

Prospects of avian influenza virus passing from birds to humans, and the possibility of a subsequent pandemic, has gripped both the scientific community and the wider population over recent months. Clearly, we cannot be complacent when dealing with this adaptable pathogen, as Ron Cutler explains.

Avian influenza

Is it strictly for the birds?

The name 'influenza' originated in 15th-century Italy, where an epidemic was attributed to the 'influence of the stars', although the disease predates this time. The first pandemic fitting the clinical description of influenza was in 1580. At least four pandemics of influenza occurred in the 19th century and three in the 20th century.^{1,2}

Today, humanity again worries about the potential of a new human flu pandemic. In 1918–19, more people died of influenza in a single year than in the four year reign (1347 to 1351) of the Black Death (bubonic plague). The Spanish flu or 'La Grippe' was a global disaster.³ In the past 300 years, 10 pandemics have been identified, but Spanish flu was the most devastating with an estimated 20–40 million people killed.

Our most recent viral candidate, avian influenza A (H5N1) may have such deadly potential.^{4,5} A new influenza pandemic has been predicted for some years, as influenza viruses are uniquely capable of causing recurrent epidemics and global pandemics. Each year, the global death toll from influenza-related disease is over a million. So, why is this virus so deadly?

Influenza viruses have two features that enable them to be successful pathogens: an ability to circulate in animals and spread to man; and to undergo rapid and unpredictable antigenic change. Studies on influenza A viruses have revealed species-specific lineages for viral genes and genes that have crossed species barriers. Aquatic birds have been shown to be the source of all influenza A viruses, and avian influenza viruses have been transmitted to a wide range of other species including pigs, horses, cats and sea mammals (Fig 1).

Classification and structure of the Orthomyxoviridae

Negative-strand RNA viruses are classified into seven families. Influenza belongs to the

family Orthomyxoviridae (Greek: *orthos*, straight or correct; *myxa*, mucus), which has four genera: influenza virus A, B, and C, and Thogotovirus. Genera are classed on antigenic differences in their nucleoprotein (NP) and matrix (M1) proteins. All avian influenza viruses are classified as group A.

Subtyping is based primarily on the antigenicity of the two main surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). Influenza C viruses lack NA but contain instead a haemagglutinin-esterase fusion protein (HEF).^{6,7} Orthomyxoviruses contain a segmented, linear negative-sense (complementary to messenger RNA [mRNA]), single-stranded RNA. There are eight RNA segments in influenza A and B, and seven in influenza C.

Influenza A viruses are classified into subtypes based on their HA and NA molecules. There are 15 recognised HA subtypes (H1–H15) and nine NA subtypes (N1–N9). The full nomenclature for each new isolate should include the type of virus, the host of origin (except for human), the geographical site of isolation, the type strain number (if available) and the year of isolation. The antigenic description of the HA and NA is given in parentheses. For example, a type strain isolated from a turkey in Toronto in 1966 may be called A/Turkey/Ontario/7732/66 (H5N9). The original animal source often relates to the viral type, all HA types however are found in birds, with humans the second most common host (Table 1).^{8,9}

The physical structure of all influenza A viruses is similar. The virions or virus particles are enveloped and can be either spherical or filamentous in form. In clinical isolates that have undergone limited passages

in eggs or tissue culture, there are more filamentous than spherical particles, whereas passaged laboratory strains consist mainly of spherical virions (Fig 2).

Covering each influenza A virion are surface projections of about 500 spikes.¹⁰ The HA spike is rod-shaped and protrudes from the envelope, allowing the virus to attach specifically to host-cell surfaces, and is responsible for the viruses' haemagglutinin activity. The NA spike also protrudes from the envelope and is topped by a mushroom-shaped tetramer. This is a sialidase that promotes the release of the virus particles after budding.¹¹

Clinical manifestations and pathogenesis

Clinical manifestations

In man, symptoms range from mild respiratory disease with rhinitis or pharyngitis to viral or secondary bacterial pneumonia leading to death. During an epidemic, however, rates of asymptomatic infection in patients can be as great as those with symptomatic infection and this will help the spread of the disease.

