FROM ZOONOTIC INFLUENZA TO FULL SCALE INFLUENZA PANDEMIC: A WINDOW OF OPPORTUNITY NOT TO BE MISSED

Jean-Claude Manuguerra
Laboratory for Urgent Response to Biological Threats, Institut Pasteur, 75724 Paris, France

Abstract
Avians are the cornerstone of the world of influenza viruses. However other species such as pigs and humans can also be infected by influenza viruses (IV), which established themselves in their mammalian hosts. At irregular intervals, we observed the introduction in pigs or men of new IV, resulting either from an *in toto* transmission of an avian virus or from a reassortment one involving at least some genomic segments of an avian IV. In humans, such events have been associated with pandemics. The phase of the introduction of a new influenza A virus subtype in humans from the avian reservoir, with or without an intermediate species such as pigs, represents a time for urgent intervention as soon as it is detected. Measures to avoid human cases of avian influenza can be implemented such as culling sick bird domestic flocks or as implementing personal physical protective equipment for some categories of farm/veterinary workers for example.

Text
In 2003/2004, a panasian outbreak of avian influenza A(H5N1) devastated the whole poultry industry of a number of countries located on an bow linking South-Korea through to Thailand including Japan, mainland China, Vietnam, Laos and Cambodia. In Thailand as well as in Vietnam, these outbreaks were associated with human cases of influenza A(H5N1) acquired from direct transmission from sick birds.
The spectre of a new human influenza virus sprung in our collective memory as a remembrance of influenza pandemics, which plagued the earth throughout our history. It is difficult to assess whether epidemics of acute respiratory febrile illness, which occurred before the sixteenth century, were influenza outbreaks or not. From that point in time, influenza pandemics or great epidemics occurred in Europe in 1510, 1557, 1729-1733, 1781-1782, 1829-1833 et 1889-1890 and in 1900 (for review) (Potter, 1998).
The twentieth century started with the Great War and the biggest influenza pandemic ever described, *Spanish Influenza*, with casualties as numerous as 20 to 40 millions (Oxford, 2000). In total, half of the world human population was affected. In France, the first cases were reported in April 1918 among the armed forces located in Normandy. Influenza then spread to the rest of Europe and reached the United States and India. The first wave stopped in August 1918 and the second wave started in September 1918 to reach its peak at the end of October 1918, except in Australia where it continued to increase until January 1919.
The Spanish Influenza then stimulated the mind of microbiologists and René Dujarric de la Rivière, at the Institut Pasteur showed for the first time that influenza was due to an ultrafiltrable agent. In 1931, Richard Shope, puzzled by the coincidence of Spanish Influenza in men and outbreaks of a similar disease in pigs, isolated the first influenza virus, labelled as such, from pigs (Shope, 1931a, Shope, 1931b). The first human influenza virus was isolated two years later in North London, using an unexpected animal species to propagate the virus: the ferret (Smith *et al*., 1933). Two other kinds of influenza viruses, which were serologically different from what is now labelled type A, were later identified as type B (Francis, 1940) and type C viruses (Taylor, 1949). Only type A viruses are known to be associated with pandemics when a new of its subtypes is introduced in humans.
In 1957, a new pandemic virus appeared with the *Asiatic Influenza*. Although it shared common antigenic characteristics, the virus differed from its predecessors by its surface antigens. The initial outbreaks of the unfolding pandemic were first described in the south of China in February 1957 but it is possible that the new virus, identified as A(H2N2), appeared sometime sooner in this area. The virus later spread to the Yuan Nan Province and Hong Kong in April 1957, then to Singapore, Japan and the rest of the far East. The Middle-East was affected in July before Africa was hit. The new virus reached Europe during the summer 1957 but the epidemic started during the following autumn (1957). Although the number of deaths was high (2 millions worldwide (Oxford, 2000)), it never reached the scale of that of the *Spanish Influenza*. During about ten years, it circulated instead of its predecessor the A(H1N1) subtype.
Again, sometime before 1968, a new virus subtype of influenza A virus arose causing the *Hong Kong Influenza pandemic*. The outbreak of influenza A virus observed in July 1968 in Hong Kong was preceded by similar outbreaks in South-East China and was followed by influenza epidemics in Singapore, the Philippines, Taiwan, Vietnam, Malaysia (August 1968) and finally India and the North of Australia in September 1968. After this, the epidemic halted for a while, except in the United Sates where it reached California in October and then the rest of this country. Tropical countries such as Brazil, Kenya and Indonesia were hit between the last quarter of 1968 and the first one of 1969. Countries in the Southern hemisphere were affected by moderate epidemics between March and May 1969. The first episode stopped in April 1969 and in Europe influenza activity during the 1968/1969 season was usual and caused by A(H2N2) viruses. The second episode caused by the new A(H3N2) virus started in western Europe in the autumn 1969, peaked in December and stopped in March 1970. There, the epidemic was more severe than elsewhere before.
Sequence data suggest that the A(H3N2) viruses inherited six genome segments from its predecessor the human A(H2N2) virus and two other segments from a duck virus, including its haemagglutinin (H3). The scale of this last pandemic was even smaller than the previous one with a number of case fatalities less than one million.
Since then, influenza A(H3N2) viruses come back every year or so to cause epidemics in humans. Since 1977, with the re-emergence of the A(H1N1) subtype in humans, this virus has been circulating together with

