

# Human and animal influenzas: forced coexistence

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Influenza is a fascinating topic because of the nature of the causative agent and its presentation in the form of annual endemic cycles, epidemic outbreaks, and pandemics with great impact on public health due to direct morbidity and mortality or to the aggravation of other underlying diseases. Different variants of the influenza virus emerge every 1 to 2 years as the result of point mutations (antigenic drift) or major genetic reassortment (antigenic shift), which involves mixing animal and human viruses. These characteristics oblige the establishment of worldwide early warning systems so that the location of each new variant can be ascertained. The emergence of avian influenza viruses H5N1 and recently of the swine influenza virus H1N1 compel us to study the pathogenic mechanisms of these viruses and their propagation between animal species as well as to determine how the interspecies barrier is broken. A variety of clinical pictures are associated with influenzas of animal origin and in general an etiologic diagnosis is not obtained. However, such a diagnosis must be taken into consideration when the results will affect clinical, therapeutic, and epidemiologic decisions. Multiple approaches will be required if we are to cope with a future influenza pandemic, which will probably come from an animal source. Epidemiologic surveillance, antiviral therapy, vaccinations, and non-pharmacologic measures all become important public health tools for containing the impact of the virus on society as a whole and avoiding sustained transmission. [Emergencias 2009;21:203-212]

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Influenza is a fascinating disease because of the nature of its causal agents (genetic reassortments, mutations etc.), its mode of appearance with annual outbreaks, epidemics or pandemics, and the abundant historical literature on it. Its importance for Public Health lies in its association with high morbidity, as well as high mortality either directly or by aggravation of other underlying diseases in elderly people over 65 years of age and in patients with chronic cardiorespiratory diseases.

Epidemic waves of flu affect large numbers of people over short periods of time and, depending on the variety, may have a significant impact on mortality, albeit difficult to quantify. The disease has serious consequences for national economic activity, the cost of which is estimated at \$187 per capita with an attack rate of 35%<sup>1</sup>.

This evaluation includes the costs of patient care (medical consultations, treatments, hospital admissions, overcrowding of emergency departments, etc.), as well as the costs of absenteeism, which a recent study estimated as between 3.7 and 5.9 days after the diagnosis of flu<sup>2</sup>. To these

costs should be added those derived from the impact of an animal virus, or one from a tourist destination, on the industries involved<sup>3</sup> or the impact on wildlife and ecology in general<sup>4</sup>.

In Spain, influenza is the direct or indirect cause of 3,000 to 6,000 deaths per year, especially affecting risk groups. It is a seasonal disease with a mean 7.79 reported cases for every 100 inhabitants. However, given the under-reporting of these cases, the National Institute of Statistics estimates the annual incidence as almost 15%<sup>5</sup>.

## Virology

Influenza viruses belong to the family Orthomyxoviridae, genera Influenzavirus A (IVA), B (IVB) and C (IVC). Viruses B and C are essentially human pathogens, whereas the virus A naturally infects numerous mammals (pigs, horses, dogs and more rarely cats, seals, whales and mink) and various species of birds (certain wild birds are the natural reservoir and therefore do not suffer the disease, especially duck-like birds). Domestic birds

often suffer the most serious and fatal forms of the disease. The variation within Type A allows classification into subtypes by their membrane antigens: hemagglutinin (H) and neuraminidase (N). The influenza A virus is capable of causing pandemics in the human species, resulting in the death of millions of people through the centuries. From waterfowl, viruses occasionally cross the inter-species barrier to infect swine, and from here they are transmitted to humans, horses and domestic birds, causing infections that range widely in severity, from negligible to very severe.

Influenza viruses are categorized, to distinguish different strains, according to certain characteristics:

- Antigenic Type: A or B.
- Animal isolate (if human, this is omitted).
- Geographical origin.
- Number of strain in the laboratory of origin.
- Year of isolation.
- Formula of their surface antigens: Subtype H and subtype N.

For example:

- A/Beijing/262/95 (H1N1).
- A/New Caledonia/20/99 (H1N1).
- A/turkey/Iowa/13/85 (H5N9).

