Recent zoonoses caused by influenza A viruses

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Summary

Influenza is a highly contagious, acute illness which has afflicted humans and animals since ancient times. Influenza viruses are part of the Orthomyxoviridae family and are grouped into types A, B and C according to antigenic characteristics of the core proteins. Influenza A viruses infect a large variety of animal species, including humans, pigs, horses, sea mammals and birds, occasionally producing devastating pandemics in humans, such as in 1918, when over twenty million deaths occurred world-wide. 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, fifteen antigenically distinct HA subtypes and nine NA subtypes are recognised 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, has occurred on four occasions, as follows, in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1), each resulting in a pandemic. Frequent epidemics have occurred between the pandemics as a result of gradual antigenic change in the prevalent virus, termed antigenic drift. Currently, epidemics occur throughout the world in the human population due to infection with influenza A viruses of subtypes H1N1 and H3N2 or with influenza B virus. The impact of these epidemics is most effectively measured by monitoring excess mortality due to pneumonia and influenza. Phylogenetic studies suggest that aquatic birds could be the source of all influenza A viruses in other species. Human pandemic strains are thought to have emerged through one of the following three mechanisms:

- genetic reassortment (occurring as a result of the segmented genome of the virus) of avian and human influenza A viruses infecting the same host
- direct transfer of whole virus from another species
- the re-emergence of a virus which may have caused an epidemic many years earlier.

Since 1996, the viruses H7N7, H5N1 and H9N2 have been transmitted from birds to humans but have apparently failed to spread in the human population. Such incidents are rare, but transmission between humans and other animals has also been demonstrated. This has led to the suggestion that the proposed reassortment of human and avian viruses occurs in an intermediate animal with subsequent transference to the human population. Pigs have been considered the leading contender for the role of intermediary because these animals may serve as hosts for productive infections of both avian and human viruses and, in addition, the evidence strongly suggests that pigs have been involved in interspecies transmission of influenza viruses, particularly the spread of H1N1 viruses to humans. Global surveillance of influenza is maintained by a network of laboratories sponsored by the World Health Organization. The main control measure for influenza in human populations is immunoprophylaxis, aimed at the epidemics occurring between pandemics.

Keywords

Introduction and history

Influenza in humans is a highly contagious, acute, primarily respiratory illness. The symptoms and epidemiological characteristics are sufficiently distinct that accounts of epidemics can be dated back to ancient times. These accounts show clearly the occurrence of fairly regular epidemics in most populations, with occasional severe episodes in which the disease not only appeared to be more serious and to infect a larger proportion of the population, but also appeared to spread world-wide, thus reaching pandemic status. The three types of influenza virus, namely: A, B and C, are all capable of causing infections of humans. Influenza type C viruses are relatively mild, causing only common cold-like symptoms. Influenza type B viruses cause significant flu-like illnesses which are nevertheless milder than influenza type A infections. Only influenza A viruses have been known to cause the devastating infections which spread throughout the world and this review is limited to these viruses.

The first influenza pandemic for which more than descriptive reports exist, was that of 1889. Retrospective research has partially identified the virus responsible by testing for influenza antibodies in the serum of people who were alive at that time (174). By far the worst pandemic of those recognised to date began in 1918. During the pandemic, an estimated 20 to 40 million people died. In well-developed countries, such as the United States of America (USA), approximately 0.5% of the population died. However, in some communities in Alaska and the Pacific islands, half the population perished (85). Subsequent influenza pandemics occurred in 1957, 1968 and 1977; while these resulted in far less loss of life, in world-wide terms, than the 1918 pandemic, significant deaths did occur and the impact on society in terms of morbidity and consequent economic factors was enormous. During the interval between these pandemics, lesser epidemics occurred in most human populations, often with significant impacts on those populations in terms of morbidity, mortality and economy.

Despite the importance of influenza as a disease of humans, the earliest recognition of influenza virus in animals related to infections of poultry. A disease capable of causing extremely high mortality in infected domestic fowl was first defined in 1878 and became known as 'fowl plague'. As early as 1901, the causative organism of this disease was shown to be an ultra-filterable agent (i.e. a 'virus'), although not until 1955 was the close relationship between this agent (and other, milder, viruses isolated from birds) and mammalian influenza A viruses demonstrated (137).

Isolation of influenza virus as the causative organism of a disease termed 'swine influenza', which was applied to a new disease of pigs with clinical signs similar to those in humans first described at the time of the 1918 human pandemic (39), also preceded the isolation of human influenza virus. In the late 1920s, Shope demonstrated the ability to transmit swine influenza between pigs using ultrafiltered material (148). A virus demonstrated to be related to the pig virus was eventually isolated from a human patient in 1933, by inoculating a filtrate of throat washings into the noses of ferrets (160).

The demonstration, in 1955, that 'fowl plague' virus was an influenza A virus, was followed in 1956 by evidence that an emerging respiratory disease in horses was also caused by an influenza type A virus (69, 137, 162). In the next two years, respiratory disease caused by this virus in horses became widespread in Europe. The similarities and association of these viruses was noted by influenza scientists, and the World Health Organization (WHO) encouraged and co-ordinated work on the epidemiology of animal viruses, particularly in relation to human influenza (84). However, the vast reservoirs of influenza viruses that exist in animals, particularly birds, were not fully discovered until the 1970s.

Importance for public and animal health

Public health

Although infection with influenza A viruses may be considered a serious illness with potentially fatal consequences for susceptible individuals, the incidence of influenza A infections in human populations varies significantly from year to year. Incidence ranges from years of devastating pandemics to years when the number of cases in a given population does not reach epidemic status.

On average, each individual case of influenza in the USA is associated with an estimated five to six days of restricted activity, three to four days of bed disability and approximately three days lost from work or school (140). The overall effect on public health will therefore be generally related to the number of individuals that become infected, although this in turn may be related to the degree of immunity of individuals in the population which can also modify the severity of the clinical signs. Clinical attack rates during an epidemic are difficult to estimate due to the problems in making a clinical diagnosis, especially when cases of influenza A infection occur at a time when other respiratory viruses, including influenza type B, may be circulating. In the United Kingdom (UK) during the 1918 pandemic, an estimated 23% of the population developed influenza, in 1957 and 1958, 12% (8 million cases) and in 1969 and 1970, 8% (10). During the pandemic of 1969 and 1970, serological evidence suggested that a larger proportion of the population of the UK was infected, presumably often subclinically. In interpandemic years, clinical strike rates are highest in the elderly, although the highest infection rates are in school children. However, in the pandemics of 1918-1919 and 1957, the groups showing...
the highest proportions of clinical disease were children under fourteen years old (10).

Influenza A infections significantly affect overall mortality in human populations, and until the occurrence of human immunodeficiency virus (HIV) infections, were the only viruses to have this effect. Excess mortality due to pneumonia and influenza (P&I) is usually a reliable marker for assessing the beginning and end of an influenza epidemic, although this is probably an underestimation of influenza-associated deaths (158). Although mortality rates during the 1918 pandemic were extremely high (3,000 per 1,000,000 in 1918 and 1,170 per 1,000,000 in 1919 in England and Wales [10]), significant mortality also occurs during the interpandemic epidemics. The combined pandemics of 1957 and 1968, in the USA, accounted for approximately 98,000 excess deaths; however, the epidemics from 1957 to 1975, excluding the pandemic years, accounted for over twice that number of excess deaths (41). The impact of the pandemics is amplified by the difference in the distribution of deaths by age group compared to the interpandemic epidemics. Simonsen et al. observed that during the pandemic years of 1968-1970, approximately 50% of the excess P&I deaths in the USA were recorded in people less than 65 years old. In contrast, over the entire decade, only 0%-7% of the excess P&I deaths occurring each year were in the under 65 age group (158). Similar figures were obtained for the pandemic of 1957. Thus, while the 1918 pandemic was particularly noted for greater mortality in young adults than in the more elderly, this may be a feature of pandemics in general.

Human influenza epidemics may also affect public health as a result of the socio-economic impact and the monopolisation of healthcare resources during an epidemic. In the past, pandemics and significant epidemics have had enormous effects on hospital resources. During the epidemic caused by the 1957 pandemic virus, hospital admissions in the UK rose by as much as 250% in some areas, while at the peak of the epidemic, up to one third of nurses were absent from work (10).

Influenza infections may leave a legacy once the epidemic or pandemic is over. Ravenholt and Foege have advanced a compelling argument that the global pandemic of a Parkinson-like disease termed encephalitis lethargica, affecting more than 1,000,000 people between 1919 and 1928, was a direct consequence of the influenza pandemic of 1919 (128).

Animal health

Pigs

The disease caused by influenza viruses in pigs is essentially similar to that recorded in humans, although generally somewhat milder, consisting of an acute febrile, respiratory disease characterised by fever, apathy, anorexia, coughing, laboured breathing, sneezing, nasal discharge, low mortality rate and rapid recovery, usually five to seven days after the onset of clinical signs (148, 115, 43). The extremely high morbidity has serious economic consequences due to the increased time needed to attain slaughter weight as a result of the influenza virus infection. The cost has been estimated at up to £7 per pig, accounting for a financial loss to the pig industry in the UK of approximately £65 million each year (89).

Horses

Equine influenza is a clinical disease of horses, donkeys and mules caused by influenza A viruses of subtypes H7N7 and H3N8. The disease in fully susceptible animals is similar to that seen in pigs and humans, presenting as a severe respiratory infection with a harsh cough, nasal discharge and pyrexia. Infections with subtype H3N8 are generally considered to be more severe than H7N7 infections (65). When equine influenza has been introduced into susceptible populations, characteristic rapid spread and high morbidity have occurred.

In highly developed countries, equine species are largely kept for sport or as companion animals. Thus, while influenza infections can be a nuisance or, for the horse racing industry, an economic problem, the disease is managed largely by vaccination and by resting clinically infected animals (111). However, in many poorer parts of the world, horses, donkeys and mules remain the principal working animals. As a consequence, vaccination and rest may not be an option, and this in turn, results in more severe disease, often with secondary bacterial infections. In relatively recent years, such severe infections have occurred three times, in India, in 1987 (176), and in the People's Republic of China from 1989 to 1990 (60) and from 1993 to 1994 (154). In each case, the disease spread rapidly and widely. In the 1993-1994 outbreak in the People's Republic of China, an estimated 2,245,000 horses were affected clinically and 24,600 (1%) died (154). In the epidemic of 1989-1990 in the north-east of the People's Republic of China, morbidity was estimated at 81%, with mortality varying in different herds, but reaching 20% or more in some (60).

