Swine influenza: a zoonosis

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History and classification of the influenza virus

Influenza viruses are the cause of outbreaks of acute respiratory disease, known as influenza or 'flu', which has afflicted man and animals since ancient times. The name influenza has its origin in early fifteenth century Italy and was adopted in Europe to explain the sudden appearance of an epidemic disease thought to be under the influence of the stars. The viruses are classified as members of the family *Orthomyxoviridae* (from the Greek *orthos*, 'standard, correct', and *myxo*, 'mucus') because of their ability to bind to mucus, and to distinguish them from a second family of enveloped negative-strand RNA viruses, the *Paramyxoviridae* (reviewed in [22]). The *Orthomyxoviridae* are divided into two genera: influenza A and B viruses, and influenza C virus. The three virus types can be distinguished from one another on the basis of antigenic differences between their nucleoproteins (NP) and matrix (M) proteins.

Influenza B and C viruses are almost exclusively isolated from man, although influenza C virus has also been isolated from pigs and influenza B has recently been isolated from seals. Influenza A viruses, in contrast, infect a wide range of avian and mammalian species, with the latter group including man, pigs, horses and aquatic mammals [13]. The type A viruses are further divided into subtypes, based on the antigenic nature of their surface glycoproteins haemagglutinin (HA) and neuraminidase (NA). So far, 15 different HAs (H1 to 15) and 9 NAs (N1 to 9) have been identified among all influenza viruses.

Despite influenza being an important disease of man, the virus was first isolated from poultry. A disease causing extremely high mortality in domestic fowl was first described in 1878 and became known as 'fowl plague'. As early as 1901 the causative agent was shown to be an ultra-filterable agent (i.e. 'virus'), although it was not until 1955 that the close relationship between this agent and mammalian influenza A viruses was demonstrated. Isolation of influenza virus from pigs also preceded that from man. The virus was the causative agent of a 'new' disease of pigs, termed 'swine influenza'. It gave clinical signs similar to those observed in man, which were described for the first time during the 1918 human pandemic. In 1930, Shope demonstrated that swine influenza virus could be transmitted between pigs using ultrafiltered material [33], and three years later a virus shown to be related to that from the pig was eventually isolated from a human patient. The virus was cultured by inoculating a filtrate of the patient's throat washings into the noses of ferrets, which are highly susceptible to influenza virus [34]. The isolate was later classified as influenza A virus and was followed in 1940 and 1947 by the isolation and classification of influenza B and C viruses, respectively.

The current system of nomenclature of influenza viruses was introduced in 1980 and designates the type, host, place, strain number (if any), year of isolation and antigenic subtype of a virus. For example, a swine influenza virus isolated in Wisconsin in 1984 would be designated A/Swine/Wisconsin/1/84(H1N1).

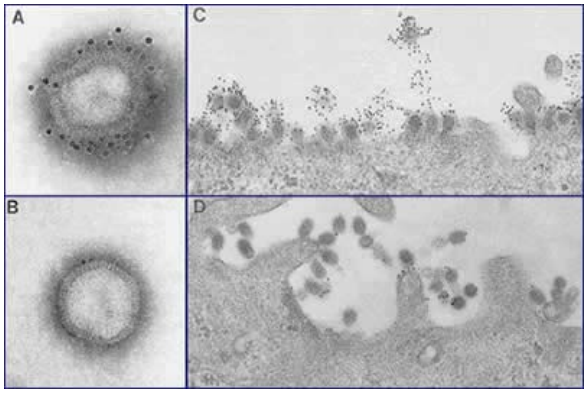

Figure 1. Electron micrographs of purified influenza virions (A and B, magnification: x 159,250) and virions budding from the surface of Madin-Darby canine kidney (MDCK) cells (C and D, magnification: x 40,600). The influenza virus has been stained with 10 nm gold labelled antibodies to (A) the surface glycoprotein haemagglutinin (HA) and (B) the transmembrane protein matrix protein 2 (M2). Courtesy of George Leser, Northwestern University, Evanston, IL.

The virus

An enormous amount of information is available the antigenic, genetic, structural and biological characteristics of influenza A viruses (for review, see [22, 27]). They have a

spherical or filamentous morphology and are medium-sized, with a diameter of 80 to 120 nm (Figure 1). The virus is enveloped, and the lipid membrane of the virion is derived from the host cell in which the virus replicated. From the surface of the envelope extend the two transmembrane glycoproteins HA and NA, which are commonly called 'spikes' (Figure 2). A third transmembrane protein, matrix protein M2, also exists but only 20-60 molecules per virion are present. The matrix protein M1 forms a layer beneath the envelope and so gives structure to the virus and encapsidates the ribonucleoprotein (RNP) complexes. RNP complexes consist of ribonucleic acid (RNA) associated with nucleoprotein (NP) as well as the polymerases PA, PB1 and PB2 that are responsible for RNA replication and transcription. Two non-structural proteins are also associated with the virus: NS2 is found in the virion while NS1 is found only in infected cells. The influenza virus genome consists of eight unique segments of single-stranded RNA which have negative polarity. Each RNA strand encodes only one protein, except for strands 7 and 8 which encode two (Table 1).

