Pigs and pandemic influenza: myths versus facts

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Introduction: the nature of influenza viruses

Influenza A viruses are enveloped, single stranded RNA viruses in the family Orthomyxoviridae. They are further classified into subtypes based on the antigenic properties of the external glycoproteins hemagglutinin (HA) and neuraminidase (NA). Sixteen antigenically different HAs (H1-H16) and 9 different NAs (N1-N9) have been recognized so far, and their combination designates the subtype of the virus. Influenza is the classic example of a genetically unstable virus and it undergoes antigenic “drift” and “shift”. Antigenic drift involves the gradual accumulation of small mutations in the virus genome, especially in the genes encoding the HA and/or NA. This may result in subtle antigenic changes, leading to decreased recognition of the virus by the immune system and thus a greater chance for an influenza epidemic. Antigenic shift is a much more dramatic antigenic change and it refers to the introduction of a virus of another HA and/or NA subtype. There are thought to be 2 main mechanisms through which such a shift is generated: the introduction of a novel virus from an animal reservoir, or genetic “reassortment”. The latter can occur when 2 different influenza viruses simultaneously infect the same host cells. The genome of an influenza virus is “segmented” – it comprises 8 single-stranded negative-sense RNAs – and the 8 gene segments of one virus can then mix and match with those of the other virus to form a new combination virus. But there are 254 possible new gene combinations and a shift will only result if the reassortment involves a change in the HA and/or NA proteins. When such a novel influenza virus subtype enters the human population, it can overcome the existing immunity and spread worldwide to cause a pandemic.

Influenza A viruses infect humans and animals as diverse as pigs, horses, seals, ferrets and a variety of wild and domestic bird species. Research indicates that wild aquatic birds are the source of all influenza viruses in other species. Birds differ from mammals in that they are susceptible to influenza viruses belonging to any H or N subtype and that the virus replicates both in the respiratory and in the intestinal tract. Periodically, influenza viruses are introduced from wild birds to domestic species (chickens, turkeys, quail), in which there are 2 pathotypes of influenza: low pathogenic and highly pathogenic avian influenza. In contrast to the mild low pathogenic viruses, highly pathogenic viruses cause a generalized infection involving all vital organs with up to 100% mortality. The causative viruses have been restricted to subtypes H5 or H7, but not all H5 or H7 viruses are highly pathogenic. Highly pathogenic avian influenza viruses appear to arise in poultry as a result of mutations in the viral HA after the introduction of H5 or H7 low pathogenic viruses from wild birds.

The pathogenesis and clinical signs of influenza are in fact remarkably similar in humans and in pigs. The virus replicates in epithelial cells of the entire respiratory tract, notably the nasal mucosa, trachea and lungs. The infection and disease are very transient, and virus excretion in nasal swabs and virus replication in the lungs last for 6-7 days at most. Though the virus does not replicate outside the respiratory tract, the disease is associated with a variety of systemic symptoms. Typical outbreaks of swine influenza (SI) are characterized by a rapid onset of high

fever, dullness, loss of appetite, labored abdominal breathing and coughing. The typical signs of human influenza include fever, chills, cough, headache, myalgia, malaise, anorexia and sore throat. Still, asymptomatic infections are common in both species, and pigs in particular become frequently infected with swine influenza viruses (SIVs) without showing clinical signs. Influenza viruses are potent inducers of inflammatory cytokines locally in the respiratory tract. Examples of such cytokines include the interferons, tumor necrosis factor-alpha, the interleukins-1 and -6, and several chemokines. It is now clear that these cytokines are important mediators of flu-like symptoms in pigs as well as in humans. But the same cytokines are most likely also responsible for the extremely rapid and solid immune response to influenza viruses.

Compared to other influenza virus hosts, pigs are also closer to humans when it comes to the nature and origins of their influenza viruses, and the same virus subtypes - H1N1, H3N2 and H1N2 – are circulating in humans and in swine. But while influenza viruses from humans and pigs frequently have a common origin, they are not 100% identical at the antigenic and genetic level. Also, the SIVs in Europe differ significantly in their antigenic and genetic make-up from those circulating in North America, and still other variants are circulating in various Asian countries. The predominant H1N1 SIVs in Europe, for example, are entirely of avian origin. They were introduced from wild ducks into the pig population in 1979 and are unrelated to the human H1N1 viruses causing seasonal influenza. Two types of H1N1 SIV, in contrast, are circulating in North America. The so-called “classical” H1N1 viruses have been present since the early 20th century and they probably emerged by transfer of the human 1918 pandemic virus to swine. These swine H1N1 viruses therefore have a similar origin as the seasonal human H1N1 viruses, but the latter viruses have shown a different and basically more rapid evolution. A second type of North American H1N1 viruses are reassortants with the surface glycoproteins of this classical swine H1N1 virus and internal proteins of more recently emerged H3N2 or H1N2 SIVs. These “triple reassortant” viruses are important progenitors of the current novel H1N1 2009 pandemic virus, as explained further. It should be noted here that most SIVs are a result of genetic reassortment and their genes are composed of human and/or avian and/or swine virus genes. Viruses of both other subtypes, H3N2 and H1N2, also have a different history and constitution in Europe and in the USA, but it is beyond the scope of this paper to discuss this in detail. The bottom line is that the epidemiology and evolution of influenza viruses in pigs are extremely complex and, unlike for human influenza viruses, different in different regions of the world.

