

# Avian influenza viruses in humans

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## Summary

Past pandemics arose from low pathogenic avian influenza (LPAI) viruses. In more recent times, highly pathogenic avian influenza (HPAI) H5N1, LPAI H9N2 and both HPAI and LPAI H7 viruses have repeatedly caused zoonotic disease in humans. Such infections did not lead to sustained human-to-human transmission. Experimental infection of human volunteers and sero-epidemiological studies suggest that avian influenza viruses of other subtypes may also infect humans. Viruses of the H7 subtype appear to have a predilection to cause conjunctivitis and influenza-like illness (ILI), although HPAI H7N7 virus has also caused fatal respiratory disease. Low pathogenic H9N2 viruses have caused mild ILI and its occurrence may be under-recognised for this reason. In contrast, contemporary HPAI H5N1 viruses are exceptional in their virulence for humans and differ from human seasonal influenza viruses in their pathogenesis. Patients have a primary viral pneumonia progressing to acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome. Over 380 human cases have been confirmed to date, with an overall case fatality of 63%. The zoonotic transmission of avian influenza is a rare occurrence, but the greater public health concern is the adaptation of such viruses to efficient human transmission, which could lead to a pandemic. A better understanding of the ecology of avian influenza viruses and the biological determinants of transmissibility and pathogenicity in humans is important for pandemic preparedness.

## Keywords

Avian influenza – Clinical – Epidemiology – Experimental – H5N1 – H7N7 – H9N2 – Pandemic – Pathogenesis – Risk assessment – Transmission.

## Background

Influenza viruses are an important cause of human disease. Seasonal influenza epidemics are regular occurrences and are caused by influenza type A (subtypes H3N2 and H1N1) and type B viruses. However, at unpredictable intervals, human influenza viruses undergo antigenic shift and acquire novel surface antigens (haemagglutinin [HA], with or without a novel neuraminidase [NA]) to which the human population has no prior immunity, leading to an influenza pandemic. Such pandemic viruses are generated either in part (by genetic reassortment with a prevailing influenza virus) or completely (through an avian influenza virus adapting to efficient human-to-human transmission)

from avian influenza viruses (63). In addition, avian influenza viruses occasionally transmit zoonotically to humans. While such zoonotic transmissions are generally self-limited and of variable clinical severity (see below), adaptation of such viruses to efficient human transmission poses an ongoing pandemic threat. Strong host barriers prevent avian influenza viruses from infecting humans or vice versa. Such barriers to inter-species transmission are believed to be multi-factorial and include receptor specificity, temperature sensitivity of the viral polymerase as well as contributions from other viral genes (40).

Our understanding of human infection and disease caused by avian influenza viruses arises from a number of sources, including reports of human disease, experimental

infections of humans with avian influenza viruses, sero-epidemiological studies and studies on relevant animal models and *in vitro* or *ex vivo* cultures.

## Experimental infection of human volunteers with avian influenza viruses

Healthy human volunteers were infected by the intra-nasal route with nine different avian influenza virus isolates belonging to subtypes H1N1, H3N8, H3N2, H6N2, H6N1, H9N2, H4N8 and H10N7 (5). Of 40 volunteers infected with H4N8, H10N7 or H6N1 viruses, 11 (28%) had virus isolated in nasal washings at days three and four post-inoculation, 6 had significant though mild clinical symptoms (respiratory with or without generalised symptoms), while 11 more had trivial discomfort. It was notable that none of these volunteers had antibody responses to the infecting virus detectable by haemagglutination inhibition (HI) test. Neutralising antibodies were not analysed in these studies. It is now known that micro-neutralising antibodies are a more reliable indicator of avian influenza virus infection of humans (see below) and, thus, the lack of HI antibodies is not surprising. Attempts to artificially transfer H6N1 infection from one infected human to another using virus isolated from human nasal washes were unsuccessful. Of the 41 other volunteers infected with avian viruses of subtypes H1N1, H3N2, H3N8, H6N2 or H9N2, none had evidence of virus excretion, two had significant respiratory or generalised symptoms, while six others had trivial discomfort. Six of these volunteers seroconverted to the infecting virus. The authors speculated that the lack of detectable virus excretion and the higher rate of seroconversion in the latter group of viruses may be attributable to immunological memory to H1, H3 and N2 subtypes from prior infections with human seasonal influenza being boosted by the avian viruses carrying surface antigens of the same subtype. Over all, these findings demonstrated that avian viruses can infect humans, albeit inefficiently.