Birds

In birds, the clinical signs and disease observed following infection with influenza A viruses vary according to the host species, age, the presence of other micro-organisms and environmental factors. In susceptible avian species, uncomplicated infections can be divided into two forms based on the severity of the clinical disease produced. 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, Seventeen primary isolates of such viruses have been reported in domestic poultry since 1959 (5). Apart from sudden onset of mortality, clinical signs associated with HPAI vary enormously and none are pathognomonic. Generally, all or some of the following may be observed: cessation of egg laying, respiratory signs,
excessive lachrymation, sinusitis and, more characteristically, oedema of the head face and neck and cyanosis of the unfeathered skin (46). Infections of poultry with HPAI are often self-limiting or rapidly controlled by slaughter. However, on three occasions in recent years, in Pennsylvania, USA, from 1983 to 1984 (187), in Mexico from 1994 to 1995 (31, 179) and in Pakistan from 1994 to 1995 (113), the viruses have become widespread in chickens and have had severe implications for animal health and the economy of the country or region in which they occurred. The impact of these outbreaks is best measured in losses of birds and financial terms. For example, in the epidemic in Pennsylvania, more than 17,000,000 birds were slaughtered or died. Lasley estimated that this cost the Government of the USA US$60,000,000, with a further US$15,000,000 borne by the farmers, and that subsequent rises in food prices cost the consumer US$349,000,000 (99).

All other viruses cause a much milder disease consisting primarily of respiratory disease, depression and egg production problems in laying birds. In addition, these non-pathogenic avian influenza viruses may replicate in the epithelial cells of the intestine of birds without inducing signs of disease (83), although virus may be shed in high concentrations in the faeces (159, 83, 90). Infections such as Pasteurella multocida, avian pneumovirus, avian paramyxovirus type 3, infectious bronchitis virus and even live bacterial or virus vaccines or environmental conditions may result in significant severe disease, sometimes with mortalities sufficiently high to be mistaken for HPAI. Typical of these extremes was the infected turkey breeder flock reported by Alexander and Stackman as infected with virus of H7 subtype, in which the only sign was 2% white, misshapen eggs (7); compared to the outbreaks caused by a virus of H4N8 subtype in flocks of chickens in Alabama, USA, in 1975, in which up to 69% mortality was reported, including one flock with 31% mortality in a single day (80). Viruses of low pathogenicity may become widespread in a given area, incurring substantial economic losses. During 1995, 178 turkey farms in Minnesota, USA, were affected by influenza A virus of H9N2, resulting in the worst economic loss due to influenza infections recorded in a single year in Minnesota (approximately US$6,000,000) (63).

Molecular biology

The genomes of influenza A and B viruses consist of eight unique segments of single-stranded ribonucleic acid (RNA) which are of negative polarity. Influenza C viruses possess seven segments of RNA. The viral RNA is transcribed to complementary messenger RNA by a virus-associated polymerase complex (designated PB1, PB2 and PA). To be infectious, a single virus particle must contain each of the eight unique RNA segments. It is likely that the incorporation of RNA into the virion is at least partly random. The random incorporation of RNA segments allows the generation of progeny viruses containing novel combinations of genes when cells are concurrently infected with two different parent viruses. This phenomenon is referred to as genetic reassortment (74, 182).

Influenza A virus particles appear roughly spherical or filamentous, with diameter of 80 nm to 120 nm. The nucleocapsid shows helical symmetry and is enclosed within a matrix. External to the matrix is a lipid membrane, the surface of which is covered by two types of glycoprotein projections, or spikes, with which haemagglutinin (HA) and neuraminidase activities are associated. These two surface glycoproteins, particularly the HA, appear to be the most important antigens in terms of stimulation of protective immunity in the host. Consequently, considerable antigenic variation is seen in these polypeptides while other polypeptides are antigenically more stable.

The classification and nomenclature of influenza viruses take account of the antigenic variation that exists (193). Influenza viruses are grouped into types A, B and C on the basis of the antigenic nature of the internal nucleocapsid or the matrix protein. Both these antigens are common to all viruses of the same type (138). Viruses of influenza A type are further divided into subtypes on the basis of the HA and NA antigens. At present, fifteen HA (H1-H15) and nine NA (N1-N9) subtypes are known; a virus possesses one HA and one NA subtype, apparently in any combination (72). The system of nomenclature for influenza viruses consists of several component parts. The information is presented as follows: type, host of origin (for non-human species), geographical origin, strain number and year of isolation. This is followed by the antigenic subtype in parentheses, e.g. A/duck/Alberta/35/76 (H1N1).

Aetiology

Taxonomy

The influenza viruses are members of the Orthomyxoviridae family which includes three genera, one consisting of types A and B influenza viruses, a second containing type C influenza viruses, and a third containing 'Thogoto-like viruses' (78). Influenza virus types A, B and C infect humans but, except for occasional reports, infections of other animals are restricted to type A influenza viruses. Only influenza A viruses have been isolated from birds.
the HA does not affect the antigenic or receptor-binding properties of the protein (105), but is essential for the virus to be infectious (93) and is an important determinant in pathogenicity (134). Molecules of HA form homotrimers during maturation. The three-dimensional structure of the complete trimer has been determined, consisting of a globular head (HA1) on a stalk (HA1/2). The head contains the receptor-binding cavity in addition to most of the antigenic sites of the molecule. The carboxy-terminus of HA2 anchors the glycoprotein in the cell or virion membrane. The HA is subject to a high rate of mutation due to error prone viral RNA polymerase activity. Selection for amino acid substitutions in the variants produced is driven at least in part by immune pressure, as the HA is the major target of the host immune response. The amino acids making up the receptor binding site are highly conserved but the remainder of the HA molecule is highly mutable. The fifteen subtypes of HA recognised currently differ by at least 30% in the amino acid sequence of HA1 and are not cross-reactive serologically.

Subtypes may include several variant strains which are only partially cross-reactive in serological assay.

The NA is the second major surface antigen of the virus which, like HA, is an integral membrane glycoprotein. The protein functions to free virus particles from host cell receptors, to enable progeny virions to escape from the cell in which they arose, and so facilitate virus spread. This activity destroys the HA receptor on the host cell (27), preventing progeny virions reabsorbing to the host cell (123). Like HA, the NA is highly mutable with variant selection driven by host immune pressure. The nine subtypes identified in nature to date are not cross-reactive serologically, although variants within subtypes are partially cross-reactive serologically.

Ecology

Influenza A viruses infect a large variety of animal species, including humans, pigs, horses, sea mammals and birds (Tables I and II) (2, 189). Recent phylogenetic studies of influenza A viruses have revealed species-specific lineages of viral genes and have demonstrated that the prevalence of interspecies transmission is dependent on the animal species involved (189). In the early 1970s, the WHO initiated long-term global studies on the influenza viruses of lower animals and birds to determine as far as possible whether a finite number of influenza A viruses existed in nature and whether it was possible to isolate a future pandemic strain of virus from among these viruses, in advance of the appearance of a new strain in humans. A vast number of viruses have been isolated from a wide variety of birds and a range of terrestrial and sea mammals. Although an enormous diversity of animal species have been shown to be susceptible to influenza A virus infections, three groups of animals appear to be far more important in terms of numbers and the epidemic/endemic nature of the disease than other animals, namely: birds, pigs and horses.

Influenza viruses in humans

Despite the recognition of fifteen distinct haemagglutinin subtypes (H1-H15) and nine neuraminidase subtypes (N1-N9), the number of subtype combinations found to be

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Humans</th>
<th>Examples(4) of viruses of the subtype isolated from the specified host group</th>
<th>Pigs</th>
<th>Horses</th>
<th>Birds</th>
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<td>Duck/Australia/341/63 (H15N8)</td>
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</tbody>
</table>

(4) The reference strains of influenza viruses, or the first isolates of the subtype from the host group
(4) Not found in this host group
FPV: fowl plague virus
prevalent in humans and other mammals at any one time appears to be severely limited. Pandemics of influenza occur when a virus is introduced which is fully capable of replication and spread, and for which all or a large proportion of the population have had no immunological experience of at least the functionally important haemagglutinin. This is termed antigenic shift and has occurred four times during the 20th Century, each time resulting in a true pandemic. The first of these shifts was, almost certainly, in 1918, when virus of H1N1 entered the human population, resulting in the most devastating pandemic known to man. The second was in 1957, when H2N2 virus appeared. The third, in 1968, with a H3N2 virus and the fourth in 1977, with the re-emergence of the H1N1 virus. In 1957 and 1968, the introduction of a new subtype, but this did not occur in 1977, and currently both H1N1 and H3N2 viruses are circulating.

The emergence of new subtypes of influenza virus and the ensuing pandemics are unmistakable, but the epidemics occurring between the pandemics may nevertheless be severe. These are a result of the gradual change of the antigenicity of the circulating virus by the accumulation of point mutations until the virus is antigenically sufficiently different from earlier strains that a large proportion of the population is susceptible and cases reach epidemic levels. This accumulative variation is termed antigenic drift, and the size, severity and spread of the virus will depend on the degree to which the virus is antigenically different from viruses already experienced by the population.

Examination of serum samples taken from elderly people prior to the pandemics of 1957 or 1968 has been used to assess virus subtypes present in humans before H1N1. ‘Sero-archaeology’ studies have indicated that the two prevalent viruses preceding 1918 were H2N2 in 1889 and H3N8 in 1900 (189), further confirming the limited range of subtypes known to cause influenza epidemics in humans. Influenza viruses in pigs

Swine influenza (SI) was first observed at the time of the pandemic in humans in 1918 and the viruses responsible are known to be closely related, possibly having derived from a common ancestor (130). Although the disease was described in pigs during the years which followed, not until 1930 was the virus isolated and identified. This H1N1 virus was the prototype strain of a group of viruses now known as classical viruses, which have been reported in pig populations world-wide.

Influenza A viruses of subtypes H1N1 and H3N2 have been reported widely in pigs and have been frequently associated with clinical disease. These include classical swine H1N1, avian-like H1N1 and human- and avian-like H3N2 viruses. Influenza is widespread and endemic in pig populations world-wide and is responsible for one of the most prevalent respiratory diseases in pigs. Occasionally, influenza infections of pigs may result in epidemics through the introduction of virus to an immunologically naïve population or due to significant antigenic drift in the HA or NA of endemic viruses.