Influenza pandemics in the 20th century and the presumed role of pigs

In theory, pandemic influenza viruses must have a novel HA subtype to which the human population has no immunity, have the capacity to cause serious human disease and, importantly, spread efficiently from human to human. The introduction of a novel influenza virus in the human population per se is insufficient start a human pandemic, because the virus must undergo genetic changes - either by mutation or genetic reassortment - to become adapted to efficient replication and transmission in humans. Three influenza pandemics have occurred during the 20th century: the Spanish flu (H1N1) of 1918, the Asian flu (H2N2) of 1957 and the Hong Kong flu (H3N2) of 1968.

The Spanish influenza pandemic was the most extreme and killed more people, at least 20 million, than World War I. The first clinical observations of SI were also made at that time and investigators were impressed with the clinical and pathological similarities of human and swine influenza in 1918. Years thereafter, phylogenetic analyses indicated that the 1918/1919 human and swine viruses were genetically similar and likely originated from a common ancestor. The

most widely disseminated hypothesis is that the virus jumped from birds to humans shortly before the start of the pandemic. But some researchers recently dispute this hypothesis. They argue that the virus evolved in people or pigs for an unknown period of time before the pandemic started and that it most likely was a reassortant and not a pure avian virus. Because of a lack of sequence data for swine and other influenza viruses from these periods, the exact origin of the 1918 virus will probably remain controversial and we may never know whether the virus first emerged in people or in pigs.

Both the 1957 and 1968 pandemics were caused by reassortants between the circulating human influenza virus of that time and an avian virus that provided a novel HA and one or 2 other genes. For many years it was believed that the reassortment did not directly occur in humans but in pigs, which served as an intermediate host to transfer the viruses to humans. The textbooks continue to quote this hypothesis, but there is in fact no direct evidence for the role of pigs in the generation of the 1957 or 1968 pandemics. Why then have pigs been blamed so often? Much of the hypothesis is based on experimental studies from the 1980s and '90s and on epidemiologic data from that time. One key finding was that reassortants between human and avian influenza viruses frequently emerge in pigs. The notion of the pig as a unique mixing vessel for these viruses was further supported by the discovery, in 1998, that epithelial cells of the pig trachea contain both human- and avian-type receptors. It was also shown that continued replication of an avian virus in pigs in nature may lead to variants that preferentially recognize human-type receptors and that are thus better adapted for replication in humans. Furthermore, infection of humans with influenza viruses from birds appeared to be extremely rare in the past, with only 3 reported cases from 1959 to 1996. Influenza viruses derived form swine, on the other hand, had been shown to transmit to humans on several occasions since the 1950s. All this has led to the central dogma that pigs are uniquely susceptible to both avian and human influenza viruses and that they serve as mixing vessels and intermediate hosts for the transmission of avian viruses to humans. The next paragraph will clarify why many of these older theories have now become outdated.

Outbreaks of highly pathogenic H5N1 avian influenza: a pandemic scare

Since 2003, outbreaks of H5N1 avian influenza have been reported in poultry in many different countries on several continents, including Asia, Africa and Europe. These are the largest and most devastating outbreaks of highly pathogenic avian influenza in history, and millions of chickens and ducks have been culled at a high economic cost. A total 467 human H5N1 infections have been reported to the World Health Organization from the start of the outbreaks in 2003 to December 2009, and 282 of these were fatal. This exceptionally high case-fatality rate is most likely due to a massive replication of the H5N1 virus in the lungs of humans, which results in a "cytokine storm" and acute respiratory distress syndrome. Still, the total number of human infections remains small compared to the numbers of infected poultry. Most, if not all, of the human cases were in people with close contact with infected poultry and direct exposure to large amounts of H5N1 virus, questioning the so popular hypothesis of the pig as an intermediate host. Fortunately, the virus has not become adapted for human-to-human transmission and this is a major reason why it did not cause a pandemic.

