Influenza: current threat from avian influenza

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Influenza is an infectious respiratory pathogen causing annual outbreaks and infrequent pandemics, resulting in significant morbidity, mortality and burdens on the delivery of health care. The geographical spread of highly pathogenic avian influenza (HPAI) H5N1 among poultry and wild bird populations is unprecedented. Growing numbers of sporadic avian influenza infections are occurring in humans, increasing the threat of the next influenza pandemic. Vaccines are the principle means of combating influenza, and a number of studies with H5N1 vaccine candidates are underway. Antiviral agents can be used to treat influenza infection and can be taken as chemoprophylaxis during influenza outbreaks. Oseltamivir has been stockpiled as part of influenza pandemic preparedness planning; however, the emergence of drug resistance may limit its clinical use.

Keywords: avian influenza, influenza, vaccines, antivirals

Introduction

Virology and structure

Influenza viruses are enveloped segmented RNA viruses belonging to the Orthomyxoviridae family. They are classified by their core proteins into three types: A, B and C [1]. Influenza A viruses infect a range of mammalian and avian species, whereas types B and C are essentially human pathogens. Influenza A causes annual epidemics and occasional pandemics, whereas influenza B causes outbreaks every 2–5 years but is not associated with pandemics. Influenza C is poorly defined and associated with sporadic infection. Influenza strains are classified according to species of origin, geographic site, year of isolation and serial number and, for influenza A, by serological properties of subtypes of haemagglutinin and neuraminidase. The main antigenic determinants of influenza A and B are two surface glycoproteins: haemagglutinin and neuraminidase. The haemagglutinin is involved with virus entry into cells by attaching to sialic acid receptors on the host cell and facilitating virus–cell membrane fusion. As the major antigenic determinant, it is the key ingredient of influenza vaccines. Neuraminidase promotes release of virus progeny from infected cells, prevents viral aggregation and aids movement of
virus through the respiratory tract. As neuraminidase sialidase activity is conserved across virus subtypes, it presents an important target for antiviral therapy. Another virus membrane protein, the M2 ion channel, is a tetrameric membrane channel important for virus replication and is another target for antiviral therapy.

**Avian influenza**

Birds, especially water birds, are the natural reservoir of influenza A viruses, in which a total of 16 haemagglutinin (H1–H16) and nine neuraminidase (N1–N9) subtypes are identified. The principal site of avian influenza virus replication in birds is the gastrointestinal tract, where infection typically causes minimal disease and viruses are transmitted faeco-orally. Both H5 and H7 subtypes have the ability to evolve into highly pathogenic forms and cause systemic infection. Over the last 30 years, sporadic outbreaks of highly pathogenic avian influenza (HPAI) viruses among poultry have occurred throughout the world causing significant economic damage to agriculture. Since 2003, over 100 million birds have been destroyed or died during outbreaks, and economic costs are estimated to run into several billion dollars [2].

**Antigenic drift and shift**

Influenza A H1 and H3 subtypes circulating in humans continuously undergo antigenic variability. Ineffective proofreading by viral RNA polymerase leads to a high rate of transcription errors in surface glycoproteins. Some of these virus variants have substitutions in the antibody-binding sites of the haemagglutinin and are capable of evading pre-existing humoral immunity. They can reinfect individuals and are responsible for interpandemic outbreaks. This is termed ‘antigenic drift’. The segmented RNA genome allows for a second form of antigenic variation called ‘antigenic shift’. The simultaneous infection of a host cell by two different influenza viruses may allow recombination of their viral RNA segments and result in the emergence of a ‘novel’ reassortant virus containing a mixture of genes. Pandemic influenza may arise by this process if a virus with a new haemagglutinin emerges and spreads efficiently through the human population [3]. Table 1 summarizes features of pandemic and epidemic influenza.

**Possible origins of pandemic viruses**

Despite the variety of viruses, only a few haemagglutinin (H1, H2 and H3) and neuraminidase subtypes (N1 and N2) have caused widespread
respiratory disease in humans. The influenza haemagglutinin binds to sialic acid receptors on the host cell. These sialic acid receptors have receptor-binding characteristics defined in part by the presence of certain linkages. Although the molecular mechanisms that define host-species specificity of influenza are unclear, it is generally believed that the haemagglutinin of avian origin must acquire human receptor-binding specificity to generate strains capable of human-to-human transmission. The haemagglutinin of human influenza preferentially binds to sialic acid receptors containing α2,6-galactose linkages, whereas avian influenza viruses preferentially bind to those containing α2,3-galactose linkages. These binding differences correlate with the predominance of sialic acid α2,6-galactose linkages on human respiratory epithelial cells and α2,3-galactose linkages on avian intestinal epithelial cells. As swine trachea contains receptors for both avian and human influenza viruses, pigs can support the replication of avian and human influenza viruses. Thus, it has been proposed that genetic reassortment between avian and human viruses may occur in pigs and that they represent a source for the evolution of new, potentially pandemic influenza strains [3, 4].