The flu virus can survive in the environment for long periods of time: in water, for up to four days at 22°C and more than 30 days at 0°C. Frozen, it can probably survive indefinitely, but it cannot survive heat (60° C for 30 minutes), common disinfectants or bleach<sup>6</sup>. It can survive for 24-48 hours on stainless steel or plastic surfaces, but less than 8-12 hours on clothing, paper or tissues. The virus survives for 5 minutes on the hands after transfer from a contaminated surface<sup>7</sup>.

The most important transmission is from person to person, via droplets (> 5 µm in diameter) originating from the nasopharynx of a person who coughs or sneezes. The distance reached by the drops rarely exceeds 1-1.5 m. Transmission may occur by direct skin-to-skin contact or contact with surfaces contaminated by respiratory droplets.

Adults are infectious from 24 hours before the onset of symptoms to over 7 days, while children and immunocompromised individuals may continue to spread the virus up to 21 days later<sup>8,9</sup>. Knowledge of the physical properties of these viruses is the basis of non-pharmacological prevention measures.

## Structure of influenza viruses

The virions are pleomorphic and rod-shaped, although spherical forms with a diameter of 80-120

nm predominate. The viral genome consists of a single-stranded RNA molecule with negative polarity, so it is not infectious because it can not act directly as an RNA messenger in protein synthesis and is divided into 8 segments that are associated with the RNA polymerase complex. Each segment genome encodes a protein, except that three of them encode two peptides (total 10 proteins). The most abundant internal protein is nucleoprotein (NP), which supports the helicoidal structure of the nucleic acid segments. In smaller quantities, the polymerases (PB1, PB2 and PA) are found, which are the viral enzymatic complex responsible for the synthesis of RNA. The segmented genome of influenza viruses allows genetic reassortment (or exchange of genetic material) when two different viruses infect the same cell<sup>9</sup>. This circumstance allows influenza viruses to generate genetic diversity for interspecies transmission and to evade the host immune response through what is known as antigenic shift or total change of one external protein for another<sup>11</sup>.

The outer membrane or envelope is derived from lipid bilayer membrane of the host cell and under electron microscope is shown to be surrounded by radial projections formed by HA and NA glycoproteins, which are inserted in the double lipid layer and enable subtyping of the influenza virus<sup>12</sup>. Mutations in the genes that encode these glycoproteins are selected by the selective pressure exerted by the immunity group of the host, leading to antigenic change over time (antigenic drift), which explains the successive waves of seasonal epidemics<sup>13</sup>. Internally, the envelope or outer surface is covered by protein membrane (M1), which provides consistency. The same genomic fragment also produces small quantities of another protein, called M2, which forms ion channels in the viral membrane that can be inhibited by amantadine, which constitutes the basis of this product's activity against influenza virus type A.

Finally, NS1 is a non-structural protein, which carries out important functions in the virus replication cycle, since it can interact with many cellular or viral factors and NS2, which is found in small amounts in the viral particle with no known functions. In 2001 a small protein encoded by the gene corresponding to the PB1 was discovered, called PB1-F2, which appears to induce protein cell apoptosis<sup>14</sup>.

## Ecology of influenza viruses

Sixteen different types of hemagglutinin have been described,(called H1 to H16 sequentially)

and nine types of neuraminidase (N1 to N9) in aquatic birds<sup>15,16</sup>. In mammals, only a few of these subtypes and their combinations have been found and recorded. In human H1, H2 and H3 and N1 and N2 in swine H1 and H3 and N1 and N2, and H3N8 in horses, dogs, H3N8 and H7N7. Interspecies transmission of influenza A viruses is a fact, but species specificity is important. Well documented cases of human infection by a virus of animal origin are rare. Both avian and human viruses have been able to establish enduring lineages in swine, which shows that this animal has receptors for both types of virus in its epithelium<sup>17</sup>. Indeed, hemagglutinin, a protein responsible for target cell recognition and virion adhesion to it, joins to galactose receptors with sialic acid terminations. In humans, this union is  $\alpha$ -2.6, while in birds it is  $\alpha$ -2.3<sup>18</sup>. Both co-exist in pigs, as mentioned, making this animal a possible intermediate host for the generation of influenza pandemics, due to the possibility of genetic reassortment. However, the 1918 pandemic appears to have been due to a direct adaptation of an avian virus with high capacity of interhuman transmission, although lack of relevant genetic information about the precursors and pre-1918 viruses does not allow valid conclusions to be drawn<sup>19</sup>.

