1

Avian influenza viruses and influenza in humans

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Abstract

Influenza-A viruses cause natural infections of humans, some other mammals and birds. Few of the 15 haemagglutinin and 9 neuraminidase subtype combinations have been isolated from mammals, but all subtypes have been isolated from birds. There are enormous pools of influenza-A viruses in wild birds, especially migratory waterfowl.

In the 20th century there were 4 pandemics of influenza due to the emergence of antigenically different strains in humans: 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1). The RNA of influenza-A viruses is segmented into 8 distinct genes and as a result genetic reassortment can occur in mixed infections with different viruses. The 1957 and 1968 pandemic viruses differed from the preceding viruses in humans by the substitution of some genes that came from avian viruses, indicating that pandemic viruses may arise by genetic reassortment of viruses of human and avian origin.

In poultry some influenza-A viruses cause highly pathogenic avian influenza [HPAI], with 100% mortality in infected flocks. The virulence of HPAI viruses is related to the presence of multiple basic amino acids at the precursor HA0 cleavage site, which enables it to be cleaved, and the virus rendered infectious, by a ubiquitous protease (e.g. furin), causing a systemic infection, instead of being restricted to cleavage by trypsin-like proteases. Humans also have furin, but none of the pandemic viruses have had HA0 cleavage sites with multiple basic amino acids.

Up to 1995 there had been only three reports of avian influenza viruses infecting humans, in 1959, 1977 and 1981. All three viruses were H7N7 and two of these infections were the result of laboratory accidents. However, since 1996 there have been regular reports of natural infections of humans with avian influenza viruses. Isolations of avian influenza viruses from humans in England in 1996 [H7N7], Hong Kong in 1997 [H5N1] and 1999 [H9N2] caused concern that a new influenza pandemic could begin. The H5N1 virus was especially alarming, as it possessed multiple basic amino acids at the HA0 cleavage site and 6/18 of the people infected died. In 2003 further human infections with H5N1 virus were reported in Hong Kong with two associated deaths and in The Netherlands a total of 82 people were confirmed as infected with the H7N7 virus responsible for a series of HPAI outbreaks in poultry, one death was reported. Although these infections seem to have been limiting, with very little human to human transmission, they are a cause for alarm since, if people infected with an 'avian' virus were infected simultaneously with a

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'human' influenza virus, reassortment could occur with the potential emergence of a virus fully capable of spread in the human population, but with an HA for which the human population was immunologically naive.
Keywords: Avian influenza; human influenza; interspecies transmission; pandemics; reassortment; zoonosis

Introduction

Influenza is a highly contagious, acute illness in humans for which there are recognizable accounts of epidemics dating back to ancient times. Influenza viruses have negative-sense RNA genomes and are placed in the *Orthomyxoviridae* family; they are grouped into types A, B and C on the basis of the antigenic nature of the internal nucleocapsid or the matrix protein, these types are now recognized as genera. Influenza-A viruses infect a large variety of animal species including humans, pigs, horses, sea mammals and birds, occasionally producing devastating pandemics in humans. The two surface glycoproteins of the virus, haemagglutinin (HA) and neuraminidase (NA), are the most important antigens for inducing protective immunity in the host and therefore show the greatest variation. For influenza-A viruses 15 antigenically distinct HA [H1-H15] and 9 NA [N1-N9] subtypes are recognized at present; a virus possesses one HA and one NA subtype, apparently in any combination. Although viruses of relatively few subtype combinations have been isolated from mammalian species, all subtypes, in most combinations, have been isolated from birds.

In the 20th century the sudden emergence of antigenically different strains in humans, termed *antigenic shift*, occurred on 4 occasions, 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1), resulting in pandemics (Nguyen-Van-Tam and Hampson 2003). In 1957 and 1968 the new viruses completely replaced the previous virus in the human population, but in 1977 this did not occur and currently H3N2 and H1N1 viruses both circulate.

Frequent epidemics have occurred between the pandemics as a result of accumulated point mutations in the prevalent virus leading to gradual antigenic change, termed *antigenic drift*, which in turn results in infections in a proportion of the population that has become immunologically susceptible. The inter-pandemic influenza epidemics may have a considerable impact on a given population as a result of significant mortality, especially amongst the elderly and other vulnerable groups, and the severe economic cost associated with debilitating illness in a large portion of the population. Occasionally the degree of antigenic drift is sufficient that a very large proportion of the population is susceptible and severe epidemics occur with world-wide spread.