During the last century, an H1N1 virus in 1918, an H2N2 virus in 1957 and an H3N2 virus in 1968 caused influenza pandemics [3]. Human–avian reassortant viruses caused the pandemics of 1957 and 1968. The 1957 H2N2 virus differed by three genes (haemagglutinin, neuraminidase and the PB1 polypeptide of the RNA polymerase complex) from the H1N1 virus that infected humans between 1918 and 1957. The 1968 H3N2 virus differed by two genes (haemagglutinin and PB1) from the H2N2 virus that infected humans between 1957 and 1968 [3]. In both the cases, avian influenza viruses contributed at least the haemagglutinin gene to the pandemic strain.

Pandemic viruses could also emerge directly from birds. Chickens support a reservoir of influenza viruses, indicating a possible role as intermediate hosts. Some avian H9 viruses established in poultry are capable of two-way transmission between domestic ducks, where they are able to generate multiple reassortants with other co-circulating viruses [5]. Some of these reassortant viruses have haemagglutinin receptor-binding

### Table 1 Features of pandemic and epidemic influenza

<table>
<thead>
<tr>
<th>Type</th>
<th>Virus</th>
<th>Immunity in population</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pandemic influenza</td>
<td>Antigenic shift: emergence of novel subtype of influenza A</td>
<td>Little or no background immune protection in adults or children</td>
<td>High attack rates, excess mortality and morbidity in all age groups</td>
</tr>
<tr>
<td>Epidemic influenza</td>
<td>Antigenic drift: evolution of existing influenza (A or B) strains</td>
<td>Little immunity in infants</td>
<td>Annual outbreaks or epidemics with variable morbidity and mortality, usually in elderly and young with H3N2 cause of greatest severity</td>
</tr>
</tbody>
</table>

Partial immunity in adults by cross-reacting antibody to previous infection and vaccination
sequences potentially capable of human infection, suggesting that potential pandemic viruses may emerge directly from birds. The 1918 ‘Spanish’ influenza H1N1 pandemic remains the greatest outbreak of any infectious disease in history, accounting for up to 40 million deaths worldwide. Genomic sequencing and recreation of the 1918 virus by reverse genetics have revealed insights into its origins [6, 7]. Unlike the pandemic viruses of 1957 and 1968, the 1918 H1N1 virus does not appear to have originated by reassortment between human and avian influenza viruses. All of its genes are closely related to known avian influenza virus genes, suggesting that an avian virus adapted to allow human-to-human transmission. Sequencing of influenza viruses implicates amino acid changes in the RNA polymerase genes (PB1, PB2 and PA), as well as the haemagglutinin, as important in conferring the ability to infect and transmit between humans.

Whilst the genetic determinants of receptor-binding and host-species specificity are polymorphic in nature, molecular surveillance of avian and human H5N1 strains isolated during the current outbreaks is important to track and identify changes that might herald the onset of transmissibility and emergence of a pandemic virus. The close proximity of people to waterfowl, poultry and swine in Southeast Asia has identified this area to be a potential source of pandemic influenza.

**Surveillance**

Influenza surveillance monitors morbidity and mortality, identifies periods of virus activity and characterizes viral isolates to modify future vaccine composition. The World Health Organization (WHO) influenza network comprises four reference collaborating centres and around 120 national centres where viral isolates are identified and typed. Although influenza activity occurs during most winters in temperate regions of the southern and northern hemispheres, the impact of influenza on health care can be difficult to assess accurately, as it lacks pathognomonic features, causes non-specific complications, such as exacerbations of chronic illness, and co-circulates with other respiratory pathogens. Networks linking virological and epidemiological surveillance based on sentinel primary care practices are well established in some countries. In England and Wales, the Royal College of General Practitioners’ network reports weekly consultation rates for influenza-like illness and provides nasal specimens for analysis.