Pigs can and do become infected with H5N1 under natural and experimental conditions, but all evidence indicates that the currently circulating H5N1 viruses are not well adapted for replication in pigs. The incidence of H5N1 infection in pigs in Asia has remained very low, even in areas where the virus remains endemic in poultry. The few available experimental infection studies indicate that the H5N1 virus replicates in the respiratory tract of pigs, but to a much

lower extent than typical SIVs and mainly in the lungs. Remarkably, the pigs showed only mild or no clinical signs, while the viruses were extremely virulent for poultry. Also, the infected pigs failed to transmit the virus to uninfected littermates. Unfortunately, all these studies involved very few pigs and a limited number of examinations, which is inherent to pig infection experiments under biosafety level-3 conditions.

In our lab we have performed a detailed pathogenesis study in pigs with a low pathogenic avian influenza virus, subtype H5N2. This virus is structurally very similar to the higher mentioned H5N1 viruses, but it is not dangerous for poultry or humans. We compared the extent and sites of replication of the avian H5N2 virus with that of an enzootic European swine H1N1 virus. Both viruses showed a similar organ tropism and duration of infection: they were found in epithelial cells along the entire porcine respiratory tract, and mainly in the lungs, for 6 consecutive days after the intranasal inoculation. The avian virus, however, infected proportionally fewer cells than the swine virus at all levels of the respiratory tract. As a result of this, the nasal cavity, which is a major portal of entry for influenza viruses, appeared to be extremely difficult to infect for the avian virus. This finding may explain why pigs apparently need exposure to high doses of avian influenza viruses to become infected, why they have lower amounts of avian than swine viruses in their nasal secretions after experimental infection, and why it is so hard for avian viruses to spread between pigs.

We have also compared the replication capacities of various swine and avian influenza viruses in vitro, in porcine respiratory organ cultures. This was combined with detailed studies of influenza virus receptor expression along the entire upper and lower respiratory tract, which were entirely novel. Like in the pig infection experiments, the avian viruses were strongly hampered in their replication, especially in the nose and trachea, but they started to replicate better in the bronchioles and lungs. The preference of the avian viruses for the lungs corresponded with the expression of influenza virus receptors. The “human” receptor, which is also used by SIVs, was found in the upper and lower portions of the airways, the “avian” receptor was almost exclusively present in the lungs. In sharp contrast with earlier beliefs, there is now agreement that the respiratory tract of humans also contains both types of receptors. The avian receptor, however, was only found deep in the lungs, the preferential replication site for avian viruses in humans, as in pigs. All this fits with the recent realization that genetic reassortment can also occur in humans, and not only in pigs. Simply put: the pig may be less unique and more similar to humans than previously thought. Wholly avian viruses are clearly hampered in their replication in either pigs or humans. The genetic changes that are required for adaptation to mammals may still occur in the pig, but they could just as well take place in humans or even some bird species.

A new pandemic that is not bird flu: the novel swine-origin influenza virus

While many feared an avian influenza H5N1 pandemic emerging from Asia, the first pandemic since 41 years is caused by a swine-origin influenza virus and it started in North America. Against all expectations, this novel H1N1 virus belongs to a subtype that has been circulating in the human population from 1918 to 1957 and again from 1977 until now. Still the novel H1N1 virus differs radically from the human seasonal H1N1 viruses, and it is a reassortant of at least 2 circulating SIVs. Six gene segments are similar to ones previously found in triple reassortant SIVs circulating in pigs in North America, from which the pandemic virus has derived a classical H1 HA. These swine viruses already have a mix of avian, human and swine virus genes. The second virus, from which the genes encoding the N1 neuraminidase and matrix proteins are derived, is circulating in swine populations in Europe or Asia.

It has long been known that SIVs sporadically jump to humans. There are some 70 documented cases of swine flu in humans since 1958. Almost any of the established North American or European SIV genotypes has occasionally been isolated from humans. Most of these people had close contact with pigs and the human disease was usually clinically similar to disease caused by infections with human influenza viruses. A few cases were fatal, but these were mainly in persons with underlying medical conditions. Most important is that all of the SIVs so far lacked the critical property to spread further in the human population, and this is a major difference with the novel 2009 H1N1 pandemic virus. It will also be clear that the total number of proven SIV cases in humans remains small compared to the total number of humans with occupational exposure to pigs. Serologic studies, on the other hand, suggest that these few documented cases are only the tip of the iceberg. These studies consistently found higher seroprevalence rates and higher antibody titers against SIVs in those working with pigs than in non-swine-exposed controls. Unfortunately, the interpretation of such studies remains very difficult: the elevated antibody titers do not necessarily result from infection with SIVs, because of the possibility of partial serologic cross-reactivity between human and swine influenza viruses of the same subtype in the serologic assays used. One certainty, therefore, is that the true incidence of SI in humans is unknown.

