Influenza Pandemics

Ruth Elderfield and Wendy Barclay

1 Introduction

Influenza is a seasonal respiratory illness associated with more serious consequence and even death in the very young, old and immunocompromised. Annual epidemics are predictable and affect a relatively small percentage of the global population at any one time. Pandemics differ from epidemics in that they are a global phenomenon, affecting large numbers of people in multiple countries simultaneously. Pandemics tend to arise swiftly often out of the normal season, and affect a wider age group and spectrum of individuals than seasonal influenza. The first recorded influenza pandemic was in 1510 [1]. Since then human populations have been subjected to at least 15 pandemics, most notably in 1918 when estimates of the human deaths that resulted vary between 40 and 100 million [1, 2].

2 The Influenza Virus

The influenza virus, the etiologic agent, is a member of the family *Orthomyxoviridae*. There are three categories of influenza known as types A, B and C. Only type A causes pandemics and thus is the most widely studied. Influenza B viruses cause typical seasonal infections restricted to humans. Influenza C viruses also infect humans and have been isolated from clusters of children, but are often not recognised and may be dismissed as an untyped influenza like illness, due to the lack of diagnostic tests [3].

The virus particle is enveloped, whereby the genome is protected by a lipid bilayer derived from the host cell membrane. The appropriated membrane is studded with viral glycoproteins: the attachment spike protein haemagglutinin (HA) which binds to sialic acid (SA) receptors on the cell surface; the integral M2 protein, an ion channel involved in the uncoating of the virus inside the infected cell, and the neuraminidase protein (NA) which cleaves the cell membrane SAs that would otherwise tether the budding nascent virus particle to the infected cell.

Lining the inside of the virion membrane is the abundant matrix protein (M1), surrounding the eight genomic negative-sense RNA segments which are intertwined with nucleoprotein (NP) and each associated with one set of the three viral polymerase subunit proteins (PB1, PB2 and PA) (Fig. 1).

R. Elderfeild • W. Barclay (✉)
Barclay Influenza Group, Imperial College London, St. Mary's Campus, London, UK
e-mail: ruth.elderfield07@imperial.ac.uk; w.barclay@imperial.ac.uk

N. Curtis et al. (eds.), *Hot Topics in Infection and Immunity in Children VIII*,
Advances in Experimental Medicine and Biology 719, DOI 10.1007/978-1-4614-0204-6_8,

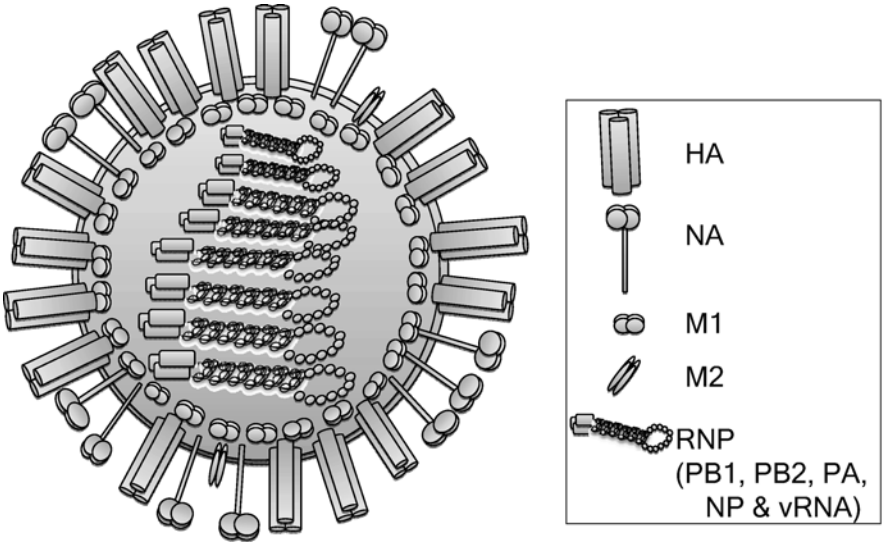

Fig. 1 Schematic cross section of the influenza A virus

The viral ribonucleoproteins (vRNPs) are the replicative units that are transported into the nucleus of the host cell after virus entry. There, the virus co-opts a number of host factors to assist the polymerase in transcribing viral mRNA, and replicating new genomes via cRNA intermediates. Finally the newly synthesized viral proteins and genomes are transported to the host cell's external membrane where the progeny virions assemble and bud.

3 The 1918 Influenza Pandemic

The 1918 pandemic dealt a devastating impact on a global population. Several features of the era, in addition to the extraordinary virulence of the virus itself that will be discussed below, contributed to the impact of the 1918 pandemic. At that time, although influenza was known as an infectious disease in terms of symptoms, the virus itself had not yet been identified. Indeed for a good proportion of the pandemic, *Hemophilius influenzae* (known as *Bacillus influenzae* at the time) was suggested as the causative agent. The influenza virus was eventually isolated from pigs by Richard Shope in 1931 [4, 5], then from humans by Andrewes, Laidlaw and Wilson Smith in 1933 [6, 7].

At the start of the influenza pandemic in 1918, the world was at war. The situation in America is absorbingly described in John M. Barry's "The Great Influenza" (Penguin, 2004) complied from military records, personal papers, oral history and newspapers of the period. There is some epidemiological and historical evidence that the outbreak may have begun in army camps either in the USA or in Europe where large numbers of young susceptible hosts were living in very crowded conditions. There is also evidence of at least two waves of disease, and indications that the second wave was more virulent than the first. For example, by the second wave historical accounts by the medical and scientific staff at the time describe symptoms such as: *cyanosis which started as mahogany spots over the cheek bones, and could expand until the patient turned black, caused by the lack of oxygen transfer in the lungs, leading to blue unoxygenated blood; extreme chills and fever, severe joint pains, vomiting and abdominal pains; earaches, headaches often localised around the eyes; and disturbing blood loss from nose, stomach, intestine and eyes*. At post mortem the lungs were often filled with the debris of destroyed cells and blood, which today would be diagnosed as Acute Respiratory Distress Syndrome (ARDS).

In Philadelphia, where the virus had been introduced from the local port, health workers and scientists were requesting widespread restrictions on gatherings and provision of information to the media. Unheeding, the governor and the senior health official sanctioned a large city wide Liberty Loan parade in order to gather funds for the war effort. Within 72 h of the parade every bed in the city's 31 hospitals was filled. The daily death rates for the city rose at an alarming rate, 3 days after the parade 117 died in one day, on day 11 more than 400 people died.

The second and third waves of the 1918 pandemic resulted in a cumulative case fatality rate (expressed as a ratio of the number of people infected to the number of people who died) of >2.5%. Later pandemics of the twentieth and twenty-first century only reached case fatality rates of less than 0.1% [8].

3.1 Reconstituting the 1918 Influenza Virus

Remarkably, although it has not been possible to isolate infectious virus directly from stored samples of that era, we do today have access to the causative agent of this pandemic following the elegant application of modern science. In 2005, Jeffrey Taubenberger and colleagues used the polymerase chain reaction to amplify small fragments of viral RNA isolated from formalin-treated post-mortem pathological slides and also from frozen lung tissue obtained from a person who died in Alaska and was buried in the permafrost, from this material the nucleotide sequence of the 1918 virus was deduced [9, 10]. Taubenberger joined forces with Terence Tumpey and colleagues at the CDC. They used the 1918 virus sequence information to generate plasmids containing the viral cDNA which when transfected into suitable mammalian cells, allowed the recovery of infectious 1918 virus [9, 11–16].

The reconstituted virus was more virulent in animal models than any other influenza virus strains studied previously [11, 16–23]. Thus although there is strong evidence that secondary bacterial infection contributed significantly to deaths from 1918 virus in humans [24, 25], the virus itself, in the absence of bacteria, is remarkably pathogenic to animals. Studies have been carried out to map the genetic determinants of this virus in the hope that this will help us to predict the virulence of future influenza strains as they emerge. The polymerase genes and the virus HA gene have been implicated in the extreme virulence of this virus, but work continues to understand the mechanisms by which this particular influenza strain is so deadly [26–29].

Phylogenetic studies suggest that the genome of the 1918 virus is most similar to viruses found in birds. However there are a number of key amino acid changes that indicate that, although it originated in an avian host, the virus underwent adaptation in order to replicate and transmit within human and swine hosts [8]. Interestingly the 1918 virus exhibits low pathogenicity in experimentally infected swine [20].

4 Twentieth Century Influenza Pandemics

The 1918 pandemic virus has been called the 'Mother of all pandemic viruses' [8] as all of the twentieth Century pandemics are derived from virus lineages descended from the 1918 virus. After 1918, viruses derived from that outbreak continued to circulate in humans causing annual epidemics of moderate or mild severity [30]. However, because of their segmented genomes, influenza viruses are particularly prone to a special form of recombination known as reassortment that occurs if one host is coinfected by two different viruses. Such mixing events allow the introduction of genetic material from viruses that usually circulate in birds with the human adapted viruses, and new viruses thereby created may be able to cause a novel outbreak. We know that the two major pandemics in the second half of the twentieth century were formed in this way: The 'Asian' pandemic of 1957 was caused by the emergence of an H2N2 sub-type virus that retained the M, NP, PB2, PA and the

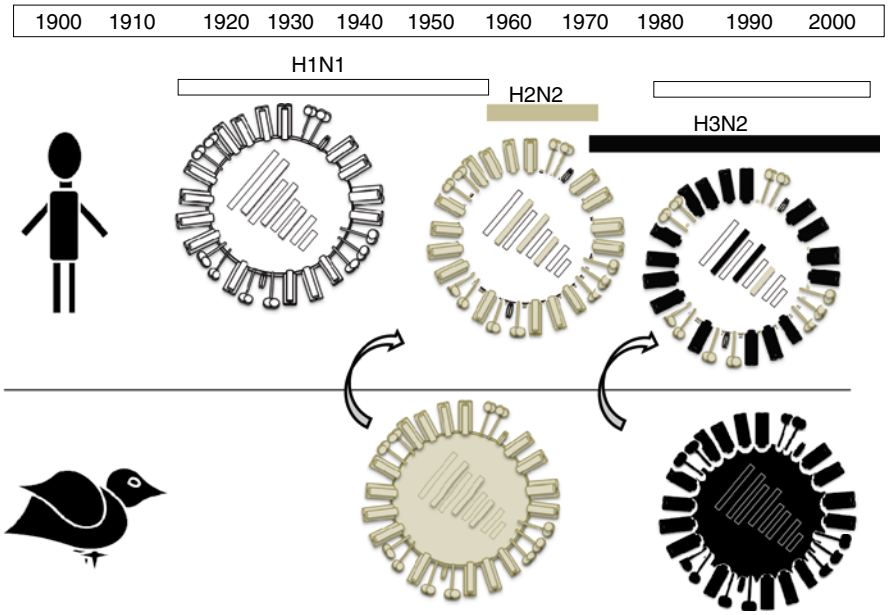

Fig. 2 A time line illustrating the emergence of the three twentieth century influenza pandemic subtypes. The H1N1 virus accepted alternative genes in recombination events with avian viruses, giving rise to the H2N2 and H3N2 subtypes

NS of viruses derived directly from the 1918 virus, but obtained a new HA, NA and PB1 from an avian H2N2 virus. Because the human population had not experienced infection with a virus of H2 antigenic type, the entire world was susceptible and the virus spread rapidly.