By far the worst influenza pandemic for which there are accurate records was the one beginning in 1918. It has been estimated that during the pandemic more than 40 million people died (Nguyen-Van-Tam and Hampson 2003). In well-developed countries such as the USA about 0.5% of the population died, but in some communities in Alaska and the Pacific islands half the population perished. Since 1918 the central theme in the study of human influenza has been to understand how antigenic shift occurs and to predict when and how it will next occur.

Human/avian influenza link

The RNA of influenza viruses is segmented into 8 distinct genes, which code for 10 proteins. Because the viral RNA is segmented and is packed at random, genetic reassortment can occur in mixed infections with different strains of influenza-A viruses. This means that when two viruses infect the same cell, progeny viruses may inherit sets of RNA segments made up of combinations of segments identical to those of either of the parent viruses. This gives a theoretical possible number of 2^8 (=256) different combinations that can form a complete set of RNA segments from a dual infection, although in practice only a few progeny virions possess the correct gene constellation required for viability. Demonstration that the H3N2 1968 pandemic virus differed from the 1957-1968 H2N2 virus in the substitution of two genes, PB1 and the important surface-glycoprotein HA gene, with genes almost certainly from an influenza virus of avian origin, led to the suggestion that antigenic shift occurred as a result of reassortment of genes in dual infections with viruses of human and avian origin (Fang et al. 1981; Gething et al. 1980; Kawaoka, Krauss and Webster 1989; Scholtissek et al. 1978). As a result systematic surveillance studies into the presence of influenza viruses in avian species were undertaken. These revealed enormous pools of influenza-A viruses in wild birds, especially migratory waterfowl. In a series of surveillance studies involving over 20,000 birds during 1973-1986, virus was isolated from about 10%, with an isolation rate of approximately 15% from ducks and geese and 2% from other birds (Alexander 2000). In addition, unlike mammals, where the number of subtypes that have been established appears to be limited, all 15 H and 9 N subtypes recognized currently have been recorded in birds in most possible combinations.

This wealth of influenza viruses in the bird population brought into question the reassortment theory for the origins of pandemic virus, as transfer and reassortment would seem likely to occur much more frequently than subtype changes have appeared in the human population. However, volunteer experiments had shown that only transitory infections resulted when humans were infected with viruses of avian origin (Beare and Webster 1991) and at that time few natural infections of humans with avian viruses had been reported (see below). It was clear that there was some barrier to the establishment of avian influenza viruses in the human population that was related to one or more of the gene segments. Both human and avian viruses are known to infect pigs readily and it was suggested that pigs acted as ‘mixing vessels’ in which reassortment between human and avian influenza viruses could take place with the emergence of viruses with the necessary gene(s) from the virus of human origin to allow replication and spread in the human population, but with a different haemagglutinin surface glycoprotein, so that the human population could be regarded as immunologically naive. This theory was also thought to account for the apparent emergence of pandemics in the 20th century in the Far East where agricultural practices mean that high concentrations of people, pigs and waterfowl live closely together (Shortridge and Stuart-Harris 1982).

The emergence of pandemic virus may be even more complicated and two hypotheses have been proposed for the rhythm of occurrence of human influenza A viruses, which were termed by Shortridge (1992) as an influenza circle or cycle and an influenza spiral, respectively. The circulation theory suggests there is simply a recycling of H1, H2 and H3 subtypes. If this is so, the HA subtype of the next pandemic virus would be H2. The spiral theory presupposes that humans are capable of being infected with all HA subtypes of influenza-A viruses providing a specific

constellation of the other genes is present and that it is a lottery as to which of the 15 recognized will emerge next by reassortment. The theories are not mutually exclusive and since the circulation theory does not state how the next pandemic virus will arise, it could be by reassortment.

Avian influenza pathogenicity

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 a disease formerly known as fowl plague and now termed highly pathogenic avian influenza [HPAI] in which mortality may be 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. Sometimes other infections or environmental conditions may cause exacerbation of influenza infections leading to much more serious disease.

The main functional glycoprotein, the haemagglutinin, for influenza viruses is produced in a precursor form, HA0, which requires post-translational cleavage by host proteases before the protein is functional and the virus particles are infectious. It has been demonstrated that the HA0 precursor proteins of avian influenza 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 (Stieneke-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.

Comparisons of the amino-acid sequences at the HA0 cleavage site of avian influenza viruses of high and low pathogenicity revealed that while viruses of low virulence have a single basic amino acid (arginine) at the site, 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 (Senne et al. 1996; Vey et al. 1992; Wood et al. 1993). The additional basic amino acids result in a motif recognized and cleavable by the putative ubiquitous protease(s). Mammals, including humans, also have furin-like proteases capable of cleaving at multiple basic amino-acid motifs.