Swine influenza is related to the movement of animals from infected to susceptible herds, clinical disease generally appears with the introduction of new pigs into a herd. Once a herd is infected, the virus is likely to persist through the production of young susceptible pigs and the introduction of new stock. Outbreaks of disease occur throughout the year but usually peak in the colder months (45). Infection is frequently subclinical, and often typical signs are seen in only 25% to 30% of a herd. Swine husbandry practices influence directly the evolution of SI viruses through reduced immune pressure and constant availability of susceptible hosts, generally leading to reduced genetic drift in the genes encoding HA and NA, compared to those of similar viruses in the human population. However, mixing of pigs from multiple-sources and the high frequency of contact with other

<table>
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<th>Subtype</th>
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<th>Pigs</th>
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<td>Duck/Memphis/545/74 (H1N9)</td>
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</table>

**Table II**

Neuraminidase (N) subtypes of influenza A viruses isolated from humans, pigs, horses and birds

**a)** The reference strains of influenza viruses, or the first isolates of the subtype from the host group

**b)** Not found in this host group

FPV: fowl plague virus

Influenza viruses in pigs

Swine influenza (SI) was first observed at the time of the pandemic in humans in 1918 and the viruses responsible are known to be closely related, possibly having derived from a common ancestor (130). Although the disease was described in pigs during the years which followed, not until 1930 was the virus isolated and identified. This H1N1 virus was the prototype strain of a group of viruses now known as classical viruses, which have been reported in pig populations world-wide.

Influenza A viruses of subtypes H1N1 and H3N2 have been reported widely in pigs and have been frequently associated with clinical disease. These include classical swine H1N1, avian-like H1N1 and human- and avian-like H3N2 viruses. Influenza is widespread and endemic in pig populations world-wide and is responsible for one of the most prevalent respiratory diseases in pigs. Occasionally, influenza infections of pigs may result in epidemics through the introduction of virus to an immunologically naïve population or due to significant antigenic drift in the HA or NA of endemic viruses.

Swine influenza is related to the movement of animals from infected to susceptible herds, clinical disease generally appears with the introduction of new pigs into a herd. Once a herd is infected, the virus is likely to persist through the production of young susceptible pigs and the introduction of new stock. Outbreaks of disease occur throughout the year but usually peak in the colder months (45). Infection is frequently subclinical, and often typical signs are seen in only 25% to 30% of a herd. Swine husbandry practices influence directly the evolution of SI viruses through reduced immune pressure and constant availability of susceptible hosts, generally leading to reduced genetic drift in the genes encoding HA and NA, compared to those of similar viruses in the human population. However, mixing of pigs from multiple-sources and the high frequency of contact with other
species, particularly humans, provides an opportunity for co-circulation of viruses and genetic reassortment.

Serological studies of pigs worldwide have shown that classical swine H1N1 influenza virus is prevalent throughout the major pig populations, with approximately 25% of animals demonstrating evidence of infection (24, 70). Classical swine H1N1 influenza virus isolates in the USA have remained conserved both genetically (104) and antigenically (146), but viruses which are antigenically distinguishable, although closely related, have been reported by Olsen et al. (120) and Wentworth et al. (190). In Canada, an H1N1 virus was associated with a new and distinctive pathology in pigs which appeared in 1990. However, the virus was most closely related to classical swine influenza viruses (131). In Europe, classical viruses reappeared in 1976 (116), but have been largely replaced since 1979 by 'avian-like' swine H1N1 viruses which are antigenically and genetically distinguishable from the classical swine H1N1 influenza viruses of North America (125, 142).

Influenza A viruses of H3N2 subtype, related closely to early human strains, have been widely reported from pigs, particularly in Europe and Asia where they continue to circulate long after disappearing from the human population. These viruses, following years of adaptation to pigs, are associated with clinical signs in pigs which are typical of SI (61). Seroprevalence is usually in the range 30% to 50% (98, 132), but can be as high as 75% (175). This apparently high level of H3N2 infections in Europe is in sharp contrast to the low prevalence in pigs in North America. Some of the H3N2 viruses isolated from pigs in Asia are entirely avian-like (91), but repeated introductions from humans appear to occur regularly.

The prevailing human H1N1 strains are transmitted frequently to pigs and are occasionally associated with respiratory epizootics in pigs (24, 87). However, most strains are not readily transmitted among pigs in the field and are not therefore maintained entirely independently of the human population (70).

Influenza A H1N2 viruses, derived from classical swine H1N1 and 'human-like' swine H3N2 viruses have been isolated in Japan (169) and France (55). In Japan, these viruses appear to have spread widely within pig populations and are associated frequently with respiratory epizootics (121). Subsequently, an H1N2 influenza virus related antigenically to human and 'human-like' swine viruses has emerged and become endemic in pigs in the UK, often in association with respiratory disease (23).

Influenza viruses in horses

Influenza is a common disease of horses throughout the world. Apart from rare reports of isolations or serological evidence of infection with other subtypes, only two subtypes of influenza A virus, H7N7 and H3N8, have been identified as infecting and causing disease in horses. Subtype H7N7 viruses may have disappeared largely from the horse population, as no substantiated reports of virus isolated from horses have been recorded since 1980 (180). However, recent world-wide serological surveillance has suggested that H7N7 may be circulating in Eastern Europe (111, 106) and Central Asia (189). Phylogenetic analyses of H3N8 (equine-2) viruses has revealed the existence of two distinct evolutionary lineages, European and American, the circulation of which was originally centred largely on the geographical origin (112). However, in Europe, both lineages now appear to be co-circulating, with the American type viruses predominant in some areas (122). These findings have importance for control, since vaccines including one H3N8 virus type may not provide protection against infection with viruses from the heterologous group. Recently, equine influenza outbreaks due to infection with H3N8 virus were observed in South Africa, India, the People's Republic of China, Hong Kong and Nigeria, where equine influenza viruses were not known to be circulating. Recent outbreaks in the People's Republic of China have been due to both conventional strains of H3N8 virus, and viruses which contained the same surface antigens as the other equine viruses of this subtype, but which had genetic features that were avian-like, indicating that the virus had been introduced from birds (60, 188). Sporadic outbreaks typically occur and may, in part, be due to antigenic drift, which may compromise the efficacy of the vaccines available (18, 112).

Influenza viruses in birds

Wild birds

The first isolation of influenza virus from feral birds was in 1961, from common terns (Sterna hirundo) in South Africa (14). However, the enormous pools of influenza viruses which were present in the wild bird population were not revealed until systematic investigation of influenza in feral birds was undertaken in the 1970s, following the 1968 pandemic in humans.

Isolations of virus from other wild birds have been completely overshadowed by the number, variety and widespread distribution of influenza viruses in waterfowl, order Anseriformes. In the surveys listed by Stallknecht and Shane, a total of 21,318 samples from all species resulted in the isolation of 2,317 (10.9%) viruses (163). Of these samples, 14,303 were from birds of the order Anseriformes and yielded 2,173 (15.2%) isolates. The next highest isolation rates were 2.9% and 2.2% from the Passeriformes and Charadriiformes. The overall isolation rate from all birds other than ducks and geese was 2.1%. In a three-year study of waterfowl congregating on lakes in Alberta, Canada, prior to migration, Hinshaw et al. found an overall isolation rate of 26%, with 60% of juveniles excreting virus in the first year of the life of the bird (71). The large numbers and concentration of waterfowl on such lakes, coupled with the facts that ducks have been reported to excrete up to $10^8$ mean egg infectious doses of virus per gram of faeces (184), and that this virus
remains viable for considerable periods in lake water (164),
must represent a unique level of viral contamination of a large
environment.

Caged ‘pet’ birds
Since 1975, when the first isolates from caged birds were
recorded, isolates from all sources have been mainly of H4 or
H3 subtypes. The majority of influenza viruses from caged
birds come from passerine species and only rarely are
psittacines infected. Although the presence of influenza
viruses in birds held in quarantine is monitored continually in
several countries around the world, periods when no
isolations have been made have been recorded, often lasting
several years.

Domestic poultry
At the end of the 19th Century and in the early 20th Century,
‘fowl plague’, the highly pathogenic form of avian influenza,
was often reported in chickens and was probably enzootic in
several countries. However, in the second half of the 20th
Century, reports of influenza infections of chickens have been
rare compared to infections of other domestic poultry, despite
the much higher populations of chickens. For example in the
USA, despite frequent influenza epizootics in turkeys in some
States, between 1964 and 1982, only three outbreaks were
recorded in chickens (127).

Despite the low incidence of influenza infections of chickens
throughout the world, twelve outbreaks of HPAI have
occurred since 1959, with significant spread of infection in
Pennsylvania and neighbouring States of the USA during
1983 and 1984, and in Mexico and Pakistan in 1994 and
1995. Outbreaks since 1959 have shown no or extremely
limited spread. Eight HPAI outbreaks in backyard poultry
flocks infected with H5N2 virus were reported in Italy in
1997 and 1998. Outbreaks of H5N1 HPAI occurred on three
farms in Hong Kong from March to May 1997, with
70%-100% mortality and subsequent spread to live bird
markets (35).

Turkeys tend to be reared in less substantial accommodation
than chickens or on open range and most of the major
turkey-producing countries have had disease problems
associated with influenza infections. In the USA, in California
and Minnesota, where turkey farms are not only heavily
concentrated but are also situated on migratory waterfowl
flyways and are reared on range, influenza virus infections
have been seen regularly and may represent a significant
economic loss (63). In other countries where these birds are
generally reared indoors, infections of turkeys are not seen
regularly and in the years when infection has occurred, these
have usually been restricted to one or two isolated incidents.

During the period between 1994 and 1999, infections of
poultry with influenza viruses of H9N2 subtype appear to
have been common world-wide. Outbreaks occurred in Italy
in 1994, Germany from 1995 to 1996, Ireland in 1997, South
Africa in 1995, and the Republic of Korea in 1996 (9). Since
1997, serious problems associated with H9N2 virus have
been reported in Iran, Saudi Arabia, Pakistan, the People's
Republic of China and other countries of Asia. In Minnesota,
USA, during 1995, 178 turkey farms were infected with virus
of subtype H9N2 (63).

The influenza status of commercial ducks in most countries is
poorly understood or has not been investigated fully.
However, commercial ducks, especially fattening ducks, are
generally reared on ponds or fields outdoors and when
surveillance of commercial ducks has been undertaken,
enormous pools of virus and many subtype combinations
have been detected (3).