Nonetheless, perhaps because antibiotics were available by that time or perhaps the H2 virus itself had a milder phenotype, the death toll of this pandemic was much lower at only ~two million people. The 1968 H3N2 'Hong Kong' pandemic that followed just 11 years later resulted in between one and two million deaths. This virus was a recombinant between the circulating human H2N2 virus and an avian strain with H3 HA. The reassortant virus still retained five segments originally derived from the 1918 strain, but acquired the HA and PB1 from the avian virus. Each of the H2 and H3 pandemic events were so universal that the virus displaced the previously circulating strains [31, 32]. In 1977, the H1N1 strain re-emerged as a circulating human strain (not to be confused with the 1976 swine H1N1 outbreak at Fort Dix). The colourfully named 'Red' or 'Russian' flu appeared initially in China in May of 1977 with isolates found in Russia soon after [33]. This strain produced a relatively mild disease mainly in young children. However, subsequent genetic analysis indicated a 27 year gap in the evolutionary history of this virus. In fact it was genetically similar to virus isolated in 1950. The eventual conclusion drawn was that this virus had been deep frozen in a laboratory and its release was accidental. The H3N2 and H1N1 subtypes have continued to co-circulate and to cause human seasonal influenza outbreaks into the twenty-first century (Fig. 2) [32–36].

5 Birds Are the Natural Host for Influenza Viruses

The influenza A virus naturally circulates in aquatic birds, where it replicates in the gut [37–39]. All of the 16 HA and 9 NA subtypes have been isolated from either or both of the *Anseriformes* (an order which includes ducks and geese) and from the *Charadriiformes* (the order to which shore

birds and gulls belong). No other species has been infected by all the influenza types. Different subtypes predominate in the different orders of birds; H3 and H6 for example, are found mainly in *Anseriformes*, whereas in *Charadriiformes* H4, H9, H11 and H13 are the predominant HA subtype. The virus is excreted in high titres into the water bodies that are home to domestic and migratory birds [40, 41]. The virus can then be picked up by migratory birds and spread along the migratory routes. However, the relative geographic isolation of some flocks has been proposed as one cause of genetic divergence within the HA and NA subtypes [42].

Whilst the disease caused by influenza is mainly asymptomatic in aquatic birds, some isolates are capable of developing from low pathogenic avian influenza (LPAI) into a highly pathogenic influenza (HPAI) capable of killing domestic poultry. The two subtypes that are prone to change pathogenicity are H5 and H7, as seen in Eurasia with the HPAI H5N1 viruses and the Netherlands with the H7N7 virus. Economically, the now widely-distributed H5N1 virus has been responsible for the death of over a billion head of poultry either directly through the disease or indirectly through preventative culling measures [39] Other subtypes H9, H1, H3, H4 and H14, whilst still appearing mild in aquatic birds, can be fatal in domesticated flocks [39].

Only viruses of the H1, H2 or H3 subtypes are known to have circulated among humans or pigs.

5.1 Antigenic Shift and Drift

The drastic recombination events that result in novel pandemic viruses described above are called antigenic shift.

After the introduction of the new subtype and its wide circulation in humans, the increasing prevalence of specific immunity among human hosts exerts selection pressure that drives evolutionary change in the HA protein via the accumulation of point mutations that block the antibody recognition through conformational changes or glycosylation events on the antigenic epitopes. This process is called antigenic drift.

5.2 Cyclical Nature of Seasonal Influenza Since the Hong Kong Pandemic

As the virus continues to circulate in humans in the interpandemic periods, mutations accumulate that confer antigenic drift as well as other adaptive mutations that alter the nature of the virus and may be associated with loss of virulence. This, along with increased wide vaccination campaigns for the elderly and immuno-compromised populations, could explain the gradual decrease in influenza-like illness since the Hong Kong pandemic of 1968, particularly in recent years (Fig. 3) [43, 44].

The ability of influenza A viruses to recombine so readily has worried virologists and public health planners alike, because of the risk that a seasonal strain of influenza with human adapted components might recombine with one of the highly pathogenic avian influenza (HPAI) viruses such as the notorious H5 or H7 subtypes. These two subtypes are lethal in poultry because of an extended tropism conferred by mutation in the HA gene that allows them to infect and propagate in many organs and tissues, rather than being restricted to areas where the appropriate host cell proteases exist. Consequently infection with these viruses carries a high mortality in humans of more than 60%. H5N1 virus has been responsible for over 500 cases of human infection and 300 deaths (as of 31st August 2010 according to the World Health Organisation avian influenza surveillance system) but thankfully has not yet reassorted with a human-adapted influenza virus, nor given rise to a pandemic outbreak [45].

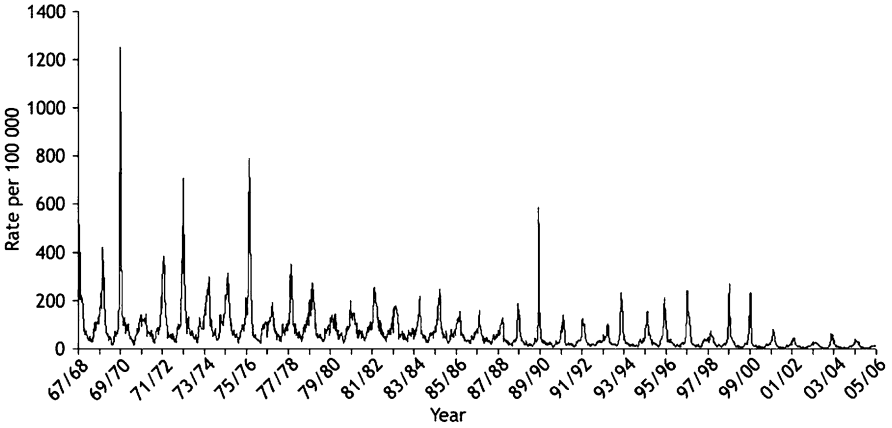

Fig. 3 Influenza-like illness incidence in England and Wales since the Hong Kong pandemic [46]

Because the research community was so focused on surveillance of and strategies to control H5 and H7 infections, the outbreak of the 2009 'swine' influenza was a surprise. We had largely overlooked the idea that the next pandemic would originate in pigs even though an outbreak in Fort Dix, New Jersey in 1976 associated with the death of a soldier from infection with an H1N1 swine flu had led to mass vaccination campaigns at that time. The Fort Dix incident did not give rise to a pandemic, the virus remained contained within the military and transmission of the virus had fizzled out by the time the now-infamous vaccination campaign began [47, 48].

5.3 Swine as a Recombining Mixing Pot

Most text books propose that swine are the mixing vessel in which influenza viruses of avian and human origin reassort. It has been evident that pigs can be infected with influenza since the early days of virus isolation. Indeed, Shope initially isolated influenza from a pig [4, 5] and Kida et al. showed that pigs could be infected by many different subtypes of avian influenza [49].

The 2009 pandemic virus illustrates just how good a mixing pot the swine host can be. The origins of its eight gene segments come from at least four different sources and three different hosts. The PB2 and PA segments appear to have originated in an avian reservoir and transmitted to the swine host around 1998. The PB1 segment derives from a human virus but was transferred to swine in 1998. The HA, NP and NS segments once again can trace their lineage back to the 1918 pandemic influenza, when the virus infected pigs and subsequently circulated through the years to become a classical swine virus. The NA and M segments are from circulating swine viruses, of the Eurasian lineage, believed to have transferred from birds in 1979 (Fig. 4) [50, 51].

Why do swine make such good mixing vessels? Ito et al. showed in 1998 that the pig respiratory tract displayed SA receptors that are bound by viruses isolated from birds as well as those used by human-adapted viruses, implying that the pig was capable of being infected by an avian and a human-adapted virus at the same time [52]. In addition, the co-expression of the avian-like and human-like receptors in swine potentially allows for the selection of avian viruses with small mutations that adapt them to bind to and replicate in mammalian cells, a process known as 'receptor switching.'

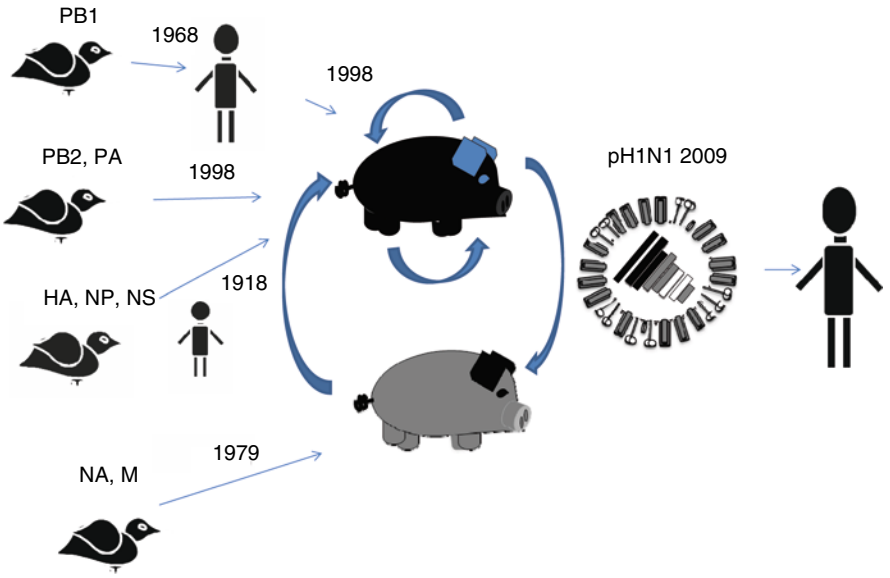

Fig. 4 The emergence of the pH1N1 2009 influenza virus. A series of avian viruses transferred into swine hosts. There multiple recombination events of classical swine influenza (*black*) resulted in a triple reassortant (TRIG), when recombined with Eurasian swine influenza (*grey*) generated the pH1N1 virus [51]

5.4 Receptor Switching

For avian influenza viruses to adapt to and transmit between humans, it is now apparent that in addition to the reassortment events that occur during antigenic shift, their HA proteins must also undergo modifications that alter their fine receptor binding specificity. The influenza HA protein binds to SA residues on the host cell surface as a prelude to cell entry. In the avian gut, these are predominantly α-2,3 linked receptors but in the human upper respiratory tract α-2,6 linked receptors predominate. Avian influenza viruses would therefore preferentially bind α-2,3 linked receptors and human-adapted influenza viruses have changed key residues at the receptor binding site allowing greater affinity for α-2,6 linked receptors (Fig. 5) [42].