## Naturally acquired avian influenza virus infections in humans

### Sero-epidemiology of avian influenza viruses

The HI test, which is the standard test for detecting mammalian serological responses to mammalian influenza viruses, lacks sensitivity for detecting mammalian

serological responses to avian influenza virus infection. Ferrets and pigs experimentally infected with human and pig influenza viruses make good HI antibody responses to these viruses, but make poor or undetectable antibody HI responses to avian influenza, in spite of efficient virus replication in these animals (28). The poor HI antibody responses of human volunteers experimentally infected with avian influenza viruses has been discussed above (5). Patients with H5N1 infection showed poor antibody responses in conventional HI tests, but satisfactory responses as detected by microneutralisation assays with kinetics similar to those found with human (H3N2 or H1N1) influenza virus infections (31, 53). More recently, modified HI tests using horse erythrocytes were found to provide improved serological sensitivity and specificity in humans infected or vaccinated with avian influenza viruses (60). Testing for antibodies to NA antigen may also be a more sensitive method for detecting avian influenza infection in mammals, though there may be problems with cross-reacting antibody due to related human NA antigens (28, 51).

Antibodies to influenza virus subtypes H4, H5, H6, H7, H10, and H11 were demonstrated by single radial haemolysis (SRH) tests in sera of humans resident in southern China collected in the late 1970s and early 1980s (57). With the exception of H7, the same subtypes were also most commonly isolated from domestic ducks. Since no highly pathogenic avian influenza (HPAI) viruses (including H5N1) had been isolated from poultry during this period, the serological responses to H5 that were generated (up to 2.3% in Jiangsu Province) were probably the result of exposure to low pathogenic avian influenza (LPAI) viruses.

### Human disease caused by avian influenza viruses

Avian influenza viruses of subtypes H5, H7 and H9 have been repeatedly associated with clinical disease of varying severity (Table I).

#### H7 subtype infections

An H7N7 virus was isolated in the United States of America (USA) from the blood clot of a 46-year-old man with clinical features of acute hepatitis (10, 15). However, the interval between the putative exposure and virus isolation was beyond that usually associated with influenza. His convalescent serum failed to neutralise virus infectivity in embryonated eggs. The evidence for avian influenza as a cause for disease in this case must remain equivocal.

Accidental laboratory exposure to allantoic fluid infected by H7N7 virus led to a follicular conjunctivitis with muco-

**Table 1**  
**Summary of avian influenza viruses associated with human disease**

Subtype	Year/Place	Source	No. of cases (fatal)	Clinical syndrome	Reference
H7N7	1980, USA	Seal	3 (0)	Conjunctivitis	73
H7N7	1995, UK	Domestic duck	1 (0)	Conjunctivitis	34
H5N1 (HPAI) Clade 0	1997, Hong Kong	Poultry	18 (6)	ILI, pneumonia	76
H9N2 (LPAI)	1999, Hong Kong	NK	2 (0)	ILI	49
H9N2 (LPAI)	1998, Shaoguan, Shantou, PR China	Poultry	5 (0)	ILI	23
H7N7 (HPAI)	2003, Netherlands	Poultry	89 (1)	Conjunctivitis, ILI, pneumonia	32
H5N1 (HPAI) Clade 1	2003, Hong Kong (recent travel to Fujian, China)	NK	2 (1) (one additional related fatal case, undiagnosed, died in Fujian)	Pneumonia	48
H5N1 (HPAI) Clade 7	2003, China	NK	1 (1)	Pneumonia	78
H9N2 (LPAI)	2003, Hong Kong	NK	1 (0)	ILI	8
H7N2 (LPAI)	2003, New York, USA	Poultry	1 (0)	Upper and lower respiratory infection	11
H7N2 (LPAI)	2002, Virginia, USA	Poultry (turkey)	1 (0)	ILI	11
H7N3	2004, Canada	Poultry	2 (0)	Conjunctivitis	66
H9N2 (LPAI)	2007, Hong Kong	NK	1 (0)	ILI	*
H7N3 (LPAI)	2006, UK	Poultry	1 (0)	Conjunctivitis	43
H7N2 (LPAI)	2007, UK	Poultry	1 (0)	Conjunctivitis	17
H5N1 (HPAI) Clades 1, 2.1, 2.2, 2.3.4	December 2003 to 10 September 2008, 15 countries in Asia and Africa	See text: 'H5N1 infections'	387 (245) (63%)	Pneumonia, ILI	Reviewed in 1

ILI: influenza-like illness  
 HPAI: highly pathogenic avian influenza  
 LPAI: low pathogenic avian influenza  
 NK: not known

PR China: People's Republic of China  
 UK: United Kingdom  
 USA: United States of America  
 \* W.L. Lim, personal communication

purulent discharge progressing to multiple small intra-epithelial opacities, and H7N7 virus was isolated from a conjunctival swab. Serological response in HI tests was equivocal, but it is now recognised that conventional HI tests are sub-optimal for detecting antibody responses to avian haemagglutinins. The patient made an uneventful and complete recovery within three weeks (64).