Ratites
The increase in trade in ostriches and other ratites during the
1990s led to the movement of large numbers of such birds
around the world. The testing for viruses, including influenza,
has resulted in the regular isolation of influenza viruses from
these birds. Since these birds are mainly kept in open fields,
influenza infections probably represent spread from feral
birds.

Pathogenesis
Differences in pathogenicity among influenza viruses result in
the production of a wide spectrum of clinical diseases that
range in severity from fatal systemic to mild, sometimes
inapparent, respiratory disease (43, 108). Severity of the
disease is also determined by the host species infected (6, 8,
77) and in part by factors such as host age and sex, virus dose,
environment and concurrent infections with other pathogens
(46).

The pathogenesis of infection with influenza virus is difficult
to define precisely, since it is complicated frequently by the
effects of infection with secondary bacteria. Severe damage to
the epithelium of the respiratory tract is the main feature in
infections of mammals with influenza virus. The resulting
damage to the epithelium facilitates secondary infection by
bacterial respiratory pathogens.

Humans
In humans, the disease incubation period will vary between
one and three days, depending on the virus strain and dose.
Typically, the onset of clinical signs is rapid, characterised by
malaise, fever, nasal symptoms, an unproductive cough,
myalgia and headache. Initially, typical symptoms are rhinitis
followed by tracheobronchitis and, infrequently, an interstitial
pneumonitis (40). The disease process usually damages the
respiratory tract from the nose to the small bronchi, but rarely
damages alveolar cells (100). The illness is rapidly prostrating
and usually lasts from three to five days, being most severe in
children and the elderly. Complications include primary viral
or secondary bacterial pneumonia (68, 101) and these often
confuse the respiratory pathology following infection with influenza virus. Significant pathological changes in other organs have not been observed consistently.

Influenza A virus infection of humans and other mammals can result in gross lung lesions which are patchy and randomly distributed throughout the lobes. The altered lung areas are depressed and consolidated, dark red or purple red in colour, contrasting sharply with normal lung tissue. These changes are observed in a large number of animals of all ages kept within a single unit, following an incubation period of one to three days. In pigs, morbidity is high, resulting in serious economic implications for the farmer. Secondary bacterial infections in both pigs and horses can often increase the severity of the illness and may result in complications such as pneumonia, in addition to strangles in horses (81).

In pigs, the bronchi and bronchioli are dilated and filled with exudate. Bronchial and mediastinal lymph nodes are usually hyperemic and enlarged (148, 177). Histologically, widespread degeneration and necrosis of the epithelium occurs in the bronchi and bronchioli. The lumen of bronchi, bronchioli and alveoli are filled with exudate containing desquamated cells and neutrophils progressing to mainly monocytes. Furthermore, dilatation of the capillaries and infiltration of the alveolar septae with lymphocytes, histiocytes and plasma cells occurs. Widespread interstitial pneumonia and emphysema accompany these lesions, although the severity of the former is dependent on the infecting strain (21). In North America, a proliferative necrotising pneumonia of pigs is characterised by widespread hyperplasia of type II epithelial cells (51).

Influenza infection of other animals, such as ruminants, sea mammals, dogs and mustelids usually results in subclinical disease. However, H7N7 and H4N5 influenza A viruses were associated with a high mortality in Harbour seals at Cape Cod, USA, in 1979 (97) and 1982 (167), respectively, and were isolated from the lungs and brains of dead animals.

Avian species

In birds, the disease signs can vary considerably as described earlier. Infection of birds with highly virulent avian viruses is characterised by haemorrhagic, necrotic, congestive and transudative changes. Haemorrhagic changes are frequently severe in the oviducts and intestines. Sometimes, despite a high titre of virus, no lesions occur in the lung (135). Encephalitis may develop in the cerebrum and cerebellum, especially in broilers (1). Alterations to myocardial tissues have been observed following infection with highly pathogenic strains (126).

The haemagglutinin glycoprotein for influenza viruses is produced as a precursor, HAO, which requires post-translational cleavage by host proteases before it is functional and virus particles are infectious (134). The HAO precursor proteins of avian influenza viruses of low virulence for poultry have a single arginine at position -1 from the cleavage site and another at position -4. These viruses are limited to cleavage by host proteases such as trypsin-like enzymes and are thus restricted to replication at sites in the host where such enzymes are found, i.e. the respiratory and intestinal tracts. The HPAI viruses possess multiple basic amino acids (arginine and lysine) at the HAO cleavage sites, either as a result of apparent insertion or apparent substitution (145, 178, 191), and appear to be cleavable by a ubiquitous protease(s), probably one or more proprotein-processing subtilisin-related endoproteases of which furin is the leading candidate (165). These viruses are able to replicate throughout the bird, damaging vital organs and tissues which results in disease and death (134). Typical cleavage site amino acid sequences for H5 viruses of high and low virulence are shown in Table III.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Amino acids at HAO cleavage site (marked by *)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5 viruses low pathogenicity</td>
<td>100 isolates</td>
</tr>
<tr>
<td>H5 viruses high pathogenicity</td>
<td>1994/1995 Mexican isolates (H5N2)</td>
</tr>
<tr>
<td></td>
<td>Chicken/Scotland/59 (H5N1)</td>
</tr>
<tr>
<td></td>
<td>Turkey/England/90-92/91 (H5N1)</td>
</tr>
<tr>
<td></td>
<td>Ck/HK/97 (H5N1)</td>
</tr>
<tr>
<td></td>
<td>O1/N/97 (H5N1)</td>
</tr>
</tbody>
</table>

HAO: uncleaved haemagglutinin gene

Date from: Genbank or viruses sequenced at the Veterinary Laboratory Agency-Weybridge
Arginine (R) and lysine (K) are basic amino acids
**a) Direct transmission**

Phylogenetic evidence suggests that an influenza A virus possessing eight gene segments from avian influenza reservoirs may have been transmitted directly to humans and pigs before 1918. This virus is believed to have caused the severe Spanish influenza pandemic of 1918. Direct transmissions of H5N1 and H9N2 viruses from chickens to humans, whilst not resulting in a pandemic to date, demonstrate that avian species may be a direct source of virus for humans.

**b) Genetic reassortment**

In 1957, the Asian pandemic virus H2N2, appears to have acquired three genes (PB1, HA and NA) from the avian influenza gene pool in wild ducks by genetic reassortment with the circulating human strain from which it retained five other genes. Following the appearance of this virus, the H1N1 strains disappeared from humans. In 1968, the Hong Kong pandemic virus H3N2 probably originated by a similar mechanism. It has been suggested that the reassortment event leading to the production of Asian and Hong Kong pandemic viruses may have occurred in an intermediate host such as the pig, since the latter is receptive to and allows productive replication of both human and avian influenza viruses.

**Fig. 1a & Fig. 1b**

Theoretical origin of influenza A viruses circulating in humans since 1918
Epidemiology

Theories of pandemic human influenza

Three major pandemics occurred in the 20th Century, caused by viruses antigenically 'new' to the host population (antigenic shift), interspersed with both minor and major epidemics. Pandemic strains generally appear through genetic reassortment. Because viral RNA is segmented, genetic reassortment can readily occur in mixed infections with different strains of influenza A viruses (182, 183). 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 theoretically possible number of $2^8$ (256) different combinations that can form a complete set of RNA segments from a concurrent infection, although in practice, only a few progeny virions possess the correct gene constellation required for viability (74, 189). The new subtypes of influenza viruses which appeared in humans in 1957 (Asian influenza), 1968 (Hong Kong influenza) and 1977 (Russian influenza), had several features in common. The appearance of these subtypes was sudden, the viruses were antigenically distinct from the influenza viruses then circulating in humans, they were confined to H1, H2 and H3 subtypes and the first outbreaks occurred in South-East Asia. Phylogenetic evidence suggests that these pandemic strains were derived from avian influenza viruses either after reassortment (Fig. 1a) or by direct transfer (Fig. 1b) (189). The appearance of the H2N2 and H3N2 subtypes was paralleled by the disappearance from the human population of the subtypes circulating previously, H1N1 and H2N2, respectively. This phenomenon probably occurred in 1918 when emerging H1N1 viruses replaced H3-like viruses. The reasons for the sudden disappearance of human strains circulating previously are unknown, but the earlier strain is possibly disadvantaged compared with the new strain because it has already elicited widespread immunity in the human population. This may explain the failure of the H1N1 virus to replace H3N2 on re-emergence in 1977 (Fig. 1c), as a large proportion of the population would have been infected with H1N1 prior to 1957, and would have retained some immunity.

Fig. 1c
Theoretical origin of influenza A viruses circulating in humans since 1918

c) Re-emergence
In 1977, the Russian influenza virus H1N1 that had circulated in humans prior to 1950 reappeared and has continued to co-circulate with H3N2 viruses in the human population. The origin of the virus is a mystery and a number of sources have been proposed, including the possibility that the virus re-emerged following escape from a laboratory.
The emergence of pandemic strains following genetic reassortment

The detection of vast pools of influenza viruses of many different subtypes among animals, particularly aquatic birds, gave considerable impetus to research aimed at determining where new subtypes, particularly those that cause pandemics, emerge. A number of theories has been suggested, of which the most widely accepted is that by adaptation, sometimes involving genetic reassortment, transference of virus from animals to humans occurs, resulting in an antigenically novel virus with the ability to infect and spread in humans (incidents are summarised in Table IV). Following genetic reassortment, viruses may arise that possess the genes necessary to enable infection of humans but may have surface antigens new to the host immune system.

Evidence for the pig as a mixing vessel of influenza viruses of non swine origin has been demonstrated in Europe by Castrucci et al., who detected reassortment of human and avian viruses in pigs in Italy (32). Novel reassortant viruses of H1N2 subtype derived from human and avian viruses (26) or H1N7 subtype derived from human and equine viruses (22) have been isolated from pigs in the UK. The H1N2 viruses derived from a multiple reassortant event and spread widely within pigs in the UK. Other studies of influenza viruses isolated from pigs in North America (195) and the south of the People’s Republic of China (156) failed to detect any reassortant viruses containing internal gene segments of non swine origin, although genetic heterogeneity of the HA of swine H3 influenza viruses occurs in nature in the People’s Republic of China (91). Since 1998, H3N2 viruses isolated from pigs in the USA have contained combinations of human, swine and avian genes. Furthermore, the HA gene of these viruses was derived from a human virus circulating in the human population in 1993 (181).