Early symptoms in adults are a high fever (38–40°C, usually peaking in 24 hours), chills, headache, sore throat and a dry cough. Pyrexia can last for up to five days, while the cough and malaise may persist for weeks. In an examination of 27 H5N1 infections in patients from Vietnam, Thailand and Hong Kong, all patients had fevers >38°C, 23 had a cough and 21 had a sore throat. In all cases, lower respiratory tract manifestations can develop early in the course of the illness, and progression to respiratory failure is

'A new influenza pandemic has been predicted for some years'

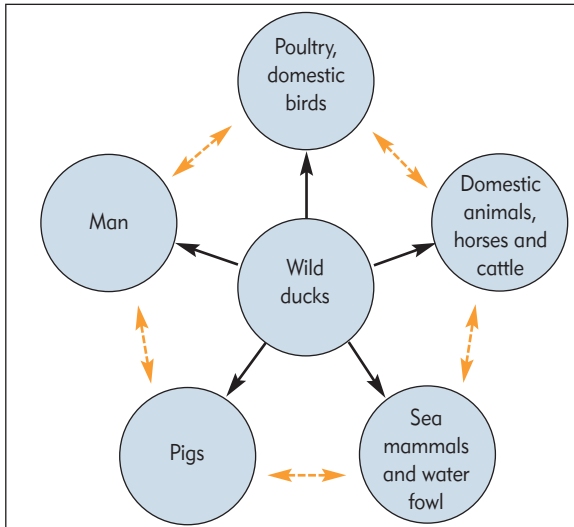


Fig 1. The wide range of species that can be infected by or carry Influenza A. Central to all are aquatic wildfowl.

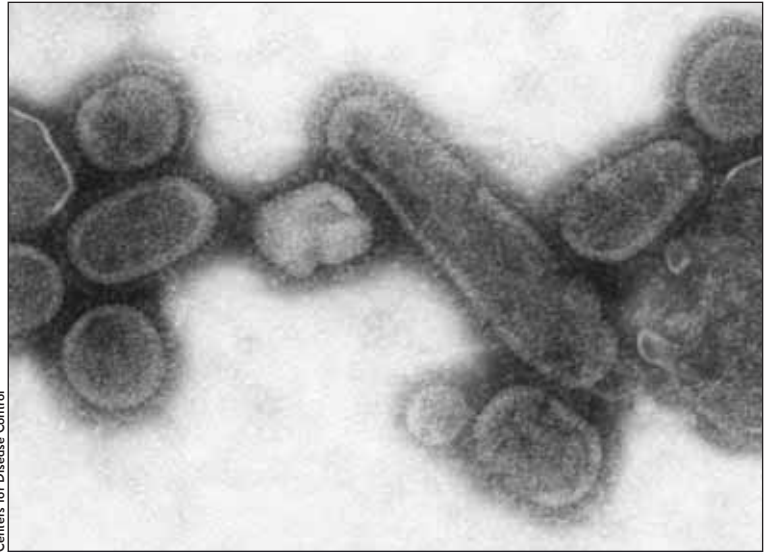


Fig 2. Transmission electron micrograph of influenza A virions.

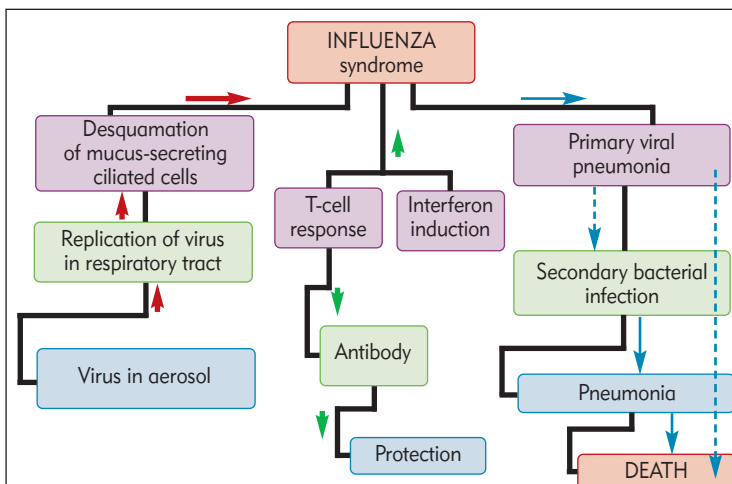


Fig 3. Pathogenesis of the influenza virus.