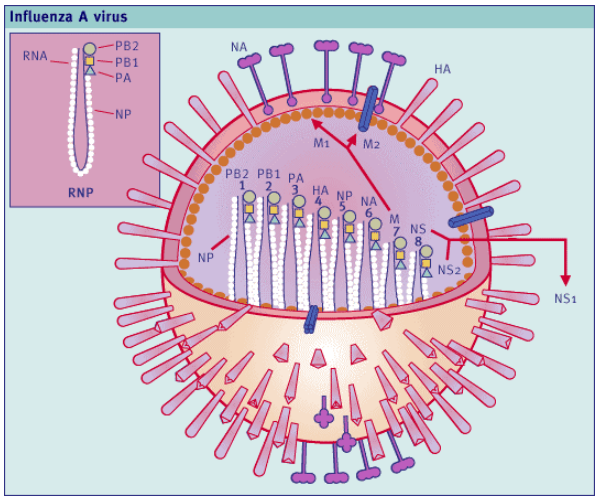

Figure 2. Schematic representation of influenza A virus. (Reproduced with permission from Fields Virology. Third edition. Lippincott Williams and Wilkins, Philadelphia [27]).

Influenza A virus gene segments and encoded proteins

RNA segment	Nucleotides	Protein	Amino acids	Molecules per virion
1	2341	polymerase PB2	759	30-60
2	2341	polymerase PB1	757	30-60
3	2233	polymerase PA	716	30-60
4	1778	haemagglutinin HA	566	500
5	1565	nucleoprotein NP	498	1000
6	1413	neuraminidase NA	454	100
7	1027	matrix protein M1	252	3000
		matrix protein M2	97	20-60
8	890	non structural protein NS1	230	-
		non structural protein NS2	121	130-200

Table 1. Influenza A virus gene segments and encoded proteins (adapted from Lamb and Krug, 1996 [22])

The replication cycle of influenza virus starts with the cleavage of HA into HA1 and HA2 by enzymes present in the respiratory tract. The enzymes are produced by the host but may also be derived from bacteria, which, therefore, can promote the influenza infection. Virus grown in cells that lack a cleavage enzyme can be activated by treatment with trypsin. After HA cleavage, the receptor-binding site of HA1 can attach to a terminal sialic acid residue of a cell surface receptor; once attached to the host cell the virus is endocytosed (receptor-mediated endocytosis) (Figure 3). NA functions as a receptor-destroying enzyme by cleaving terminal sialic acid residues from the receptor. Thus, NA releases progeny virions from the host cell in which they arose and facilitates virus spread. The progeny virions can infect other cells or can be transmitted to another individual.

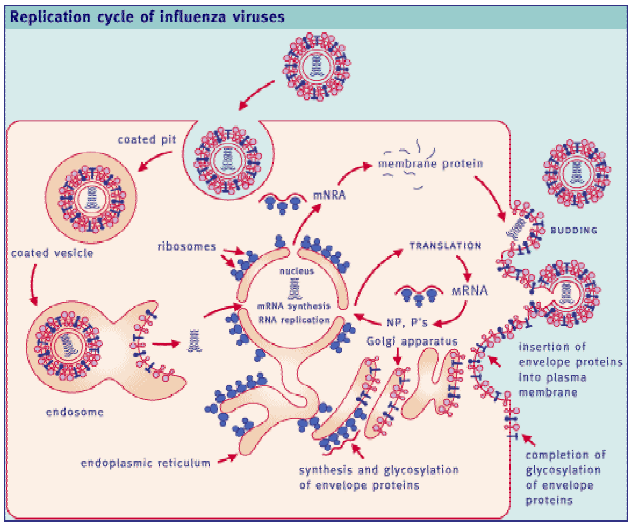

Figure 3. Schematic diagram depicting the replication cycle of influenza viruses. (Reproduced with permission from Fields Virology. Third edition. Lippincott Williams and Wilkins, Philadelphia [27]).

Host range and interspecies transmission

Ducks and other waterfowl are the principal natural hosts of influenza A viruses (Figure 4), with all 15 HA and 9 NA virus subtypes circulating among them. Unlike in other species, influenza viruses target the gastrointestinal tract of waterfowl rather than the respiratory tract and the infections are almost without exception completely subclinical, some ducks shedding virus for as long as 30 days. This, together with the migratory behaviour of waterfowl and the ability of influenza viruses to persist in cold lake water, contributes to the fact that waterfowl form an immense reservoir for influenza viruses in nature. From this natural reservoir viruses are sometimes transmitted to other host species in which they less often

continue spreading and sporadically cause high mortality. There are reports of direct virus transmission from waterfowl to pigs, horses, mink, domestic poultry and aquatic mammals, with associated infections of varying severity.

Occasionally, avian-derived influenza A viruses are transmitted directly to man and one recent example is the H5N1 virus isolated in 1997 from patients in Hong Kong [7, 9, 35]. Six out of eighteen people to become infected died. The same H5N1 virus caused an influenza outbreak on Hong Kong's chicken farms and resulted in a high mortality of birds. In an attempt to eradicate the disease, all of Hong Kong's poultry (approximately 1.5 million birds) were slaughtered, which may well have prevented adaptation of the avian H5N1 virus to man and, thus, a subsequent human pandemic. Another example of direct interspecies transmission of a virus was reported recently when an H9N2 virus of avian origin infected two children in Hong Kong and five people in China. Perhaps the greatest threat these infections pose is the risk of a dual infection with a human influenza virus. The result can be a reassortant virus with H5 or H9 haemagglutinin, combined with all or some of the genes from the human virus, thereby enabling transmission between humans (see below).