In H3 HA proteins, receptor switching occurs if there is a change from glutamine (Q) at position 226 to leucine (L) (H3 numbering) and is enhanced by glycine (G) at 228 to serine (S) [53, 54]. For other subtypes the changes required for human adaptation are not exactly the same. Some H2 viruses still bind to α-2,6 human-like receptors even when the Q226 is present, a trait shared by avian H6 and H9 proteins. The H1 subtype tends to show changes at residues 225 (aspartate [D] to G) and 190 (D to glutamic acid [E]) rather than 226 and 228 but they achieve the same end [9, 10, 53–55].

Fortunately, in the case of H5 HA, none of the changes found in other subtypes have completely mediated a receptor binding switch, suggesting that the barrier to human adaptation may be particularly high for this subtype [56, 57].

One important difficulty in understanding these adaptive events is that the nature of the influenza virus receptor is not completely clear, but it is certainly more complex than a single sugar moiety [58]. The nature of carbohydrate to which influenza virus might attach has been recently studied using glycan arrays. Glycan arrays present hundreds of different carbohydrates. Different viruses or expressed HA proteins are then given the opportunity to bind to a favourite residue [59–63]. This type of experimental procedure was used recently to elucidate the receptor binding preferences of the novel pandemic H1N1 2009 virus. Interestingly, this virus along with two other swine viruses tested, was able to bind both α-2,6 and α-2,3 SA, whereas seasonal H1N1 influenza virus had a strong preference to bind carbohydrates with α-2,6 linkages and showed no binding to those with α-2,3 [63]

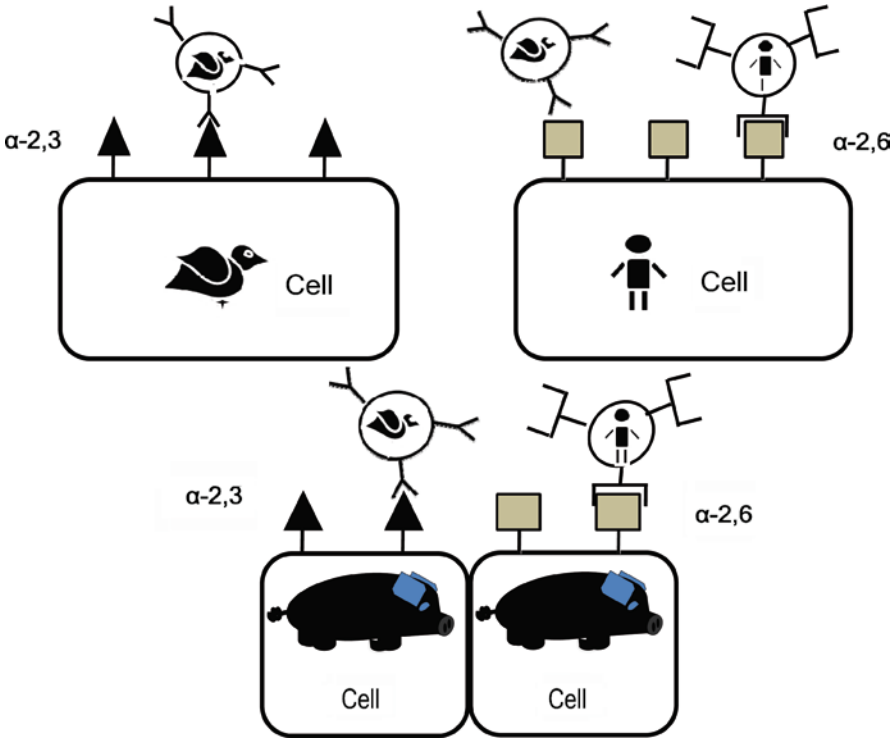

Fig. 5 A schematic representing the putative sialic acid linked receptor distribution on cells in the human upper airway, the avian gut and the swine respiratory system, with cognisant avian-like or human-like HA attachment proteins

6 The Emergence of Swine Origin Influenza (pH1N1)

The swine origin 2009 pandemic influenza virus appears to have emerged from San Luis Potosi, Mexico in late February 2009 [31], though it has been suggested that the virus was circulating at low levels in humans for some months prior to this. Indeed the most common ancestor may have emerged between August 2008 and January 2009 [31, 51, 64]. The pandemic threat of the new virus was realised as the first wave peaked in Mexico in late April 2009. The virus quickly spread across the globe. WHO moved to pandemic phase 4 after confirming human to human transmission on the 27th April, and just two days later, phase 5 was declared as the outbreak was found in two or more countries within one WHO region. Finally passage of the virus into a second WHO region triggered escalation to phase 6 on the 11th June.

6.1 How Could an H1 Virus Cause a Pandemic?

The H1N1 virus responsible for the 2009 pandemic is not the same H1N1 virus that had been circulating in humans in recent years causing seasonal H1N1 outbreaks. Both viruses have HA proteins originating from the 1918 pandemic virus. However the HA of the seasonal H1 had been under antigenic and other selective pressure as it circulated in the human population over a total of seven decades. On the other hand, since pigs are short lived, they exert little antigenic pressure to

drive evolution of HA because the likelihood that a pig will be re-infected by the same influenza virus during its brief life time is very low. The rather genetically static 1918-derived pig virus became known as 'classical' swine influenza virus and it was the predominant influenza virus of swine on the North American continent throughout the twentieth century.

At the time this classical swine H1 HA recombined into what was to become the 2009 pandemic virus, it still retained 90% amino acid sequence identity to its 1918 progenitor. However, the human seasonal virus had changed so dramatically that it shared only 79% amino acids with the 1918 HA protein, and this did not allow for any antigenic cross protection for humans who had been infected with seasonal H1 in recent years against the novel 2009 pandemic strain.

6.2 The Course of the Outbreak in the UK

In the UK, the first reported case was on the 26th April 2009, brought back by those returning from holidays in Mexico. By May 29th there were 215 UK cases, of whom 52 were returning travellers, 39 were direct contacts of those travellers and 108 were people who had links to the secondary cases. Already at this early stage there were eight sporadic cases which were not linked to travel. On 21st June, the influenza-like-illness (ILI) incidence baseline was crossed and the first wave in the UK was clearly underway. This wave peaked in July and then fell well below baseline by mid-August, after schools closed for the summer holidays. Nevertheless 17% of the deaths in the UK occurred during this wave. The second wave was far shallower, but endured for a longer period from September 2009 until February 2010, and was responsible for the remaining 83% of fatal cases [65, 66].

The height of the peak of ILI cases in the second wave was much lower than had been predicted. Several factors contribute to the explanation for this: a proportion of the population (>30%) most vulnerable to seasonal influenza infection, the elderly, were already immune. In blood samples of those over 80 years old, collected in 2008 before the 2009 pandemic virus emerged, it was possible to detect antibodies that cross react with 2009 pandemic virus' HA in >30% of the samples (haemagglutination titre 1/32). In those aged 65–79 years, the seropositive frequency drops to ~20%, whereas in the 4–14 year old age bracket, the proportion with significant HAI was just ~4% [67]. These neutralising antibodies in sera collected from the elderly exist because many people in older age groups were infected in early life by closer derivatives of the 1918 H1N1 virus.

The second factor was the surprisingly mild nature of the 2009 pandemic virus in most people. Serology conducted retrospectively detected neutralising antibodies in a far larger percentage of the population than could be expected from the reports of ILI. One in three of the children in London and Birmingham were seropositive for the virus by September 2009 [67] suggesting that many people who had the infection did not report it. This could be because the symptoms were sufficiently mild that they did not feel they needed the flu service that was on offer at the time, or they did not even realise they had the infection. In this respect, the population was fortunate; this virus spread effectively but did not produce overtly pathogenic effects in most people. The case fatality rate was 0.02%, far lower than the 2.5% of the 1918 pandemic or the 0.1% of the 1957 and 1968 pandemics. Despite that, there were over 1,500 hospitalizations due to influenza like illnesses in the UK and 474 deaths [68]. Globally mortality far exceeds 18,000 deaths [69].

6.3 Who was at Risk?

In the event of a pandemic, such as in 2009, difficult decisions need to be made by health authorities to prioritise limited supplies of drugs and vaccines. Pharmaceutical companies may generate

millions of doses of vaccine (300 million for the 2009 pandemic) [70] to protect the population, but this is still only enough to immunize a small proportion of the globe, so provision of vaccine to those most vulnerable to this virus needed to be prioritized.

6.3.1 Age

Usually vaccine is given primarily to those most susceptible to poor outcome from infection by seasonal influenza, namely the elderly. In the UK, from 2001 to 2009, 69% of those who died from seasonal influenza were >65 years old. However, with the 2009 pandemic virus only 15% of deaths were in the >65 demographic. True to form, those elderly individuals who did succumb to the 2009 pandemic influenza suffered a severe illness, with a case fatality rate of 0.9%, indicating the virus was able to cause serious morbidity in those who were immunologically susceptible [65].

The first infection with an influenza virus in children can often be quite severe. Johnson et al. showed that the high incidence of unexpected paediatric fatalities from the Fujian H3N2 seasonal drift variant in 2003 was linked with a higher than usual infection rate for seasonal influenza in the young in that year, possibly explained by a more drastic antigenic drift than in immediately previous years [71]. Similarly when a novel pandemic virus circulates widely, the incidence rate in the very young is particularly high and their clinical course in the face of lack of any relevant immunological experience is often severe. Indeed during the 2009 pandemic, the highest mortality rates were observed in those under 1 year of age [72]. In addition since school age children are major transmitters of influenza, there is good logic in targeting them in pandemic and seasonal immunization campaigns because overall community incidence may be curtailed in this way [73–76].

The 2009 pandemic virus was certainly able to infect and transmit well within the paediatric cohort. In the UK one in three children and in Hong Kong half of the children had been infected after the first wave [77, 78], supporting the global observations that school aged children and young adults were most likely to contract influenza [79]. Generally seasonal influenza causes two paediatric deaths per million people, while pH1N1, by mid-2010, had been responsible for 5–6 deaths per million in the Netherlands and the UK respectively and 11 per million in Argentina in the paediatric cohort [72, 80–82].

In their 2010 study of paediatric mortality from pandemic influenza in the UK, Sachedina and Donaldson identified 70 deaths in the 0–18 year old age group directly attributable to infection with the virus. As in similar studies across the world, they described common symptoms including fever, cough and shortness of breath [72, 79, 83–86]. The UK study observed that a combination of neurological, gastrointestinal and respiratory disease was present in more than half the deaths, an observation again echoed in other countries. Indeed pre-existing neurological disorders have frequently been listed as a co-morbidity [65, 66, 83–85, 87–89]. Half of those of school age who died in the UK attended schools for those with special needs [72]. As with adults, bacterial coinfections were often observed; in the US 43% of paediatric deaths were associated with secondary bacterial infections and in the UK 20% were associated with laboratory confirmed cases [72, 73].