## Avian Virus

The avian influenza virus can be found in a variety of domestic or wild birds. Occasionally, they are also found in humans and other mammals. Waterfowl belonging to orders Anseriformes (geese and ducks) and Charadriiformes (waders) seem to be the natural reservoir of all the known subtypes, and most infections are asymptomatic<sup>20</sup>. Poultry can be infected by a wide variety of subtypes, classified as low pathogenicity avian influenza (LPAI) viruses and high pathogenicity avian influenza (HPAI), depending on the severity of the clinical picture in these birds. To date, only H5 or H7 subtypes have caused HPAI outbreaks. In USA, a large number of isolated subtypes have been found: H3N2, H4N2, H4N6, H5N1, H5N2, H5N9, H7N1, H7N3, H9N2, H10N4 and H10N721. HPAI viruses present multiple mutations in the precursor molecule of HA, resulting in a large amount of basic amino acid residues. In the viral cycle, the rupture of this precursor (HA0) gives rise to two subunits (HA1 and HA2), which operate jointly to mediate the fusion between the virus and the host cell membrane. This rupture occurs extracellularly by trypsin-like proteases previously existing

in the tissues of the infected animal. The distribution of these proteases is naturally restricted to the respiratory and gastrointestinal tracts. However, with the abundance of basic amino acids, also modulated by the presence of carbohydrate residues near the site of proteolytic rupture, the HA0 precursor protein can be cleaved by multiple proteases widely distributed in the organism, including the brain<sup>22</sup>. Reconstruction of the H1N1 virus of the 1918 pandemic shows that this virus could have had high pathogenicity for poultry even without a several basic rupture sites, characteristic of HPAI, so the debate is still open as to the cause of the high pathogenicity of this virus<sup>12</sup>.

The influenza A virus H5N1 isolated from wild ducks in southern China between 1999 and 2003 were all antigenically similar to a precursor virus in geese. Genetic analysis of H5N1 strains isolated during 2000-2004 from poultry and cases of infection humans in China, Hong Kong, Indonesia, Thailand and Vietnam have shown that a series of phenomena of genetic reassortment from different strains of avian precursor A/Goose/Guangdong/1/96 have led to the current dominant genotype (genotype Z) responsible for the current outbreaks of highly pathogenic avian influenza in Southeast Asia and of cases of human infection. This evolution of A/H5N1 in recent years to the appearance of the Z genotype has been associated with increased virulence and spectrum of hosts, including mammals. It has been suggested that the increased ability to replicate in mammals is a consequence of transmission between ducks and pigs. The increase in the spectrum of hosts has also been highlighted with the establishment of the the cat as an animal model and the outbreak that occurred between tigers and leopards at a zoo in Thailand 2004<sup>23,24</sup>.

The current outbreak already affects 15 countries since 2003 with 421 cases and 257 deaths. In 2009, the most affected country is Egypt with 16 cases and no deaths. There have been no cases in Europe since 2006, when there were 12 cases in Turkey (4 deaths) and 8 cases in Azerbaijan (5 deaths). It is noteworthy that in 2003 there was an outbreak of conjunctivitis in Holland by H7N7, with 89 farmers affected and three possible cases of inter-human transmission. One person (a veterinarian) died of respiratory complications<sup>25</sup>.

## Swine virus

Swine viruses are found primarily in pigs, but can also be found in other animals, including humans, who acquire the infection by airborne trans-

mission, never from eating pork. The most common are H1N1, H1N2 and H3N2, H2N3 and H3N1 although two or more subtypes may circulate at the same time<sup>26</sup>. In Europe and United States (U.S.) an H1N1 swine virus with avian characteristics has been isolated in China and this subtype has replaced the classic swine H1N1 virus. Descriptions of porcine virus with avian and even human genomic segments have multiplied in recent years, producing asymptomatic or respiratory infection, and even paralysis in pig populations<sup>27,28</sup>. Until 2007, only 50 human cases of swine flu had been described, with a mortality rate of 14%. Nineteen cases were in the U.S., 6 in the Czech Republic, 4 in Holland, 3 in Russia, 3 in Switzerland, 1 in Canada and 1 in Hong Kong. In some cases (39%) there was no known contact with pigs. The 13 remaining cases occurred in an outbreak at Fort Dix (USA) in 1976 among soldiers with an average age of 18 years, with no known exposure to pigs. One man died and the rest had varying degrees of respiratory impairment.