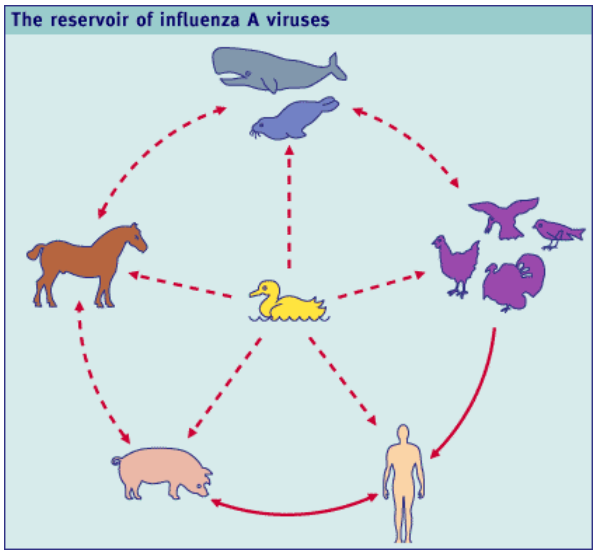

Figure 4. Schematic diagram to show the natural reservoir of influenza A viruses and the interspecies transfer of the virus. It is hypothesised that wild aquatic birds are the primordial reservoir of all influenza A viruses and interspecies transmission of the virus is known to have occurred from pigs to man, and vice versa, and from poultry to man. There is also extensive evidence of transmission between other species. (Adapted with permission from Murphy and Webster, 1996. In Fields Virology. Third edition. Lippincott Williams and Wilkins, Philadelphia [27]).

It remains uncertain which of the influenza virus proteins restrict host range. The available evidence indicates it is a polygenic trait and the receptor specificity of HA is considered an important determinant. Although influenza A viruses uniformly recognise cell surface oligosaccharides with a terminal sialic acid, their receptor-specificity varies. Most avian and equine viruses preferentially bind to the N-acetylneuraminic acid-α2,3-galactose (NeuAcα2,3Gal) linkage on sialyloligosaccharides, while human and swine influenza viruses prefer the NeuAcα2,6Gal linkage. Some amino acid substitutions responsible for this difference in receptor specificity have been identified. Substitutions identified in H1 were also found in early isolates of H1N1 human and swine viruses, suggesting they are important for the generation of H1 human pandemic strains [24]. It has also been proposed that the NP is a major determinant in host range restriction.

Reassortment, antigenic shift, antigenic drift, pandemics and epidemics

The two surface glycoproteins of the influenza virus, HA and NA, are the most important antigens for inducing protective immunity in the host and show the greatest variation. Pandemics of influenza arise from the introduction of a virus capable of replication and spread, and for which all, or a large proportion, of the population have no immunological experience, at least of the functionally important HA molecule.

The sudden emergence of antigenically different strains of influenza virus in man, termed antigenic shift (Figure 5), is thought to have occurred through one of three mechanisms: i) **Direct transfer** of whole virus from another species. This is probably what occurred in 1918 when the H1N1 'Spanish flu' virus entered the human population. It resulted in the most devastating pandemic ever known and was responsible for an estimated 20 to 40 million deaths. ii) **Genetic reassortment** of avian and human influenza A viruses infecting the same host. The genome of the influenza viruses is segmented and therefore gene segments can be exchanged in mixed infections with different strains of the viruses. When two viruses infect the same cell, progeny viruses may inherit sets of segments made up of combinations of segments of the parent viruses. As a result of reassortment, the H2N2 'Asian flu' virus, responsible for the pandemic of 1957, acquired the HA, NA and PB1 gene segment from an avian virus and kept the other five segments from the human H1N1 strain already in circulation. The H3N2

'Hong Kong flu' virus of 1968 acquired HA and PB1 segments of avian origin and six from the H2N2 virus. iii) **Re-emergence** of a virus that may have caused an epidemic many years earlier. The H1N1 'Russian flu' virus, for example, re-emerged in 1977 after having been in circulation in man prior to 1950. It is believed the virus might have escaped from a laboratory.

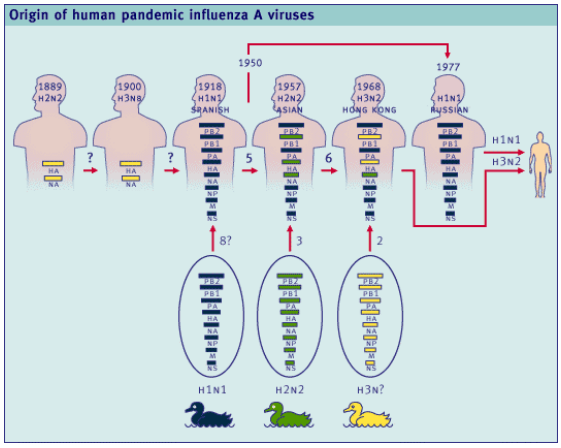

Figure 5. Schematic diagram illustrating the origin of human pandemic influenza A viruses. (Reproduced with permission from Fields Virology. Third edition. Lippincott Williams and Wilkins, Philadelphia [27]).