influenza A(H1N1), influenza B and C viruses. Influenza A and B viruses bear two surface glycoproteins: the haemagglutinin (HA) and the neuraminidase (NA). Among influenza A viruses, the subtypes are determined by antigenic and genetic characteristics of the HA and the NA. The number of HA and NA, which are or were part of human or swine viruses, are limited to H1, H2 and H3 and to N1and N2 whereas there are 15 molecular species of HA (H1 to H15) and 9 of NA (N1 to N9). These are all found in avian viruses, particularly in wild aquatic bird viruses such as ducks and geese viruses.

Influenza viruses were isolated from birds in Asia, in Oceania, In Europe and in America. The avian species harbouring the viruses are numerous and belong to various zoological orders and families. Among these birds, some migrate over long distances from the Northern hemisphere, where they establish their nest and procreate to the Southern, where they shelter during winter. During the migration, birds enjoy pauses during which they live in promiscuity with birds of various species originating from diverse Northern regions. Among the avian migrating population, a large number of young birds do the trip for the first time in their life. They represent a population naïve to many viruses and are a target for influenza virus infection. According to some data, some quite old now, the proportion of infected birds coming down south in autumn is highest compared to other time of year. In the Baie de la Somme in France, the infection can be so widely spread (Hannoun & Devaux, 1980) that virus could be recovered from water.

Ducks such as mallards belong to migrating colonies or to wild non migrating population and even to domestic flocks. This explains how easily, influenza viruses can be transmitted from the wild world to animal populations in the closest contact with men such as pigs. Infection of humans by pigs has well been documented.

Influenza A(H1N1) and A(H3N2) as well as A(H1N2) viruses have been established for years in pigs, which can be infected either by human and/or avian viruses. Pigs can therefore play the role of mixing vessel and transmission link between humans and birds, one of the reasons of this being that swine epithelial cells of the upper respiratory tract harbour both type of "*receptors for the virus*". These are terminal sialic acids borne by glycoproteins, those preferentially recognised by human viruses (alpha 2-6) and those preferentially recognised by avian viruses (alpha 2-3). Pigs can also be "conservatories" of old human influenza A viruses such as A/Victoria/3/75(H3N2)-like viruses.