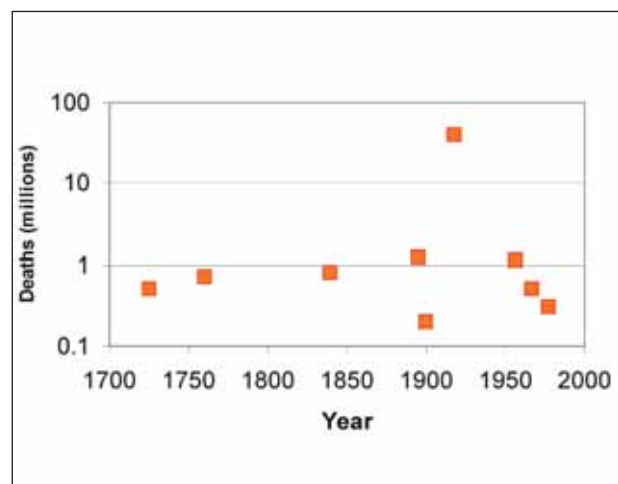


Fig 4. Influenza pandemics from 1700 to 2000 (data approximated to historical records).

associated with acute respiratory distress syndrome (ARDS).¹²

In the past, H5N1 was considered to be non-pathogenic for humans; however, in 1997, 18 humans were infected in Hong Kong and six of them died. The H5N1 virus was linked to this outbreak through a live bird market and area farms. In 2003, in The Netherlands, there were 83 confirmed cases in humans but this time only one death. Another subtype, H7N7, in South-East Asia (Indonesia, Viet Nam, Thailand and Cambodia) in 2004–05 produced 118 cases with 61 deaths.¹²

In birds, especially free flying aquatic birds, there is a worldwide distribution and they act as a reservoir. Clinical symptoms in birds develop in three to 14 days, there is a significant reduction in egg production and there are neurological signs with depression, anorexia and ruffled feathers. The combs may be swollen and cyanotic and there may be incidences of sudden death. Influenza is clinically indistinguishable from virulent

Newcastle disease and further tests are necessary for definitive diagnosis.^{5,6,13,14}

Pathogenesis

Virus particles are inoculated as an aerosol into the respiratory tract where they replicate, causing desquamation of ciliated and mucus-secreting cells. The viral neuraminidase may facilitate access to the cell surface by liquifying the mucus cover. Adherence to the cilia may also be a mechanism whereby influenza virus resists removal by mucociliary action. Virulence factors of avian influenza A (H5N1) include a highly cleavable haemagglutinin that is activated by host cellular proteases and a substitution in NS1 (Table 2) that gives increased resistance to interferon and enhanced replication.

Avian and human HAs have different abilities to bind to other forms of sialic acid.¹⁵ Avian HAs generally bind poorly to the sialic acid receptors in the human respiratory tract. These receptor affinities act as a barrier to

cross-species infection. An avian virus must adapt its HA binding before it can replicate and spread efficiently in humans.

After binding to sialic acid-containing receptors the virus is endocytosed and fuses with the vesicle membrane. Transcription and replication of the genome occurs in the nucleus. Viral proteins are synthesised and helical nucleocapsids form and associate with M1/M2 and the protein-lined membranes containing HA and NA glycoproteins. The virus buds from the plasma membrane and moves on to infect other cells (Fig 3).¹²

In birds, two types of strains have been identified. Low pathogenic avian influenza (LPAI), which includes subtypes H1–H15, and highly pathogenic avian influenza (HPAI), which involves some H5 or H7 subtypes. In infections with HPAI strains, morbidity and mortality can approach 100% in commercial poultry flocks. Death normally occurs two to 12 days after the first signs of illness. The infection is spread by aerosol, shared drinking water, faeces or fomites. Virus is

Table 1. Influenza A subtypes related to species.