6.3.2 Pregnancy

Pregnant women appear to be especially at risk from complications of influenza of either a seasonal or pandemic nature, especially during the second and third trimesters [90]. This increased susceptibility has been ascribed to mechanical changes within the body, which act to increase the pressure on the cardiovascular system, including an increased heart rate, stroke volume and oxygen consumption set against a decreased lung capacity [91, 92]. This may also account for increased risk in obesity.

In addition, hormonal changes in pregnancy cause what might be broadly termed a swing away from cell mediated immunity and a bias towards the humoral system that may affect the ability to clear the virus [92, 93]. On the other hand humoral immunity is also not complete in pregnancy and depletion in the levels of IgG2 were observed in pregnant women who died in the 2009 pH1N1 pandemic [94]. There is evidence from previous pandemics that the mortality rate was high amongst pregnant women: In the 1918 pandemic between 27% and 47% of those pregnant who contracted influenza died. In 1957 the percentage of deaths was lower, but still considerable at 20% [92]. There is also evidence of complications; of those pregnant women who developed pneumonia but survived during the 1918 pandemic, more than 50% did not carry the foetus to full term [92].

6.4 Other Co-morbidities

In the 2009 pandemic it was very evident that other co-morbidities increased susceptibility to severe influenza infection. These included obesity, asthma and chronic obstructive pulmonary disease (COPD), diabetes, immunosuppression, heart conditions and neurological complications. Whilst only a third of those who were admitted to intensive care had co-morbidities, over three quarters of those who died did [73]. In the UK, the FLUCIN database collected data from all those admitted to hospital with influenza during the pandemic first wave. Co-morbidities were described for around 50% cases, leaving a significant number of young healthy adults that suffered severe disease and even death despite no obvious prior predisposition for bad outcome [95].

6.5 Bacterial Coinfections

In the US, a study of the first 100 fatal cases caused by pH1N1 found that 25% of them had bacterial coinfections, with *Staphylococcus aureus* and *Streptococcus pneumoniae* being the most common pathogens [96]. Other fatal case studies have put the incidence of bacterial coinfections between 28% and 36% and also included *Streptococcus pyogenes* in the list of common bacterial coinfections [89, 97, 98]. In Argentina, coinfection with streptococci increased the likelihood of severe outcome with an odds ratio of 17 [99]. However in the UK, high incidence of bacterial coinfection was less evident [95]. The effect of bacterial superinfection on the outcome of infection with pandemic H1N1 2009 is likely to have been affected by differences in the bacterial strains circulating in communities around the world at the time and this may, in part, account for the widely different case fatality rates seen in different areas.

6.6 Influenza Vaccination for the Pandemic

There is a well-established system in place for the generation of seasonal influenza vaccines; the dominant circulating strains are carefully monitored and predictions are made annually about which viruses are likely to predominate in the forth-coming ‘flu season. The chosen seasonal viruses are recombined with the internal segments of the high growth A/Puerto Rico/08/34 (PR8) vaccine backbone strain, to create viruses with HA and NA antigens from seasonal strains that can be readily amplified in eggs. Similarly for the 2009 pandemic vaccine, a reassortant virus bearing the H1 HA and N1 NA genes of the pandemic strain A/California/07/2009 on a high growth body was used to generate high yield virus in eggs. However, vaccine production problems became

apparent early during production phase when manufacturers realized that the growth of the Cal/07/09 reassortant was only 30–50% of that seen for the seasonal strains. Eventually a higher growth variant was obtained. In all about 30 versions of H1N1/2009 pandemic vaccine were generated in multiple countries by different manufacturers, with either wild type or reassortant viruses grown traditionally in eggs or in cell based systems and vaccines produced as spilt (just the HA and NA genes) preparations, whole inactivated virion preparations or, in one case, a live attenuated virus. Using a strategy based on development of H5N1 pandemic vaccines, the most widely used pandemic vaccine in the UK was an inactivated vaccine generated by GSK that was administered combined with AS03 adjuvant (composed of α-tocopherol, squalene and polysorbate 80 emulsion) a chemical mix added to enhance and prolong the immunogenic response and reduce the amount of HA protein required per dose to achieve immunity (antigen sparing). The immunogenicity of the pandemic vaccine was in fact much higher than expected based on experience of clinical trials with the H5N1 equivalent. In the end, a single dose of adjuvanted vaccine was sufficient to achieve seroconversion in adults. Although it was expected that two doses would be needed in children, who usually require a prime boost regimen for effective levels of antibody to be achieved, a single dose was eventually used as it turned out to be adequately immunogenic and significantly less reactogenic after the first dose than the second [100]. This vaccine was recorded as having a 72% effectiveness despite the relatively small doses of 3.75 μg of HA protein (unadjuvanted vaccines typically contain 15–30 μg). Other adjuvants were also trialled globally including alum (aluminium hydroxide) in China and Russia and another oil-in-water adjuvant broadly similar to ASO3 called MF59 in Korea and Italy. Clinical trials were run using the proprietary Sanofi-Pasteur AF03 adjuvant in the USA, Europe and Asia [70].

Live attenuated vaccine (LAIV) for pandemic 2009 was widely administered in the US. Other vaccine strategies that were not yet licensed have been researched using the 2009 H1N1 virus as antigen. These include: the use of virosomes (lipid vesicles) that have the HA and NA proteins scattered through the bilayer, live recombinant adenovirus vaccines that express the HA protein, virus like particles in which the HA, NA and M are expressed in insect cells which are infected with a recombinant baculovirus, these are purified and self assembled into immunogenic particles, finally plants infected with a transformed Agrobacterum vector that generate HA proteins [70].

6.7 The Virulence of H1N1 2009 Virus in Animal Models

Although the outcome of infection in most people infected with pandemic 2009 virus was mild, in animal models this virus causes more severe disease than recent seasonal H1N1 viruses. Itoh et al., compared seasonal H1N1 (A/Kawasaki/UTK-4/09) and pandemic H1N1 (A/California/04/2009) viruses in a number of animal models. Interestingly, infected mini-pigs remained relatively asymptomatic. In contrast, pH1N1 virus caused severe lung pathology in mice, ferrets and macaques including lung lesions and damage caused by the infiltration of inflammatory mediators to a greater extent than was observed with infection with the seasonal viruses [17, 101, 102]. Van de Brand et al., infected ferrets intratracheally with very high doses of seasonal H1N1, pH1N1 or HPAI H5N1 virus and found that infection with pH1N1 caused pathology intermediate between seasonal influenza and H5N1 and could lead to severe pneumonia and death in this model [17]. Infection of alveolar pneumocytes, not observed with the seasonal virus [17, 102] may correlate with the more profound binding of the pH1N1 HA to α-2,3 linked SA [63] which tends to be located deeper into the lung [103]. The difference between the animal models and the epidemiology in humans suggests that a basal level of existing immunity in the human population has protected against the moderately severe disease this virus can cause in immunologically naive experimental animals.

6.8 Oseltamivir Resistance of pH1N1 Virus

There are two antiviral drug classes currently available to treat influenza. Adamantanes (amantadine and rimantadine) are directed against the ion channel M2 protein and prevent the uncoating of the virus genome early in infection. The second class of drugs, the neuraminidase inhibitors (oseltamivir -Tamiflu® and zanamivir- Relenza®) were rationally designed to block the active site of the neuraminidase of the influenza virus. NA acts to cleave the SA receptors on the surface of the host cell, allowing the release of newly formed virions which can then infect uninfected cells.

Unfortunately, influenza viruses readily developed resistance to the adamantanes through point mutations at residues 26, 27, 30, 31 or 34 in the M2 protein, with no compromise in viral fitness [104, 105]. 90% of the seasonal H3N2 isolated in the US and Asia contain a resistant phenotype and the S31N mutation was already present in the 2009 pH1N1 at the time it crossed into humans [106, 107].

The NAI drug class has therefore become a favourite for stockpiling anti-influenza therapies. The first of these drugs to reach the clinic was zanamivir (Relenza) but use of this drug is hindered by the necessity to inhale it because it is not orally bioavailable. The second NAI oseltamivir (Tamiflu®) benefits from a convenient oral formulation, good bioavailability and is suitable for use in paediatric and adult populations. Data have not yet emerged fully for its effects in those > 65 years of age or the immunocompromised [108].

According to the Cochrane review of efficacy in 2005, if zanamivir or oseltamivir are used to treat an infection 48 h after onset of symptoms, there is a 'modest' reduction in influenza symptoms within 0.78 days in adults and 1 day in children for zanamivir and 0.86 days in adults and 0.87 days in children for oseltamivir [108, 109]. As prophylactics, both drugs fare well, in control groups taking the medications during seasonal influenza, there was a reduction in incidence of 69% and 74% for zanamivir and oseltamivir respectively. For post-exposure prophylaxis there were 81% and 90% relative reductions in infection after zanamivir and oseltamivir administration respectively [108, 109].

Resistance to oseltamivir can emerge, but early experiments indicated that mutations such as H275Y (N1 numbering, H274Y in N2), would confer a fitness cost to the virus by reducing NA affinity for the SA substrate [110, 111]. Thus, it was assumed that the resistance mutation would not persist in the community. However, by 2008, ~99% of seasonal H1N1 viruses had acquired the H275Y mutation that was associated with the resistant phenotype without fitness cost [108, 112]. The lack of fitness cost can be ascribed to compensating mutations in the NA protein [113, 114].

The obvious fear was that the pH1N1 virus would develop oseltamivir resistance given the widespread use of the drug in the early waves of the pandemic. pH1N1 viruses with the H275Y mutation have been isolated, however these were predominantly in those undergoing prolonged treatment regimens (often in immunocompromised patients) or through the use of low dose prophylaxis [115, 116]. At the time of writing, there had not been widespread transmission spread of the resistant strain. Indeed the fitness cost to this strain of virus is still under debate, with different labs publishing conflicting results [117–119].

6.9 Other Mutations in pH1N1 Virus that May Impact the Course of the Pandemic

6.9.1 D225G

There is historical evidence that implies that during the 1918 pandemic, the second and third waves were more severe than the first. It has been suggested that the virus acquired mutations as it

circulated in its new human host and these 'hotted up' its virulence. Thus it is important to identify any mutations that may similarly increase virulence of the 2009 pH1N1 strain. In particular it was possible that critically ill or deceased patients had been infected with a virus variant that had more pathogenic potential than the viruses that predominated in the community. The sequence of viruses from such cases has been analysed in a number of studies [120–125]. One of the interesting mutations observed is a D225G (H3 numbering, D222G in H1) mutation in the HA protein. 18% of critical cases in Norway and 12.5% of the critically ill in Hong Kong had this mutation [123]. In Scotland this mutation was only found in patients who were critically ill (4.1%) [120].