The influenza outbreaks of 1957 and 1968 saw the introduction of a new influenza virus coincidental with the disappearance of the earlier subtype. This was not the case, however, in 1977, and means that currently both H3N2 and H1N1 viruses are in circulation. The reason for the sudden disappearance of the previously circulating subtypes is unknown, but the earlier strain is possibly disadvantaged because it has already elicited widespread immunity in the population. In addition, subtype cross-reactive (heterosubtypic) immunity may not allow the co-circulation of subtypes in a limited population.

The emergence of new subtypes of influenza virus and the ensuing pandemics are unmistakable, but epidemics that occur between pandemics may still be severe. They result from the gradual change in antigenicity of the circulating virus after successive point mutations in the HA molecule, until finally the virus is sufficiently different from earlier strains so that a large proportion of the population is susceptible and cases reach epidemic levels. This accumulative variation is termed antigenic drift, and the size and severity of the epidemic will depend on the degree to which the virus is different from those already experienced by the population.

History of swine influenza	
1918	• Swine influenza H_1N_1 described in north central USA, Hungary, and China. May have been cause of human pandemic [19], which resulted in 20-40 million human deaths.
1930	• Shope isolated influenza virus from pigs [33]. The prototype classic swine influenza H_1N_1 strain (A/Swine/Iowa/30) transmitted experimentally to pigs.
1941	• Recognised in Europe and disappeared.
1970	• Transmission of human H_3N_2 virus to pigs. Avian-like H_3N_2 in pigs in Asia.
1976	• Classical H_1N_1 reappears in European pigs.
1979	• Introduction of whole H_1N_1 virus from birds to pigs. Antigenically distinguishable from classical strains. Still circulating today (2002).
1984	• Reassortment between human H_3N_2 and avian H_1N_1 in swine resulting in reassortant H_3N_2 virus with avian internal gene segments [5]. H_3N_2 strains first associated with respiratory epizootics. Still circulating today (2002).
1986	• Classical H_1N_1 reappears in UK, similar to classical H_1N_1 in continental Europe.
1987	• Reassortant H_3N_2 associated with respiratory epizootics in UK. Related to A/Port Chalmers/73(H_3N_2).
1989	• Avian-like swine H_1N_1 is dominant and widespread in Europe.
1992-1993	• Avian-like H_1N_1 strains widespread in UK.
1993	• Infection of children with reassortant H_3N_2 virus from pigs and isolation of avian-like swine H_1N_1 virus from a pneumonia patient in the Netherlands.
1994	• H_1N_2 first isolated in pigs in UK, and later also in Belgium. Human-avian reassortant virus [3, 37].
1992-1998	• H_3N_1 (H_3 human, N_1 swine) and H_1N_7 (H_1 human, N_7 equine) also occurred in swine in the UK but failed to spread.
1998	• H_9N_2 in pigs and humans in Asia [17]. Apparently an avian virus that has adapted to pigs.
1998	• For the first time, H_3N_2 viruses cause severe disease in N. America. Viruses are triple (avian-human-classical swine) reassortants, distinct from earlier strains and European strains. H_1N_2 identical to H_3N_2, but with H_1HA from classical swine H_1N_1, also isolated.
1999	• Single case of isolation of avian H_4N_6 from pigs with pneumonia in Canada.
2002	• Current situation in Europe: avian-like H_1N_1, and reassortant human-like H_3N_2 and H_1N_2. In North America: classical swine H_1N_1, triple reassortant H_3N_2.

Table 2. Salient points in the history of swine influenza (adapted from Done and Brown, 1999 [11])

Swine influenza and public health implications

Swine influenza was first observed in 1918 at the time of the human pandemic and the virus was isolated and identified in 1930 by Shope ([33]; Table 2). This H1N1 virus was the prototype strain of a group of viruses now known as classical swine influenza viruses. Serological studies have shown that classical swine H1N1 is prevalent throughout the major pig populations of the world, with 25% of animals demonstrating evidence of infection. In the US the viruses have remained antigenically conserved, in Europe, however, they disappeared, reappeared in 1976, and were replaced in 1979 by avian-like swine H1N1 viruses that are antigenically distinguishable from classical swine H1N1 viruses (Figure 6). Around 1970, following the human 'Hong Kong' flu pandemic, the human H3N2 virus was transmitted to pigs. This human-like swine H3N2 virus continued to circulate, particularly in Europe and Asia, but only sporadically caused clinical signs. It has only started causing clinical disease since 1984, probably as a result of a reassortment with the avian-like swine H1N1 virus. The new virus was a reassortant human-like swine H3N2 virus with the HA and NA of the human virus and all the internal proteins of the avian virus (Figure 6; [5]). It has since replaced the original H3N2 virus in Europe. It was only recently that H3N2 started to circulate in the US, where it has caused serious illness and

reproductive losses in sows. The viruses evolved from reassortments involving classical H1N1 and human H3N2 viruses and are antigenically and genetically distinct from the European human-like H3N2 viruses.