In a separate incident, a seal experimentally infected with influenza A/seal MA/1/80 (H7N7) sneezed on one of the investigators and transmitted infection that resulted in a self-limiting conjunctivitis. The virus was an avian-like influenza virus associated with an outbreak in harbour seals in New England in 1979 and 1980 (73). Influenza virus of subtype H7N7 was isolated at high titre from an eye swab collected on the second day of illness, continued to be isolated with decreasing titres on days three and four of illness and was undetectable by day five. No serological response to the virus was detectable by HI or NA inhibition tests in the serum or lacrimal fluid. Previously, four other persons involved in necropsies of seals affected during this

influenza outbreak had developed episodes of self-limiting conjunctivitis and, although virological studies were not done in these patients, the illnesses were also presumed to be due to the H7N7 virus (73).

A low pathogenic H7N7 avian influenza virus was isolated from the conjunctival swab of a 43-year-old woman with self-limited unilateral conjunctivitis in the United Kingdom (UK) (3, 34). Again, there was no detectable serological response. She had exposure to pet ducks and these ducks mingled freely with wild ducks and geese at a nearby lake, providing ample opportunity for transmission of an LPAI H7N7 virus from wild birds to her pet birds and from there to the patient.

Seven of 185 poultry workers exposed to infected poultry during outbreaks of H7N3 in Italy between 1999 and 2003 had serological evidence of infection when tested by microneutralisation, HI and western blot assays. One of them had conjunctivitis (52).

Since then, LPAI H7N2 viruses have been isolated from humans exposed to infected poultry in Virginia (2002) and New York (2003) and in the UK (2007) (11, 17, 66). These patients presented with conjunctivitis or with upper and/or lower respiratory tract infections (Table I). One patient with conjunctivitis associated with LPAI H7N3 virus infection has also been reported in the UK (43). An HPAI H7N3 outbreak in poultry led to two exposed individuals acquiring conjunctivitis.

The largest outbreak of human disease caused by HPAI virus (HPAIV) H7N7 infection occurred in association with a large outbreak in poultry in the Netherlands in 2003. In individuals exposed to infected poultry and their contacts, H7N7 virus was isolated from 78 patients with conjunctivitis only, five with conjunctivitis and influenza-like illness (ILI), two with ILI only and four with other symptoms. A veterinarian who was exposed at an infected farm developed a severe viral pneumonia and died of acute respiratory distress syndrome (ARDS) 15 days after exposure. H7N7 virus was isolated from his broncho-alveolar lavage (18, 32). Three of 83 contacts tested also had evidence of H7N7 infection. A sero-epidemiological study using a modified HI test with horse erythrocytes suggests that infection rates in exposed humans were even higher, with evidence of human-to-human transmission (41).

An HPAI H7N7 virus isolated from a patient with conjunctivitis was less virulent in a mouse model when compared to the virus isolated from the fatal human case. Using reverse genetics and site-directed mutagenesis, the E627K amino acid substitution in the PB2 gene was identified as the key determinant of virulence in the mouse model. The HA of the virus from the fatal case also contributed to increased virus replication in the lung and this may be related to virus receptor interactions (42). Experimental infection of mice with different LPAI and HPAI H7 viruses isolated from humans demonstrated that they all can replicate in the respiratory tract of mice, but only the HPAI H7N7 virus from the fatal human case had the ability to disseminate to other organs, including the brain (7). The HPAI H7N7 viruses retain an affinity for the 'avian'  $\alpha$  2-3 linked sialic acid receptors and were not readily transmissible in ferrets, whereas several LPAI H7N3 viruses isolated in Canada in 2004 and LPAI H7N2 viruses isolated in the USA had affinity towards  $\alpha$  2-6 linked sialic acids, as found in the human upper respiratory tract, and were transmissible after experimental infection of ferrets (6).

### H9N2 infections

In March 1999, H9N2 viruses were isolated from two girls aged four years and one year, with mild self-limited upper respiratory tract infections (36, 49). There was no epidemiological link between the two cases, and only one

of them had had brief exposure to live chickens 11 days prior to disease onset (70). The children had high fever (39.8°C to 39.9°C), decreased appetite, vomiting, sore throat and abdominal pain. There was no conjunctivitis or diarrhoea in either child and no evidence of pneumonia on chest X-ray examination. Other laboratory findings were unremarkable. At seven weeks after the onset of clinical signs, H9N2 neutralising antibody titres of >1/80 were detectable in convalescent serum of one of these children, but the other had only an antibody titre of 1/40. However, antibody to H9 HA was detected by Western Blot, and both immunoglobulin G (IgG) and IgM antibodies to H9 were detected by enzyme-linked immunosorbent assay (ELISA). There was no serological evidence of transmission within the family or to health care workers (70). One of 150 blood donor sera from Hong Kong and none of 100 human adult sera from the UK had H9N2 neutralising antibody (49). H9N2 viruses were endemic in the poultry markets of Hong Kong (22) and were isolated from two other patients with ILI in Hong Kong again in 2003 and 2007 (8).