However it is not clear that this mutation alone is responsible for poor outcome: The D225G mutation was found in the virus from a nasopharyngeal swab and tracheal aspirate from a 25 year old man admitted to intensive care with pneumonia and ARDS. However the same virus transmitted to a contact case, but did not lead to severe illness despite the latter individual having two hallmark co-morbidities, namely obesity and diabetes [121]. Although D225G was detected predominately in viruses from critical cases in Greece, it was also isolated in two mild cases of the disease [122]. It has been suggested that some of the reported isolates with this change are the results of egg adaptation during the culture period [126]. In addition, the prevalence of this mutation in the critically ill has been ascribed to factors such as sampling bias, the critically ill being more likely to be genotyped than the mild cases.

The presence of this mutation enhances binding of H1 HA to α2,3 linked SA receptors [124]. The proposed mechanism by which such a mutation may enhance virulence is that the ability to bind more efficiently to α2,3SA receptors extends the lung tropism of the virus to bind ciliated cells that may then be unable to clear virus efficiently via the mucociliary escalator [124]. Additionally, increased binding to type II pneumocytes and macrophages in the alveoli and to submucosal glands in the trachea and bronchi may enhance lung damage [127]. However using reverse genetics to engineer this point mutation into an otherwise isogenic background, it was shown that the D225G change was not associated with an increase in virulence in the ferret or guinea pig models and remains easily transmitted between guinea pigs [127]. The mutation did result in a lower infectious dose for infection of mice who predominately express the α2,3, linked form of SA receptor [128].

6.10 Presence or Absence of Other Virulence Factors in pH1N1Virus

6.10.1 PB1-F2

The PB1 segment has a second reading frame (+1) which encodes a small protein (87-90 amino acids), PB1-F2 [129]. This protein has been assigned two functions, induction of apoptosis through its mitochondrial targeting C terminal domain and a role in lung inflammation [129–133]. A proposed third function, relating to polymerase function and reflected by the retention of the PB1 protein in the nucleus appears to be strain specific [134, 135].

Viruses with intact PB1-F2 genes cause increased pathology in the mouse model [131] and also predispose the host to secondary bacterial infections and subsequent pneumonia. Mice infected with a PR8 virus containing the full length PB1-F2 suffered greater weight loss and increased mortality when subjected to a secondary bacterial infection than mice infected with a PR8 with a truncated form of PB1-F2 [133].

However in natural isolates, particularly those from swine, the PB1-F2 gene is not always full length. Zell et al. analysed the influenza A sequences available in Genbank in 2007 [136]. They found that 96% avian strains possessed the full length PB1-F2, but in contrast only 75% of swine viruses and 81% of human viruses had the full length gene. Classical swine influenza strains have truncated forms of PB1-F2 with premature stop codons after 11, 25 and 34 residues [136].

Truncation of the PB1-F2 gene to just 57 amino acids also occurred in seasonal H1N1 viruses in the 1950s. Loss of the C terminus of PB1-F2 removes the mitochondrial targeting sequence of the protein, abrogating its interaction with host proteins ANT3 and VDAC1 and reducing its ability to trigger apoptosis in immune cells [130, 137]. In addition this region of the protein appears to harbour pro-inflammatory properties. Indeed, peptides generated to contain amino acid sequence from the C-terminal region of PB1-F2 generated an inflammatory response when administered to mouse lung. Two days post exposure mice lost up to 15% body weight. Interestingly the same peptide derived from recent H3N2 seasonal virus contains 5 amino acid differences from early H3N2 homologues that appear to abrogate the pro-inflammatory function. Inflammation triggered by PB1-F2 peptides from highly virulent strains such as 1918 influenza may predispose to secondary bacterial pneumonia [138]. Indeed, some highly virulent viruses such as H5N1 and 1918 H1N1 viruses possess a point mutation in this region of PB1-F2, N66S, that is partly responsible for their enhanced morbidity and mortality in mice [139].

The 2009 pH1N1 influenza virus has a PB1-F2 gene truncated to just 11 amino acids in length which is inactive. Using reverse genetics to engineer viruses in which full length protein was restored, Hai et al. noted no increase in virulence in mice or ferrets [140]. Even the introduction of the notorious 1918 like N66S point mutation did not affect the outcome of infection in these models. Thus acquisition of virulence by restoration of this gene to the pandemic virus seems unlikely [140].

6.10.2 Cleavage of Influenza HA Leads to Extended Tropism

The tropism of influenza virus is not only determined by its receptor use. SA is a widely distributed cell surface sugar but influenza in humans is largely restricted to the respiratory tract. The reliance on host cell proteases to cleave and thus activate the fusogenic properties of the HA protein determines the organs in which the virus can undergo productive infection. In humans the abundance of Clara Tryptase in respiratory secretions allows the virus efficient replication in the lung [150]. In highly pathogenic avian influenza viruses such as H5 and H7 strains, the insertion of a polybasic motif allows the HA to be cleaved by ubiquitous proteases such as furin, facilitating systemic infection [151]. Despite the high mortality rates of those afflicted with the 1918 virus, it does not contain the polybasic cleavage site found in the highly pathogenic H5N1 viruses. The acquisition of this virulence motif in H5 and H7 subtypes of HA occurs during amplification in poultry [152, 153]. This motif has not been seen in any pH1N1 isolates in 2009 or 2010, and pH1N1 viruses remain dependent on the addition of trypsin to growth media for their propagation in cell culture.

6.10.3 The NS1 Protein

The influenza virus counteracts the otherwise suppressive effect of the interferon response using a nonstructural protein NS1, reviewed in detail by Hale et al., [141]. NS1 works in at least two ways to prevent induction of interferon. Firstly in the cytoplasm NS1 binds dsRNA and other RNAs that are the likely triggers of innate immunity as well as forming a complex with the host cell pattern recognition receptor RIG-I and its controlling protein TRIM25. Secondly in the nucleus some NS1 proteins can bind to the host cell factor CPSF30 and in doing so they suppress the processing of newly synthesized mRNAs and prevent their export to the cytoplasm. This latter function is strain specific. It has been suggested that viruses that have enhanced ability to perform both these functions may induce a more severe disease because they can evade the innate immune response more efficiently. Indeed introduction of CPSF30 binding ability to the lab adapted PR8 vaccine strain that usually lack this function enhanced its virulence in mice [142]. The pH1N1 virus lacks CPSF

binding capacity. However Hale et al. have shown that reintroduction of this phenotype did not affect virulence of pH1N1 in ferrets or mice [143]. Despite lacking CPSF30 binding capacity, the pH1N1 virus induces very low levels of interferon in infected cells [144].

7 The Future

The 1968 and 1957 pandemic viruses both displaced the previously circulating subtypes. In contrast the re-emergence of H1N1 in humans in 1977 was not associated with subtype displacement likely because a large cohort of the older population was not susceptible to the virus and therefore remained viable hosts for the contemporary H3N2 viruses. Similarly due to residual immunity in the elderly, pH1N1 2009 has not displaced the H3N2 subtype. Initially it was believed that the seasonal H1N1 subtype may have gone extinct after pH1N1 emerged, as there was a period of many months where this virus was not isolated, however recently seasonal H1N1 isolates have been detected in Texas [145]. The trivalent vaccine administered in 2010 contains pH1N1, H3N2 and influenza B virus antigens but no seasonal H1N1 component.

Influenza is a seasonal disease. Infections peak once a year in the cold, dry season in the Northern or Southern hemispheres, although in the tropics the seasons are less clearly separated and it may be that virus continually circulates [146]. The Royal College of General Practitioners (RCGP) scheme in the UK has monitored the incidence of ILI since the emergence of the H3N2 subtype in the 1968 pandemic (Fig. 3), the re-emergence of related H3N2 and seasonal H1N1 strains has been observed year on year and is due to the capacity of the virus to accumulate small point mutations in HA and NA antigens, the process called antigenic drift. These mutations occur at antigenic sites and allow the circulating virus to evade immune suppression by throwing off antibody binding through confirmation changes or glycosylation events. Accumulated drift mutations may ultimately change the phenotype of the virus. The virus may alter its affinity or specificity for the receptors on the host cell surface in its efforts to avoid the immune response [147]. Indeed it is clear that, as it has evolved over four decades in humans, the H3N2 virus has changed its receptor binding affinities with phenotypic consequence [43, 148].

As herd immunity increases against the newly emerged pH1N1 virus, it is not in doubt that antigenic drift will occur. However, the resulting phenotypic changes are unknown and currently unpredictable. Moreover since the virus has re-infected swine, a species in which frequent reassortments occur [149], the evolution of this 2009 H1N1 virus and the consequence of reassortment events in animals for human disease remain to be observed in the coming years.

References

1. Morens DM, Taubenberger JK, Harvey HA, Memoli MJ. The 1918 influenza pandemic: lessons for 2009 and the future. Critical Care Medicine. 2010;38(4 Suppl):e10–20.
2. Johnson NPAS, Mueller J. Updating the accounts: global mortality of the 1918–1920 “Spanish” influenza pandemic. Bulletin of the History of Medicine. 2002 Jan;76(1):105–15
3. Gouarin S, Vabret A, Dina J, Petitjean J, Brouard J, Freymuth F. Study of Influenza C Virus Infection in France. IBIS. 2008;1446(April):1441–1446.
4. Shope RE. Swine Influenza : I. Experimental Transmission and Pathology. The Journal of Experimental Medicine. 1931 Jul;54(3):349–59.
5. Shope RE. The Etiology of Swine Influenza. Science (New York, N.Y.). 1931 Feb;73(1886):214–5.
6. Laidlaw PP, Smith W, with C. H. The susceptibility of mice to the viruses of human and swine influenza. Lancet, The. 1934;2(859):
7. Andrewes CH, Laidlaw PP, with W. Virus obtained from influenza patients. Lancet, The. 1933;19(66):