Serologically, there were 230 soldiers infected<sup>29</sup>. One of the latest cases described in the literature occurred in Aragon: a woman aged 50 years living in close contact with pigs acquired a respiratory infection with influenza. A blood sample was obtained and the isolate was characterized as swine influenza A H1N1. There were no more cases and a serological survey is being conducted. The patient did not require treatment or hospitalization and recovered totally<sup>30</sup>.

The swine virus identified in Mexico (A/california/04/2009) has shown, in preliminary analysis, that its closest relatives are from strains of pigs and turkeys. Six segments of the virus are associated with swine virus in North America and the other two (NA and M) with swine virus isolated in Europe and Asia in 1992. For the segment encoding HA, the nearest genetic relationship is with highly reassorted swine virus H1N2 and H3N2, isolated as far back as 1998. In particular, the H3N2 isolate presents a triple human, swine and poultry reassortment. Therefore, the new virus (never isolated in swine) has two swine ancestors and at least one of them is, in turn, linked to a triple reassortment that occurred in 1998<sup>31</sup>. Its image under electron microscope and the origin of its proteins are shown in Figure 1.

## Equine Virus

Equine viruses typically infect horses, but rarely other equines (donkeys, mules and zebras). Only

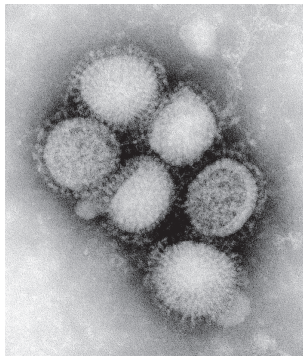
two subtypes have been described: H7N7 (equine virus 1), extinct or present at a very low level, and H3N8 (equine virus 2), which presents two different lineages circulating in Europe and America. In 1989, a new equine strain caused high mortality among horses in China, but then lost pathogenicity<sup>28,32</sup>.

## Canine Virus

Recently, an outbreak of influenza among dogs in USA was described, whose subtype H3N8 is from Equidae, which has crossed the interspecies barrier<sup>33</sup>. There is a second subtype (H3N2) isolated in South Korea which has a high capacity for transmission between dogs and whose origin is avian<sup>34</sup>.

## The interspecies barrier

The avian virus does not infect humans in an efficient manner and vice versa. There are a number of factors, some misunderstood, which determine this. HA receptors seem to differ. However, the recent discovery that the lower respiratory tract epithelium in humans also has the two receptors and that the subtype H5N1 is capable of adhering to *in vitro* cultures of lung has made us reconsider this mechanism as the only explanation<sup>35</sup>. Other mechanisms that appear to be involved in the interspecies barrier are the existence of more receptors than have so far been described, the wealth of sialic acid in the mucin layer that the virus has to cross to bind to cell receptors<sup>36</sup> and temperature sensitivity, which seems to originate at position 627 of PB2, which is lysine in humans and glycine in avian subtypes<sup>37</sup>. In the pandemic of 1957, along with the change of hemagglutinin (from H1 to H2), a change was also produced in PB1, but it seems that this transfer produces no adaptive advantage to the virus in human infection<sup>38</sup>. Under controlled laboratory conditions and experimental challenge testing in 81 human volunteers, the capacity of avian viruses to replicate and produce infection has been investigated. After challenge with the subtypes H1N1, H3N8, H3N2, H6N2, H6N1, H9N2, H4N8 and H10N7, only some volunteers exposed to H4N8, H6N1 and H10N7 were infected, always presenting very mild respiratory tract symptoms. In the remaining cases there was no evidence of infection. But what is most interesting is that, regardless of the symptoms observed or naso-



Protein	Human/animal origin	Geographic origin
Hemagglutinin	Swine (H1) 1999	N. America ⇒ related to H1N1 of the 1918
Neuraminidase	Swine (N1)	Europe
Polymerase A	Avian	N. America
Polymerase B1	Human	1993 H3N2
Polymerase B2	Avian	N. America
Nucleoprotein	Swine	N. America
Matrix M protein	Swine	Eurasia
Non-structural 1	Swine	N. America

Trifonov V, Euro surveill 2009; 14: 191932009

**Figure 1.** Electronic photograph of the new A/H1N1 virus, with antigenic structure and origin. Photo by courtesy of the Centers for Disease Control United States (CDC). H1N1 virus 80-120 nm in diameter. CDC Influenza Laboratory.

pharyngeal replication, the immune response measured by haemagglutinin inhibition was inconsistent. This means that avian viruses can infect humans, at least in experimental conditions, but conventional serological tests can underestimate these infections<sup>39</sup>.