**Burden of influenza**

In England and Wales, baseline winter weekly general practice consultations are 30–70 consultations per 10^5 population [8]. These figures rise
Influenza: current threat from avian influenza

During epidemic periods, for example, up to 800 per 10^5 population (5% of the population) in 1975–1976. US primary care surveillance suggests that 17–52% of upper respiratory illness attributed to influenza result in medical visits, with over 600 consultations per 10^5 in peak years [9]. Consultation rates for influenza-like illnesses strongly correlate with viral isolation and excess mortality even during years without major epidemics. The spread and intensity of annual epidemic influenza within communities vary. As pre-existing immunity to influenza A (H3N2) is often limited because of frequent antigenic drift, H3N2 tends to cause more severe illness, medical consultations in primary care, hospital admissions and increased mortality than H1N1 or influenza B [8]. As about 30% of elderly people suffer from at least one acute respiratory illness per winter, even low rates of morbidity and mortality have significant impact in this group, where impaired immune responses and underlying chronic conditions contribute to increased mortality and morbidity. Although the minority of influenza-related hospitalizations occur in those >65 years, about 75% of all influenza deaths and 90% of excess winter deaths occur in this age group [9, 10]. An estimated, excess 28 000 deaths were attributed to influenza deaths during the 1989–1990 epidemic [11]. Certified influenza deaths during the 1989–1990 epidemic in England and Wales show that 50% deaths and 15% hospital admissions attributed to influenza and pneumonia lived in residential homes, illustrating the vulnerability of this population. Surges in hospital admissions occur throughout influenza epidemics, placing healthcare providers under pressure. In England and Wales, 14% of the total variation in hospital admissions over 1987–1995 was attributed to influenza activity [12], and an average additional 422 000 general practitioner consultations and 9077 respiratory hospitalizations in those >65 years occurred during influenza epidemic periods between 1989 and 1998 [10]. Among children, influenza-associated hospitalization rates are highest among those aged <2 years and are similar to rates in elderly populations. For those under the age of 6 years, rates of influenza-associated hospitalizations range between 100 and 150 per 10^5 population in the United States and Europe, placing significant burdens on the delivery of paediatric care [13, 14]. In subtropical regions, inadequate surveillance underestimates the burden of influenza; however, in Hong Kong, the number of excess hospitalizations among children attributed to influenza appears to be 10-fold higher than western studies [15] possibly due to prolonged circulation of virus due to climate and crowding.

Two explosive outbreaks of influenza A (H3N2) in Madagascar and the Congo during 2002 affected over 50 000 people with case fatality rates of 3–3.5% among children and the elderly. Poor nutrition and limited healthcare provision contributed to the high-mortality rate that demonstrates the importance of the 2002 WHO Global Agenda on
Influenza, which plans to develop virological and epidemiological surveillance in resource-poor countries.

### Avian influenza

Generally, avian influenza viruses do not replicate efficiently in human. Although it is unclear whether the recent increase in transmission of human cases of avian influenza is the result of increased awareness (Table 2), the geographic spread of HPAI H5N1 is unprecedented.

**Influenza A/H5N1**

Respiratory illness in humans was first associated with H5N1 influenza in Hong Kong in 1997, when six deaths among 18 cases occurred during intense outbreaks of HPAI H5N1 among live-bird markets [16]. The high mortality rate (33%) demonstrated H5N1 to be clearly more aggressive than human influenza, with patients exhibiting rapidly progressive pneumonia and multiorgan failure. Serological surveillance revealed little evidence of secondary spread, and no further cases were identified following depopulation of poultry in Hong Kong [17]. Although further outbreaks of H5N1 occurred in farms and markets [18], no further additional human cases were identified until February 2003 when two culture-confirmed cases occurred in a family group returning to Hong Kong from China [19]. A younger family member also died of a respiratory illness, but the aetiology was undetermined.