8. Taubenberger JK, Morens DM. 1918 Influenza: the mother of all pandemics. Emerging Infectious Diseases. 2006 Jan;12(1):15–22.
9. Reid AH, Fanning TG, Hultin JV, Taubenberger JK. Origin and evolution of the 1918 "Spanish" influenza virus hemagglutinin gene. Proceedings of the National Academy of Sciences of the United States of America. 1999 Feb;96(4):1651–6.
10. Taubenberger JK. Initial Genetic Characterization of the 1918 "Spanish" Influenza Virus Science. 1997 Mar;275(5307):1793–1796.
11. Tumpey TM, Basler CF, Aguilar PV, Zeng H, Solórzano A, Swayne DE, et al. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. Science (New York, N.Y.). 2005 Oct;310(5745):77–80.
12. Reid AH, Fanning TG, Janczewski TA, Taubenberger JK. Characterization of the 1918 "Spanish" influenza virus neuraminidase gene. Proceedings of the National Academy of Sciences of the United States of America. 2000 Jun;97(12):6785–90.
13. Basler CF, Reid AH, Dybing JK, Janczewski TA, Fanning TG, Zheng H, et al. Sequence of the 1918 pandemic influenza virus nonstructural gene (NS) segment and characterization of recombinant viruses bearing the 1918 NS genes. Proceedings of the National Academy of Sciences of the United States of America. 2001 Feb;98(5):2746–51.
14. Reid AH, Fanning TG, Janczewski TA, McCall S, Taubenberger JK. Characterization of the 1918 "Spanish" influenza virus matrix gene segment. Journal of Virology. 2002 Nov;76(21):10717–23.
15. Reid AH, Fanning TG, Janczewski TA, Lourens RM, Taubenberger JK. Novel origin of the 1918 pandemic influenza virus nucleoprotein gene. Journal of Virology. 2004 Nov;78(22):12462–70.
16. Taubenberger JK, Reid AH, Lourens RM, Wang R, Jin G, Fanning TG. Characterization of the 1918 influenza virus polymerase genes. Nature. 2005 Oct;437(7060):889–93.
17. Brand JMA van den, Stittelaar KJ, Amerongen G van, Rimmelzwaan GF, Simon J, Wit E de, et al. Severity of pneumonia due to new H1N1 influenza virus in ferrets is intermediate between that due to seasonal H1N1 virus and highly pathogenic avian influenza H5N1 virus. The Journal of Infectious Diseases. 2010 Apr;201(7):993–9.
18. Memoli MJ, Tumpey TM, Jagger BW, Dugan VG, Sheng Z-M, Qi L, et al. An early "classical" swine H1N1 influenza virus shows similar pathogenicity to the 1918 pandemic virus in ferrets and mice. Virology. 2009;393(2):338–345.
19. Tumpey TM, García-Sastre A, Taubenberger JK, Palese P, Swayne DE, Basler CF. Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(9):3166–3171.
20. Weingartl HM, Albrecht RA, Lager KM, Babiuk S, Marszal P, Neufeld J, et al. Experimental infection of pigs with the human 1918 pandemic influenza virus. Journal of Virology. 2009;83(9):4287–4296.
21. Meunier I, Pillet S, Simonsen JN, Messling V von. Influenza pathogenesis: lessons learned from animal studies with H5N1, H1N1 Spanish, and pandemic H1N1 2009 influenza. Critical Care Medicine. 2010 Apr;38 (4 Suppl):e21-9.
22. Watanabe T, Watanabe S, Shinya K, Kim JH, Hatta M, Kawaoka Y. Viral RNA polymerase complex promotes optimal growth of 1918 virus in the lower respiratory tract of ferrets. Proceedings of the National Academy of Sciences of the United States of America. 2009 Jan;106(2):588–92.
23. Belser J a, Wadford DA, Pappas C, Gustin KM, Maines TR, Pearce MB, et al. Pathogenesis of pandemic influenza A (H1N1) and triple-reassortant swine influenza A (H1) viruses in mice. Journal of Virology. 2010 May;84(9):4194–203.
24. Klugman KP, Mills Astley C, Lipsitch M. Time from Illness Onset to Death, 1918 Infl uenza and Pneumococcal Pneumonia Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009 Apr;15(2):346–347.
25. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. The Journal of Infectious Diseases. 2008 Oct;198(7):962–70.
26. Brown JN, Palermo RE, Baskin CR, Gritsenko M, Sabourin PJ, Long JP, et al. Macaque Proteome Response to Highly Pathogenic Avian Influenza and 1918 Reassortant Influenza Virus Infections. Journal of Virology. 2010 Sep;
27. Billharz R, Zeng H, Proll SC, Korth MJ, Lederer S, Albrecht R, et al. The NS1 protein of the 1918 pandemic influenza virus blocks host interferon and lipid metabolism pathways. Journal of Virology. 2009 Oct;83(20):10557–70.
28. Kash JC, Basler CF, García-Sastre A, Carter V, Billharz R, Swayne DE, et al. Global host immune response: pathogenesis and transcriptional profiling of type A influenza viruses expressing the hemagglutinin and neuraminidase genes from the 1918 pandemic virus. Journal of Virology. 2004 Sep;78(17):9499–511.
29. Kobasa D, Jones SM, Shinya K, Kash JC, Copps J, Ebihara H, et al. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature. 2007 Jan;445(7125):319–23.

30. Nelson MI, Viboud C, Simonsen L, Bennett RT, Griesemer SB, St George K, et al. Multiple reassortment events in the evolutionary history of H1N1 influenza A virus since 1918. PLoS Pathogens. 2008 Feb;4(2):e1000012.
31. Lagacé-Wiens PRS, Rubinstein E, Gumel A. Influenza epidemiology–past, present, and future. Critical Care Medicine. 2010 Apr;38(4 Suppl):e1-9.
32. Cox NJ, Subbarao K. Global epidemiology of influenza: past and present. Annual Review of Medicine. 2000 Jan;51407–21.
33. Wertheim JO. The re-emergence of H1N1 influenza virus in 1977: a cautionary tale for estimating divergence times using biologically unrealistic sampling dates. PloS One. 2010 Jan;5(6):e11184.
34. Zimmer SM, Burke DS. Historical perspective–Emergence of influenza A (H1N1) viruses. The New England Journal of Medicine. 2009 Jul;361(3):279–85.
35. Scholtissek C, Hoyningen V von, Rott R. Genetic relatedness between the new 1977 epidemic strains (H1N1) of influenza and human influenza strains isolated between 1947 and 1957 (H1N1). Virology. 1978 Sep;89(2): 613–7.
36. Nakajima K, Desselberger U, Palese P. Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. Nature. 1978 Jul;274(5669):334–9.
37. Kishida N, Sakoda Y, Isoda N, Matsuda K, Eto M, Sunaga Y, et al. Pathogenicity of H5 influenza viruses for ducks. Archives of Virology. 2005 Jul;150(7):1383–92.
38. Homme PJ, Easterday BC. Avian influenza virus infections. IV. Response of pheasants, ducks, and geese to influenza A-turkey-Wisconsin-1966 virus. Avian Diseases. 1970 May;14(2):285–90.
39. Krauss S, Webster R. Avian Influenza Virus Surveillance and Wild Birds: Past and Present. Avian Diseases. 2010;(54):394–398.
40. Stallknecht DE, Kearney MT, Shane SM, Zwank PJ. Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. Avian Diseases. 1990;34(2):412–8.
41. Stallknecht DE, Shane SM, Kearney MT, Zwank PJ. Persistence of avian influenza viruses in water. Avian Diseases. 1990;34(2):406–11.
42. Donis RO, Bean WJ, Kawaoka Y, Webster RG. Distinct lineages of influenza virus H4 hemagglutinin genes in different regions of the world. Virology. 1989 Apr;169(2):408–17.
43. Thompson CI, Barclay WS, Zambon MC, Pickles RJ. Infection of human airway epithelium by human and avian strains of influenza a virus. Journal of Virology. 2006 Aug;80(16):8060–8.
44. Thompson CI, Barclay WS, Zambon MC. Changes in in vitro susceptibility of influenza A H3N2 viruses to a neuraminidase inhibitor drug during evolution in the human host. The Journal of Antimicrobial Chemotherapy. 2004 May;53(5):759–65.
45. WHO. WHO | Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO 2010.
46. Elliot AJ, et al. Surveillance of influenza-like illness in England and Wales during 1966–2006. Euro Surveill. 2006;11(10):pii=651. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=651.
47. Lessler J, Cummings DAT, Fishman S, Vora A, Burke DS. Transmissibility of swine flu at Fort Dix, 1976. Journal of the Royal Society, Interface/The Royal Society. 2007 Aug;4(15):755–62.
48. Gaydos JC, Top FH, Hodder RA, Russell PK. Swine Influenza A Outbreak, Fort Dix, New Jersey, 1976. Emerging Infectious Diseases. 2006;12(1):23–28.
49. Kida H, Ito T, Yasuda J, Shimizu Y, Itakura C, Shortridge KF, et al. Potential for transmission of avian influenza viruses to pigs. Journal of General Virology. 1994 Sep;75(9):2183–2188.
50. Garten RJ, Davis CT, Russell C a, Shu B, Lindstrom S, Balish A, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science (New York, N.Y.). 2009 Jul;325(5937):197–201.
51. Smith GJD, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature. 2009 Jun;459(7250):1122–5.
52. Ito T, Couceiro JNSS, Kelm S, Baum LG, Krauss S, Castrucci MR, et al. Molecular Basis for the Generation in Pigs of Influenza A Viruses with Pandemic Potential J. Virol. 1998;72(9):7367–7373.
53. Naeve CW, Hinshaw VS, Webster RG. Mutations in the hemagglutinin receptor-binding site can change the biological properties of an influenza virus. Journal of Virology. 1984 Aug;51(2):567–9.
54. Matrosovich M, Tuzikov A, Bovin N, Gambaryan A, Klimov A, Castrucci MR, et al. Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. Journal of Virology. 2000 Sep;74(18):8502–12.
55. Pappas C, Viswanathan K, Chandrasekaran A, Raman R, Katz JM, Sasisekharan R, et al. Receptor Specificity and Transmission of H2N2 Subtype Viruses Isolated from the Pandemic of 1957. PloS One. 2010 Jan;5(6):e11158.
56. Ayora-Talavera G, Shelton H, Scull M a, Ren J, Jones IM, Pickles RJ, et al. Mutations in H5N1 influenza virus hemagglutinin that confer binding to human tracheal airway epithelium. PloS One. 2009 Jan;4(11):e7836.

