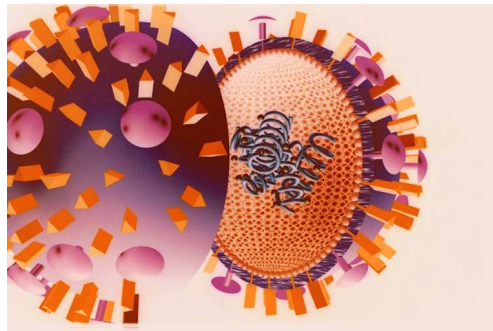


"Avian flu : the new Yellow Peril?"



A runny nose, endless bouts of sneezing and coughing that shake you from head to toe in a feverish haze... Remind you of anything? No? From Asia to Europe via the North and the South Pole no one - not even the hardest - is spared by influenza. Though seemingly trivial, influenza can be dangerous and even fatal. In the last century alone, three flu epidemics took their toll by the million. And what causes flu? A virus and... its proteins. Today, experts agree the chances are that we may expect another world epidemic in the near future.

Autumn 1918. Soon after World War I, an even greater peril threatened. A virus, later known as the "Spanish flu", was about to wipe out several million people over a period of only a few months, before disappearing without trace. Amongst those who succumbed were a number of key figures: Apollinaire, Edmond Rostand, Gustav Klimt and Egon Schiele. In the early 1900s, bacteriology was a thriving new field of research and it enjoyed a certain prestige. Indeed, towards the end of the 19th century, Pasteur had revolutionized the scientific world with his theory that bacteria were at the root of every infectious disease. This had since been unanimously accepted and indeed substantiated by a series of successes in identifying the pathogenic agents of such terrible diseases as cholera, tuberculosis and syphilis. Furthermore, in 1892, Richard Pfeiffer, a German bacteriologist, believed he had identified the pathogenic agent of influenza in bacteria he called *Haemophilus influenzae*. In this context, the epidemic of flu was quite a slap in the face of the then cocksure science of bacteriology. As the most deadly epidemic since the Black Death in

1300 raged on, scientists were finally forced to admit that *Haemophilus influenzae* was not a bacterium but a virus. With the collapse of Pfeiffer's theory, bacteriology suffered a new crisis: a world rid of infectious diseases was an illusion.

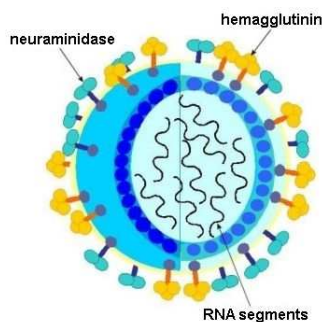
Eighty years later, the recently recurring outbursts of avian flu in Asia is causing alarm in scientific circles. Why are a few cases of human contamination by the chicken virus so disturbing? Should not progress in medicine afford us protection against a human disaster as great as the Spanish flu?

Haemophilus influenzae: clinging on

The flu was no doubt ruthless during Antiquity, but it was only in the 14th century that it was given its scientific name "influenza virus" in Florence, meaning "the influence of the stars and the cold". Its French name ("grippe") is derived from the Gothic, meaning "to cling suddenly". Such as *Haemophilus influenzae* does. Literally.

The flu is caused not only by one but several viruses that can be of three different types: A, B or C. They are very closely related but only A and B cause concern to humans; they are transmitted from person to person via microdroplets in the air we breathe.

What do these viruses look like? They are microscopic spheres coated with spikes and resemble sea urchins or lychees. Two proteins lodged in the virus' membrane - hemagglutinin and neuraminidase - play an essential part in the mechanism that leads to infection. These are also called "surface" proteins. The hemagglutinin specifically recognizes a receptor protein on the surface of the cell it is about to infect and to which it clings tightly.



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Fig.1 Diagram of an influenza virus in cross section

Inside each sphere rests the virus' genetic information: eight segments of RNA each of which contains the required information to produce a different viral protein. As with all viruses, once it has penetrated into the infected cell, *Hemophilus influenzae* makes use of the latter's machinery to multiply. The different parts of the virus (RNA and viral proteins) are made separately in many copies, and subsequently assembled. The resulting new viruses can then leave the cell. It is at this stage that the second surface protein - neuraminidase - makes an appearance. Neuraminidase is an enzyme whose function is to "cut" the receptor protein on the surface of the infected cell subsequently preventing the new viruses from clinging to it as they are freed.