Five other H9N2 influenza viruses were isolated in 1998 from patients with acute respiratory diseases in Shantou and Shaoguan in mainland China. One patient was a 22-month-old child with fever, cough and bronchitis. The mother also had high HI antibody titres to H9N2 virus, suggesting that she may also have been infected with this virus (23, 24, 25). Several of these patients reported contact with poultry. Antibody to H9 subtype viruses was detected by HI test in several of the patients from whom viruses were isolated, but these results were not confirmed by microneutralisation tests (23).

Sero-epidemiology of H9N2 virus infections is limited by evidence of cross-reacting antibody in neutralisation and HI tests detected in populations who are not expected to be exposed to these viruses (2, 59). Forty percent of healthy British volunteers for an H9N2 vaccine trial were found to have H9N2 antibodies prior to vaccination. Interestingly, antibodies were only detected in those individuals born prior to 1969, i.e. those likely to have been exposed to the H2N2 virus that circulated between 1957 and 1968 (59). The authors conclude that these cross-reactive antibodies had been generated by the H2 of this virus, but further study is needed to confirm this hypothesis. In a separate study, one third of young adults recruited to an H9N2 vaccine trial in the USA had HI antibody titres of  $\geq 16$  to A/chicken/HK/G9/97 (H9N2) prior to vaccination, and 5% had neutralising antibody titres of  $\geq 20$  (2). Since they were not expected to have natural exposure to H9N2 virus infections, these results indicate cross-reactive antibodies to the H9 and/or N2 antigens generated by exposure to H1N1 or H3N2 influenza viruses.

H9N2 viruses isolated from poultry in Hong Kong and southern China from 1997 to 2000 belonged to two virus

lineages, the A/quail/HK/G1/97-like (G1-like) lineage that contains 6 internal genes similar to H5N1/97, and the A/duck/Hong Kong/Y280/97-like (Y280-like) viruses (22). H9N2 viruses in poultry that have been found more recently have varying combinations of gene segments of these two virus lineages, sometimes with additional gene segments acquired from other avian influenza virus subtypes, including contemporary H5N1 viruses (74). The H9N2 viruses isolated from the two children in Hong Kong in 1999 belonged to the G1-like lineage (36) while that isolated from the child in Hong Kong in 2003 was a virus containing HA, NA, NP, NS genes from the Y280-like lineage, the M, PB1 and PB2 gene segments from the G1-like lineage and the PA of a different derivation. In contrast, the H9N2 viruses isolated in mainland China appear to be antigenically more similar to H9N2 viruses of the duck/HK/Y280/97 viruses. One of these viruses was fully genetically characterised and was also found to have gene segments from both G1 and Y280-lineage viruses (24, 25).

H9N2 viruses have the capacity to bind  $\alpha$  2-6 sialic acids found in the human upper respiratory tract (39). In experimentally infected ferrets, these viruses have the ability to transmit from animal to animal by direct contact, but not by aerosol. Leu226 in the HA receptor binding site was responsible for the human receptor specificity as well as the transmissibility in ferrets (71). Given the widespread distribution of these viruses and their ability to transfer to humans (see above) and pigs (47), H9N2 viruses are important candidates for new pandemic influenza viruses. While (as of November 2008) only 9 human cases of H9N2 disease have been diagnosed worldwide, in comparison to over 387 cases of H5N1, the mild nature of H9N2 illness implies that such infections are likely to be grossly under-recognised. H9N2 disease in humans will only be detected by intensive ILI surveillance. It is pertinent to note that of the 387 human H5N1 cases detected to date, most have been diagnosed by the investigation of severe pneumonic disease and only a handful have been uncovered by ILI surveillance systems.

### H5N1 infections

An outbreak of HPAIV H5N1 (clade 0) infection in the poultry farms and live poultry markets of Hong Kong led to infection and disease in 18 humans and to a fatal outcome in six of them (76). The outbreak was aborted by the slaughter of all 1.5 million poultry in farms and live poultry markets in Hong Kong. However, the precursor H5N1 virus continued to persist in geese and continued to reassort and evolve, and readapt to chickens. It was repeatedly detected in poultry markets and farms in Hong Kong (21). In February 2003, a father and son who had recently returned to Hong Kong from travel in Fujian, China, were diagnosed with H5N1 disease. The daughter had also recently died of an undiagnosed pneumonia in Fujian (48). The H5N1 virus isolated from these patients