Although events of interspecies barrier crossing seem to be sporadic and do not lead to efficient transmission in the secondary species, there is serological evidence of exposure to different animal viruses among humans, usually related to close occupational or lifestyle contact with animals. In fact, in regions of China up to 2% of the population is zero positive for the virus H9<sup>40</sup>.

## Influenza pandemics in the twentieth century

Different variants of influenza virus emerge every 1-2 years, through point mutations (antigenic drift) that can elude the defensive system, and therefore do not generally confer lasting immunity. Even many lineages of the same subtype can co-circulate and persist<sup>41</sup>.

Pandemics appear in a different way. Since the sixteenth century various influenza pandemics have been recorded, and in each century about three pandemics have occurred at intervals of between 10 and 50 years. Diffusion is very fast and spreads throughout the world in less than a year and causes flu in approximately 25% of the population. Abrupt onset and high morbidity and mortality rates limit the response capacity. During the twentieth century there were three major flu pandemics, all being caused by viruses of Type A: H1N1 subtypes (1918-19, "Spanish flu"), H2N2 (1957-58, "Asian flu") and H3N2 (1968-69, "Hong Kong flu").

The 1918 pandemic caused an estimated 50 million deaths and 25-30% of the population were infected with the disease. The first outbreaks were detected in March in Europe and in different locations of USA. This first wave was highly contagious but not particularly lethal. The second wave began in September, with greatest mortality among young people and healthy people; 99% of deaths occurred in people aged less than 65 years. Many of the deaths were caused by pneumonia due to secondary bacterial infection, but there were also deaths due to primary viral pneumonia<sup>42</sup>.

It has been possible to recover viral RNA from paraffin-embedded tissues and from the body of a victim buried in permafrost (layer of permanently frozen surface ice) and so complete the entire sequence of the eight genomic segments. According to this research, the 1918 virus was not a reassortment, like those of the 1957 and 1968 pandemics, but an entirely avian virus that adapted to human infection, possibly by successive step mutations<sup>19,42</sup>. The 1957 pandemic was caused by a less virulent virus than that of 1918, and health systems were better prepared and had already produced antibiotic and vaccines. It began in China in March; by December cases had been reported in all regions of the world. The second wave occurred one to three months after the first had ceded, and caused high rates of illness and increased mortality (estimated at 1-2 million), mainly affecting the elderly, people with underlying disease and pregnant women. Vaccines were available by August 1957 in USA, October in United Kingdom, and by November in Japan. The quantities, however, were too low for large scale use. Vaccine-producing countries had sufficient supplies to cover their risk groups, but not to cover their entire population. The last pandemic of the 20th century was in 1968. It began in July in

**Table 1.** Parameters of the transmissibility of influenza A virus in various scenarios

Parameter	Seasonal influenza	1918 Pandemic 1 <sup>st</sup> wave	1918 Pandemic 2 <sup>nd</sup> wave	1918 Pandemic 3 <sup>rd</sup> wave	1957	1968
Clinical attack rate (%)	25	–	–	–	31	21
Serological attack rate (%)	50	79	61	69	67	65
R0 (number of secondary cases per primary case)	1.39	2.00	1.55	1.70	1.65	2.5
Lethality (%)	–	0.7	3.25	2.7	–	–

Vaqué J. Epidemiology of communicable diseases. In Piédrola Gil. Preventive Medicine and Public Health, 11th edition. Barcelona. 2008.

southeast China and on August 16 the WHO issued a warning. Initial spread was similar to that of 1957. Clinical symptoms were moderate, with low mortality, and progress was slow in most countries. Some tropical countries did not experience the first outbreak until early 1969. Excess mortality is estimated at one million people and, at least in U.S.A., half the deaths occurred in people aged less than 65 years. It is unclear why the pandemic had such a low rate of mortality; one of the reasons suggested is that the virus was genetically similar to those in earlier pandemics, including 1957. As in the previous pandemic, the vaccine arrived late and in insufficient quantities<sup>43</sup>. Transmissibility parameters of the three pandemics and seasonal flu can be seen in Table 1<sup>44</sup>.