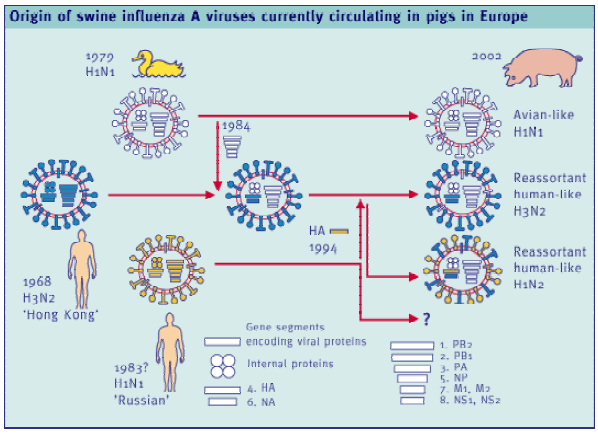

Figure 6. Schematic diagram illustrating the origin of swine influenza A viruses currently circulating in pig herds in Europe. In 1968, a H3N2 'Hong Kong' influenza virus was transmitted from man to pigs and started to circulate within the pig populations. In 1979, an H1N1 virus was transmitted from ducks to pigs. In 1984, a reassortment occurred when the H3N2 virus acquired all proteins from the H1N1 virus, except for the haemagglutinin (HA) and neuraminidase (NA) molecules. In 1994, an H1N2 virus was isolated in the UK and subsequently in Belgium. This virus has become increasingly important in the UK and to a lesser extent in Europe. The virus arose from a reassortment of a human 'Russian' H1N1 virus with a reassortant human-like swine H3N2 virus. Only the HA was derived from the human H1N1 virus, phylogenetic analysis of which has suggested it had been circulating in pigs since the early 1980s. It is possible that it is still circulating. The three viruses that resulted from these events are listed on the right hand side. They regularly cause outbreaks of respiratory disease on pig farms in Europe.

In recent years a reassortant human-like swine H1N2 virus has become increasingly important in Europe. It was first described in the United Kingdom and subsequently in Belgium. The virus resulted from a reassortment involving a human H1N1 virus left over from the 1980s and a reassortant human-like H3N2 virus. Only the HA of the reassortant human-like H3N2 was replaced. Similarly, in the US, shortly after the appearance of the reassortant H3N2 viruses, an H1N2 virus was isolated that had arisen from reassortment of a classical swine H1N1 virus and a reassortant H3N2 virus.

Today's pig husbandry systems provide easy pig-to-pig virus transmission, which together with the high frequency of contact with other species, particularly man, provides an ideal opportunity for co-circulation of viruses and genetic reassortment. Whereas reassortment and antigenic shifts occur frequently in pigs, antigenic drift is less pronounced in the species compared to man. The availability of new susceptible piglets produces relatively low immune pressure and therefore may explain the reduced antigenic drift.

Swine influenza is a zoonosis for which pigs may act as an intermediate host and 'mixing vessel' for genetic reassortment between human and avian viruses [32]. There is strong evidence to support this: i) Pigs are often in close contact with both poultry and man. Especially in China, 'the influenza epicentre' from where new virus strains originate, pigs are often kept close to people and ducks. ii) The same virus strains can infect and spread in both man and pigs, for example both the 1918 H1N1 and 1968 H3N2 human pandemic strains continued to circulate in pigs. iii) Although man can be infected with true avian viruses, they have never been known to spread in man, unlike swine viruses (Fort Dix, New Jersey, 1976). iv) Genetic reassortment of influenza viruses occurs frequently in pigs [3, 5, 40]. v) Pigs have cell surface receptors for both human and avian viruses [18] and they appear to host a broader range of NP genes in reassortant viruses than either man or birds [31]. vi) It has been reported that an avian-human reassortant from pigs in Europe caused disease in two children [6]. The involvement of reassortment in at least the human pandemics of 1957 and 1968 shows that it is a successful event from the virus' point of view and therefore can be expected to underlie the next possible human pandemic. For example, the presence in pigs of H9N2 subtype influenza viruses similar to those transmitted from birds to man [17] is a potential threat to man.

In addition to being a possible source of reassortant influenza viruses, pigs could also be involved in the 'direct transfer' of avian viruses. Influenza in swine was first observed in the US during the catastrophic 1918 to 1919 human influenza pandemic. Genetic analysis suggests that the influenza virus that caused the Spanish flu of 1918 was not a reassortant but a complete 'avian' virus. Sequences of gene segments of an influenza virus isolated from a soldier who died in 1918 revealed it to be genetically similar to swine influenza viruses. However, it remains unclear whether these viruses appeared first in man and then spread to pigs or vice versa.

Finally, pigs could also be involved in the re-emergence of a virus that many years earlier had caused an epidemic. Pigs can be a reservoir in which old human influenza strains are maintained and then re-introduced in the human population when immunity has disappeared. After the 1918 human pandemic in the US, H1N1 influenza viruses have

continued to infect pigs there ever since the 1930s (classical swine influenza H1N1) and human infections with swine influenza viruses have been documented in the US at least nine times since 1974, including fatal infections, as well as in Europe and in New Zealand. In 1976, several hundred US military recruits were infected with an influenza virus, A/NJ/8/76(H1N1), closely related to swine influenza viruses [16, 21]. Consequently, a national programme to vaccinate the population against A/NJ/8/76(H1N1) virus and a surveillance programme to monitor pigs and their human contacts were initiated. In addition, data suggest that zoonotic swine influenza infections occur more often among people in regular contact with pigs than the number of documented cases indicate [28, 30]. Influenza viruses of the H3N2 subtype have also persisted in pigs many years after their antigenic counterparts caused the 'Hong Kong' flu. Thus, pigs provide a reservoir of influenza viruses and viral gene segments which may in the future be transmitted to a susceptible human population.