belonged to what has now been designated clade 1. Another case with fatal human H5N1 disease occurred in Guangdong in late 2003 (clade 7), but was only diagnosed retrospectively (78). Between 2003 and 2005, outbreaks of HPAI were diagnosed in poultry in a number of southeast Asian countries and human cases were diagnosed in Vietnam, Thailand, Cambodia (all clade 1), Indonesia (clade 2.1) and China (clade 2.3.4). Following the detection of clade 2.2 viruses in association with mortality of wild birds in Qinghai Lake, China, in mid-2005, and the subsequent spread of clade 2.2 viruses to Central Asia, the Middle East and Africa, human H5N1 disease caused by clade 2.2 viruses was documented in Azerbaijan, Djibouti, Egypt, Iraq, Nigeria and Turkey, as well as within China itself. Human H5N1 cases caused by viruses of various clades have also been diagnosed in Laos, Myanmar, Pakistan and Bangladesh. At present (November 2008), 387 human HPAI H5N1 infections have been confirmed, with an overall mortality of 63%.

Patients with H5N1 disease, from Hong Kong in 1997 to the present day, share a similar clinical picture (reviewed in Abdel-Ghafar *et al.* [1]). The median age of H5N1 patients is approximately 18 years and only 10% of cases involve patients who are over 40. It is not clear whether the apparent under-representation of older persons is related to differences in exposure, to pre-existing immunity or to other factors. It is noted that studies in Cambodia indicated that those over 40 years old had as much exposure to sick and dying poultry as those under 40 years of age (37). Most patients were previously in good health. The incubation period is usually 2 to 5 days, but may occasionally be as long as 7 days. Disease onset is characterised by fever, cough, shortness of breath and pneumonia. Some patients may also manifest gastrointestinal symptoms (e.g. diarrhoea, abdominal pain). Radiologically, lung involvement is often bilateral and extensive, with focal consolidation, lobar collapse and air bronchograms. The pneumonia is rapidly progressing, often leading to ARDS and multi-organ failure. In fatal cases, median time from onset to death is 9 to 10 days. In addition to respiratory involvement, patients have evidence of mild or moderate liver and renal dysfunction, lymphopenia, leukopenia and thrombocytopenia (reviewed in Abdel-Ghafar *et al.* [1]). Occasionally, patients may present with predominantly gastro-intestinal or central nervous system manifestations (sometimes presenting as encephalitis), but these clinical presentations are less common. In contrast to H7-subtype virus infections, conjunctivitis is not prominent in clade 1, 2.1 or 2.3.4 virus infections, but has been reported in a proportion of patients infected with clade 2.2. viruses (1). While most cases have a fulminant clinical progression, milder cases have been reported, especially in children (30, 45, 75). The sero-epidemiological studies carried out so far (reviewed in Abdel-Ghafar *et al.* [1]) suggest that while asymptomatic infection does occur, it is rare.

There is a paucity of autopsy data on patients dying of H5N1 disease. The key findings are diffuse alveolar damage with hyaline membrane formation. Patchy interstitial infiltrates and pulmonary congestions with varying degrees of haemorrhage are found (20, 48, 68). Lymphocyte depletion is seen in the spleen and lymph nodes. Apoptosis of alveolar epithelial cells and infiltrating leukocytes are prominent (67).

Overall, oseltamivir treatment was associated with improved survival (47%) in comparison with untreated patients (12%) (reviewed in Abdel-Ghafar *et al.* [1]). In Indonesia, those treated within 4 days of disease onset had better survival rates (42%) than those receiving treatment later (23%) (29).

### Transmission

Most people with H5N1 disease acquired infection following direct or close contact with sick or dead poultry through activities such as slaughter, burial, food preparation and de-feathering, or exposure to apparently healthy chickens at cock fights or birds in live poultry markets. Occasionally, human disease was acquired from de-feathering dead wild birds (swans). However, though large numbers of humans have been massively exposed to HPAIV H5N1 via infected poultry, human disease, or even asymptomatic seroconversion, is very rare (reviewed in Abdel-Ghafar *et al.* [1]). On the other hand, while most patients with H5N1 disease were exposed to infected poultry, a quarter or more patients had no obvious poultry exposure and indirect exposure such as environment-to-human transmission is thought to be responsible. Humans with H5N1 disease have high levels of virus in the upper respiratory tract, comparable to that seen in seasonal influenza (14). While occasional instances of limited non-sustained human-to-human transmission have occurred following close, unprotected contact with an infected patient (69, 72), in general, transmission to other humans is uncommon. Approximately 25% of all human cases have occurred as clusters of more than one case, and more than 90% of these case clusters occurred within blood relatives (reviewed in Abdel-Ghafar *et al.* [1]). Thus, the epidemiological observations indicate that exposure to the virus, while necessary, is not sufficient to explain the occurrence of human H5N1 disease. Thus, it is likely that presently undefined mechanisms of host resistance, host susceptibility or unusual routes of exposure are critical in determining the final outcome.