Genetic analysis has shown that the pandemics of 1957 and 1968 were produced by a recombination of human and avian viruses. The 1957 virus (H2N2) had three avian virus genes and the remaining five were of the circulating H1N1 strain. The 1968 virus (H3N2) also had three avian genes from an avian strain and the other five were from the human H2N2 strain responsible for the previous pandemic. As can be seen, the H1N1 strain of 1918, of uncertain origin, is finally the ancestor of the human strains that produced the later pandemics and all the subsequent subtypes. In reality, 4 of its lineages persist: a human H1N1, a swine H1N1 (called classical), a human H3N2 and a swine H3N2. None of these descendants show the virulent pathogenicity of this predecessor, calculated at 100 times higher<sup>42</sup>.

The main characteristics of an influenza pandemic are:

- Unpredictability.
- Cases occur rapidly.
- The pandemic virus can cause severe disease in groups not normally affected by flu, such as young adults and pregnant women. Epidemiological monitoring is of vital importance.
- The virus tends to produce multiple waves, and those age groups and areas not affected by the first may be the most vulnerable in later waves, which often have more impact.

- The first wave has a peak incidence 3-6 months after detection of the first cases, and the second wave can peak about a year from the start.

- Public health action can delay spreading, but can not stop pandemic flu.

- In the 1918 epidemic, the first wave had a lower rate of mortality than the second; it is reasonable to think that the survivors affected by the virus during this first wave had some protection during the second. This suggests that the earlier the contact with the pandemic virus, the better. However, an analysis of the second wave tells us otherwise, the later the contact, the lower the mortality; in the U.S. the second wave hit the East Coast first, with higher mortality than the West Coast. Australia, which was not hit by the second wave until 1919, had the lowest mortality rate of all the East<sup>45</sup>.

## Symptoms of human influenza

The virus arrives at the respiratory mucosa by airborne transmission, where it can be neutralized by local antibodies established by in previous infections; nonspecific inhibitors and mucus in the mucociliary system also contribute to the defense. The infection is initiated when the virus binds to mucoproteic receptors of the columnar respiratory system epithelial cells, where intense replication takes place in the following 48-72 hours or longer in children. From here the virus is released in large infective doses by aerosol droplets (Pflügge droplets) emitted into the environment by talking, sneezing or coughing. This aspect implies a certain resistance of the virus in the environment, favoured by conditions of high relative humidity and low temperature, concentration of the virus in respiratory secretions and the size of aerosols formed. All these factors facilitate micro-droplets floating in the atmosphere and loaded with the infectious virus. If large (over 5 µm in diameter) the tendency is to fall on surfaces (floors, tables or any object within a distance of 1-1.5 m) and for this reason

hand-washing is recommended as a highly effective measure against the spread of the virus. The degree and intensity of virus replication in the respiratory mucosa, and the action of viral NA which facilitates mucoprotein breakdown of the secretions and the formation of very small virus-laden droplets, are biological properties which differentiate certain strains from others, especially between viruses of avian, mammalian and human origin.

After a short incubation period (1-4 days) the disease starts abruptly with fever and chills, general malaise syndrome, fatigue, weakness and headache, and myalgia. It is accompanied by respiratory symptoms such as non-productive cough, sore throat and rhinitis. Nausea, vomiting and earache may also be common in children<sup>46</sup>. Table 2 shows the frequency of flu symptoms in a study performed in 2470 patients with laboratory-confirmed influenza<sup>47</sup>, as well as an outbreak of New A/H1N1 influenza in a New York school<sup>48</sup>. The physical findings of uncomplicated influenza are: fever, flushed face, clammy hot skin, red watery eyes, nasal discharge, ear infection, hyperaemic mucosa and neck lymphadenopathy, especially in children. The severity of clinical presentation varies from afebrile respiratory symptoms resembling those of the common cold to severe respiratory signs, especially in the elderly and people with varying degrees of immunosuppression.