From genetic shift to genetic fault

Once an organism has been infected by a virus, it needs a little time to build a counter strategy, i.e. an immune response. During this period, the virus meets no resistance and multiplies unhindered, thus producing new viruses that can infect other organisms. Antibodies constitute one of our mechanisms of defense. They are

specialized molecules each of which recognize one type of virus. Once recognized, antibodies neutralize it before it has time to infect any cells. If the virus happens to be "variable" - like Influenza - our system will produce antibodies to combat a "subtype" of virus and that particular subtype only. Viruses that are recognized by the same antibodies are considered as originating from the same subtype. However, the same subtype can also consist of hundreds of variants, that is viruses that are very similar but not alike. In the case of the Influenza viruses, both surface proteins, i.e. hemagglutinin and neuraminidase, are recognized by the antibodies. This is why type A viruses are classified according to the subtype of each of their H (hemagglutinin) and N (neuraminidase) proteins (e.g. H1N1, H2N3 and even H5N1).

Once our body is rid of all traces of the virus, the antibodies still remain active for several years. As a consequence, if we are subsequently infected by the same virus or one which is very similar, it will not be able to multiply. This is the very principle of vaccination. Of course any virus is less than happy with this state of affairs: its survival is at stake. But viruses are resourceful and one means of side-stepping this is a virus' ability to mutate (type C viruses do not have this ability and are therefore harmless). As mentioned above, in order to multiply the flu virus must copy all its genetic information, i.e. its RNA. This is achieved by an unscrupulous viral protein, which can neither "proofread" nor "correct" and so frequently makes mistakes as it copies the viral RNA. Such errors in the new viral RNA give rise to novel mutations, some of which will have no effect on newly synthesized viral proteins. However, a number of mutations will actually end up destroying the virus since they will have also destroyed the function of a protein that is essential to its survival. Finally, certain "minor" mutations will turn out to be favorable to the virus. In which way? Minor mutations only alter the "appearance" of hemagglutinin and neuraminidase without affecting, however, their function. In the long run, the accumulation of such mutations leads to the emergence of variants of the virus, which will be able to avoid recognition by antibodies already present in humans who have been previously infected. Such a phenomenon, which is present in all flu viruses, is known as a "genetic shift".

This is why, every year, the flu virus can strike again and cause new epidemics, thus compelling researchers to update the flu vaccine regularly. However a number of antibodies can recognize variants of the same subtype, which explains how the spread of a new epidemic - caused by a mutated virus but which belongs to a same subtype as that of previous years - is constrained. It

follows that the most infectious viruses (that is those that will multiply and spread) will be those that differ the most from those of the year before; so long as the variant is of the same subtype the epidemic will be under control thanks to the antibodies mentioned above. But if a virus derived from a new subtype appears, the population is unprotected and the usual result is an epidemic of pandemic proportions. What is more, vaccinations are useless since they are outdated: the vaccines were adapted to the strain of virus of the previous year.

How can a sudden change of subtype occur? There are two possibilities. First, subtypes can have been "absent" amongst humans for several years but remained present in animals. If the animals are in contact with humans, they can transmit the virus. This is what happened with the H1N1 subtype which caused the outbreak of the Spanish flu: by 1957, it had disappeared from the human population but was still present in pigs, and was again transmitted to humans in 1977. The further back in time the outbreak of a virus is, the less natural protection people have. The second possibility is that the subtype is a new one altogether. This is due to a mechanism known as the "genetic fault". What happens is that one of the surface proteins, hemagglutinin or neuraminidase, undergoes a complete change. Such an occurrence is rare and limited, taking place every 10 to 30 years and only in type A viruses.

What is the process underlying the 'genetic fault' mechanism? Type A viruses infect several kinds of animals some of which are frequently in contact with human beings, such as pigs, horses and various species of birds for example. In fact, on the whole, animals are infected by many more subtypes than human beings are. Fortunately on rare occasions, pigs can be infected by both a human and an avian virus. When these two viruses multiply in the same cell and produce several copies of their 8 RNA segments, there can result a hybrid virus at the time when the new viruses are being assembled and this hybrid will have incorporated RNA segments from both the original viruses. If, in this new virus, the segment particular to birds contains information required for the synthesis of the surface proteins, the virus will manage to shun any recognition by the human immune system.

What is more, if it has adaptation genes specific to humans, it will be able to multiply without hindrance and will undoubtedly provoke a world epidemic. In Asia these different species prosper on poultry farms and, because of contact with humans, co-infections are frequent. Co-infections are melting pots for the genetic material of both human and avian viruses and drive the creation of new subtypes. Recent events, however, have

established that yet a third mechanism is possible: it seems that humans can actually act as the melting pot for some of the 13 subtypes of virus carried by the avian population!