SUBTYPE	HUMAN	SWINE	HORSE	BIRDS
H1	+	+		+
H2	+			+
H3	+	+	+	+
H4				+
H5	+			+
H6				+
H7	+		+	+
H8				+
H9	+			+
H10-15				+

Table 2. Functions of each virus segment.

SEGMENT	PROTEIN	FUNCTION
1	PB2	Polymerase
2	PB1	Polymerase
3	PA	Polymerase
4	HA	Haemagglutinin, fusion and attachment protein
5	NP	Nucleocapsid
6	NA	Neuraminidase (promotes virus release)
7	M (M1&2)	M1 Matrix protein M2 Membrane protein
8	NS (NS1&2)	Non-structural proteins

'Influenza pandemics have been identified as occurring in regular cycles'

found in respiratory secretions and in very high concentrations (10^7 – 10^9 particles/g) in bird faeces.^{6,13,14,16–18}

As in man, the pathogenicity of H5 and H7 influenza A viruses for poultry is determined by the amino acids at the HA cleavage site. Cleavability influences tissue specificity and is a major determinant of pathogenicity for H5 and H7 viruses.

Emergence of new viruses

In addition to the virulence factors described above, influenza A viruses continually evolve, changing antigenicity often with increased environmental stability. There are two main ways that mutations occur and these are antigenic drift and antigenic shift.^{12,18–25}

Influenza A is designed for continuous evolution. It has highly variable antigenic domains, situated at the outer end of the spike glycoproteins. This permits variability without affecting the function or the assembly of the virion.¹¹

In antigenic drift, small point mutations occur during the normal virus replication process. This type of mutation does not usually result in major changes in virulence but produces genetic variation among influenza viruses. The lack of proofreading among RNA polymerases contributes to replication errors of the order of 1 in 10^4 bases, which contrasts with the higher replication fidelity of DNA polymerases (ie errors of 1 in 10^9 bases per replication cycle). RNA virus replication therefore results in a mixed population, with many variants.¹⁸ Although most of these are not viable, some will be potentially advantageous and become dominant under the right selective pressures.

In contrast, antigenic shift results in major changes in genetic material. This occurs

Table 3. Mechanisms of antigenic shift and drift.

Antigenic drift	Antigenic shift
Gradually the virus changes enough antigenically so the host population is susceptible to re-infection.	There is a sudden emergence of new antigenically different subtypes, to which the host population is susceptible.
Size and severity of the epidemic depends on how different the virus is from previous outbreaks.	The host population has no immunity against the new subtype and a pandemic follows.
Influenza viruses are continually changing.	Antigenic shift only occurs rarely.
Both type A and type B influenza viruses can undergo changes.	Only type A influenza viruses change.
The WHO Global Influenza Programme monitors antigenic changes and adjustments required to vaccines.	Antigenic shift is of public health concern as subtypes from different species may be able to infect humans.
Gradual changes in an already circulating virus.	Direct transfer of a virus from another species. Re-assortment of avian and human influenza viruses infect the same host. The emerging virus may be an avian virus containing enough human influenza genes to allow human-to-human transmission or may be the re-emergence of a virus that previously caused an epidemic.

when cells are infected with two different strains of influenza virus at the same time. This co-infection may involve viruses from different species (human, avian, swine or equine). Movement of genes from one virus to another is possible, due to the segmented RNA. The presence of eight separate segments is an advantage, as it allows a 'mix and match' process to occur. This antigenic shift then creates a new hybrid virus, which is potentially pathogenic to a different species (Table 3).