Fever and general symptoms usually last 3-5 days, then diminish gradually, with cough and a feeling of sickness persisting for some days. Out of an epidemic context, it can be difficult to distinguish the common cold from flu, so definitive diagnosis can only really be made by a clinical microbiology service, using currently available tests (Immunochromatography, laboratory culture or polymerase chain reaction –PCR–). Without this support the clinical diagnosis is weakened and insufficient.

The most frequent complication of influenza is bacterial pneumonia, but primary viral pneumonia is more serious: this is the flu that does not resolve, with radiological findings of diffuse interstitial infiltrate, acute respiratory distress and hypoxia. In the 1918 pandemic, hemorrhagic pneumonia was a common necropsy finding. Flu can exacerbate existing disease such as chronic heart and/or lung disease and has also been associated with encephalopathy<sup>49</sup>, transverse myelitis, myositis (in case of influenza B and in boys more than in girls), myocarditis<sup>50</sup> and Reye's syndrome, especially with influenza B and simultaneous use of aspirin<sup>51</sup>.

The clinical symptoms of human influenza of animal origin are usually mild, such as in new in-

fluenza A/H1N1 of swine origin, the exception being influenza H5N1 in recent years, although its clinical spectrum has not been well established, ranging from asymptomatic cases to pneumonitis with fatal multi-organ failure. Initial symptoms include conjunctival and gastrointestinal manifestations, as well as those described for common seasonal influenza. The picture may progress to respiratory distress and varied radiological findings: interstitial infiltrates, lobular infiltrates and finally progression to a diffuse bilateral ground-glass appearance, including consolidation and air bronchogram. There is no agreement on factors associated with a fatal evolution. Most cases were attended at hospitals when the disease was already well advanced, and advanced age was a prominent factor in Hong Kong but not in Thailand, where age of less than 6 years was a risk factor<sup>52</sup>. In any case, the pneumonias were primary without evidence of secondary bacterial pneumonia. This is similar to the 1918 pandemic and relates to a massive release of cytokines or cytokine storm<sup>45</sup>.

The reason for the mildness of the current influenza of swine origin may be due to several factors: the absence of amino acids with basic residues, no mutation at position 627 of the PB1; it is the same HA as the seasonal H1N1 virus and the same genetic stability. At the time of writing this review, the reason has not yet been established.

## Diagnosis

The appropriate treatment for patients with an acute respiratory infection depends on an early

**Table 2.** Flu-like symptoms in 2470 patients with verified diagnosis of influenza and 44 cases from an outbreak of A/H1N1 in New York

Symptoms	Monto, 2000 (n = 2.470) percentage	Nueva York, Outbreak 2009 (n = 44) percentage
Fever $\geq 37,8^{\circ}\text{C}$	68	–
Feeling feverish	90	96
Cough	93	98
Nasal congestion	91	82
Weakness	94	89
Loss of appetite	92	–
Sore throat	84	82
Headache	91	82
Myalgia	94	80
Chills	–	80
Diarrhea	–	48
Arthralgia	–	46

Monto AS. Arch Intern Med 2000, 160:3243-3247. April CDC. MMWR 30, 2009/58 (Dispatch), 1-3.

**Table 3.** Predictive values of rapid diagnostic tests according to the prevalence of influenza, assuming a sensitivity of 60% and a specificity of 92%

Prevalence of influenza	Positive predictive value (%)	False positives (%)	Negative predictive value (%)	False negatives (%)
Low (2,5%)	17	83	99	1.1
Moderate (20%)	66	34	90	9.8
High (40%)	84	16	78	22

Author elaborated.

and accurate diagnosis. This can prevent the inappropriate use of antibiotics and indicate the use of antiviral treatment, when necessary; the need for hospitalization may be reduced by up to 77% if treatment is adequate<sup>53</sup>. However, numerous clinical cases of viral influenza are similar to seasonal influenza and may often overlap. It must be remembered that the diagnosis of influenza is an acute respiratory infection caused by an influenza virus determined by a series of specific tests performed by a microbiology laboratory. Diagnostic tests currently available follow logical steps from low to high complexity and availability: rapid tests (mainly Immunochromatography), real time PCR (RTPCR) and cell culture (which requires a level of Biosafety 3 for valid performance, only available in a few laboratories in Spain).

