (Guan et al. 2002). Early detection and reaction was the order again in 2002 and 2003 (Secretary for the Environment and Food 2002; Guan et al. 2003). Thus, there now lay the prospect for influenza-pandemic preparedness not only at the human level but, better still, at the baseline avian level with the ideal that if a virus could be stamped out before it infected humans, an influenza incident or pandemic will not result (Shortridge, Peiris and Guan 2003). In 1997, the world was probably one or two mutational events away from a pandemic while in 2002, with earlier detection, it was probably three or four events away.

Preparedness at the human level was put to the test in an unexpected manner in early 2003 around the start of the SARS (severe acute respiratory syndrome) outbreak in Hong Kong (Peiris et al. 2003). Two Hong Kong residents, recently returned from Fujian Province in southern China, exhibited illness with a clinical picture not dissimilar from that of SARS. They did not have SARS; instead, H5N1 viruses were isolated from them, one of whom died. A third family member died earlier in Fujian Province from acute respiratory disease that was not subject to laboratory examination (World Health Organization 2003; Guan et al. 2003). No doubt, the paths for preparedness and reaction will continue to be a learning experience. Having low-cost, widely available, reliable diagnostic tests for distinguishing infection by H5N1 virus and the SARS coronavirus is of paramount importance (Shortridge 2003a).

Nineteen ninety-seven might be considered something of a watershed in dealing with a pandemic. Given that the interpandemic periods in the 20th century ranged from 20 years for the probable H3-like virus that straddled the two centuries from

1898 to 1918 (Masurel and Marine 1973), 39 years for the H1N1 virus and 11 years for the H2N2 (Asian) virus and, as the H3N2 (Hong Kong) virus era was 29 years on in 1997, the time was apposite for a new pandemic virus to emerge. The H5N1 virus did emerge in 1997, in doing so dispelling the notion of a dependency on recycling of haemagglutinin (H) subtypes for pandemicity (Shortridge 1992). However, the virus lacked significant human-to-human transmissibility. It might have acquired this trait through reassorting with a prevailing human influenza-A virus in a human (more likely) or porcine (less likely) 'mixing vessel' but the slaughter of poultry across the SAR denied it this possibility (Shortridge et al. 2000).

## Changing virus and behaviour

The genotype of the 2003 human isolates was similar to that of a novel H5N1 virus isolated from resident waterfowl and migratory birds in two Hong Kong parks in late 2002, highlighting the potential of such birds for spreading H5N1 viruses within and beyond the influenza epicentre (Sturm-Ramirez et al. 2003). As well, antigenic analysis of the HAs of the new isolates showed that they differed considerably from those of H5N1 viruses of 1997 and 2001, potentially exacerbating an already tricky situation with H5N1 vaccine preparedness efforts. In the avian influenza-virus milieu of southern China, infection of land-based poultry such as quail by influenza viruses from domestic ducks could facilitate the generation of novel variants capable of further interspecies transmission (Perez et al. 2003; Li et al. 2003). Here, the quail as a minor poultry, possibly acts as an intermediate host or as an avian 'mixing vessel' for the generation of variants capable of infecting land-based chicken and other gallinaceous birds, in the process leading to antigenic variation of the HA. The antigenic differences seen in the 2002 and 2003 H5N1 isolates could represent antigenic drift of the H5 HA. The demonstration that HA escape mutants of an avian H5N2 virus can be selected experimentally with monoclonal antibodies is a pointer of the potential of the H5 HA to undergo antigenic drift (Kaverin et al. 2002). This emerging picture about the H5 subtype is relevant to, say, that of the commonly encountered avian H3 subtype that gave rise to the H3N2 pandemic virus of 1968. This subtype exists in domestic ducks in southern China as a range of established antigenic variants each seemingly with its own potential hierarchic capability of infecting the human host and undergoing antigenic drift in it (Shortridge, Underwood and King 1990). As well, the potential for influenza-A viruses to undergo recombination should not be overlooked (Worobey et al. 2002; Chare, Gould and Holmes 2003). The isolation of avian H7N3 viruses from chicken in Chile that had apparently converted from low-pathogenic avian influenza (LPAI) to highly pathogenic avian influenza (HPAI) forms by recombination is of interest here (Suarez et al. 2003).

As part of a momentum toward improved infectious disease intelligence, of which influenza pandemic preparedness is an intrinsic constituent, there is much to be gained by (1) carrying out extensive influenza-virus surveillance to have a better picture of the influenza viruses in nature with particular reference to domestic animals as the most immediate source of avian influenza viruses for humans and (2) conducting detailed antigenic analyses on the HAs of isolates (in conjunction with receptor-binding studies) for insight into the H subtypes and their variants likely to cross the species barrier to humans and undergo 'prolonged' antigenic drift in humans. It is a moot point whether such studies should lead genotyping and molecular analysis, much the norm nowadays, rather than the other way around. This view does not

override the pressing need for better understanding of the signatures in the genes that facilitate interspecies transmission. Here, there is much scope for fruitful international collaborative and co-operative studies in the spirit of those undertaken in the SARS outbreak (Stöhr 2003b). There is urgency in this, for each year brings us closer to the next influenza pandemic (Shortridge 1995) bearing in mind that, at this point in influenza history, pandemic influenza is a non-eradicable zoonosis (Shortridge 1992; Webster et al. 1992).