Though the novel 2009 H1N1 pandemic virus almost certainly comes from pigs, it is still a mystery how, when and where it originated. Before its detection in humans in the spring of 2009, this specific virus had never been found in pigs. But there is only limited surveillance for influenza in pigs, and the virus has probably been circulating unnoticed in swine somewhere. In any case, the novel H1N1 virus was almost certainly absent in European swine populations in the past, as the “European Surveillance Network for Influenza in Pigs” that has operated from 2001 to 2008 has never detected it. Several swine influenza research groups set out to conduct experimental pig infection studies with the novel H1N1 virus soon after its swine-origin was a fact. These studies unanimously showed that the virus is infectious to pigs and spreads rapidly in a pig trial population. The virus seemed to behave almost exactly like the enzootic SIVs. From mid May 2009 until this time of writing, occasional cases of the novel H1N1 virus on swine farms have been reported in a total 20 countries, including (Northern) Ireland, the UK, Norway, Iceland, Finland, Italy, Germany and Denmark. The pigs seem to have become infected by infected humans, and there is no evidence so far that swine are playing any role in the epidemiology or in the worldwide spread of the virus in the human population. It remains to be seen whether the novel H1N1 virus will become established in swine populations. One critical question here is whether immunity to enzootic SIVs may protect pigs against this novel virus.

To get a preliminary sense as to whether the European swine population is fully susceptible to the novel H1N1 virus, we have examined sera from pigs that had been infected or vaccinated with European SIVs. The sera were tested for antibodies against the novel H1N1 virus, as well as SIVs that have been circulating in North America over the last decade. All these viruses have a classical H1 HA that shows very low sequence identity (only 75% or less) with the H1 of European SIVs. Pigs that had undergone a single infection with European H1N1 or H1N2 SIVs lacked antibodies to the novel H1N1 and North American viruses. In contrast, serologic cross-reactivity was common after consecutive infections with 2 different European subtypes or after vaccination with commercial vaccines. These serologic data suggest that European pigs may have partial immunity to the novel H1N1 virus and that the chances that they will become involved in the spread of the virus to humans may be lower than generally believed. The next step is to test this assumption in well-controlled experimental challenge studies in pigs.

No one can predict the evolution of the novel H1N1 virus and the eventual impact of the pandemic for humans. So far, the virus is not more virulent for humans than the seasonal human influenza viruses and the mortality rate remains low. Another important fact is that there may be a certain degree of immunity to the pandemic virus in the human population. The novel H1N1 virus is antigenically very different from the contemporary seasonal human H1N1 viruses, but it is relatively similar to H1N1 viruses circulating in the human population up until the 1950s. This agrees with the finding that a substantial proportion of humans over 60 appear to have cross-reactive antibodies to the novel H1N1 virus, and it likely explains why the virus hit the young harder than the elderly. This is just one reason why the pandemic has remained relatively mild. In addition, specific vaccines against this novel virus have been developed in a very short time and they have been used widely in several European countries.

Conclusions

We owe several new insights into the generation of pandemic influenza viruses to the outbreaks of H5N1 and to the emergence of the novel 2009 H1N1 pandemic virus. Both viruses have taken the edge of some old dogmas about the role of pigs in influenza pandemics. It is clearly a myth that pigs are essential intermediate hosts for the transmission of avian influenza viruses to humans, that they are the single animal species with both avian- and human-type influenza virus receptors, or that genetic reassortment is restricted to the pig. On the other hand, it is true that pigs frequently are a platform for reassortment and they can transmit reassortant viruses to humans. Though obviously a very rare event, there is a real danger that swine-origin reassortants start to spread readily between humans and launch a pandemic, and this is perfectly illustrated by the novel H1N1 virus. What we still don't know is what is needed for efficient replication in and adaptation of influenza viruses to pigs. Similarly, it remains unknown what factors trigger transmission of influenza viruses from pigs to humans at the physiological and molecular level, or what is needed for the further transmission of such viruses between humans. These are difficult but extremely important questions that can only be solved through research. Combined with improved surveillance for influenza in animals, effective vaccines and antivirals, an understanding of these questions is critical to the control of future influenza pandemics.

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