Until 1997, the direct infection of men by avian influenza viruses associated with respiratory symptoms was thought to be impossible. In 1997, the so-called "*Chicken flu*" in Hong Kong did not lead to a pandemic although a new subtype A(H5N1) was infecting men. Data on A(H5N1) viruses isolated then from men and compared with their avian counterparts suggested that these were not adapted to humans and failed to start a chain of transmission. Such adaptation can be acquired by the accumulation of mutations in various genes and/or by reassortment between two viruses, one being already adapted to humans. This must have been the case around 1957 where, possibly in pigs, the contemporary human influenza A(H1N1) virus reassorted with an avian influenza A(H2N2) virus making up a new A(H2N2) virus with genomic segments PB1, HA and NA from the avian parental strain and the other five segments from the human A(H1N1) parental virus. Again a similar situation must have occurred around 1968, with the contemporary human influenza A(H2N2) reassorting with an avian influenza A(H3Nx) virus making up a new A(H3N2) virus with PB1 and HA from the avian parental strain and the six segments from the other parental virus, the human A(H2N2) strain. Genomic reassortments are common in human viruses as it is illustrated by the emergence of the A(H1N2) in 2001 in humans (Xu *et al.*, 2002). There are also common in birds as recently shown (Hatchette *et al.*, 2004).

Reassortment is probably the biggest risk for the emergence of new influenza A virus subtypes in humans. Between December 2003 and April 2004, a total of 34 human cases of influenza A(H5N1) symptomatic infections were reported in Vietnam and Thailand with 23 deaths (WER, 2004). The case fatality rate was very high for such respiratory infections. A first paper describing human cases in 2003/2004 showed that, in all 10 cases analysed, the infection appears to have been acquired directly from infected poultry. They also show that eight out of ten patients died although none of them had preexisting medical conditions (Tran *et al.*, 2004).

Genetic data concerning the 2003/2004 avian and human A(H5N1) isolates are currently being made available and show that reassortment had not occurred between a human A(H1N1), A(H1N2) or A(H3N2) virus and the avian current dominant A(H5N1) virus causing the vast epizootics. However, a recent study (Li *et al.*, 2004) show a series of genetic reassortment events traceable to the precursor of the H5N1 viruses that caused the initial human outbreak in Hong Kong in 1997 and avian outbreaks in 2001 and 2002. These events gave rise to a H5N1 genotype (Z) dominant in chickens and ducks, which was responsible for the 2003/2004 widespread outbreak. Interestingly, their data suggest that domestic ducks in South China played a central role in the generation and maintenance of this virus genotype. They also suggest that wild birds may have contributed to the wide spreading of the virus in Asia. Furthermore, their results advocate that influenza A(H5N1) viruses with pandemic potential have become endemic in Asia and the situation we witnessed since the beginning of 2004 in the affected countries demonstrated that this virus was not easily eradicable and carries on being a Damocles' spade over our head. A recently published model estimated that, during the epidemic circulation of human influenza A viruses with 10% of the population infected at anyone time and with a probability of reassortment of 1 in case of co-infection with two viruses, 45 human cases of infection by an avian A(H5N1) influenza virus would have corresponded to a 5% risk of reassortment (Ferguson *et al.*, 2004). This figure reached 50% if 600 A(H5N1) cases were to happened. Using this model, the calculated hypothetical risk of reassortment reached 3.4%, which is probably overestimated but was nevertheless not negligible. That was the time to cull all sensitive animals in infected farms to prevent any human/avian virus reassortant virus to appear. In the same paper, the authors suggest that instead of

considering the risk of reassortment by observing the total cumulated number of human cases of A(H5N1) infections, the size of the clusters of A(H5N1) human cases were most relevant to monitor. With various hypotheses on the reproductive rate of the infection, thresholds were calculated above which, epidemiological data would point out an unusual event corresponding, most probably to a virological event (Ferguson *et al.*, 2004) to investigate in priority.

As in 1997, in 2004 influenza A(H5N1) viruses did not established themselves in the human population and human to human transmission chain never started or, if it did it aborted very quickly. The culling of millions of poultry was probably necessary and was this far successful to avoid more human cases and possibly viral reassortment and/or cumulated mutations (2004).

Although emphasis has recently been put upon the role of some bird species such as hens for their role in the amplification and transmission of avian influenza viruses to humans, pigs should not be ignored as a zoonotic source of influenza or as a mixing vessel, which could facilitate the transmission to human of a new pandemic influenza A reassortant virus.

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