Clinical signs

The disease caused by influenza viruses in pigs is essentially similar to that in man, albeit generally milder. It is an acute febrile, respiratory disease characterised by fever (usually in the range 40.5-41.7 ºC), apathy, anorexia and laboured breathing. Coughing may be apparent during the later stages of disease. Clinical signs seen less frequently include sneezing, nasal discharge and conjunctivitis.

Morbidity may be up to 100% but the mortality rate is low and recovery rapid, usually five to seven days after the onset of clinical signs. Secondary bacterial infections can increase the severity of illness and may result in complications such as pneumonia. The gross lesions found in uncomplicated swine influenza are mainly those of a viral pneumonia and are most often limited to the apical and cardiac lobes of the lungs, although in severe cases more than half of the lung may be affected. The altered lung areas are depressed and consolidated and are dark red or purple-red in colour, contrasting sharply with normal tissue. The airways are likely to be dilated and filled with blood-tinged, fibrinous exudate. The associated bronchial and mediastinal lymph nodes are usually enlarged.

The extremely high morbidity associated with swine influenza has serious economic consequences because of the increased time needed for infected animals to attain slaughter weight. In the UK, the cost has been estimated at up to £7 per pig, accounting for a financial loss to the pig industry of approximately £65 million each year [20].

Epizootiology

Swine influenza is widespread and endemic in pig populations worldwide and is responsible for one of the most prevalent respiratory diseases in pigs. In Europe, serologic examination of finishing pigs has revealed the prevalence of the H1N1 and H3N2 strains to be, respectively, 92% and 57% in Belgium (1996), 73% and 62% in Spain (1992), 55% and 51% in Germany (1993), and 60% and 30% (1990) or 54% and 13% (2001, Loeffen, personal communication), in the Netherlands. Although many infections may be subclinical, a field survey performed in the Netherlands during the winter of 1995-96 revealed that swine influenza virus is indeed a major cause of outbreaks of acute respiratory disease in finishing pigs [23]. Swine influenza H1N1 and H3N2 infections were responsible for half of the cases of acute respiratory disease, which was confirmed in a follow-up study conducted between 1996 and 2000 (2001, Loeffen, personal communication).

Swine influenza is related to the movement of pigs from infected to susceptible herds and 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 [14]. Infection is often subclinical and typical signs are seen in only 25 to 30% of a herd. Disease transmission is primarily direct and occurs via the nasopharyngeal route through the dispersal of aerosols formed either directly during coughing or sneezing or indirectly after physical contact. Nasal secretions are laden with virus during the acute febrile stages of infection and virus excretion lasts for approximately 6 days. The severity of clinical disease is influenced by many factors but most importantly by maternal immunity, virus strain, route of inoculation and secondary bacterial infections. Maternal colostrum-derived antibodies decrease the severity of disease and typical clinical signs are generally limited to seronegative pigs [14].

Of the viruses circulating in the UK, avian-like H1N1 has caused more severe clinical disease outbreaks than either the classical H1N1 or the reassortant human-like H3N2 viruses. Classical H1N1 and reassortant H3N2

produced minimal gross lesions and mild interstitial pneumonia [12] while avian-like H1N1 produced marked gross lesions and more severe histopathological changes [2]. The amount of virus that reaches the deeper airways and the resulting production of infectious virus in the lungs determine the severity of illness. Although influenza virus replicates throughout the respiratory tract, the lungs seem to be the major target organ for its replication. It has been reported that experimental inoculation of pigs with high virus quantities via the nasal route results in subclinical infections while intratracheal or aerosol inoculation produces typical clinical signs. In man, the infectious dose administered intranasally is 127 to 320 $TCID_{50}$ and by aerosol it is 0.6 to 3 $TCID_{50}$.

Immune responses

Much of our knowledge about immune mechanisms operating against influenza virus has been gained from studies of man and rodents. However, the importance of pigs in agriculture has resulted in a substantial increase in research on the swine immune system during the past few years. Many reagents used to study immunity in man and rodents have also been developed for pigs and, therefore, the roles of antibodies and T-lymphocytes in immunity against many viral, bacterial and parasitic infections could be better investigated in pigs (reviewed in [4, 29]). Although limited differences may exist between immune responses in pigs and man, the general mechanisms are the same. Furthermore, the course of infection with influenza A virus in pigs is similar to that in man [36] and the same influenza virus strains naturally infect pigs and man. Rodents, on the other hand, are not natural hosts to the viruses and infection typically leads to lethal pulmonary infection. The pig, therefore, offers a highly valuable animal model to study pathogenesis of, and immunity to, influenza in man.