**Table 2** Confirmed cases of avian-to-human transmission of influenza A subtypes [27, 53]

<table>
<thead>
<tr>
<th>Year</th>
<th>Place</th>
<th>Virus subtype</th>
<th>Infections (deaths)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>UK</td>
<td>H7N7</td>
<td>1</td>
<td>Conjunctivitis</td>
</tr>
<tr>
<td>1997</td>
<td>Hong Kong</td>
<td>H5N1</td>
<td>18 (6)</td>
<td>Respiratory</td>
</tr>
<tr>
<td>1999</td>
<td>Hong Kong</td>
<td>H9N2</td>
<td>2</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2003</td>
<td>Hong Kong</td>
<td>H5N1</td>
<td>2 (1)</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2003</td>
<td>The Netherlands</td>
<td>H7N7</td>
<td>83</td>
<td>Conjunctivitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Respiratory (1)</td>
</tr>
<tr>
<td>2003</td>
<td>Hong Kong</td>
<td>H9N2</td>
<td>1</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2004</td>
<td>Canada</td>
<td>H7N3</td>
<td>2</td>
<td>Conjunctivitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Respiratory (1)</td>
</tr>
<tr>
<td>2004–2006</td>
<td>Vietnam</td>
<td>H5N1</td>
<td>93 (42)</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2004–2006</td>
<td>Thailand</td>
<td>H5N1</td>
<td>22 (14)</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2005–2006</td>
<td>Cambodia</td>
<td>H5N1</td>
<td>4 (4)</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2005–2006</td>
<td>Indonesia</td>
<td>H5N1</td>
<td>29 (22)</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2005–2006</td>
<td>China</td>
<td>H5N1</td>
<td>15 (10)</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2006</td>
<td>Turkey</td>
<td>H5N1</td>
<td>12 (4)</td>
<td>Respiratory</td>
</tr>
<tr>
<td>2006</td>
<td>Iraq</td>
<td>H5N1</td>
<td>2 (2)</td>
<td>Respiratory</td>
</tr>
</tbody>
</table>
Influenza: current threat from avian influenza

In late 2003 and continuing into 2006, HPAI H5N1 has caused extensive and unprecedented multiple outbreaks among poultry across Eurasia [20–23]. It appears that H5N1 has become established as an endemic virus in poultry and ducks [20]. Between April and August 2005, poultry outbreaks of H5N1 in western China, Mongolia, Kazakhstan and Russia appeared to be associated with deaths of wild waterbirds in local nature reserves and lakes [24]. Molecular analysis of H5N1 isolates from Russian outbreaks revealed antigenic similarity to viruses isolated in China, leading to concerns that migratory birds are capable of spreading avian influenza [23]. Further evidence for this route of spread came in October 2005 when large outbreaks of H5N1 affected poultry in eastern Turkey [25]. Despite attempts to control the outbreaks by culling of domestic and agricultural flocks, outbreaks in multiple provinces were reported, and the first fatal human cases outside southeastern Asia (four deaths of 12 cases) were detected among Turkish poultry farmers in early 2006 [26]. Since the beginning of February 2006, the rapid geographical spread of H5N1 virus has been reported with 14 new countries detecting cases in wild and domestic birds. These countries, in order of reporting, are Iraq, Nigeria, Azerbaijan, Bulgaria, Greece, Italy, Slovenia, Iran, Austria, Germany, Egypt, India, France and Israel [27]. Of particular note, the disease has now been isolated in several European Union countries and continued its spread into Africa [28].

Human H5N1 infections have been confirmed by WHO in Thailand, Vietnam, Indonesia, Cambodia, China, Turkey and Iraq [27]. A total of 177 cases have been reported to the WHO with a mortality rate of greater than 50%. Additional suspected human cases are under investigation in countries affected by agricultural outbreaks. The isolation of H5N1 from throat, cerebrospinal fluid (CSF) and stool specimens in two Vietnamese children presenting with severe diarrhoea and coma suggest that the spectrum of disease may be wider than previously thought, potentially underestimating numbers of human cases [29]. Most human H5N1 cases are associated with direct exposure to infected poultry, and to date, there is little evidence of human-to-human transmission [17, 30]. The acquisition of characteristics enabling the H5N1 virus to sustain transmission between people would have devastating global consequences.

Since its emergence in humans in 1997, influenza H5N1 has undergone changes in antigenicity and virulence [19, 20, 22, 23, 31]. Sequencing and antigenic characterization of the haemagglutinin gene of the 2005 human H5N1 viruses reveal significant antigenic differences between those isolated from humans in 1997 and 2003 [19, 22]. Furthermore, genetically and antigenically distinct sublineages of H5N1 virus have become established in poultry in different regions of southeast Asia [20]. Therefore, vaccines prepared from the earlier or currently
circulating strains may be suboptimal in protecting against a future H5N1 pandemic.