Occasional transmission of influenza viruses from pigs to humans has been demonstrated (see the sub-section below, entitled ‘Pigs’ in the section ‘Spread between humans and mammalian species’). The internal protein genes of human influenza viruses share a common ancestor with the genes of some swine influenza viruses. A number of authors have proposed the nucleoprotein (NP) gene as a determinant of host range which can restrict or attenuate virus replication, thereby controlling the successful transmission of virus to a ‘new’ host (143, 172, 161). These observations support the potential role of the pig as a mixing vessel of influenza viruses from avian and human sources. The pig appears to have a broader host range in the compatibility of the NP gene in reassortant viruses than both humans and birds (143).

## Table IV

<table>
<thead>
<tr>
<th>Influenza host</th>
<th>Contact with humans</th>
<th>Infections with human influenza viruses</th>
<th>Spread from host to humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs</td>
<td>Yes</td>
<td>Yes: H1N1, H3N2</td>
<td>Yes: natural</td>
</tr>
<tr>
<td>Horses</td>
<td>Yes</td>
<td>None known</td>
<td>None known</td>
</tr>
<tr>
<td>Birds</td>
<td></td>
<td>Indirectly&lt;sup&gt;a&lt;/sup&gt;</td>
<td>None known&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waterfowl</td>
<td></td>
<td>None known</td>
<td>None known&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caged birds</td>
<td>Yes</td>
<td>None known</td>
<td>Yes: H5N1, H9N2, H7N7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Domestic poultry</td>
<td></td>
<td>None known</td>
<td>Yes: laboratory</td>
</tr>
<tr>
<td>Mustelids</td>
<td>Yes</td>
<td>Yes: laboratory</td>
<td>Yes: laboratory</td>
</tr>
<tr>
<td>Whales</td>
<td>No</td>
<td>None known</td>
<td>None known</td>
</tr>
<tr>
<td>Seals</td>
<td>No</td>
<td>None known</td>
<td>Yes: laboratory</td>
</tr>
</tbody>
</table>

<sup>a</sup> Contact may occur via lake water and other media contaminated by infective faeces
<sup>b</sup> The H7N7 virus was isolated from the eye of a woman who kept ducks with which feral waterfowl mingled

Genetic and biochemical studies have shown that the pandemic viruses of 1957 and 1968 arose by genetic reassortment. The 1957 Asian H2N2 strain obtained HA, NA and PB1 genes from an avian virus and the remaining five genes from the preceding human H1N1 strain (49, 88, 141). The 1968 Hong Kong H3N2 strain contained HA and PB1 genes from an avian donor (47, 88) and the NA and other five genes from the Asian H2N2 strain.

The role of the pig

Pigs serve as major reservoirs of H1N1 and H3N2 influenza viruses and are frequently involved in interspecies transmission of influenza viruses. The maintenance of these viruses in pigs and the frequent introduction of viruses from other species may be important in the generation of ‘new’ strains of influenza, some of which may have the potential to transmit to other species, including humans.
Pigs have been considered the logical intermediate host for reassortment of influenza A viruses, for they can serve as hosts for viruses from either birds or humans. This susceptibility is due to the presence of receptors for both avian (α2-3-galactose sialic acid) and human (α2-6-galactose sialic acid) influenza viruses which, following infection of pigs with an avian virus, can result in receptor modification on the HA to that characteristic of human viruses, thereby providing a potential link from birds to humans. The segments in the centre of each virus particle represent the genome. The subtype of some emerging reassortant viruses may differ from their progenitors through exchange of HA (white in reassorted virus) and/or NA genes. These viruses which are distinct antigenically may be able to spread in an immunologically naïve human population if transmission from pigs occurs.

HA: haemagglutinin
NA: neuraminidase

**Fig. 2**
The potential role of the pig as an intermediate host

The causative agent was an H1N1 influenza A virus which was possibly derived from the same avian ancestor (52, 83, 130). For this reason, the avian-like H1N1 viruses circulating in pigs in Europe since 1979 (125), and in South-East Asia since 1993, have been implicated as the precursors of the next human pandemic virus (56, 103). Although a pandemic has not resulted to date, the transmissions of H5N1 and H9N2 viruses from chickens to humans in Hong Kong in 1997 and 1999, respectively, further demonstrate that avian species may directly be the source of human pandemic strains (35, 151). Initially, following the transmission of these viruses from aquatic birds to pigs or chickens, the viruses appeared genetically unstable (103, 198), and in the case of transmission of avian H1N1 virus to pigs, apparently became stable after approximately twelve years (103).

Re-emergence of pandemic strains

The appearance of pandemic virus may be in fact the re-emergence of a virus which caused an epidemic many years earlier. The appearance of Russian influenza (H1N1) provides support for this concept. The virus, which reappeared in the People's Republic of China in 1977, and subsequently spread world-wide, was genetically identical to the virus which caused a human influenza epidemic in 1950 (114). Webster et al. suggested that this virus was most likely reintroduced to humans from a frozen source, possibly from a laboratory (189), whilst Shoham has proposed a biotic mechanism for the preservation of influenza viruses (147). Influenza viruses of the H3N2 subtype persist in pigs many years after their antigenic counterparts have disappeared from humans (61, 151).
viruses have accumulated many mutations and are therefore genetically distinct from the precursor viruses. Pandemic strains may also be antigenically conserved in the avian reservoir, since counterparts of the Asian pandemic strain of 1957 continue to circulate with increased prevalence in wild ducks, domestic fowl and live bird markets and are coming into closer proximity to susceptible human populations (136).

An ‘influenza epicentre’
The majority of pandemic strains have apparently originated in the People’s Republic of China, raising the possibility that this region is an influenza epicentre (153, 150). In the tropical and subtropical regions of the People’s Republic of China, human influenza occurs throughout the year (129). In the People’s Republic of China, influenza viruses of all subtypes are prevalent in ducks and in water frequented by ducks (59).

Agricultural practices provide close contact between wild aquatic birds, domestic ducks, pigs and humans, thereby presenting the opportunity for interspecies transmission and genetic exchange among influenza viruses, with the pig acting as an intermediary between domestic ducks and humans. Aquatic birds migrating or over-wintering in the region might provide a source of virus for domestic ducks. Yasuda et al. have shown that domestic ducks harbour H3 influenza viruses antigenically and genetically similar to those in pigs, suggesting these birds may play a role in the transfer of avian influenza viruses from feral ducks to pigs (196).

Rhythm of occurrence
Two hypotheses have been proposed for the rhythm of occurrence of human influenza A viruses, namely: an influenza circle or cycle, or an influenza spiral (149). The circle or cycle theory suggests a recycling of H1, H2 and H3 subtypes. If this is so, the HA subtype of the next pandemic virus would be expected to be H2. The spiral theory presupposes that humans are capable of being infected with all known HA subtypes of influenza A viruses; these presently number fifteen. There are serological grounds for this in that rural dwellers in the influenza epicentre have been found to possess antibodies to the avian subtypes H4 to H13 examined (149). It is possible that the hypotheses are not mutually exclusive, as there is no reason that recycling should not occur within the spiral.

Spread between humans and mammalian species

Pigs
Early theories suggesting that the pandemic of 1918 was a result of the transmission of virus from pigs to humans were at the time speculative and it was not until 1976 that further evidence for such transmissions became available. Pigs were implicated as the source of infection when an H1N1 virus was isolated from a soldier who had died of influenza at Fort Dix, New Jersey, USA. The virus was identical to viruses isolated from pigs in the USA. Furthermore, virus isolation demonstrated that five other servicemen were infected, and serological evidence suggested that some 500 personnel at Fort Dix were, or had been, infected with the same virus (76, 173).

The incident at Fort Dix cannot be regarded as evidence of zoonosis, since although pigs were the likely source of the virus, this was never established. However, considerable evidence exists to suggest that transmission from pigs to humans does occur, following the detection of antibodies to swine H1 viruses in people who have had close contact with pigs (95, 139). Final confirmation of the zoonotic nature of H1N1 influenza viruses from pigs came in 1976 when, following an influenza epizootic in pigs, viruses isolated from the pigs and a human contact were shown to be both antigenically and genetically identical swine H1N1 influenza viruses (44, 70). Subsequently, there have been several reports from North America of swine virus being isolated from humans with respiratory illness (38, 42), occasionally with fatal consequences (133, 190). All cases examined followed contact with sick pigs and were due to viruses related most closely to classical swine H1N1 influenza virus. Perhaps of greater significance for humans is a report in the Netherlands during 1993 of two distinct cases of infection of children with H3N2 viruses whose genes encoding internal proteins were of avian origin (34). Genetically and antigenically related viruses had been detected in pigs in Europe, raising the possibility of potential transmission of avian influenza virus genes to humans following genetic reassortment in pigs (32). These concerns were substantiated further by the results of serological studies in Italy which indicated that these ‘swine’ H3N2 viruses had apparently been transmitted to young, immunologically naïve people (30).

Influenza viruses of subtype H3N2 are ubiquitous and endemic in most pig populations world-wide, but no apparent evidence exists of pigs being infected with this subtype prior to the pandemic in humans in 1968. Indeed, the appearance of a H3N2 subtype variant strain in the pig population of a country appears to coincide with the epidemic strain infecting the human population at that time (11, 24, 117).

Further evidence of the spread of influenza viruses from humans to pigs was the appearance in pigs of H1N1 viruses (or antibodies to H1N1), related to those circulating in the human population since 1977 (11, 24, 53, 118). Genetic analysis of two strains of H1N1 virus isolated from pigs in Japan revealed that the HA and NA genes were most closely related to those of human H1N1 viruses circulating in the human population at that time (87). In addition, reassortant viruses with some characteristics of human H1 viruses have been isolated from pigs in the UK (23, 26).
Horses

In historical accounts of pandemics in humans, frequent reference is made to similar disease in horses, which occurs either simultaneously or preceding that in humans. Beveridge and others noted such references in the accounts of twelve pandemics which occurred during the 18th and 19th Centuries (17).

Experimental infection of human volunteers with H3 (equine-2) viruses has produced an influenza-like illness with virus-shedding and seroconversion (36, 86). No evidence exists of infection of humans with the other subtype of influenza, H7 (equine-1), which has caused widespread epizootics in horses.

Several isolated cases of infection of horses with subtypes H1N1, H2N2 and H3N2 have been reported, usually associated with human infections (174). Experimental infections of horses with human-derived H3N2 virus have confirmed the susceptibility of horses to this virus (19).