History of human and avian influenza

Naturally, due to its importance to man, the history of human influenza can be traced further back in time than can avian influenza. However, identifying the epicentre of

influenza pandemics and links to animal and avian sources of the disease is very important for future planning to combat influenza pandemics.

As the first human influenza virus was not isolated until 1932, evidence prior to that of influenza epidemics and pandemics has been provisionally identified from observational data. Influenza outbreaks occur without warning, with sudden onset of fever, muscle pains and prostration. Hippocrates (412 BC) is often quoted as reporting the first influenza outbreak but it is unsubstantiated. Prior to 1700, the data are difficult to interpret, although the first influenza pandemic agreed by several authors was in 1580. This spread from Asia and Africa and infected the whole of Europe over a six-month period.^{2,8,17}

Table 4. Outbreaks of highly pathogenic avian influenza strains (Data from WHO website).

YEAR	COUNTRY	STRAIN	YEAR	COUNTRY	STRAIN
1959	Scotland	H5N1	1999	Italy	H7N1
1991	England	H5N1			
1997	Hong Kong	H5N1	1963	England	H7N3
2002	Hong Kong	H5N1	1994	Australia	H7N3
			2002	Chile	H7N3
1983	USA	H5N2	2004	Pakistan	H7N3
1994	Mexico	H5N2	2004	Canada	H7N3
1997	Italy	H5N2			
2004	USA	H5N2	1997	Australia	H7N4
1983	Ireland	H5N8	1976	Australia	H7N7
			1979	England	H7N7
1966	Canada	H5N9	1979	England	H7N7
			1985	Australia	H7N7
			2003	Netherlands	H7N7

Since 1700, pandemics have been identified as occurring in regular cycles of around 30–60 years (Fig 4), although the number of deaths in the 1918–19 pandemic were far greater than those previously recorded. There has been some controversy as to the original source of the outbreak. Was it in Spain (hence the name Spanish flu) or in the USA, where an outbreak was documented in an army camp in 1918?

The most recent hypothesis points to an outbreak in 1916 in a huge military camp in Northern France. Here, the mixture of overcrowding, stress, gas attacks and open markets with live pigs, ducks, geese and horses produced conditions in which the transfer of avian influenza A virus could occur. This outbreak showed the same pathology that characterised patients in the 1918 outbreak.^{26,27} The trigger for the 1918 pandemic may have been the return of millions of soldiers to their homelands around the world. This would also fit with the emergence of the first outbreak in the USA, occurring later in an American army camp.²

Several factors, such as age and nutritional status, can affect susceptibility to influenza infections. The effect of external agents on the lungs is also important, and recent evidence has shown exposure to air pollutants, such as diesel exhaust, can effect respiratory virus infections in rodent models. This would also fit with the hypothesis suggested above relating to the emergence of the 1918 pandemic.^{1,2,26-29}

There is historical evidence that can link influenza carriage in animals to occurrence in man. Fowl plague, the disease we now recognise as avian flu, was first described in Italy in 1878. The causative agent was isolated from a chicken in 1902, but was not finally identified as a member of an influenza A group, A/Chicken/Brescia/1902 (H7N7), until 1955.

Two types of avian strains have been identified: highly virulent types (H5 and H7) and avirulent types. Non-pathogenic strains have been isolated from a wide variety of birds, including wild, captive and caged birds and domestic ducks, chickens and turkeys. The highly pathogenic strains (H5 and H7) were both identified originally in chickens.^{6,13,30,31}

The H5N1 subtype currently causing such problems was first identified over 40 years ago and was classified as A/Chicken/Scotland/59 (H5N1). It was identified again in 1963 in England, although without genetic subtyping it is not possible to identify how close these two strains actually were.^{6,13,32}

Influenza A and its subtypes have been found in over 90 species of wild birds, all of which were apparently healthy. Wild ducks are the most frequent carriers of a large variety of influenza viruses. Wild waterfowl are a natural reservoir of influenza A viruses and can carry them over large distances and excrete huge numbers of virions in faeces. Viruses causing highly lethal disease are restricted to H5 and H7 subtypes (Table 4).