Human infections with avian influenza viruses (Table 1)

There were three reports of human infections with avian influenza virus in the literature prior to 1996. In 1959 an HPAI virus of was obtained from a patient with hepatitis (Campbell, Webster and Breese Jr. 1970). The second related to a laboratory worker in Australia who developed conjunctivitis after accidental exposure directly in the eye with a HPAI virus (Taylor and Turner 1977). The third also related to conjunctivitis as the result of infection with an avian LPAI virus, which spread to an animal handler from an infected seal (Webster et al. 1981). Interestingly all three of these viruses were of H7N7 subtype.

In 1996 an 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 8

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 (Banks, Speidel and Alexander 1998).

Table 1. Reports of human infections with avian influenza viruses

Year	Subtype	HPAI/LPAI[1]	Number of people infected	Symptoms
1959	H7N7	HPAI	1	hepatitis?
1977	H7N7	HPAI	1	conjunctivitis
1981	H7N7	LPAI	1	conjunctivitis
1996	H7N7	LPAI	1	conjunctivitis
1997	H5N1	HPAI	18	influenza-like illness, 6 deaths
1999/98	H9N2	LPAI	2 (+5?)	influenza-like illness
2003	H5N1	?	2 (+1?)	influenza-like illness, 1 (+1?) death(s)
2003	H7N7	HPAI	82	conjunctivitis, some cases of influenza-like illness, 1 death

[1]HPAI or LPAI in chickens. See text for source.

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. 2000). There was evidence of very limited human-to-human spread of this virus (Buxton Bridges et al. 2000), but clearly the efficiency of transmission must have been extremely low. 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 (Suarez et al. 1998).

In recent years outbreaks in poultry due to viruses of H9 subtype, usually H9N2, have been widespread. During the second half of the 1990s outbreaks, due to H9N2 subtype had been reported in Germany, Italy, Ireland, South Africa, USA, Korea, China, the Middle East, Iran and Pakistan (Banks et al. 2000) and this virus continues to spread. In March 1999 two independent isolations of influenza virus subtype H9N2 were made from girls aged one and 4 who recovered from flu-like illnesses in Hong Kong (Peiris et al. 1999a; 1999b). Subsequently, 5 isolations of H9N2 virus from humans on mainland China in August 1998 were reported.

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 had become ill and died while visiting the Chinese mainland; it is not known if she was infected with H5N1virus. There were reported to be some genetic differences between the 1997 and the 2003 H5N1 viruses (WHO website: http://www.who.int/mediacentre/releases/2003/pr17/en/).

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).

Five of the eight reports of avian-influenza infections in humans have been with H7N7 subtype viruses. The significance of this is not known.

Conclusions

The high mortality, 6/18, amongst the people infected with the H5N1 virus in Hong Kong was worrying in case the virus was capable of systemic infection due to the presence of multiple basic amino acids at the HA0 cleavage site allowing cleavage to be mediated by (a) furin-like protease(s). However, evidence that this was the case is lacking. Generally, the 18 patients presented with severe respiratory symptoms and for those who died – several of whom were vulnerable due to complicating medical conditions present prior to infection – pneumonia appeared to be the main cause as it often is in deaths occurring as a result of infections with influenza viruses 'normally' in the human population. Similarly the single death amongst those infected with the HPAI H7N7 virus in The Netherlands in 2003 was also the result of pneumonia. Infections of other mammals with avian influenza viruses also give few clues to the significance of multiple basic amino acids at the HA0 cleavage site. An infection of harbour seals during 1978-80 off the Northeast coast of the United States of America with H7N7 avian influenza resulted in death of an estimated >20% of the population. While this mortality rate is comparable to that occurring in humans in Hong Kong, the HA0 cleavage site of the H7N7 virus did not have a motif containing multiple basic amino acids (Webster et al. 1992). Conversely, H7N7 viruses responsible for equine influenza type 1, for which A/equine/Prague/56 is the type strain, do have multiple basic amino acids at the HA0 cleavage site and yet in infections of horses with this strain, virus replication is invariably restricted to the respiratory tract (Gibson et al. 1992).

The demonstration of direct natural infections of humans with avian viruses suggests that pandemic viruses could emerge as a result without an intermediate host. However, for the human population as a whole the main danger is probably not directly the viruses that have spread from avian species, but if the people infected with the avian influenza viruses had been infected simultaneously with a 'human' influenza virus, reassortment could have occurred with the potential emergence of a virus fully capable of spread in the human population, but with H5, H7 or H9 HA, resulting in a true influenza pandemic.

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