Multiple host factors restrict interspecies transmission of H5N1 to humans (40). While ferret-to-ferret transmission by the contact and droplet route is readily demonstrated with human influenza H3N2 viruses, H5N1 viruses are not transmissible under similar circumstances. Furthermore, reassortants with differing combinations of H5N1 and human H3N2 gene segments were also not transmissible in

ferrets, suggesting that reassortment with a human virus by itself is insufficient to lead to the emergence of a virus that is efficiently transmitted between mammals (38).

Previous H5 HPAI outbreaks were not known to be associated with transmission to humans. There was no evidence of seroconversion by the HI test in individuals involved in the depopulation of chickens during the outbreak of HPAIV H5N2 in Pennsylvania in 1983, even though some of them did carry the virus for a short time in the nasal cavity (4). Since neutralising antibody activity was not analysed in these sera the results remain inconclusive. A serosurvey using neutralisation tests carried out on humans exposed to HPAIV H5N2 infected poultry in Italy also failed to document any evidence of infection (16).

### Pathogenicity

While other LPAI and HPAI viruses may cause human disease, none of them is comparable in clinical severity to the currently circulating HPAIV H5N1. The reasons for this unusual virulence in the mammalian host are poorly understood. The key target cells in the lung are alveolar epithelial cells and alveolar macrophages (20, 44). However, HPAIV H5N1 has the potential to replicate in tissues beyond the respiratory and gastro-intestinal tracts. Clearly, such dissemination of the virus is key to its virulence in poultry, and dissemination involving multiple organs including the central nervous system sometimes also occurs in humans. The virus has been repeatedly detected by reverse transcription-polymerase chain reaction (RT-PCR) and, occasionally, also by culture in the gastro-intestinal tract and peripheral blood. In some autopsy cases, virus has also been detected in the brain (20). However, in most cases, fatality is still largely due to the respiratory pathology associated with ARDS.

When compared with seasonal human influenza viruses (H1N1, H3N2), patients with H5N1 disease have higher serum levels of a number of cytokines and chemokines including IP-10, MCP-1, IL-8, IL-6 and IL-10 (14). Since these elevated cytokine levels are directly correlated with increased viral load in the upper respiratory tract, it is possible that they are merely reflecting the increased viral activity in tissues. However, when primary human macrophages and alveolar epithelial cell cultures are infected *in vitro* with identical doses of H5N1 and H1N1 viruses, a markedly higher cytokine response is seen in the H5N1 virus-infected cells, suggesting that this virus has an inherent capacity to hyper-induce cytokines and chemokines (12, 13). The interaction of inflammatory mediators secreted by H5N1 infected macrophages contributed to the induction of an enhanced inflammatory cascade in alveolar epithelial cells (35). Thus, the hyper-induction of cytokines may play an accessory role in pathogenesis.

Mice and ferrets are commonly used as experimental models for H5N1 influenza. However, there are important caveats with both model systems with regard to pathogenesis. H5N1 viruses differ in lethality for mice and ferrets. Some viruses are highly lethal for mice (e.g. A/Hong Kong/483/97; A/Vietnam/1203/04), with virus dissemination to the brain, while others are less so (e.g. A/Hong Kong/486/97) (19, 62, 65). The more rapidly neurotropic viruses tend to be more widely used as experimental models, since they give rapid and clear endpoints in animal studies. However, as these mice die mainly from encephalitis rather than pneumonia, their relevance to human pathogenesis is questionable. It is relevant to keep these caveats in mind when considering available data from studies in mice.

In experimentally infected ferrets and mice, the viral polymerase complex is a major contributor to viral virulence (54). A single Glu627Lys amino acid substitution in the H5N1 PB2 gene was associated with adaptation to mammals and virulence in mice (26, 27). Indeed, Gly627Lys or Asp701Asn substitutions in PB2 appear to be associated with adaptation of H5N1 virus to replication in humans (14). However, the H5N1 clade 2.2 wild-bird viruses, which consistently carry the PB2 627Lys amino acid substitution, do not cause more severe human disease (reviewed in Abdel-Ghaffar *et al.* [1]). The multibasic cleavage site in the haemagglutinin was independently associated with virulence in mice (26). While the Asp92Glu in the NS1 gene was associated with increased virulence of the 1997 H5N1 viruses in pigs (56), this change has not been seen in any of the contemporary H5N1 isolates from poultry or from humans (14, 58).