Highly pathogenic strains have no natural reservoir but may emerge by mutation when a wild bird introduces the virus into poultry, where it mutates to a lethal version of the original strain. Prior to 2004, outbreaks of highly pathogenic strains were relatively rare, but 10 of the 24 outbreaks in the past 46 years have occurred since 1997, six of which have involved H7 strains and four have involved H5 strains, although the H5N1 outbreak of 2004 was by far the largest outbreak.^{2,6,22,24,25,33-35}

Diagnostics

Rapid diagnostic tests are used increasingly as they yield results in a clinically relevant time frame (approximately 30 minutes); however, the reference standard for diagnosis

of influenza remains virus culture.

Most of the rapid influenza tests are >70% sensitive and >90% specific compared with virus culture. Most tests with positive results correctly identify infection, but up to 30% of negative test results may be false negatives. The predictive values of influenza tests depend on the level of influenza activity in the community; exposure of the patient to a contagious person; susceptibility of the patient; sensitivity and specificity of the tests; and adequacy of specimen collection.

Inappropriate and inadequate specimens will yield false-negative results. Tests are most reliable when there is known influenza activity in the community and when they are performed on patients who have signs and symptoms consistent with influenza (eg fever, cough, sore throat, muscle aches, headache and malaise). However, not all patients manifest typical signs and symptoms. Nasopharyngeal and nasal specimens (swab, aspirate, wash) are preferred over other samples for diagnostic testing, as they contain higher quantities of detectable virus. Specimens should be collected within the first three to four days of illness.

Assays available for the diagnosis of influenza A virus infections include virus culture, rapid antigen detection methods, and polymerase chain reaction (PCR) and real-time PCR assays.³⁶

Virus culture

Virus culture can produce results in two to 10 days. Both shell-vial and standard cell culture methods are used to detect influenza viruses. Positive cultures may or may not exhibit cytopathic effects in cell culture systems. It is necessary to further identify the virus by immunofluorescence of cell cultures or haemagglutination-inhibition (HI) assay of the cell culture medium supernatant.

Rapid antigen detection methods

Rapid antigen detection methods include point-of-care (POC) tests, immunofluorescence assays and enzyme immunoassays, with results obtainable in 15–30 minutes. Point-of-care tests are commercially available.³⁷⁻³⁹

Immunofluorescence assays are a sensitive method for diagnosis of influenza A and B virus infections and five other clinically important respiratory viruses, and are used widely.⁴⁰⁻⁴³ Enzyme immunoassays mainly target influenza A nucleoprotein.³⁶

Polymerase chain reaction and real-time PCR assays

Primer sets specific for the haemagglutinin (HA) gene of currently circulating influenza A/H1, A/H3 and B viruses are used widely. Results are available in a few hours, either from clinical swabs or infected cell cultures.^{13,36,44-46}

Specimens that give a positive result using any the above approaches for influenza A virus and are suspected as avian influenza infection must be tested further and verified