Not all animal viruses are transmissible to humans however and, in normal circumstances, avian viruses do not infect species other than birds or pigs. What is more, a new subtype can infect a number of people but will not be infectious from one person to another. This was the case in 1977 when an epidemic of avian flu struck the poultry in Hong Kong. The strain of virus - derived from subtype H5N1 - infected 18 people causing severe respiratory disorders, six of whom died. It was the first case of human contamination by an avian strain to be noted, but it proved to be innocuous from man to man. Later, genetic studies revealed that the virus had been transmitted directly from bird to man. Early in 2003, there was a new outbreak of the virus with a few cases of human contamination, but the most recent alert was in January 2004 when laboratory reports confirmed the presence of H5N1 in patients suffering from severe respiratory disorders in North Viet Nam.

Great pandemics of the 20th century

Over the course of the 20th century, the H protein of the Influenza virus type A has been subjected to major modifications on three occasions thus giving rise to three new subtypes. These subtypes triggered off devastating world epidemics, the most notorious of which was the Spanish flu in 1918-1919 - which incidentally was not Spanish at all. Indeed, Spain was a neutral country at the time but was the first to admit publicly that there was an epidemic. Its neighbors who were at war preferred to keep the information secret. Today, it is believed that this type of flu came from America. The H1N1 virus caused the outbreak and it is reckoned that 50% of the world population was infected, causing the death of 40 to 50 million people. 1957 was the year of the Asian epidemic, which was caused by simultaneous changes in H and N, and H2N2 replaced H1N1. In 1968-69, virus H alone was modified and H3N2 appeared, and was subsequently called the Hong Kong flu. The sick and dying were countless during these two epidemics and throughout the world the number of deaths was estimated at about 6 million. Although severe or even fatal forms of flu normally affect elderly people or those with respiratory problems and pregnant women, it was the young and healthy who suffered most in these last two epidemics.

However, the number of deaths never reached the same proportions as those during the Spanish

flu, during which the death rate was in fact highest amongst the young.



Courtesy of the National Museum of Health and Medicine, Armed Forces Institute of Pathology, Washington, D.C. (NCP 1603)

Fig.2 Patients with the Spanish flu in 1918

What is it that happened in 1918 to cause such a disaster? Two factors were involved but only one is well known to us. To start with, the virus subtype was new. Until then, the existing viruses were either type H2N8 or H3N8; this particular one was H1N1, very close to the pig virus. Secondly, this virus was 10 times more deadly than any of those listed in the previous 150 years. In particular, it caused bronchitis/bronchiolitis which brought on death by suffocation. What is more, it struck down people aged 20 to 50, whose mortality rate was normally lowest. And this is what is not yet well understood, although a mutation in one of the virus's proteins could be the cause. Hence, the numerous attempts to find an intact copy of the original virus. The last attempt was made in the Arctic in August 1998. Fifteen scientists from four countries - Canada, the United States, Great Britain and Norway - went to the Arctic Circle to uncover the bodies of seven Norwegian miners, seven young men who had died of the Spanish flu in October 1918 on Longyearbyen Island, one of the coldest regions in the world. They had been buried in the permafrost and were likely to harbor the frozen virus still intact. Macabre, you might say. Perhaps. But this was done in the hope that if ever a similar virus were to resurface, it would be rapidly identified as extremely dangerous and the medical profession would be prepared for it. As for the Americans, they also isolated the "killer virus" from samples taken from patients' lungs at the time of their death and have preserved them. But, for all that, analyses of the original virus have still not been able to lift the veil of mystery surrounding it.

Since the first outbreak of the H1N1 virus, there have been three more: in 1947, in 1957 and then again in 1977, and scientists now know that the virus has been cohabiting - world-wide - with subtype H3N2 since 1969.

Could there be another world epidemic?

Historical evidence seems to point at three or four world epidemics every century due to the emergence of new viral subtypes that are easily transmitted from person to person. For virus type A, 13 hemagglutinin subtypes (H1 to H13) and 9 neuraminidase subtypes (N1 to N9) have been identified. Consequently, there are virtually 117 possible subtypes! All these subtypes are found in birds, but only a few have been identified in humans and pigs. Yet because of A virus's ability to reshuffle its genetic material, the variety of hemagglutinin and neuraminidase available for the creation of new variants seems to be all too probable. In fact, experts in the matter agree that another world epidemic is inevitable and may be impending. Though naturally, it is impossible to predict when and where.