The H5N1 incident in Hong Kong involving, as it did, chicken, might be viewed as part of a changing global pattern that had been building up in recent years, particularly with H5 and H7 subtype viruses, following transitions from non-pathogenicity to LPAI and to HPAI viruses. In the last 100 years or so, outbreaks of disease originally described in the 19th century as 'fowl plague' because of their widespread occurrence and severity, but now known as HPAI, were becoming increasingly rare. In the period 1959-1999, there were 18 outbreaks. However, from 1999, there have been seven major outbreaks involving H5 or H7 viruses (excluding those from Mexico and Pakistan) with around 38 million birds affected (Scientific Committee on Animal Health and Animal Welfare 2000; Capua and Alexander 2003). Apparently non-pathogenic H5 viruses had been isolated in Hong Kong from ducks and a goose in a study some 20 years prior to 1997; isolation of influenza viruses from chicken was rare (Shortridge 1992). Could the H5N1/97 virus incident and follow-on H5N1 events in Hong Kong be the East-Asian dimension of an H5/H7 AI continuum? The isolation of H5N1 HPAI viruses from geese in northern Vietnam in 2001 as part of the wider region of East Asia and their genetic relatedness to recent human and avian H5N1 isolates in Hong Kong, are therefore of as much interest as they are of concern (Nguyen et al. 2003). Could transmission of H7N7 HPAI viruses from poultry to humans in The Netherlands in 2003 giving rise to conjunctivitis and a case fatality (Osterhaus et al. 2003) be a signal of a further interspecies transmissions of H7 virus to humans?

The Hong Kong and Dutch incidents caused by H5N1 and H7N7 HPAI viruses, respectively, show a link between high pathogenicity in chicken with ability to cause disease in humans possibility leading to pandemic scenarios. Attractive as this lead is to a virus's potential for pandemicity, it may be misleading in the sense that the H2N2 and H3N2 pandemic viruses of 1957 and 1968, respectively, lacking significant basic amino-acid motifs at the cleavage points of their HAs, are unlikely to have been pathogenic for chicken. The factors giving rise to each pandemic virus may be different, pandemicity probably being a polygenic trait (Webster et al. 1992). These factors are really not known although there is evidence that the PB1 gene may be a facilitator gene in this (Kawaoka, Krauss and Webster 1989; Lin et al. 1994). To the present, the geographic source of viruses appears to be important. Avian influenza viruses may be divided into two lineages: (1) Eurasian including African and Australasian viruses and (2) North-American, although it remains to be see whether there is a distinct South-American clade (Suarez et al. 2004). These two broad groupings presumably reflect migratory bird routings and virus seedings. In keeping with the historical record and modern genetic information, it might be reasonable to expect avian viruses of the wider East-Asian region encompassing as it does southern China to have, or be capable of having, the necessary factors or signatures for pandemicity. Genetic analysis of avian isolates from southern China is a pointer toward this (Lin et al. 1994). However, insufficient viruses have been genotyped to know whether separate European and Asian sublineages exist with the Eurasian lineage and whether one or other or both hold the secrets of pandemicity. Exploration

of this would be a good move within the wider ambit of infectious-disease intelligence and, optimistically, an additional step toward baseline influenza-pandemic preparedness.

The intensification of the poultry industry worldwide seems to be a key element in causing influenza viruses of aquatic origin to undergo 'more rapid' adaptation to land-based poultry as proffered earlier. This has been aptly demonstrated with H9 viruses from southern China whose HAs retain amino-acid signatures compatible with a receptor-binding preference for human tissue (Perez et al. 2003; Li et al. 2003). The spread of H9N2 viruses to almost panzootic proportions (Alexander 2001) is not only a threat to the poultry industry worldwide but a threat to human health. The ability of H9N2 viruses to infect and cause respiratory illness in humans in Hong Kong and China (Peiris et al. 1999; Guo et al. 1999) against the background that H9N2 viruses isolated from poultry in Hong Kong have a cell receptor specificity similar to that of human H3N2 viruses (Matrosovich, Krauss and Webster 2001) underlines the importance of this virus subtype as a potential pandemic virus (Shortridge et al. 2001). The co-circulation of H9N2 viruses and human H3N2 variants in pigs, i.e. the porcine mixing vessel, in southern China takes this potential a step forward (Peiris et al. 2002).