to be complacent when dealing with such an adaptable pathogen. ■

REFERENCES

- 1 Potter CW. A history of influenza. *J Appl Microbiol*, 2001; **91**(4): 572–9.
- 2 Belshe RB. The origins of pandemic influenza – lessons from the 1918 virus. *N Engl J Med* 2005; **353**(21): 2209–11.
- 3 Crawford R. *The Spanish flu, stranger than fiction: vignettes of San Diego history*. San Diego Historical Society, 1995.
- 4 Horimoto T, Kawaoka Y. Pandemic threat posed by avian influenza A viruses. *Clin Microbiol Rev* 2001; **14**(1): 129–49.
- 5 Gordon S. Avian influenza: a wake-up call from birds to humans. *Cleve Clin J Med* 2004; **71**(4): 273–4.
- 6 Alexander DJ. A review of avian influenza in different bird species. *Vet Microbiol* 2000; **74**(1): 3–13.
- 7 Mackie PL. The classification of viruses infecting the respiratory tract. *Paediatr Respir Rev* 2003; **4**(2): 84–90.
- 8 Sharp PM. Origins of human virus diversity. *Cell* 2002; **108**(3): 305–12.
- 9 Hilleman MR. Realities and enigmas of human viral influenza: pathogenesis, epidemiology and control. *Vaccine*, 2002; **20**(25): 3068–87.
- 10 Klenk HD *et al*. Orthomyxoviridae virus taxonomy. Vienna: Springer-Verlag, 1995: 293–9.
- 11 Lamb R, Krug RM. Orthomyxoviridae: the viruses and their replication. In Knipe D, Howley PM eds. *Field's virology* 4th ed. Philadelphia: Lippincott Williams & Wilkins, 2001.
- 12 Beigel JH, Farrar J Han AM *et al*. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 2005; **353**(13): 1374–85.
- 13 Kallio-Kokko H, Uzcategui N, Vapalahti O, Vaheri A. Viral zoonoses in Europe. *FEMS Microbiol Rev* 2005; **29**(5): 1051–77.
- 14 Neurology TL. Avian influenza should be ruffling our feathers. *Lancet Infect Dis* 2004; **4**(10): 595.
- 15 Olofsson S *et al*. Avian influenza and sialic acid receptors: more than meets the eye? *Lancet Infect Dis* 2005; **5**(3): 184–8.
- 16 Monto AS. The threat of an avian influenza pandemic. *N Engl J Med* 2005; **352**(4): 323–5.
- 17 Tracy CS, Upshur REG, Daar AS. Avian influenza and pandemics. *N Engl J Med* 2005; **352**(18): 1928.
- 18 Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. *Microbiol Rev* 1992; **56**(1): 152–79.
- 19 Puthavathana P, Auewarakul P, Charoenying PC *et al*. Molecular characterization of the complete genome of human influenza H5N1 virus isolates from Thailand. *J Gen Virol* 2005; **86**(2): 423–33.
- 20 Tufaro F. *Viral strategies for breaching host defenses: tipping the balance towards survival*. *Curr Opin Microbiol* 2002; **5**(4): 393–4.
- 21 Boni MF, Gog JR, Andreason V, Christiansen FB. Influenza drift and epidemic size: the race between generating and escaping immunity. *Theor Popul Biol* 2004; **65**(2): 179–91.
- 22 Cunha BA. Influenza: historical aspects of epidemics and pandemics. *Infect Dis Clin North Am* 2004; **18**(1): 141–55.
- 23 Treanor J. Influenza vaccine--outmaneuvering antigenic shift and drift. *N Engl J Med* 2004; **350**(3): 218–20.
- 24 Ferguson NM, Galvani AP, Bush RM. Ecological and immunological determinants of influenza evolution. *Nature* 2003; **422**(6930): 428–33.
- 25 Capua I, Alexander DJ. Avian influenza and human health. *Acta Trop* 2002; **83**(1): 1–6.
- 26 Oxford JS, Sefton A, Jackson R, Innes W, Daniels RS, Johnson NP. World War I may have allowed the emergence of "Spanish" influenza. *Lancet Infect Dis* 2002; **2**(2): 111–4.
- 27 Oxford JS, Lambkin R, Sefton A *et al*. A hypothesis: the conjunction of soldiers, gas, pigs, ducks, geese and horses in Northern France during the Great War. *Vaccine* 2005; **23**(7): 940–5.
- 28 Reid AH, Taubenberger JK. The origin of the 1918 pandemic influenza virus: a continuing enigma. *J Gen Virol* 2003; **84**(9): 2285–92.