During late 2002 and early 2003, significant numbers of waterfowl in Hong Kong died from H5N1 infection [32]. This was a notable event, as avian influenza virus infection does not typically cause disease in their natural hosts, suggesting that changes in phenotypic virulence had occurred. Subsequently, H5N1 has extended its avian range and caused lethal infections in a wide variety of birds previously unknown to be susceptible including herons, egrets and birds of prey. Mammalian mouse and ferret models have demonstrated that H5N1 viruses isolated from humans in 2003 and 2004 exhibit increased virulence in challenge experiments compared with earlier H5N1 strains [31], suggesting molecular changes in the virus are occurring. In addition to infecting birds and humans, H5N1 has extended its host range among mammals, with reports of fatal infections in cats and stone martens, probably acquired by feeding on infected dead birds [33, 34]. Outbreaks of severe respiratory illness in tigers and leopards at Bangkok zoo, which were fed on infected poultry carcasses, were identified as H5N1 [35].

**Influenza A/H9N2**

In Hong Kong, in 1999 and again in 2003, avian influenza H9N2 viruses were isolated from children suffering mild, self-limiting respiratory illnesses [36, 37]. No secondary cases were identified. The 1999 human H9N2 viruses contained internal genes homologous to those of the 1997 human H5N1 viruses, suggesting that genetic reassortment between virus subtypes is occurring. Avian H9N2 viruses are now widespread in poultry and have also been recovered from pigs in Hong Kong and China [38, 39]. Some avian H9 viruses have acquired receptor-binding characteristics typical of human strains, increasing the potential for reassortment in both human and pig respiratory tracts [40].

**Influenza A/H7N7 and H7N3**

In 2003, extensive outbreaks of HPAI H7N7 among poultry occurred in the Netherlands causing destruction of millions of birds. There were 83 confirmed cases of viral conjunctivitis, including five with associated influenza-like illness and two cases of isolated respiratory infection among poultry workers involved in control of the outbreak [41]. Human-to-human transmission was confirmed in three households. A veterinarian developed fatal respiratory infection highlighting a significant public health threat. Destruction of infected birds, strict quarantining and antiviral prophylaxis to workers terminated the outbreak and
prevented further transmission. In 2004, cases of H7N3 viral conjunctivitis occurred in workers involved with control of HPAI H7N3 virus outbreaks among poultry in British Columbia despite personal protection [42]. Serological evidence of infection was observed in 7 of 158 (4.4%) serum samples obtained from Italian poultry workers involved in control of low-pathogenic avian influenza H7N3 outbreaks during 2003, suggesting that additional infections with H7 have occurred [43].

Pathogenesis of avian influenza

Understanding the basis of virulence is important for vaccine design, so that viruses can be safely attenuated. Both influenza H5 and H7 subtypes have the ability to evolve into highly pathogenic forms. The precise molecular determinants that confer virulence of avian and human viruses and the circumstances under which virulent phenotypes emerge remain unclear. However, the ease with which the haemagglutinin is cleaved and activated is a major contributing factor. The haemagglutinin of low-pathogenic avian viruses is cleaved by proteases essentially restricted to the avian intestinal tract resulting in limited pathology. In contrast, acquisition of multiple basic amino acids at the cleavage site in HPAI H5 or H7 haemagglutinin enables cleavage by widespread tissue proteases, resulting in systemic spread including respiratory and central nervous system infection. In humans, infection with H5N1 virus results in a severe and an unusually aggressive illness with high mortality and complication rate when compared with infection with epidemic human influenza. Infection of human macrophages in vitro with HPAI H5N1 viruses induces high levels of cytokines compared with some human influenza strains [44]. Additionally, H5N1 viruses appear relatively resistant to the inhibitory effects of host antiviral cytokines, such as interferons [45]. Thus, the severity of human H5N1 infection may be related to the induction of excessive proinflammatory responses that can accompany a primary infection and viraemia.

Vaccines against influenza

Inactivated influenza vaccines afford the principal means of prophylaxis against influenza. Current inactivated vaccines are produced from influenza viruses grown in eggs that possess the two desired surface glycoprotein genes (haemagglutinin and neuraminidase) and the remaining genes from an attenuated human strain (A/PR/8/34) that confers high growth properties in eggs. These egg-grown viruses are inactivated and formulated into one of three types of vaccine. Most are detergent-treated
virus particles (split-product) or purified haemagglutinin and neuraminidase (subunit) vaccines. Both the formulations are well tolerated with excellent safety profiles. Whole-virus vaccine formulations are generally more immunogenic but are associated with increased adverse effects and are little used.