Cattle

Influenza infections of cattle with human viruses of H3N2 and H1N1 subtypes have been reported regularly, based largely on the detection of antibodies (82), occasionally in association with respiratory epizootics in cattle (25). The prevailing human strains appear to transmit to cattle frequently and viruses have been isolated from cattle infected naturally (48, 110), although whether or not these viruses are able to persist in cattle remains unclear. Brown et al. reported the apparent maintenance of human influenza viruses in cattle some ten years after the disappearance of the viruses from the human population, raising the possibility that cattle may provide a reservoir for virus which may be able to transmit back to humans when a susceptible population becomes available (25).

Avian influenza infections of mammals

Pigs

The probable introduction of classical swine H1N1 influenza viruses to turkeys from infected pigs has been reported from North America, and in some cases, influenza-like illness in pigs has been followed immediately by disease signs in turkeys (62, 109, 127). Genetic studies of H1N1 viruses from turkeys in the USA has revealed a high degree of genetic exchange and reassortment of influenza A viruses from turkeys and pigs in the former species (195). In Europe, avian H1N1 viruses were transmitted to pigs, became established, and have subsequently been reintroduced to turkeys from pigs, causing economic losses (102, 192). An independent introduction of H1N1 virus from birds to pigs occurred in Asia in the early 1990s; these viruses are genetically distinct from the viruses in Europe (36). Recently, H9N2 viruses have been introduced to pigs in South-East Asia, apparently from poultry, although the potential of these viruses to spread and persist in pigs remains unknown (20).

Horses

Apart from occasional isolated reports of evidence of infection of horses with viruses of subtypes H1N1, H2N2 and H3N2 (174), influenza infections of horses have been restricted to H7N7 and H3N8 subtypes of influenza A and these viruses form distinct lineages in phylogenetic studies (see earlier). However, examination of H3N8 viruses isolated from severe epizootics in horses occurring in the Jilin and Heilongjiang Provinces in the north-east of the People’s Republic of China in 1989 and 1990, showed these to be antigenically and genetically distinguishable from other equine H3N8 viruses circulating in the world. Guo et al. concluded that this virus was of recent avian origin and had probably spread directly to horses without reassortment (60). Despite the rapid geographical spread locally in 1989 and 1990, and the high morbidity, this virus does not appear to have become established in the horse population; more widespread epidemics which occurred in the People’s Republic of China from 1993 to 1994 were caused by ‘classical’ H3N8 virus (154).

Marine mammals

During 1979 and 1980, approximately 500 deaths occurred in harbour seals (Phoca vitulina) around the Cape Cod Peninsula in the USA, representing about 20% of the population. Deaths were primarily due to acute haemorrhagic pneumonia, and influenza A viruses of H7N7 subtype were isolated repeatedly from the lungs or brains of dead seals (97). The virus infecting the seals was shown to be closely related both antigenically and genetically to avian influenza viruses (185) and would appear to represent direct transmission to the seals without reassortment.

In 1983, further deaths (2%-4%) occurred in harbour seals on the New England coast of the USA and, on this occasion, another influenza A virus of subtype H4N5 was isolated. While distinguishable from the H7N7 virus, once again, all eight genes of this virus were demonstrably of avian origin (189). Further surveillance of seals on the Cape Cod peninsula was undertaken and Callan et al. reported isolates of two influenza A viruses of H4N6 subtype made in 1991 and three of H3N3 subtype in 1992 from seals found dead with apparent viral pneumonia (28). Following antigenic and genetic characterisation, the authors concluded these too were avian viruses that had entered the seal population.

Two viruses of H13N2 and H13N9 subtypes were isolated from a single beached pilot whale, and genetic analysis indicated that the viruses had been introduced recently from birds (33, 73).

Mink

In October 1984, outbreaks of respiratory disease affected approximately 100,000 mink on 33 farms situated in close proximity along a coastal region of southern Sweden, with 100% morbidity and 3% mortality (94). Influenza A viruses of H10N4 subtype were isolated from the mink and genetic
analysis indicated that the viruses were of avian origin and were very closely related to a virus of the same subtype isolated from chickens and a feral duck in England in 1985 (16). Earlier experimental infections had suggested that mink were susceptible to infection with avian influenza viruses (119).

Humans

**Historical background**

Until 1996, no reports existed of naturally acquired infections of humans with avian influenza viruses by direct transmission from birds. Evidence of antibodies to influenza subtypes known only to infect birds had been detected in serum samples taken from families with household ducks and pigs in the south of the People's Republic of China (149, 157), but this may have occurred following passage of the virus through pigs. Beare and Webster performed experimental infections on human volunteers and reported only transitory infections with little or no clinical signs and no antibody production (13). However, reports of ostensibly avian viruses infecting humans or infections as a result of laboratory accidents have been made on several occasions.

Campbell et al. reported the isolation of a virus of H7N1 subtype from a man suffering from hepatitis, but with no antibody response to that virus (29). A female laboratory worker in Australia developed keratoconjunctivitis after accidentally splashing infective allantoic fluids containing A/chicken/Victoria/76 (H7N7) on her face. Virus was isolated by swabbing the conjunctiva, but significant antibody titres were not obtained (171). A harbour seal infected experimentally with A/seal/Massachusetts/1/80 (H7N7), and known to be shedding that virus, sneezed directly into the face and right eye of an investigator who subsequently developed conjunctivitis (186). The virus was isolated from the affected eye up to four days after the incident, but no antibody response was detected. As discussed above, the virus was known to be of avian origin. Four field workers autopsying seals during the outbreak of 1980 also developed conjunctivitis, but no virology was performed (185). Since 1996, three accounts have been recorded of avian influenza viruses apparently affecting humans as a result of spread directly from birds.

**H7N7 in England**

Kurtz et al. reported the isolation of an influenza virus, A/England/268/96 (H7N7), from the eye of a forty-three-year-old woman with conjunctivitis (96). Partial nucleotide sequence of the haemagglutinin gene of 268/96 showed all seven genes to have closest homology with an H7N7 virus isolated from turkeys in Ireland in 1995 (12). Kurtz et al. considered the most likely source of the virus to be waterfowl, as the woman tended a collection of twenty-six ducks of various breeds which mixed freely with feral waterfowl on a small lake. The infected woman did not have measurable antibodies to H7 five weeks after the appearance of conjunctivitis. Cloacal swabs taken from her ducks one month after the isolation of virus from the affected eye did not yield any virus.

**H5N1 in Hong Kong**

Outbreaks of HPAI, caused by virus of H5N1 subtype, occurred on three chicken farms in Hong Kong from March to May 1997, with mortality ranging from 70%-100%, and subsequent spread to live bird markets in Hong Kong (35, 155). In May 1997, a three-year-old child died of apparent viral pneumonia with severe complications after admission to hospital in Hong Kong (197). An influenza virus of H5N1 subtype was isolated from respiratory specimens taken from this patient and similar virus infections were confirmed by virus isolation and/or serology in seventeen other cases in humans which occurred in Hong Kong during November and December 1997; a further five patients died (155). Nucleotide sequencing of the virus from the index case showed all eight genes to be of avian origin (35), and demonstrated 99% homology of all eight genes with those of the H5N1 virus from chickens in Hong Kong (168). Analysis of the haemagglutinin and neuraminidase proteins of the sixteen viruses isolated from the eighteen cases indicated that all were essentially similar and appeared to have spread directly from poultry without cumulative changes that would indicate adaptation to the human host (15). All human isolates had the same multiple basic amino acids as the chicken isolates (15).

Investigations of the human infections with H5N1 in Hong Kong revealed no evidence of human-to-human transmission and each case was assumed to represent spread from domestic poultry (155). One of the surprising features of these infections was the severe clinical disease caused in humans. Both human and poultry H5N1 viruses, in common with most avian influenza viruses, were shown to bind preferentially to the N-acetylneuraminic acid-α2,3-,galactose linkages on sialyloligosaccharides (107), whilst conventional human influenza viruses bind to N-acetylneuraminic acid-α2,6Gal linkages; apparently this did not prevent penetrative virus replication in some of the infected humans. There was considerable fear that the virus would mutate to become transmissible either by mutation in one of the infected people or by reassortment with human influenza in a person infected with human influenza earlier or subsequently. To prevent this occurring, it was decided to remove the apparent source of the H5N1 virus, and all chickens in Hong Kong, approximately 1,500,000, were slaughtered.

**H9N2 in Hong Kong and other regions of the People's Republic of China**

Although no H5N1 viruses have been isolated from humans since December 1997, the problems of bird to human spread in the area were raised again in March 1999, when two young girls aged one and four years were admitted to hospital in Hong Kong with typical influenza-like symptoms. In both regions of the People's Republic of China.
cases, influenza A viruses of H9N2 subtype were isolated from respiratory tract samples. Both children made uneventful recoveries.

Subsequent to these reports from Hong Kong, it was revealed that five cases of human H9N2 infection had been recorded on the mainland of the People's Republic of China in August 1998.

Genetic analyses of the two Hong Kong H9N2 isolates have shown that these, like the H5N1 viruses, appear to be avian viruses which have infected humans directly (57, 124). As described earlier, influenza A viruses of H9N2 subtype have been widespread in domestic poultry throughout the world in recent years, but especially in Asia, including the People's Republic of China. Transfer to humans could quite possibly have occurred in other areas, but gone unobserved, as the level of monitoring in Hong Kong and the People's Republic of China had been considerably raised as a result of the H5N1 virus isolations from humans. The greatest threat of these infections is probably the risk of a dual infection with a conventional human virus, resulting in a reassortant virus with H9 subtype haemagglutinin, combined with all or some of the other genes from the human virus, thereby allowing transmission between humans.

Surveillance

Humans

Global surveillance of influenza is maintained by a network of laboratories sponsored by the WHO. The two primary goals of the programme are to gain an understanding of the epidemiology of influenza and to promptly isolate influenza viruses from new epidemics and distribute them for vaccine production (64). The programme is implemented through three WHO Collaborating Centres for Influenza Reference and Research located in London, Atlanta and Melbourne. The functions of these centres are as follows:

a) to collect and distribute information on the types of influenza virus which prevail in various countries world-wide
b) to advise on the strains to be included in influenza vaccines
c) to collect, store and study strains from various outbreaks and distribute these strains to interested laboratories
d) to educate visiting workers in research techniques.