Immune responses to infectious agents can be divided into non-specific innate responses and specific acquired responses. Non-specific immune responses to influenza virus infection include the production of cytokines, particularly interferons, and the activation of natural killer (NK) cells. NK cells kill infected cells, so limiting replication and spread of the virus. Interferons are produced early in infection and may reduce viral spread by inducing an antiviral state in host cells and by activation of CTLs and NK cells, thereby contributing to recovery from infection.

In addition to these non-specific immune mechanisms, influenza specific antibody responses and cellular immune responses are needed for the ultimate elimination of the virus. Antibodies contribute in two ways: i) By binding to infected cells and thereby reducing the production of progeny virus; this is termed cell-targeting (CT) activity; and ii) by binding to released progeny virus and thereby inhibiting the spread of the infection; this is known as virus neutralising (VN) activity [26]. Most of the antibodies produced are directed against the HA, NA, NP and M proteins. Neutralising antibodies against the HA of influenza virus are highly effective in clearance of and resistance to infection and/or illness, whereas antibodies to NP and M1 are not. Non-neutralising antibodies to the HA and NA, as well as to the extracellular part of the M2, also contribute. It has been suggested that the synergism between CT and VN activity underlie the high efficacy of HA-specific VN antibodies in combating influenza infection [26]. Not surprisingly, epitopes on the HA molecule that are recognised by virus neutralising antibodies are the most variable.

After primary viral infection, antibodies of the IgM isotype are the first to be produced, followed by those of antibodies of the IgA and IgG isotypes. IgM antibodies are highly efficient in aggregating virions and in mediating lysis of infected cells by complement via the classical pathway. IgA antibodies function mainly by binding to released virions (reviewed in [8]), and aggregated IgA promotes phagocytosis by binding to polymorphonuclear neutrophils (PMN). In addition, IgA can mediate cellular lysis by complement via the alternative pathway. IgG antibodies can bind to released virions, promoting phagocytosis by PMN and macrophages, and mediate cellular lysis by complement via the classical pathway. They also mediate antibody dependent cellular cytotoxicity (ADCC) by NK cells.

Antibodies are transferred from the sow to the newborn piglets by means of colostrum. This passively acquired maternal immunity protects the piglets from clinical signs when infected during the first months of their life. The antibodies concerned are mainly of the IgG isotype and generally do not prevent infection with influenza. Although maternal immunity is beneficial to the piglet by allowing it to acquire active immunity without developing disease, it is disadvantageous for vaccination because it inhibits the induction of active immunity and can greatly reduce vaccine efficacy.

The cellular immune response to influenza

infection includes influenza virus-specific T helper (Th) cells. They contribute to the clearance of the infection primarily by stimulating antibody and cytokine production and proliferation of cytotoxic T lymphocytes (CTLs). It has been reported that Th cells specific for NP or M proteins can stimulate B cells specific for HA. Although a subset of $CD4^+$ cells has been shown to be cytotoxic to cells infected with influenza virus, Th cells alone cannot clear a virus infection. CTLs kill virus-infected cells when they recognise the viral peptides that are presented to them via MHC class I. They help to clear virus from the respiratory tract and accelerate recovery from infection. CTL epitopes are less abundant than B and Th cell epitopes and are mainly concentrated in the more conserved influenza virus proteins, such as the NP. CTLs directed against conserved antigens have been shown to confer protection against heterosubtypic influenza A viruses. In addition, numerous experiments in mice have demonstrated that CTLs can clear as well as protect against virus infection in the absence of B and Th cells. A recent study showed that just as mutations in HA and NA allow escape from antibody-mediated immunity, mutations in CTL epitopes occur to avoid CTL-mediated immunity, giving credence to the belief that CTLs have a significant protective role [38]. However, despite the fact that heterosubtypic CTLs are induced following infection, they do not confer long-lived immunity against infection in man [25].

Long-lived immunity against the infecting virus is established after recovery from primary infection. The immune system has adapted its effectors to optimally combat the virus and some of them will continue to circulate for a considerable time, in particular IgG antibodies, while the quantity of IgA antibodies in the respiratory tract and the number of circulating Th and CTL will gradually drop. Virus-specific IgA (and IgG) antibody secreting cells (ASC) as well as Th and CTLs will be present in the tissues lining the respiratory tract and in the local lymph nodes. The immune system has by now built up a memory that will immediately recognise the influenza virus upon secondary contact, leading to a faster, stronger, more localised and more accurate secondary response. The individual is likely to have life-long protection from clinical signs of a secondary infection with the same strain. When, in 1977, the H1N1 virus reappeared in the human population, the people who had been infected 20 years previously with H1N1 were still resistant to infection or disease [15].

Vaccination strives to establish long-lived immunity by artificially exposing individuals to the influenza virus without causing the clinical signs of a natural infection. However, as discussed above, influenza viruses change gradually or abruptly depending on whether it is by antigenic drift or shift. While immunity protects against re-infection with the same or a closely related virus, it is less protective or absent when a more distant virus is encountered. In other words, immunity to influenza wanes over time, depending on the speed of antigenic drift of the virus, and little, if any, immunity exists to a virus of another subtype. The fact that heterosubtypic immunity is weak in man, as became clear during influenza pandemics of the last century, indicates that long lasting protective immunity after natural infection is mediated primarily by responses, particularly VN antibody responses, to the surface glycoproteins. It is possible that infection induces responses that are optimal for protection against homologous, but not heterologous or heterosubtypic infections. However, by surveillance and by determining the cross-reactive antigens it might be possible to guide the immune response in the right direction. The cross-reactivity of post-infection immune responses might be improved by developing vaccines that target epitopes other than those recognised by VN antibodies.