One study has compared the effects of both the lethal A/Hong Kong/483/97 and non-lethal A/Hong Kong/486/97 challenge in mice with defined defects in innate immune function, i.e. TNF- $\alpha$ , IL1 receptor, IL-6 and MIP-1. The effect of passive antibody treatment with anti-TNF- $\alpha$  was also investigated. Mice deficient in IL1-R had increased weight loss, viral load in lung, and mortality (though effect on survival was not statistically significant) when challenged with A/HK/486/97. Mice deficient in TNF- $\alpha$  infected with A/HK/483/97 virus had significantly less weight loss than wild type mice, suggesting that the innate immune defect apparently improved mortality, although it did not have a statistically significant effect on survival. A comparable phenotype was seen with mice treated with anti TNF- $\alpha$  (61). In another study, TNFR1 and TNFR2 double knock out mice appeared to have less weight loss compared to wild type mice following challenge with A/Vietnam/1203/04 virus, although the number of mice tested was too small ( $n = 3$ ) to allow statistical significance. However, corticosteroid treatment did have a significant improvement on weight loss. Survival was not affected in either group of mice (55).

COX2 activation has been shown to contribute to the cytokine cascade in HPAIV H5N1 infected macrophages and epithelial cells, and COX2 inhibitors reduced this inflammatory cascade (35). In mice challenged with HPAIV H5N1, COX2 inhibitor in combination with mesalazine and the antiviral drug zanamivir improved survival when compared to antiviral treatment alone (77). Gene expression profiling of the lung of H5N1 or H3N2 virus infected ferrets showed that H5N1 viruses differentially activated innate immune responses, including CXCL-10. AMG487, an inhibitor of signalling via the cognate receptor CXCR3, reduced symptoms and delayed mortality associated with H5N1 virus (9).

There are similarities in the clinical course and pathology observed with HPAIV H5N1 and the 1918 pandemic H1N1 virus, and interestingly, there are also similarities in lung pathology. Both viruses induced massive infiltration of macrophages and neutrophils into the lung and had higher levels of pro-inflammatory cytokines when compared with corresponding low pathogenic viruses (50).

Other animals have also been investigated as models for understanding the pathogenesis of H5N1 disease and for investigating the efficacy of vaccines and antivirals. 1997 HPAIV H5N1 causes a necrotising broncho-alveolar pneumonia in cynomolgous macaques (*Macaca fascicularis*) associated with diffuse alveolar damage, comparable to that seen in humans. There is no dissemination beyond the respiratory tract (33).

Early and appropriate antiviral therapy remains paramount for the management of human H5N1 disease. However, the results above suggest that a better understanding of the pathogenesis of H5N1 disease may provide novel therapeutic options for better management of this disease in future.

## Conclusion

H5N1, H9N2 and H7 subtype viruses repeatedly cause zoonotic disease in humans. Although HPAI H5N1 viruses have high mortality rates in poultry and in humans, and continue to pose a pandemic threat, it is relevant to note that the last pandemics arose from LPAI, rather than HPAI viruses. Thus, pathogenicity in chickens and virulence in humans are not pre-requisites for pandemicity, and LPAI viruses which may cause minimal disease in poultry are at least as credible as pandemic candidate viruses. However, since human infections with contemporary HPAIV H5N1 are associated with disease of unprecedented severity and because acquisition of transmissibility in humans may not necessarily lead to attenuation of its virulence in the short term, H5N1 viruses deserve special attention in terms of pandemic preparedness (46).

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## Les virus de l'influenza aviaire chez l'homme

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### Résumé

Les pandémies survenues dans le passé ont eu pour origine des virus de l'influenza aviaire faiblement pathogènes (IAFP). Plus récemment, le virus de l'influenza aviaire hautement pathogène (IAHP) de sous-type H5N1, le virus de l'IAFP de sous-type H9N2 et les virus de l'IAHP et de l'IAFP de type H7 ont été à l'origine de foyers zoonotiques répétés chez l'homme. Ces foyers n'ont pas révélé de transmission interhumaine régulière. L'infection expérimentale pratiquée chez des sujets humains volontaires et les résultats de diverses études séroépidémiologiques indiquent que l'homme est également sensible à d'autres sous-types des virus de l'influenza aviaire. Les virus de type H7 sont surtout responsables de conjonctivite et de maladies similaires à la grippe (ILI) ; dans un cas toutefois, le sous-type H7N7 du virus de l'IAHP a provoqué une maladie respiratoire à issue fatale. Les virus de l'IAFP de sous-type H9N2 provoquent des formes modérées d'ILI ; leur incidence réelle est donc probablement sous-estimée. En revanche, les virus H5N1 de l'IAHP qui circulent actuellement présentent une virulence exceptionnelle chez l'homme ; leur pathogénie se distingue également de celle des virus de la grippe saisonnière humaine. Les sujets atteints présentent une pneumonie virale primaire évoluant vers un syndrome respiratoire aigu sévère (SRAS) et un syndrome de dysfonctionnements organiques multiples. Plus de 380 cas humains ont été confirmés à ce jour, avec un taux de mortalité global de 63 %. La transmission zoonotique de l'influenza aviaire reste un événement rare ; en revanche, le risque que ces virus s'adaptent pour faciliter la transmission humaine représente un plus grand danger pour la santé publique ; dans ce cas en effet, une pandémie n'est pas à exclure. Une meilleure connaissance de l'écologie des virus de l'influenza aviaire et des facteurs biologiques déterminant la transmissibilité et la pathogénicité de ces virus chez l'homme est essentielle pour préparer l'éventualité d'une pandémie.