## China

What is special about China as a source of epidemics and pandemics in times past (Potter 1998), the events of the last century including H5N1 and H9N2 focusing attention on southern China? Quite simply, the region has a permanent gene pool of avian influenza viruses year-round (Shortridge 1992). This is as a consequence of the domestication of the duck as a source of these viruses around 4500 years ago in the fertile southeastern region of the country and subsequent intensification and spread of duck raising as an adjunct to rice farming around the start of the Ching Dynasty in 1644 A.D. (Needham 1986). The high human population density in the countryside and preference of the population generally for its food to be as fresh as possible have provided on-going opportunities for human (and porcine) exposure to avian influenza viruses (Shortridge and Stuart-Harris 1982; Shortridge 1992; 2003b). The influenza gene pool in southern China is already established and, more than likely, is not dependent upon introductions from migratory birds. Obviously, introductions could re-vitalize it from time to time and place to place, but movements within southern China or the wider region would help to maintain the pool indigenously. Interestingly, virus isolations from migratory birds at a wetland migratory-bird resting point at the edge of Hong Kong over a number of years yielded few isolates (unpublished data) whereas birds in a roughly parallel route through Taiwan to the East yielded a diverse range of virus subtypes (Cheng et al. 2003). By contrast, an avian influenza gene pool similar to the one in southern China does not exist anywhere else in the world. Virus introductions into poultry are usually the result of the presence of migratory waterfowl in the vicinity and subsequent infection of chicken and turkey, especially if the turkey are raised in the open (Alexander 2001). It is worth noting here that, while the avian-influenza milieu in southern China is complex, it could become even more complex should turkey be raised there commercially.

What is not known about the avian-influenza milieu of southern China is whether there have been outbreaks of AI in chicken and other gallinaceous birds given that up to the last 20 or so years, poultry were scattered widely across the region in villages and small holdings. More recently, larger operations have come on-stream for an increasing population particularly in the cities and for export. Due to the limitations of

virus surveillance, it is conjectural whether the H5N1/97 virus in Hong Kong would have existed as an LPAI virus before converting to an HPAI virus in the manner of AI outbreaks in chicken in Europe and elsewhere (Osterhaus et al. 2003; Campitelli et al. 2003). As a triple reassortant involving precursor H5N1, H9N2 and H6N1 viruses and goose and quail hosts (Guan et al. 2002) the possibility that the H5N1/97 virus was an HPAI virus at the outset cannot be excluded. The recognition that the precursor H5N1 virus was associated with the death of geese on a farm in Guangdong Province prior to the 1997 incident (Tang et al. 1998; Zu et al. 1999) and in late 2002 H5N1-like viruses were isolated from dead, resident waterfowl and migratory birds in two Hong Kong parks (Sturm-Ramirez et al. 2003) suggests that a range of genotypes and antigenic variants of H5N1 viruses may be lurking in the region with 'smouldering virulence'. A similar, but more insidious situation may be brewing with certain H9N2 viruses following adaptation from aquatic to land-based poultry and back again as double or triple reassortants (Perez et al. 2003; Li et al. 2003). The fact that these viruses are able to retain a receptor-binding specificity for human tissue while exhibiting limited or no pathogenicity for poultry means that their hypothetical 'smouldering virulence' could go unnoticed as could an incipient pandemic situation. Thus, while there is an obvious need to clarify the definitions of LPAI and HPAI for H5 and H7 virus subtypes as well as taking on board pathogenicity changes in other subtypes (Scientific Committee on Animal Health and Animal Welfare 2000), the situation with the influenza viruses in southern China seems to be far more complex in some cases for clear-cut definitions.

## Comment

This meeting was a timely recognition of the need to thread a path of understanding through the complex maze of influenza epidemiology and ecology and its impact on the poultry industry. This has a value-added effect on human health in preparedness for influenza pandemics. Appreciation of the animal dimension of human influenza came of age with the H5N1 incident in Hong Kong in 1997. In concordance with these developments, the World Health Organization (WHO) set about establishing an Animal Influenza Network (AIN) through an inaugural meeting in Hong Kong in 2000 taking as its model the highly successful WHO Influenza Surveillance Network with a view to expanding animal-influenza surveillance in a more co-ordinated fashion and integrating it with human-influenza surveillance. Animal influenza formally took its place in the wide ranging "Global Agenda on Influenza Surveillance and Control" at a "WHO Consultation on Global Priorities in Influenza Surveillance and Control" in Geneva, May 2002 (Stöhr 2003a). An important component of the animal agenda is the need to establish close links between the Office International des Epizooties (OIE), the world organization that oversees animal health, and WHO networks. In doing so, it is highlighting the desirability, if not the moral responsibility, of nations to make available to the best of their circumstances information on influenza and its viruses for the common good of man and animal.

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