Because of continuous antigenic change and ample availability of naive individuals in natural hosts, influenza viruses, unlike most other pathogens, have not evolved mechanisms to evade specific acquired immunity. Immunity induced by the virus is highly effective, both in clearing the virus after primary infection and in protecting against a secondary infection. Nonetheless, the influenza virus may have a mechanism to escape innate immunity. An important function of NS1 seems to be to prevent induction of interferon α and β, thus removing their early inhibitory effect on viral replication [1, 39]. The absence of evasion mechanisms for specific immunity makes the influenza virus an ideal virus to study the proper function of acquired immune mechanisms.

Diagnosis

Influenza can be diagnosed by virus isolation or by detection of viral antigen, viral RNA or specific antibody. Virus can be isolated by inoculating fertilised chicken eggs or cell cultures with nasal or pharyngeal mucus samples, collected by swabbing the nasal passage or throat. The cytopathic effect of the virus can be observed in cell culture, and

allantoic fluid or cell lysates can be tested for their ability to agglutinate chicken red blood cells, presumptive evidence for the presence of an influenza virus. The haemagglutinin and neuraminidase subtypes are determined by the haemagglutination-inhibition (HI) and neuraminidase-inhibition (NI) assays.

Other methods used to detect virus or viral components include immunofluorescence of lung tissue, nasal epithelial cells or bronchioalveolar lavage contents, immunohistochemistry of fixed tissue samples, enzyme linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), cell culture and immunoperoxidase staining for determination of virus type and subtype, and a rapid enzyme-immunoassay membrane test (Directigen FLU-A; Zyme Tx Inc., Oklahoma, USA).

The most common serologic assay for diagnosis of swine influenza is the HI assay. One of its advantages is that it can discriminate between different subtypes and antigenic variants within a subtype. It also has several disadvantages, however. It is relatively insensitive and may not detect a response if the infecting strain is antigenically different from the virus used in the assay. Thus, infections with new subtypes or antigenic variants within the subtype will not be detected. For this reason, the virus strains used in the assay need to be updated regularly. Furthermore, it requires the use of paired serum samples, one obtained during the acute phase of the disease and the second 3-4 weeks later, to demonstrate an increase in antibody titre. In addition, the assay is very laborious, needs many standardised reagents, and is consequently prone to variation.

Diagnosis of the disease by virological and serological methods is complicated in piglets with maternal antibodies. Virus recovery and the severity of signs of disease are inversely related to the level of maternal antibody, and, depending on this level, it may be very difficult to isolate virus. Maternal antibodies also inhibit active antibody production by the piglets and so the serological response may not be detected.

Vaccines

Influenza vaccines currently in use for pigs are based on inactivated and disrupted ('split') virus suspended in an oil adjuvant. In man, inactivated whole or split virus, or purified preparations containing the surface glycoproteins HA and NA, are used in vaccines, but as yet no adjuvant is licensed. Pig vaccines are bivalent, i.e. they contain representatives of both influenza A viruses (H1N1 and H3N2), while those for man additionally contain influenza B virus. The WHO reviews the composition of the human vaccine biannually, for both the northern and southern hemispheres, and relies on advice from influenza centres all over the world where circulating viruses are monitored. Representative viruses are selected as vaccine strains and propagated in the allantoic cavity of embryonated chicken eggs.

In pigs, antigenic drift of influenza viruses seems to be more limited than in man. This is probably because pigs have a much shorter life span, covering no more than one influenza epidemic, and vaccines are not widely applied. Nevertheless, antigenic drift of the swine influenza A H3N2 viruses was detected in the Netherlands and Belgium and has led to a loss of cross-reactivity of recent field isolates with the human A/Port Chalmers/1/73 (H3N2) virus strain that is currently used in the swine vaccine. As a result, replacement of this strain by a more recent swine H3N2 isolate has been recommended [10].

In man, live-attenuated virus vaccines have been tested extensively as an alternative to those currently available, but they are still not licensed. An advantage of these vaccines is that, when administered intranasally, they mimic natural infection and thus, in addition to serum antibodies, induce local secretory IgA (and local IgG) and CTLs. For pigs, however, injection remains the only practical method for immunisation. One disadvantage of the live-attenuated vaccines is the risk that attenuated viruses will revert to their wild type. Furthermore, the segmented genome of the virus means that if an individual is vaccinated while at the same time being naturally infected with an influenza virus, the attenuated vaccine strain can acquire wild type gene segments of the other virus strain, and at the same time many other reassortant viruses can also arise.

In addition to vaccination, antivirals can be used for the control of influenza in man. For example, amantadine and rimantadine, which inhibit viral replication by blocking the M2 ion channel, and the neuraminidase inhibitors zanamivir and oseltamivir are effective against all influenza A virus subtypes. Vaccination, however, will remain the major means to control influenza in man and the only means in pigs because antiviral treatments are not licensed for use against influenza in swine, and anyway are unlikely to be economically viable.

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