Current annual influenza vaccines are trivalent, containing 15 μg each of two influenza A subtypes (H1N1 and H3N2) and one influenza B strain. They induce a relatively strain-specific antibody response, display reduced efficacy against antigenically drifted viruses and are ineffective against unrelated strains. Antigenic content is updated biannually depending on prevalent circulating subtypes to provide antigenically well-matched vaccines. Protective efficacy of 70–95% in healthy younger adults is obtained when there is a good match between the vaccine and circulating strains [46]. In the elderly, studies focusing on the effectiveness of influenza vaccine in preventing hospitalization for respiratory conditions and deaths have demonstrated benefits from influenza vaccination. As other pathogens can cause these outcomes, estimates of vaccine effectiveness generally underestimate the true protection. Nonetheless, vaccination of elderly people is associated with 19–63% reductions in hospitalization for pneumonia and influenza, 17–39% reductions for all respiratory conditions and 27–75% reductions in all causes of mortality [47]. However, numerous outbreaks of influenza have occurred in well-immunized elderly populations, prompting attempts to enhance vaccine immunogenicity.

Adjuvants and virosomes

Various adjuvants have been used with influenza vaccines in an attempt to enhance and broaden immunogenicity, but this has typically been associated with increased reactogenicity and costs. MF59 is an oil-in-emulsion adjuvant licensed for use in some European countries. The addition of MF59 to influenza vaccine significantly increases post-vaccine serum antibody responses, particularly in older people and those with underlying chronic illnesses, and may provide greater protection against clinical illness when compared with plain subunit vaccines [48].

Virosomes consist of phospholipid bilayers containing haemagglutinin and neuraminidase embedded in the bilayer. Virosomal formulated influenza vaccines are licensed in Europe [49]. Clinical studies have found that they are well tolerated and induce higher seroconversion rates and more durable antibody titres after vaccination than conventional vaccines.
**Live attenuated vaccines**

Although intramuscular inactivated influenza vaccines induce strain-specific serum immunoglobulin G (IgG), they are poor at inducing mucosal responses. By mimicking natural infection, live attenuated virus vaccines may offer broader immune protection against drifted variants by stimulating secretory IgA that displays heterotypic cross-reactivity to influenza virus strains. Live attenuated viruses are widely used in Russia and are now licensed in the United States for those aged between 5 and 45 years. They are generated by genetic reassortment between a wild-type virus expressing target haemagglutinin and neuraminidase and a cold-adapted attenuated strain. Protective efficacy of live attenuated vaccines against influenza infection and complications such as otitis media has been demonstrated in young children even when the circulating strain is antigenically different to the vaccine strain [50]. Studies in nursing home populations have suggested that a combination of live and inactivated vaccines may increase protection [51].

**Vaccines for influenza H5N1**

Following the H5N1 outbreak among humans in Hong Kong in 1997, up to three doses of conventional surface antigen H5N3 vaccine were found to be poorly immunogenic in unprimed humans and unlikely to afford protection against H5N1 infection [52]. The addition of MF59 adjuvant significantly boosted immune responses and induced cross-reactive antibodies to antigenically unrelated H5 strains. This approach relied on the availability of a non-pathogenic but antigenically well-matched virus from which vaccine could be produced. However, no such strain exists for the current H5N1 viruses. The safe production of vaccines from highly virulent strains (such as H5 and H7 subtypes) requires changes in manufacturing procedures [53]. Vaccine preparation using HPAI viruses requires enhanced biocontainment to protect staff from infection and limit risk of environmental contamination, and this is unachievable for large-scale production. Reverse genetics has been used to genetically engineer safe and attenuated H5N1 viruses suitable for vaccine production.

A reverse genetic subunit H5N1 vaccine, based on A/Vietnam/1203/04 (H5N1), was assessed in a randomized trial among healthy adults in the United States [54]. Early analysis indicates that although vaccine was well tolerated, immune responses were disappointing and induced antibodies only at higher doses, with 11, 4, 48 and 67% of 117 recipients seroconverting after two doses containing 7.5, 15, 45 and 90 μg of antigen, respectively. Annual influenza vaccine manufacturing capacity delivers approximately 300 million doses of trivalent vaccine containing
15 µg haemagglutinin per strain. This is equivalent to 900 million doses of monovalent vaccine, enough for 15% of the world’s population. As individuals will be immunologically naïve to a new pandemic strain, it is likely that at least two doses, possibly containing greater antigen content, will be required. If two doses of 90 µg H5N1 vaccine were needed to achieve seroprotection, only about 50 million people could be protected per year.