- 29 Ligon BL. Avian influenza virus H5N1: a review of its history and information regarding its potential to cause the next pandemic. *Semin Pediatr Infect Dis* 2005; **16**(4): 326–35.
- 30 Claas ECJ. Pandemic influenza is a zoonosis, as it requires introduction of avian-like gene segments in the human population. *Vet Microbiol* 2000; **74**(1): 133–9.
- 31 Köhler M, Köhler W. Zentralblatt für bakteriologie – 100 years ago an outbreak of fowl plague in Tyrol in 1901. *Int J Med Microbiol*, 2001; **291**(5): 319–21.
- 32 Hiromoto Y, Yamazaki Y, Kukushima T *et al*. Evolutionary characterization of the six internal genes of H5N1 human influenza A virus. *J Gen Virol* 2000; **81**(5): 1293–303.
- 33 Fenner F. The nature and classification of viruses of man. *Pharmacol Ther* 1979; **4**(1): 35–55.
- 34 Donatelli I, Campitelli L, Di Trani L *et al*. Characterization of H5N2 influenza viruses from Italian poultry. *J Gen Virol* 2001; **82**(3): 623–30.
- 35 Iwatsuki-Horimoto K, Kanazawa R, Sugii S, Kawaoka Y, Horimoto T. The index influenza A virus subtype H5N1 isolated from a human in 1997 differs in its receptor-binding properties from a virulent avian influenza virus. *J Gen Virol* 2004; **85**(4): 1001–5.
- 36 Gavin PJ, Thomson RB. Review of rapid diagnostic tests for influenza. *Clin Appl Immunol Rev* 2003; **4**(3): 151–72.
- 37 Wood JM *et al*. A single radial haemolysis assay for antibody to H5 haemagglutinin. *Int Congr Series* 2001; **1219**: 761–6.
- 38 Wood JM, Nicholson KG, Stephenson I *et al*. Experience with the clinical development of influenza vaccines for potential pandemics. *Med Microbiol Immunol (Berlin)* 2002; **191**(3): 197–201.
- 39 Nicholson KG, Wood JM, Zambon M. Influenza. *Lancet*, 2003; **362**(9397): 1733–45.
- 40 Daisy JA, Lief FS, Friedman HM. Rapid diagnosis of influenza A infection by direct immunofluorescence of nasopharyngeal aspirates in adults. *J Clin Microbiol* 1979; **9**(6): 688–92.
- 41 Stokes CE, Bernstein JM, Kyger SA, Hayden FG. Rapid diagnosis of influenza A and B by 24-h fluorescent focus assays. *J Clin Microbiol* 1988; **26**(7): 1263–6.
- 42 Waner JL, Todd SJ, Shalaby H, Murphy P, Wall LV. Comparison of Directigen FLU-A with viral isolation and direct immunofluorescence for the rapid detection and identification of influenza A virus. *J Clin Microbiol* 1991; **29**(3): 479–82.
- 43 Schmidt NJ, Ota M, Gallo D, Fox VL. Monoclonal antibodies for rapid, strain-specific identification of influenza virus isolates. *J Clin Microbiol* 1982; **16**(4): 763–5.
- 44 Tam JS. Influenza A (H5N1) in Hong Kong: an overview. *Vaccine* 2002; **20**: S77–S81.
- 45 Daum LT, Canas LC, Schadler CA *et al*. A rapid, single-step multiplex reverse transcription-PCR assay for the detection of human H1N1, H3N2, and B influenza. *J Clin Virol* 2002; **25**(3): 345–50.
- 46 Clewley JP. A role for arrays in clinical virology: fact or fiction? *J Clin Virol* 2004; **29**(1): 2–12.
- 47 Tollis M, Trani LD. Recent developments in avian influenza research: epidemiology and immunoprophylaxis. *Vet J* 2002; **164**(3): 202–15.
- 48 Moscona A. Neuraminidase inhibitors for influenza. *N Engl J Med* 2005; **353**(13): 1363–73.
- 49 Peters PJ, Gravenstein S, Norwood P *et al*. Long-term use of oseltamivir for the prophylaxis of influenza in a vaccinated frail older population. *J Am Geriatr Soc* 2001; **49**: 1025–31.
- 50 World Health Organization. *Avian influenza: assessing the pandemic threat*. 2005.

Dr Ronald R Cutler ^{CS} FIBMS is a principal lecturer in infectious diseases and pathology in the School of Health and Bioscience, University of East London.