The diagnosis is important for epidemiological control in times like the present (period of containment of a possible pandemic), to carry out epidemiological surveillance or to monitor antiviral sensitivity.

Under "normal" circumstances, seasonal influenza only requires a clinical diagnosis with an acceptable degree of uncertainty. The sensitivity and specificity of any test can vary depending on the skill and ability of the microbiology department, but the critical aspect is the sample used. Samples of nasopharyngeal secretions are more effective and preferable to a scraping or swab, and must always be taken before the administration of antivirals, as all diagnostic tests are based on viral load which decreases soon after treatment is initiated<sup>54</sup>.

For the diagnosis of influenza, the first step is the evidence from rapid immunochromatography tests that can detect influenza A and B viruses (and are usually able to differentiate between them) within fifteen minutes. Technical complexity is variable, but they are often simple tests. They do not distinguish the subtypes of influenza A, and with samples of nasopharyngeal secretions they present a sensitivity of 50-70% and specificity of 90-95%<sup>55,56</sup>. They tend not to produce false positive results (more frequent when the prevalence of the disease is low), but they do produce false negatives (especially when the prevalence of

the disease is high). Table 3 shows expected predictive values according to the prevalence of the influenza.

A second diagnostic step involves the use of RT-PCR, which requires somewhat more complex technical support; testing a sample of nasopharyngeal excretion provides a diagnosis of the presence of the genetic material of the influenza virus A (or B, if sought), and even A/H1. If the technology is commercial, the duration of the test can be 3-4 hours. If not, the process is somewhat slower. In any case, evaluation of validation parameters is complicated and periodically checked by each laboratory. Viral culture is the last diagnostic step, only available at a few reference centres owing to the level of bio-safety required for this type of work with infectious agents.

The definitive diagnosis of the subtype of the virus is made by reference centres, using RT-PCR techniques and sequencing.

Preliminary unpublished data suggest that rapid immunochromatographic tests lack the validity parameters suitable for the diagnosis of new influenza A/H1N1 of swine origin.

In general, we can say that we must use etiological diagnostic techniques beyond the clinical syndrome when the results affect clinical and therapeutic decision making, and that the choice of technique depends on the clinical and epidemiological setting.

In conclusion, we can say that the approach to a future influenza pandemic (probably of animal origin) should encompass epidemiological surveillance, early warning networks worldwide, antiviral treatment and vaccines, as well as the use of non-pharmacological measures. All these aspects are important public health measures to mitigate and contain the impact of such pandemics on society as a whole.

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## Gripe humana y gripes animales: una convivencia forzada

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La gripe posee un gran atractivo por las características que tiene su agente causal, por su forma de presentación mediante epidemias anuales, brotes epidémicos y pandemias con gran repercusión en Salud Pública, ya que presentan una elevada morbilidad y mortalidad directa o por agravamiento de otras enfermedades de base. Las diferentes variantes del virus de la gripe surgen cada 1-2 años mediante mutaciones puntuales (deriva antigénica) o grandes reorganizaciones genéticas (cambios antigénicos mayores) que involucran mezcla de virus animales y humanos. Estas particularidades obligan a establecer sistemas de alerta temprana a nivel mundial para poder localizar cada nueva variante del virus. La aparición de gripes de origen aviar H5N1 y recientemente de origen porcino H1N1 nos obligan a estudiar de forma detenida los mecanismos de patogenicidad de este virus, su propagación entre las diferentes especies animales y cómo se produce el salto de la barrera interespecie. Los cuadros clínicos de estas formas de gripe de origen animal son muy variados y, de forma general, no se suele estudiar un diagnóstico etiológico en los cuadros clínicos que generan, pero sí es necesario plantearse los cuando los resultados vayan a afectar la toma de decisiones clínicas, terapéuticas y epidemiológicas. La mejor manera de afrontar una futura pandemia gripal (de probable origen animal) es hacerlo desde una perspectiva múltiple. La vigilancia epidemiológica, el tratamiento antiviral y las vacunas, así como el uso de medidas no farmacológicas, se convierten en importantes medidas de Salud Pública para contener el impacto de la misma sobre la sociedad en su conjunto y evitar la transmisión sostenida de la misma. [Emergencias 2009;21:203-212]

**Palabras clave:** Gripe. Salud Pública. Pandemia.