It is clear that dose-sparing formulations are urgently needed, and a range of clinical trials assessing H5N1, H7N7 and H9N2 vaccine candidates are planned. Inactivated egg-grown subunit and whole-virion vaccine, cell-culture-derived vaccine, virosomal, adjuvanted (aluminium hydroxide, aluminium phosphate and MF59) and live attenuated virus formulations are planned for clinical evaluation in 2006/2007.

**Antiviral therapy for influenza**

Two classes of antiviral agents are available: the M₂ channel inhibitors, amantadine and rimantadine, and the neuraminidase inhibitors, zanamivir and oseltamivir.

**Amantadine and rimantadine**

If commenced within 24 h of onset of illness, amantadine and rimantadine are effective for the treatment of acute influenza A, reducing fever and symptoms by 1–2 days and allowing earlier return to work when compared with placebo [55]. Chemoprophylaxis with amantadine or rimantadine, or for post-exposure prophylaxis of household contacts, can reduce infection rates by 50–90% during influenza outbreaks. However, their use is limited by inactivity against influenza B, significant adverse effect profile such as insomnia, dizziness, falls and lowered seizure threshold, particularly in the elderly and, most importantly, the rapid emergence of drug resistance following treatment of acute influenza A. The genetic basis for resistance is single amino acid substitutions in the transmembrane portion of M₂. Widespread use of amantadine in some parts of the world has contributed to increasing resistance rates of >60 and >90% among currently circulating human H3N2 and avian H5N1 viruses, respectively, rendering this class of drug ineffective [22, 56].

**Neuraminidase inhibitors**

The neuraminidase represents an attractive target for antiviral therapy, as it is essential for viral replication and is conserved across subtypes
including influenza B and all non-human and avian influenza A strains. Two agents are available: oseltamivir, an orally active agent, and zanamivir, delivered through an inhaler device thereby limiting its use in some elderly and infant populations [55]. Both the agents are generally well tolerated, although zanamivir has been associated with bronchospasm and oseltamivir is associated with mild gastrointestinal upset.

In pooled analysis of studies, treatment of acute influenza with neuraminidase inhibitors has been demonstrated to reduce the duration of clinical symptoms and shorten the time to return to normal activities by around 1.3 and 1.6 days respectively [55] (Table 3) and to cause significant reductions in antibiotic use and pneumonia. In addition, treatment of confirmed influenza illness among community-living patients with oseltamivir reduces the risk of hospitalization, otitis media, bronchitis, pneumonia and related antibiotic use when compared with placebo. A retrospective cohort study of patients with influenza-like illness from a US health database of 176 000 people suggests that oseltamivir treatment reduced the risk of pneumonia by 32% \( (P < 0.001) \) and of death by 91% \( (P < 0.05) \) [57].

Meta-analysis and review of seasonal prophylaxis of mainly unvaccinated healthy adults with neuraminidase inhibitors suggest 74–81% protection against confirmed influenza when compared with placebo [55]. In households with index cases of influenza, post-exposure prophylaxis with oseltamivir protected around 80% contacts including children from infection [58].

**Resistance to neuraminidase inhibitors**

The therapeutic use of any antiviral therapy is compromised by the emergence of drug-resistant mutants. Influenza viruses with reduced susceptibility to neuraminidase inhibitors have been recovered *in vitro* following multiple passages in the presence of the drug. Haemagglutinin substitutions confer resistance by decreasing sialic acid binding, thus reducing dependency on neuraminidase activity required for viral release. Mutations in the neuraminidase-active site, most frequently the H274Y substitution, alter its sensitivity to the drug. The first report of

<table>
<thead>
<tr>
<th>Table 3 Meta-analyses of zanamivir and oseltamivir for the treatment of confirmed influenza [55]</th>
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<tbody>
<tr>
<td>Reduction (in days) in treatment groups compared with placebo (95% CI)</td>
</tr>
<tr>
<td>Time to alleviation of symptoms</td>
</tr>
<tr>
<td>😹</td>
</tr>
<tr>
<td>Zanamivir</td>
</tr>
<tr>
<td>Healthy persons (12–65 years)</td>
</tr>
<tr>
<td>At-risk persons (&gt;65 years)</td>
</tr>
<tr>
<td>Healthy children</td>
</tr>
<tr>
<td>All individuals</td>
</tr>
</tbody>
</table>
resistance emerging as a result of clinical use was an influenza B virus recovered from a bone marrow transplant recipient during 14 days of treatment with zanamivir. Although low rates of virus isolates exhibiting reduced drug susceptibility were found during oseltamivir clinical trials, a recent Japanese study identified that about one-fifth of the children treated with oseltamivir for acute influenza developed resistance and in many cases continued to shed high titres of oseltamivir-resistant virus after treatment [59].