57. Chutinimitkul S, Riel D van, Munster VJ, Brand JMA van den, Rimmelzwaan GF, Kuiken T, et al. In vitro assessment of attachment pattern and replication efficiency of H5N1 influenza A viruses with altered receptor specificity. Journal of Virology. 2010 Jul;84(13):6825–33.
58. Nicholls JM, Chan RWY, Russell RJ, Air GM, Peiris JSM. Evolving complexities of influenza virus and its receptors. Trends in Microbiology. 2008 Apr;16(4):149–57.
59. Stevens J, Blixt O, Glaser L, Taubenberger JK, Palese P, Paulson JC, et al. Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. Journal of Molecular Biology. 2006 Feb;355(5):1143–55.
60. Stevens J, Blixt O, Chen L-M, Donis RO, Paulson JC, Wilson I a. Recent avian H5N1 viruses exhibit increased propensity for acquiring human receptor specificity. Journal of Molecular Biology. 2008 Sep;381(5):1382–94.
61. Blixt O, Head S, Mondala T, Scanlan C, Huflejt ME, Alvarez R, et al. Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proceedings of the National Academy of Sciences of the United States of America. 2004 Dec;101(49):17033–8.
62. Liao H-Y, Hsu C-H, Wang S-C, Liang C-H, Yen H-Y, Su C-Y, et al. Differential Receptor Binding Affinities of Influenza Hemagglutinins on Glycan Arrays. Journal of the American Chemical Society. 2010 Sep;286–291.
63. Childs RA, Palma AS, Wharton S, Matrosovich T, Liu Y, Chai W, et al. Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray. Nature Biotechnology. 2010 Feb;28(2):178–178.
64. Trifonov V, Khiabanian H, Rabadan R. Influenza A (H1N1) Virus. Emerging Infectious Diseases. 2009; 115–119.
65. Pebody RG, McLean E, Zhao H, Cleary P, Bracebridge S, Foster K, et al. Pandemic Influenza A (H1N1) 2009 and mortality in the United Kingdom: risk factors for death, April 2009 to March 2010. Euro Surveillance : Bulletin Européen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin. 2010 Jan;15(20):1–11.
66. HPA. The role of the Health Protection Agency in the "containment" phase during the first wave of pandemic influenza in England in 2009. 2010.
67. Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. The Lancet. 2010 Apr;375(9720): 1100–1108.
68. HPA. Weekly epidemiological update 2010;36[cited 2010 Sep 13] Available from: http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1243928258560.
69. WHO. WHO | Pandemic (H1N1) 2009 - update 112 World Health Organisation, Global Alert Response. 2010;
70. Girard MP, Katz J, Pervikov Y, Palkonyay L, Kieny M-P. Report of the 6th meeting on the evaluation of pandemic influenza vaccines in clinical trials World Health Organization, Geneva, Switzerland, 17–18 February 2010. Vaccine. 2010 Oct;28(42):6811–20.
71. Johnson BF, Wilson LE, Ellis J, Elliot AJ, Barclay WS, Pebody RG, et al. Fatal cases of influenza a in childhood. PloS One. 2009 Jan;4(10):e7671.
72. Sachedina N, Donaldson LJ. Paediatric mortality related to pandemic influenza A H1N1 infection in England: an observational population-based study. The Lancet. 2010 Oct;6736(10):1–7.
73. Rothberg MB, Haessler SD. Complications of seasonal and pandemic influenza. Critical Care Medicine. 2010;38(4 Suppl):e91-7.
74. Ferguson NM, Cummings DAT, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature. 2006;442(7101):448–452.
75. Kawaguchi R. Influenza (H1N1) 2009 Outbreak and School Closure, Osaka Prefecture, Japan. Emerging Infectious Diseases. 2009 Oct;15(10):2009-2009.
76. Wu JT. School Closure and Mitigation of Pandemic (H1N1) 2009, Hong Kong. Emerging Infectious Diseases. 2010 Mar;16(3):10–13.
77. Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. The Lancet. 2010;375(9720):1100–1108.
78. Wu JT, Ma ESK, Lee CK, Chu DKW, Ho P, Shen AL, et al. The Infection Attack Rate and Severity of 2009 Pandemic H1N1 Influenza in Hong Kong. Clinical Infectious Diseases. 2010 Nov;51(10):1184–1191
79. Reyes L, Arvelo W, Estevez A, Gray J, Moir JC, Gordillo B, et al. Population-based surveillance for 2009 pandemic influenza A (H1N1) virus in Guatemala, 2009. Influenza and Other Respiratory Viruses. 2010 May;4(3):129–40.
80. Libster R, Bugna J, Coviello S, Hijano DR, Dunaiewsky M, Reynoso N, et al. Pediatric hospitalizations associated with 2009 pandemic influenza A (H1N1) in Argentina. The New England Journal of Medicine. 2010;362(1):45–55.

81. 't Klooster T van, Wielders C, Donker T, Isken L, Meijer A, Den Wijngaard C van, et al. Surveillance of Hospitalisations for 2009 Pandemic Influenza A(H1N1) in the Netherlands, 5 June – 31 December 2009 Eurosurveillance. 2010;15(2):4.
82. Pitman RJ, Melegaro A, Gelb D, Siddiqui MR, Gay NJ, Edmunds WJ. Assessing the burden of influenza and other respiratory infections in England and Wales. The Journal of Infection. 2007 Jun;54(6):530–8.
83. Zhao C, Gan Y, Sun J. Radiographic study of severe Influenza-A (H1N1) disease in children. European Journal of Radiology. 2010 Oct;1–5.
84. Zheng Y, He Y, Deng J, Lu Z, Wei J, Yang W, et al. Hospitalized children with 2009 influenza a (H1N1) infection in Shenzhen, China, november-december 2009. Pediatric Pulmonology. 2010 Oct;(July):1–7.
85. Halasa NB. Update on the 2009 pandemic influenza A H1N1 in children. Current Opinion in Pediatrics. 2010 Feb;22(1):83–7.
86. Feiterna-Sperling C, Edelmann A, Nickel R, Magdorf K, Bergmann F, Rautenberg P, et al. Pandemic Influenza A (H1N1) Outbreak among 15 School-Aged HIV-1-Infected Children. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. 2010 Nov;518–12.
87. Falagas ME, Koletsi PK, Baskouta E, Rafailidis PI, Dimopoulos G, Karageorgopoulos DE. Pandemic A(H1N1) 2009 influenza: review of the Southern Hemisphere experience. Epidemiology and Infection. 2010 Oct;5:1–14.
88. Sasbón JS, Centeno M a, García MD, Boada NB, Lattini BE, Motto E a, et al. Influenza A (pH1N1) infection in children admitted to a pediatric intensive care unit: Differences with other respiratory viruses. Pediatric Critical Care Medicine: A Journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2010 Apr;12(1):1–5.
89. Bautista E, Chotpitayasunondh T, Gao Z, Harper S a, Shaw M, Uyeki TM, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. The New England Journal of Medicine. 2010 May;362(18): 1708–19.
90. Siston AM, Rasmussen S a, Honein M a, Fry AM, Seib K, Callaghan WM, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. JAMA : The Journal of the American Medical Association. 2010 Apr;303(15):1517–25.
91. Goodnight WH, Soper DE. Pneumonia in pregnancy. Critical Care Medicine. 2005 Oct;33(Supplement): S390-S397.
92. Rasmussen S a, Jamieson DJ, Bresee JS. Pandemic influenza and pregnant women. Emerging Infectious Diseases. 2008 Jan;14(1):95–100.
93. Jamieson DJ, Theiler RN, Rasmussen S a. Emerging infections and pregnancy. Emerging Infectious Diseases. 2006 Nov;12(11):1638–43.
94. Gordon CL, Johnson PDR, Permezel M, Holmes NE, Gutteridge G, McDonald CF, et al. Association between severe pandemic 2009 influenza A (H1N1) virus infection and immunoglobulin G(2) subclass deficiency. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. 2010 Mar;50(5):672–8.
95. Nguyen-Van-Tam JS, Openshaw PJM, Hashim a, Gadd EM, Lim WS, Semple MG, et al. Risk factors for hospitalisation and poor outcome with pandemic A/H1N1 influenza: United Kingdom first wave (May-September 2009). Thorax. 2010 Jul;65(7):645–51.
96. Shieh W-J, Blau DM, Denison AM, Deleon-Carnes M, Adem P, Bhatnagar J, et al. 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. The American Journal of Pathology. 2010 Jul;177(1):166–75.
97. Gill JR, Sheng Z-M, Ely SF, Guinee DG, Beasley MB, Suh J, et al. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. Archives of Pathology & Laboratory Medicine. 2010 Feb;134(2): 235–43.
98. Mauad T, Hajjar LA, Callegari GD, Silva LFF da, Schout D, Galas FRBG, et al. Lung pathology in fatal novel human influenza A (H1N1) infection. American Journal of Respiratory and Critical Care Medicine. 2010 Jan;181(1):72–9.
99. Palacios G, Hornig M, Cisterna D, Savji N, Bussetti AV, Kapoor V, et al. Streptococcus pneumoniae coinfection is correlated with the severity of H1N1 pandemic influenza. PloS One. 2009 Jan;4(12):e8540.
100. Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. BMJ (Clinical research ed.). 2010 Jan;340c2649.
101. Munster VJ, Wit E de, Brand JM a van den, Herfst S, Schrauwen EJ a, Bestebroer TM, et al. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. Science (New York, N.Y.). 2009 Jul;325(5939):481–3.
102. Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature. 2009;460(7258):1021–1025.

103. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian flu: influenza virus receptors in the human airway. Nature. 2006 Mar;440(7083):435–6.
104. Bright RA, Medina M-jo, Xu X, Perez-Oronoz G, Wallis TR, Davis XM, et al. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. The Lancet. 2005 Oct;366(9492):1175–1181.
105. Sweet C, Hayden FG, Jakeman KJ, Grambas S, Hay a J. Virulence of rimantadine-resistant human influenza A (H3N2) viruses in ferrets. The Journal of Infectious Diseases. 1991 Nov;164(5):969–72.
106. Nelson MI, Simonsen L, Viboud C, Miller M a, Holmes EC. The origin and global emergence of adamantane resistant A/H3N2 influenza viruses. Virology. 2009 Jun;388(2):270–8.
107. Rungrotmongkol T, Intharathep P, Malaisree M, Nunthaboot N, Kaiyawet N, Sompornpisut P, et al. Susceptibility of antiviral drugs against 2009 influenza A (H1N1) virus. Biochemical and Biophysical Research Communications. 2009 Jul;385(3):390–4.
108. Moss RB, Davey RT, Steigbigel RT, Fang F. Targeting pandemic influenza: a primer on influenza antivirals and drug resistance. The Journal of Antimicrobial Chemotherapy. 2010 Jun;65(6):1086–93.
109. Jefferson T, Jones M, Doshi P, Del Mar C. Neuraminidase inhibitors for preventing and treating influenza in healthy adults: systematic review and meta-analysis BMJ. 2009 Dec;339(dec07 2):b5106-b5106.
110. Carr J, Ives J, Kelly L, Lambkin R, Oxford J, Mendel D, et al. Influenza virus carrying neuraminidase with reduced sensitivity to oseltamivir carboxylate has altered properties in vitro and is compromised for infectivity and replicative ability in vivo. Antiviral Research. 2002 May;54(2):79–88.
111. Ives J a L, Carr J a, Mendel DB, Tai CY, Lambkin R, Kelly L, et al. The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and in vivo. Antiviral Research. 2002 Aug;55(2):307–17.
112. Meijer A. Oseltamivir-Resistant Influenza Virus A (H1N1), Europe, 2007–08 Season. Emerging Infectious Diseases. 2009 Apr;15(4):552–560.
113. Collins PJ, Haire LF, Lin YP, Liu J, Russell RJ, Walker P a, et al. Structural basis for oseltamivir resistance of influenza viruses. Vaccine. 2009 Oct;27(45):6317–23.
114. Rameix-Welti M-A, Enouf V, Cuvelier F, Jeannin P, Werf S van der. Enzymatic properties of the neuraminidase of seasonal H1N1 influenza viruses provide insights for the emergence of natural resistance to oseltamivir. PLoS Pathogens. 2008 Jul;4(7):e1000103.
115. Chen H. Oseltamivir-Resistant Influenza A Pandemic (H1N1) 2009 Virus, Hong Kong, China Emerging Infectious Diseases. 2009 Dec;15(12):1970–1972.
116. Baz M, Abed Y, Papenburg J, Bouhy X, Hamelin M-E, Boivin G. Emergence of oseltamivir-resistant pandemic H1N1 virus during prophylaxis. The New England Journal of Medicine. 2009 Dec;361(23):2296–7.
117. Duan S, Boltz DA, Seiler P, Li J, Bragstad K, Nielsen LP, et al. Oseltamivir-resistant pandemic H1N1/2009 influenza virus possesses lower transmissibility and fitness in ferrets. PLoS Pathogens. 2010 Jan;6(7):e1001022.
118. Seibert CW, Kaminski M, Philipp J, Rubbenstroth D, Albrecht RA, Schwalm F, et al. Oseltamivir-resistant variants of the 2009 pandemic H1N1 influenza A virus are not attenuated in the guinea pig and ferret transmission models. Journal of Virology. 2010 Aug;
119. Hamelin M-È, Baz M, Abed Y, Couture C, Joubert P, Beaulieu É, et al. Oseltamivir-Resistant Pandemic A/H1N1 Virus Is as Virulent as Its Wild-Type Counterpart in Mice and Ferrets. PLoS Pathogens. 2010 Jul;6(7):e1001015.
120. Miller RR, MacLean a R, Gunson RN, Carman WF. Occurrence of haemagglutinin mutation D222G in pandemic influenza A(H1N1) infected patients in the West of Scotland, United Kingdom, 2009–10. Euro Surveillance: Bulletin Européen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin. 2010 Jan;15(16):19534–19534.
121. Puzelli S. Transmission of Hemagglutinin D222G Mutant Strain of Pandemic (H1N1) 2009 Virus. Emerging Infectious Diseases. 2010 May;16(5):2009–2011.
122. Melidou A, Gioula G, Exindari M, Chatzidimitriou D, Diza E, Malisiovas N. Molecular and phylogenetic analysis of the haemagglutinin gene of pandemic influenza H1N1 2009 viruses associated with severe and fatal infections. Virus Research. 2010 Aug;151(2):192–9.
123. Chen H, Wen X, To KKW, Wang P, Tse H, Chan JFW, et al. Quasispecies of the D225G substitution in the hemagglutinin of pandemic influenza A(H1N1) 2009 virus from patients with severe disease in Hong Kong, China. The Journal of Infectious Diseases. 2010 May;201(10):1517–21.
124. Liu Y, Childs RA, Matrosovich T, Wharton S, Palma AS, Chai W, et al. Altered receptor specificity and cell tropism of D222G haemagglutinin mutants from fatal cases of pandemic A(H1N1) 2009 influenza. Journal of Virology. 2010 Sep
125. Antón A, Marcos MA, Martínez MJ, Ramón S, Martínez A, Cardeñosa N, et al. D225G mutation in the hemagglutinin protein found in 3 severe cases of 2009 pandemic influenza A (H1N1) in Spain. Diagnostic Microbiology and Infectious Disease. 2010 Jun;67(2):207–8.

126. WHO. Preliminary review of D222G amino acid substitution in the haemagglutinin of pandemic influenza A (H1N1) 2009 viruses. Relevé épidémiologique hebdomadaire/Section d'hygiène du Secrétariat de la Société des Nations = Weekly epidemiological record/Health Section of the Secretariat of the League of Nations. 2010 Jan;85(4):21–2.
127. Chutinimitkul S, Herfst S, Steel J, Lowen AC, Ye J, Riel D van, et al. Virulence-associated substitution D222G in hemagglutinin of 2009 pandemic influenza A(H1N1) virus affects receptor binding. Journal of Virology. 2010 Sep
128. Ning Z-Y, Luo M-Y, Qi W-B, Yu B, Jiao P-R, Liao M. Detection of expression of influenza virus receptors in tissues of BALB/c mice by histochemistry. Veterinary Research Communications. 2009 Aug;895–903.
129. Chen W, Calvo PA, Malide D, Gibbs J, Schubert U, Bacik I, et al. A novel influenza A virus mitochondrial protein that induces cell death. Nature Medicine. 2001;7(12):1306–1312.
130. Zamarin D, García-Sastre A, Xiao X, Wang R, Palese P. Influenza virus PB1-F2 protein induces cell death through mitochondrial ANT3 and VDAC1. PLoS Pathogens. 2005 Sep;1(1):e4.
131. Zamarin D, Ortigoza MB, Palese P. Influenza A virus PB1-F2 protein contributes to viral pathogenesis in mice. Journal of Virology. 2006 Aug;80(16):7976–83.
132. Gibbs JS, Malide D, Hornung F, Bennink JR, Yewdell JW. The Influenza A Virus PB1-F2 Protein Targets the Inner Mitochondrial Membrane via a Predicted Basic Amphipathic Helix That Disrupts Mitochondrial Function. Journal of Virology. 2003 Jul;77(13):7214–7224.
133. McAuley JL, Hornung F, Boyd KL, Smith AM, McKeon R, Bennink J, et al. Expression of the 1918 influenza A virus PB1-F2 enhances the pathogenesis of viral and secondary bacterial pneumonia. Cell Host & Microbe. 2007 Oct;2(4):240–9.
134. McAuley JL, Zhang K, McCullers JA. The effects of influenza A virus PB1-F2 protein on polymerase activity are strain specific and do not impact pathogenesis. Journal of Virology. 2010 Jan;84(1):558–64.
135. Mazur I, Anhlan D, Mitzner D, Wixler L, Schubert U, Ludwig S. The proapoptotic influenza A virus protein PB1-F2 regulates viral polymerase activity by interaction with the PB1 protein. Cellular Microbiology. 2008 May;10(5):1140–52.
136. Zell R, Krumbholz A, Eitner A, Krieg R, Halbhuber K-J, Wutzler P. Prevalence of PB1-F2 of influenza A viruses. The Journal of General Virology. 2007 Feb;88(Pt 2):536–46.
137. Henkel M, Mitzner D, Henklein P, Meyer-Almes F-J, Moroni A, Difrancesco ML, et al. The Proapoptotic Influenza A Virus Protein PB1-F2 Forms a Nonselective Ion Channel. PloS One. 2010 Jan;5(6):e11112.
138. McCullers JA, English BK. Improving therapeutic strategies for secondary bacterial pneumonia following influenza. Future Microbiology. 2008 Aug;3397–404.
139. Conenello GM, Zamarin D, Perrone LA, Tumpey T, Palese P. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. PLoS Pathogens. 2007 Oct;3(10): 1414–21.
140. Hai R, Schmolke M, Varga ZT, Manicassamy B, Wang TT, Belser JA, et al. PB1-F2 expression by the 2009 pandemic H1N1 influenza virus has minimal impact on virulence in animal models. Journal of Virology. 2010 May;84(9):4442–50.
141. Hale BG, Randall RE, Ortín J, Jackson D. The multifunctional NS1 protein of influenza A viruses. The Journal of General Virology. 2008 Oct;89(Pt 10):2359–76.
142. Steidle S, Martínez-Sobrido L, Mordstein M, Lienenklaus S, García-Sastre A, Stäheli P, et al. Glycine 184 in the non-structural protein NS1 determines virulence of influenza A virus strain PR8 without affecting the host interferon response. Journal of Virology. 2010 Oct
143. Hale BG, Steel J, Medina RA, Manicassamy B, Ye J, Hickman D, et al. Inefficient control of host gene expression by the 2009 pandemic H1N1 influenza A virus NS1 protein. Journal of Virology. 2010 Jul;84(14): 6909–22.
144. Osterlund P, Pirhonen J, Ikonen N, Rönkkö E, Strengell M, Mäkelä SM, et al. Pandemic H1N1 2009 influenza A virus induces weak cytokine responses in human macrophages and dendritic cells and is highly sensitive to the antiviral actions of interferons. Journal of Virology. 2010 Feb;84(3):1414–22.
145. Texas State Health services. Texas Influenza surveliance Report 2010–2011 Season MMWR Week 42 Texas Influenza surveliance Report 2010–2011 Season MMWR Week 42. 2010
146. Russell C a, Jones TC, Barr IG, Cox NJ, Garten RJ, Gregory V, et al. The global circulation of seasonal influenza A (H3N2) viruses. Science (New York, N.Y.). 2008 Apr;320(5874):340–6.
147. Hensley SE, Das SR, Bailey AL, Schmidt LM, Hickman HD, Jayaraman A, et al. Hemagglutinin receptor binding avidity drives influenza A virus antigenic drift. Science (New York, N.Y.). 2009 Oct;326(5953):734–6.
148. Thompson CI, Barclay WS, Zambon MC. Changes in in vitro susceptibility of influenza A H3N2 viruses to a neuraminidase inhibitor drug during evolution in the human host. The Journal of Antimicrobial Chemotherapy. 2004 May;53(5):759–65.
149. Vijaykrishna D, Poon LLM, Zhu HC, Ma SK, Li OTW, Cheung CL, et al. Reassortment of pandemic H1N1/2009 influenza A virus in swine. Science (New York, N.Y.). 2010 Jun;328(5985):1529.

150. Kido H, Yokogoshi Y, Sakai K, Tashiro M, Kishino Y, Fukutomi a, et al. Isolation and characterization of a novel trypsin-like protease found in rat bronchiolar epithelial Clara cells. A possible activator of the viral fusion glycoprotein. The Journal of Biological Chemistry. 1992 Jul 5;267(19):13573–9.
151. Stieneke-Grober A, Vey M, Angliker H, et al. Influenza virus hemagglutinin with multibasic cleavage site is activated by furin, a subtilisin-like endoprotease. EMBO J 1992;11:2407–2414.
152. Kawaoka Y, Nestorowicz A, Alexander DJ, Webster RG. Molecular analyses of the hemagglutinin genes of H5 influenza viruses: origin of a virulent turkey strain. Virology 1987 May;158(1):218–27.
153. Bosch FX, Von Hoyningen-Huene V, Scholtissek C, Rott R. The overall evolution of the H7 influenza virus haemagglutinins is different from the evolution of the proteolytic cleavage site. The Journal of General Virology. 1982 Jul;61 (Pt l):101–4.