The surveillance programme has now grown to include 110 designated National Influenza Centres in 80 countries. National centres are involved in the isolation and preliminary characterisation of influenza viruses from suspect cases of infection in humans and, if appropriate, in collection of viruses from regional laboratories within the country. The Centres provide the WHO with regular epidemiological reports and forward virus isolates for further analysis to one of the three Collaborating Centres.

Morbidity and mortality associated with influenza is used as an indicator of influenza activity and impact. Precise quantification of the impact of influenza morbidity and mortality is problematic, since laboratory confirmation is required for exact diagnosis. Many methods have been developed to provide estimates of the mortality or morbidity associated with influenza. These methods usually use baseline rates of mortality or morbidity to calculate excess rates of mortality or morbidity attributed to circulating influenza virus.

During recent years, the operation of the network has become increasingly focused on the annual consultation on influenza vaccine formulation (see the subsection entitled 'Immunoprophylaxis' below). In addition, surveillance of human and animal influenza in the proposed 'influenza epicentre' has been intensified and improved, thereby contributing to the early identification of two subtypes of influenza virus not recorded previously in humans.

Animals

Demonstration that the H2N2 and H3N2 pandemic viruses contained genes from an influenza virus of avian origin led to systematic, long-term global surveillance studies on the influenza viruses of birds and mammals, to determine the diversity of influenza A viruses in nature and whether a future pandemic strain of virus could be isolated from these animals in advance of the appearance of the strain in humans. A vast number of viruses have now been isolated from a wide variety of birds and a range of terrestrial and sea mammals. These studies are ongoing but are generally more localised and less formally structured than the equivalent schemes for the human population. However, international and national reference laboratories with a similar remit to those in the human sector have been established for avian and equine influenza viruses. These facilities have provided invaluable information for dealing with emergencies such as the early identification of a putative vaccine strain from birds following the infection of people in Hong Kong with H5N1 virus, and after vaccine failure in controlling equine influenza.

Prophylaxis and treatment

Two control measures are currently available for influenza in humans, namely: immunoprophylaxis with vaccines and chemoprophylaxis or therapy with antiviral drugs. Since the late 1940s, the principal preventive measures against influenza have been inactivated virus vaccines. The efficacy of vaccines has varied between 60% and 90% and has been dependent on the closeness of the 'antigenic match' between the vaccine virus and the epidemic virus. However, even for those not completely protected, vaccination reduces the severity of the disease, thereby reducing costs and mortality.
Immunoprophylaxis

Vaccination of people at high risk before each annual influenza season is currently the most effective measure for reducing the impact of human influenza. When vaccine and epidemic strains of virus are well matched, achieving high vaccination rates among closed populations can reduce the risk of outbreaks by inducing population immunity. This occurs when the overall number of susceptible people in a population becomes too low for virus to spread and infect a significant number of the susceptible individuals.

To maximise protection of people at high risk, they and their close contacts should be targeted for organised vaccination programmes. Influenza vaccination is strongly recommended for any person over six months of age who, because of age or an underlying medical condition, is at increased risk for complications of influenza. The high-risk group consists of the following: people over sixty-five years of age, residents of nursing homes and other chronic-care facilities, people with chronic disorders of the pulmonary or cardiovascular systems, and people who have suffered chronic metabolic diseases or immunosuppression (including immunosuppression as a result of medication) within the past year. In addition, people who may transmit influenza to those at high risk, i.e. hospital and nursing home personnel and household members of people in high-risk groups, should also be vaccinated. Other groups may be included on individual merit, such as pregnant women, people infected with HIV and foreign travellers.

Currently, in the UK and the USA, less than 30% of people in high-risk groups are vaccinated each year and more effective strategies are required for delivering vaccine to members of high-risk groups. In general, successful vaccination programmes have combined education for healthcare workers, publicity and education targeted towards potential recipients, and a plan for identifying people at high risk.

Two basic approaches for immunisation have been pursued, namely: the use of inactivated virus preparations and the use of live, attenuated viruses. At present, only vaccines prepared with inactivated or killed virus particles are licensed for use in the European Union and the USA. Numerous refinements in the production processes over many years have resulted in vaccines that are more highly purified and more predictable in their reactogenicity and immunogenicity (194).

Each year, the influenza vaccine is redefined to reflect changes in the antigenicity of circulating virus strains, thereby containing virus strains representative of influenza viruses believed likely to circulate in the forthcoming ‘influenza season’. Annual vaccination using the current vaccine is therefore necessary because of potential changes in the circulating viruses, but also due to immunity from previous vaccinations being relatively short-lived. At present, the vaccine consists of two type A viruses, H1N1 and H3N2, and one type B virus. The exact strains of these viruses to be used are identified by an international network of laboratories that maintain surveillance for new influenza virus variants throughout the world.

One dose of inactivated vaccine is generally sufficient to induce a protective immune response, although children occasionally require a booster (37). Unless the antigen used is entirely new, most adults will mount a booster antibody response, even to strains whose antigens are marginally different. Vaccine antibodies react against the HA and NA and the level of serum antibody has been shown to correlate inversely with the occurrence of established infection with influenza viruses (75). The composition of the vaccine rarely causes systemic or febrile reactions.

The effectiveness of influenza vaccine in preventing or attenuating illness varies, depending primarily on the age and immunocompetence of the vaccine recipient and the degree of antigenic similarity between the virus strains included in the vaccine and those circulating during the influenza season. When the match between vaccine and circulating viruses is close, influenza vaccine has been shown to prevent illness in approximately 70% of healthy children and young adults, whilst preventing hospitalisation for pneumonia and influenza among elderly people living in the community.

Chemoprophylaxis

Antiviral drugs, such as amantadine and rimantadine are effective against all type A influenza viruses (including H5N1 and H9N2). If employed at the beginning of an outbreak, these drugs can prevent disease and spread of the virus. Of those receiving these antivirals within 24 to 48 hours of the onset of the disease, 70% to 90% have greatly reduced symptoms. However, effectiveness is limited by the rapid emergence of resistant viral strains and by the adverse effects of the drugs themselves (66, 166). The recent addition of neuraminidase inhibitors (GG167 and GS4104) to the portfolio of antiviral drugs is an important development. Zanamivir (GG167) is efficacious against influenza virus (including H5N1) infection in humans and animals without apparent side-effects or the induction of resistant influenza A viruses under clinical trial conditions (58, 67). Antivirals offer the advantage of not interfering with antibody production since infection is not completely prevented.

Perspectives

The next pandemic

Prediction of the next pandemic strain of influenza is important to allow greater preparedness and successful vaccine prophylaxis. However, implicit in predicting the next virus subtype, or at least the next HA subtype, is a knowledge of how pandemic viruses emerge. As discussed earlier, several theories exist regarding how antigenic shifts occur and pandemics arise. The problem in correctly assessing the actual
mechanisms is that relatively little data is available, as viruses from only three pandemics exist, although recently at least some molecular data on the 1918 pandemic virus has become available (130, 170). As this important work progresses, further understanding of pandemics in general, and of the singular virulence of the 1918 virus in particular, is anticipated. The current consensus opinion appears to be that pandemic viruses arise as a result of transfer to humans of all or part of the genome of viruses adapted to another animal species, where the virus is capable or becomes capable of spread in a fully susceptible population, or at least in that proportion of the population that is susceptible. However, this does not explain why this transference appears to happen so rarely and why so few subtypes appear to circulate. Equally, if pigs are important as an intermediate host for the emergence of pandemic virus as a result of reassortment between avian and human viruses, an explanation is required to describe why the number of subtypes of avian influenza virus isolated naturally from pigs is limited to H1 and H3. The H5 and H9 viruses isolated from humans in Hong Kong indicate that direct avian influenza virus infection and reassortment with a human virus could also result in the emergence of pandemic virus. This in turn raises the question of why this appears to occur so rarely and why three natural transmissions were recorded between 1996 and 1999, but none appeared to be recorded before that date. Hosts of influenza A viruses and the potential for recombination and/or spread to humans are summarised in Table IV.

Data is insufficient to confirm any of the current popular theories on the emergence of pandemic influenza viruses or to dismiss any of the less likely theories. In addition, none of the theories are necessarily mutually exclusive, and it could be that these viruses arise by several different mechanisms.

Virulence

As discussed earlier in regard to susceptible birds, a very clear distinction exists between influenza viruses of low virulence which produce disease similar to that of mammals, and the viruses which cause HPAI which may result in mortality rates approaching 100% in infected flocks. The primary reason for this difference is the presence of multiple basic amino acids at the cleavage site of the haemagglutinin precursor, which in turn allows the virulent viruses to replicate systemically, while those low virulence viruses are restricted to replication in the respiratory and intestinal tracts (134). This finding in birds does have some implications for human infections. While it is clear that trypsin-like enzymes may bring about cleavage of the viruses with a single arginine, furin is the prime candidate for a ubiquitous protease responsible for cleaving HA0 with multiple basic amino acids, allowing systemic replication and ultimately death. Mammals, including humans, also have furin-like proteases capable of cleaving at multiple basic amino acid motifs. However, the question as to whether or not viruses with multiple basic amino acids at the HA0 cleavage site could cause systemic infections and highly pathogenic disease in humans remains unanswered, because the viruses known to have infected humans (H1, H2 and H3 subtypes) all have motifs at the cleavage site indicating they would only be cleaved by trypsin-like enzymes. The high mortality associated with the 1918 pandemic virus was thought to have been the result of a multiple basic amino acid HA0 cleavage site, but this proved not to be the case (130).

The obvious inference was that the very high mortality (6/18), amongst the people infected with the H5N1 virus in Hong Kong occurred because the virus was capable of systemic infection due to the known presence of multiple basic amino acids at the HA0 cleavage site, allowing cleavage to be mediated by one or more furin-like proteases. However, evidence that this was the case is lacking. Generally, the eighteen patients presented with severe respiratory symptoms and pneumonia appeared to be the principal cause of death, not unlike infections with 'common' influenza viruses. Similarly, infections of other mammals with avian influenza viruses give few clues to the significance of multiple basic amino acids at the HA0 cleavage site. An infection of harbour seals from 1978 to 1980 off the north-east coast of the USA, with H7N7 avian influenza, resulted in the death of an estimated 20% of the population. While this mortality rate is comparable to that which occurred in humans in Hong Kong, the HA0 cleavage site of the H7N7 virus did not have a motif containing multiple basic amino acids (189). Conversely, H7N7 viruses responsible for equine influenza type 1, for which A/equine/Prague/56 (H7N7) 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 (50).