Use of oseltamivir for avian H5N1 influenza

The increasing numbers of fatal H5N1 infections among humans have led to national and international stockpiling of oseltamivir as an important part of pandemic preparedness planning. Although the clinical benefits of treating epidemic influenza have been studied, caution must be shown in extrapolating results to severe primary H5N1 infection. Infection with H5N1 is associated with severe systemic disease including gastrointestinal symptoms. The bioavailability and tissue penetration of drug, administered orally or by inhalation, in H5N1-infected humans are unknown. As such, data on the efficacy of treatment of H5N1 infection are scarce. In mice, oseltamivir has a dose-dependent antiviral effect against H5N1 infection. However, 2005 antigenic variants of H5N1 isolates exhibit increased pathogenicity in mice and require also increased doses and duration regimens to protect experimentally infected mice compared with 1997 H5N1 strains [60]. In human cases of H5N1 infection, the efficacy of oseltamivir is likely to be suboptimal whether used late in the course of the illness or whether patients present with overwhelming disease as may be common in resource-poor settings. In a series of eight patients with H5N1 infection in Vietnam, oseltamivir at normal doses completely suppressed viral replication in six patients [61]. However, in two fatal cases, including one who was treated promptly with oseltamivir, continued viral replication led to the emergence and recovery of H294Y oseltamivir-resistant mutant viruses. This has led to suggestions that increased doses and prolonged duration of oseltamivir treatment regimens should be considered and evaluated.

Pandemic control

Strategies to control H5N1 in domestic poultry have been implemented in some areas including Hong Kong, Thailand, Vietnam and other affected countries. These include mass slaughter of birds during outbreaks, removal of ducks, geese and quail from live-bird markets (species thought
to introduce avian influenza viruses into poultry) and improvements in sanitation and have had limited effect. The use of inactivated H5N1 vaccine in chickens has been advocated in some countries, but this strategy must be used with caution. Standardization of agricultural vaccines is not rigorous and may lead to the inadvertent use of subpotent vaccines that, whilst protecting against clinical H5N1 illness, do not reduce virus shedding following infection. The persistence of undetected H5 infection in a partially immune flock may accelerate virus evolution and increase threats to human health.

The development of national pandemic plans has strengthened surveillance, strengthened diagnostic support and outlined public health interventions [62]. Non-medical interventions will be the principal initial control measures. These may include case isolation, contact tracing, travel restrictions (domestic or international) and curtailment of mass gatherings, for example, school closures. Following declaration of a pandemic, sufficient production of vaccine from the responsible strain to cover the general population may take 6–8 months, leaving antiviral agents as an alternative response. As H5N1 strains are already resistant to adamantanes, the role of neuraminidase inhibitors has been explored [63]. Although oseltamivir may be of benefit when given prophylactically to an exposed population, unrealistic quantities would have to be stockpiled for use in this way. Many national authorities, including the United Kingdom, are stockpiling oseltamivir to treat individuals infected during the first pandemic wave. However, reports of resistance to oseltamivir are concerning, and different regimens may be needed for the treatment of H5N1 infection. The WHO is creating a mobile stockpile that could be flown to and targeted on the epicentre of an emerging pandemic in an attempt to limit spread [62, 63], but this may also require countries able to afford the purchase of a stockpile to share their drugs.

Once manufactured, the logistics of vaccine delivery to priority groups and then to general populations will be challenging. Although many H5 vaccine trials are underway, the optimal formulation and dose remain unknown. It would be impossible for manufacturers to begin large-scale H5 vaccine production for stockpiling—for an event that might never occur—without compromising their ability to produce annual trivalent interpandemic vaccine. In 2004, the abrupt withdrawal of 50% of the United States’ annual influenza vaccine supply due to contamination in a single manufacturing facility demonstrates the fragility of current infrastructure. Although further data from vaccine and treatment trials should be available later this year, it may be entirely possible that the pandemic erupts before we have sufficient information to optimize our pandemic strategy.
References