Of the 13 subtypes of avian virus, H5N1 is the most disturbing for several reasons. First, it mutates rapidly. Second, it is known to acquire genes from viruses which infect other species. Thirdly, on two occasions, it has been the cause of acute infections in man (Hong Kong in 1977 and Viet Nam in 2004), which have been confirmed by laboratory tests. Finally, birds that survive the infection spread the virus for about 10 days at least, either orally or via their excreta - which makes the propagation of the virus so easy in live poultry markets and within flocks of migratory birds. This is why the highly pathogenic avian flu (due to virus H5N1) that broke out in December 2003 in the Republic of Korea and which spread to other Asian countries is arousing so much concern. If the number of people contaminated increases, the probability that people are simultaneously contaminated by both human and avian strains also does and the conditions are ripe for a new subtype to develop.

Several steps could be taken to try and reduce as much as possible the chances of a world epidemic of avian flu. First priority: putting a stop to the spread of infection amongst fowls, thus limiting human exposure to the virus. Indeed, most experts agree that the rapid decision to slaughter all the fowl in Hong Kong in 1977 probably avoided a pandemic. Second priority: administering vaccinations to those who carry human viral strains and who are in close contact with infected fowl. Such an action would weaken the chances of human contamination by both a human and an avian virus, and also reduce the risk of the exchange of genes. Finally, although the flu virus is found all over the world, some regions like Asia and China in particular, are prime reservoirs because of the life style and especially the population's promiscuity with animals, which encourage mutations and the development of new strains. It is therefore likely that if a number of habits and

conditions were changed in those regions, certain types of flu would regress as a result. Traveling as we know it today also intensifies the chances of infection and mixing strains. However, air and wind currents are also potential carriers, and exchanges with the rest of the world would happen anyway. So, even if a number of precautions - without doubt - reduce the possible appearance of a new strain which could cause a world epidemic, we do not know for certain that it could be avoided.

The flu...symptoms that must be taken seriously

Vaccination is the best way of course to guard against flu. In February of each year, the constituents of a vaccine are recommended by WHO (World Health Organization) surveillance missions, which are found all over the world. In this way, over a hundred national centers monitor the progress of the virus in their country and can evaluate the level of immunity of the population to the most recent viruses. In Switzerland, the Laboratory of Immunology of the HUG (Hôpitaux universitaires de Genève) is in charge of this type of evaluation. The data are then sent to four centers in London, Atlanta, Melbourne and Tokyo. The WHO is keeping watch. However, acquiring knowledge on the evolution of the virus does not predict the outbreak of a world epidemic.

In the last few years, new anti-viral medications have been developed. These are medications that actually attack the virus itself - unlike other products that only relieve the disagreeable symptoms of flu - by preventing its multiplication at different stages of development. In particular, a new class of molecule was recently discovered, which acts both on type A and B viruses (which was not the case before) by blocking the activity of one of their surface proteins: neuraminidase. By binding to it, the molecules prevent the neuraminidase from functioning and thereby delay viral proliferation. There are two types of molecule. One is Zanamivir, marketed by GlaxoWellcome under the name of Relenza. It is thanks to bioinformatics and the known structure of neuraminidase, that this drug was designed in such a way that it could bind to the surface protein with a perfect fit. The second molecule is known as Oseltamivir and marketed under the name of Tamiflu by Hoffman-La Roche. In normal times, these two drugs - naturally - do not replace a vaccine. However, in the event of a new subtype appearing, and against which the vaccine would be ineffective, such drugs could be essential in arresting a pandemic. The trouble is that they must be taken within 48 hours of the first symptoms.

So what is to be done? It is now clear that, at any moment, a new subtype may appear or reappear in the human population. History has shown that pandemics are recurring phenomena that happen at regular intervals. After those of 1918, 1957 and 1968, two more examples prove it: the return of H1N1 in 1977 and H5N1 in 1997. So the next outbreak could well be expected within the next few years. H5N1 was already a warning. However, there has to be a combination of factors for such a virus to cause as great a disaster as the Spanish Flu, i.e. a high incidence of infection in humans as well as a high death rate. Nothing like this has occurred since but, unfortunately, it is very likely that such a combination of factors will happen again...

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Illustrations:

- Fig.2, Source: National museum of health and medicine:
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- Hemagglutinin, Influenza A virus: P03436
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