Although the question of the virulence of the 1918 pandemic virus could not be answered by the HA0 cleavage site amino acid motif, another mechanism for cleaving viral HA0 that confers increased virulence of the virus, proposed by Goto and Kawaoka, could have some relevance (54). The authors investigated a variant human H1N1 virus which is neurotropic for mice and discovered that the NA binds and sequesters the ubiquitous protease plasminogen, leading to the production of high levels of plasmin at the surface of the virus. This in turn ensures efficient cleavage of the HA, enabling the virus to invade a wide variety of tissues. Plasminogen sequestration has not been found in any other human influenza virus, but it will be interesting to see if future genetic analyses reveal that the 1918 virus neuraminidase had the genetic markers for this property, namely: a carboxyl-terminal lysine and lack of a glycosylation site at position 146.

Surveillance

Clearly, careful surveillance could lead to the early identification of viruses representing candidates for a new pandemic virus. However, some problems arise in achieving the benefits of unrestricted surveillance in all species, and targeted surveillance therefore seems more appropriate. Nevertheless, no distinct markers for identifying a panzootic...
virus or a potential progenitor pandemic virus are currently known. Consequently, identifying a specific, narrow target for surveillance is difficult.

The numbers and variety of influenza viruses isolated from surveillance of wild birds have not been particularly helpful in the prediction of human influenza patterns, although this surveillance may be of importance from the point of view of disease in domestic poultry. Infections and trends of infection in domestic poultry may be of greater significance and importance to humans. The current widespread infections of commercial poultry flocks in many countries of Asia with H9N2 (see the subsection entitled 'Domestic poultry' in 'Ecology') may be extremely significant in view of the known infections of humans with virus of this subtype. Surveillance studies in other domestic animals may also be particularly pertinent, especially in pigs, in view of their known susceptibility to both avian and human viruses.

Given the apparent origin of three of the four pandemics which occurred in the 20th Century, and possibly some of the earlier pandemics, in the south of the People's Republic of China, Shortridge and Stuart-Harris proposed the concept of an influenza epicentre, i.e., a geographical area where birds, other animals and humans live closely together in conditions where viruses have the greatest opportunity to pass from one species to another (153). Surveillance and monitoring of influenza viruses in various species would seem prudent in that geographical region, although not to the exclusion of other countries and geographical areas.

Contingency plans

Pandemics of influenza exert extreme pressures on all aspects of society in terms of both public health and economy. Even the relatively mild pandemic of 1968 had enormous impacts in developed countries through absenteeism, loss of earnings, pressures on hospital services and costs of palliative medication. It is essential that governments draw up practicable contingency plans in advance of the next pandemic to deal with obvious essential planning such as the production and stockpiling of vaccine and antiviral chemicals, prioritisation of treatment and arrangements for the functioning of hospitals, emergency services and even government in the face of mass incapacitation of the working force. In addition, should mortality be comparable to the 1918 pandemic, the potential for hysteria and social upheaval amongst the population must not be overlooked. It is essential that any contingency plan for pandemic influenza should be reviewed frequently and regularly to assimilate new scientific knowledge in this fast-moving field.

Zoonoses récentes dues aux virus influenza A

D.J. Alexander & I.H. Brown

Résumé

La grippe est une maladie aiguë très contagieuse qui frappe les hommes ainsi que les animaux depuis des temps reculés. Les virus influenza, qui font partie de la famille des Orthomyxoviridae, sont répartis en trois types : A, B et C, selon les caractéristiques antigéniques de la nucléoprotéine. Les virus influenza de type A infectent une grande variété d'espèces animales (porcins, équidés, mammifères marins, oiseaux); chez l'homme, ils sont à l'origine de pandémies parfois devastatrices, comme celle qui fit plus de vingt millions de victimes dans le monde en 1918. Les deux antigènes de surface du virus, l'hémagglutinine et la neuraminidase, sont les plus importants par les propriétés immunologiques qu'ils confèrent à l'hôte et sont donc soumis aux plus fortes variations. S'agissant des virus influenza A, quinze sous-types de l'hémagglutinine et neuf sous-types de la neuraminidase, antigéniquement distincts, sont actuellement répertoriés; un virus possède un sous-type de l'hémagglutinine et un sous-type de la neuraminidase, combinés de manière apparentem aléatoire. Les virus isolés chez les mammifères présentent relativement peu de combinaisons de sous-types, tandis que chez les oiseaux, tous les sous-types, dans la plupart des combinaisons, ont été isolés. Au XXe siècle, des souches antigéniquement
différentes sont apparues soudainement chez l’homme, phénomène désigné comme cassure antigénique (shift) : en 1918 (H1N1), en 1957 (H2N2), en 1968 (H3N2) et en 1977 (H1N1) ; ces mutations ont chacune été à l’origine d’une pandémie. Des épidémies surviennent fréquemment entre deux pandémies, sous l’effet d’une dérive antigénique (drift) du virus prévalent. Les épidémies qui sévissent actuellement dans le monde sont dues aux sous-types H1N1 et H3N2 du virus influenza A ou au virus influenza B. Pour mesurer l’impact de ces épidémies la meilleure méthode reste le suivi épidémiologique des épisodes meurtriers de pneumonie et de grippe. Des études phylogénétiques montrent que des oiseaux aquatiques pourraient être la source de tous les virus influenza A affectant les autres espèces. On pense que l’apparition des souches responsables de pandémies chez l’homme est le résultat de l’un des trois mécanismes suivants :
- le réassortiment génétique (dû au fait que le génome du virus comporte plusieurs segments) de virus influenza A aviaires et humains ayant infecté le même hôte ;
- le transfert direct de l’ensemble du virus d’une autre espèce ;
- la réémergence d’un virus ayant déjà provoqué une épidémie dans le passé. Depuis 1996, les virus H7N7, H5N1 et H9N2 se sont transmis des oiseaux à l’homme, apparemment sans se propager dans la population humaine. De rares cas de transmission de l’homme aux animaux ont également été établis. Ces observations ont conduit à l’hypothèse que le réassortiment éventuel des virus humains et aviaires intervient chez une espèce intermédiaire, avec transfert ultérieur à l’homme. Les porcins jouent probablement ce rôle d’intermédiaire ; en effet, ces animaux peuvent servir d’hôtes à des virus pathogènes aviaires et humains ; de plus, il a été prouvé que les porcins sont impliqués dans la transmission entre espèces des virus influenza, notamment la transmission du virus H1N1 à l’homme. La surveillance mondiale de la grippe est assurée par un réseau de laboratoires placé sous l’égide de l’Organisation mondiale de la santé. La principale mesure prophylactique de la grippe chez l’homme est la vaccination, qui a pour objectif d’éviter l’apparition d’épidémies entre deux pandémies.

Mots-clés

Zoonosis recientes causadas por virus de la influenza A
D.J. Alexander & I.H. Brown

Resumen
La influenza (o gripe) es una enfermedad aguda y extremadamente contagiosa que viene afectando a hombres y animales desde tiempos remotos. Los virus causantes de esa enfermedad, pertenecientes a la familia Orthomyxoviridae, se clasifican, según las propiedades antigénicas de sus proteínas de nucleocápside, en tres tipos distintos: A, B y C. Los virus de la influenza de tipo A infectan a un amplio abanico de especies animales, que comprenden el cerdo, el caballo,
mamiferos marinos y aves, y provocan tambien ocasionalmente en el hombre pandemias de consecuencias devastadoras, como la de 1918 que causó la muerte de más de veinte millones de personas en todo el mundo. Las dos glucoproteínas de superficie del virus, la hemaglutinina y la neuraminidasa, son los principales antígenos que inducen inmunidad protectora en el huésped, y exhiben por consiguiente el mayor nivel de variación. Para los virus de la influenza A se conocen actualmente quince subtipos de la hemaglutinina y nueve subtipos de la neuraminidasa antigénicamente distintos. Un virus posee un solo subtipo de cada una de las proteínas, que parecen presentarse en cualquier combinación. Pese a que en especies de mamíferos se han aislado sólo unas pocas combinaciones de subtipos, en las aves se han obtenido todos los subtipos, asociados en casi todas las combinaciones posibles. El siglo XX ha presenciado en cuatro ocasiones la aparición repentina de cepas antigénicamente distintas en el ser humano, un fenómeno llamado “salto antigénico” (shift) que en cada ocasión ha dado lugar a una pandemia: en 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) y 1977 (H1N1). En los intervalos entre esas grandes pandemias también se han declarado frecuentes epidemias, producto de cambios antigénicos graduales en el virus prevalente, proceso conocido como “deriva antigénica” (drift). En la actualidad, las poblaciones humanas de todo el mundo sufren epidemias causadas por los subtipos H1N1 y H3N2 del virus de la influenza A y por el de la influenza B. Uno de los procedimientos más eficaces para medir los efectos de esas epidemias consiste en registrar el exceso de mortalidad debida a neumonías o gripes. Ciertos estudios filogenéticos sugieren que tal vez las aves acuáticas sean la fuente de todos los virus de la influenza A que afectan a las demás especies. Se piensa que las cepas causantes de pandemias en el hombre han surgido por uno de los tres mecanismos siguientes:
- reordenamiento genético (proceso resultante de la segmentación del genoma vírico) entre virus aviares y humanos de la influenza A presentes en un mismo hospedador;
- transferencia directa del virus entero desde otra especie al hombre;
- resurgimiento de un virus que pudo haber causado una epidemia muchos años antes.

Desde 1996, los virus H7N7, H5N1 y H9N2 se han transmitido de las aves al hombre, aunque en apariencia no han conseguido difundirse entre las poblaciones humanas. Aunque está demostrado que el virus puede transmitirse de animales a humanos, los episodios de este tipo son poco frecuentes. Ello ha llevado a pensar que el hipotético reordenamiento entre virus aviares y humanos tiene lugar en un animal intermediario, con posterior transmisión del virus resultante a la población humana. Muchos ven en el cerdo el principal candidato a esa función intermediaria, pues no sólo puede ejercer de hospedador de infecciones productivas de virus tanto humanos como aviares sino que, además, hay sólidas pruebas de su intervención en episodios de transmisión interespecífica de virus de la influenza, y particularmente en la transmisión de virus H1N1 al hombre. La labor de vigilancia de la enfermedad a escala mundial recae en una red de laboratorios patrocinados por la Organización Mundial de la Salud. La principal medida de lucha contra la gripe en el ser humano es la inmunoprofilaxis, destinada sobre todo a combatir la aparición de epidemias entre pandemias.

**Palabras clave**
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