### Mots-clés

Clinique – Épidémiologie – Évaluation du risque – Infection expérimentale – Influenza aviaire – Pandémie – Pathogénie – Sous-type H5N1 – Sous-type H7N7 – Sous-type H9N2 – Transmission.

■



## Los virus de la influenza aviar en el ser humano

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### Resumen

Aunque hasta ahora las pandemias han venido causadas por virus de la influenza aviar levemente patógena (IALP), en fechas recientes algunos virus de las cepas H5N1 (influenza aviar altamente patógena: IAAP), H9N2 (IALP) y H7 (tanto IALP como IAAP) han provocado repetidos episodios de enfermedades zoonóticas en el ser humano. Tales infecciones no han dado lugar a una transmisión sostenida entre personas. De la infección experimental de voluntarios humanos y de varios estudios seroepidemiológicos parece deducirse que otros subtipos del virus de la influenza aviar también tienen la capacidad de infectar al ser humano. Los virus del subtipo H7 muestran tendencia a causar conjuntivitis y afecciones de tipo gripal, aunque en un caso la infección por virus H7N7 (IAAP) provocó una enfermedad respiratoria mortal. Los virus H9N2 (levemente patógenos) han causado afecciones poco graves de tipo gripal, y quizá por este motivo su presencia haya pasado a veces inadvertida. Los virus H5N1 (IAAP) contemporáneos, en cambio, muestran una excepcional virulencia en el ser humano y difieren en su patogénesis de los virus de la gripe humana estacional. Los enfermos presentan una neumonía vírica primaria que degenera en un síndrome de dificultad respiratoria aguda (SDRA) y un síndrome de disfunción orgánica múltiple. Hasta la fecha se han confirmado más de 380 casos en el ser humano, con un índice global de letalidad del 63%. Aunque la transmisión zoonótica de la influenza aviar se produce rara vez, la mayor preocupación desde el punto de vista de la salud pública reside en la posible adaptación de esos virus a una transmisión humana eficiente, lo que podría desencadenar una pandemia. La preparación para esa eventual pandemia exige entender mejor la ecología de los virus de la influenza aviar y los determinantes biológicos de su transmisibilidad y patogenicidad en el ser humano.

### Palabras clave

Epidemiología – Evaluación de riesgos – Experimental – H5N1 – H7N7 – H9N2 – Influenza aviar – Pandemia – Patogénesis – Procedimientos clínicos – Transmisión.



## References

1. Abdel-Ghafar A.N., Chotpitayasunondh T., Gao Z., Hayden F.G., Nguyen D.H., de Jong M.D., Naghdaliyev A., Peiris J.S. *et al.* (2008). – Update on avian influenza A (H5N1) virus infection in humans. *N. Engl. J. Med.*, **358**, 261-273.
2. Atmar R.L., Keitel W.A., Patel S.M., Katz J.M., She D., El Sahly H., Pompey J., Cate T.R. & Couch R.B. (2006). – Safety and immunogenicity of nonadjuvanted and MF59-adjuvanted influenza A/H9N2 vaccine preparations. *Clin. infect. Dis.*, **43**, 1135-1142.
3. Banks J., Speidel E. & Alexander D.J. (1998). – Characterisation of an avian influenza A virus isolated from a human: is an intermediate host necessary for the emergence of pandemic influenza viruses? *Arch. Virol.*, **143**, 781-787.
4. Bean W.J., Kawaoka Y., Wood J.M., Pearson J.E. & Webster R.G. (1985). – Characterization of virulent and avirulent A/chicken/Pennsylvania/83 influenza A viruses: potential role of defective interfering RNAs in nature. *J. Virol.*, **54**, 151-160.

5. Beare A.S. & Webster R.G. (1991). – Replication of avian influenza viruses in humans. *Arch. Virol.*, **119**, 37-42.
6. Belser J.A., Blixt O., Chen L.M., Pappas C., Maines T.R., Van Hoenen N., Donis R., Busch J. *et al.* (2008). – Contemporary North American influenza H7 viruses possess human receptor specificity: implications for virus transmissibility. *Proc. natl Acad. Sci. USA*, **105**, 7558-7563.
7. Belser J.A., Lu X., Maines T.R., Smith C., Li Y., Donis R.O., Katz J.M. & Tumpey T.M. (2007). – Pathogenesis of avian influenza (H7) virus infection in mice and ferrets: enhanced virulence of Eurasian H7N7 viruses isolated from humans. *J. Virol.*, **81**, 11139-11147.
8. Butt K.M., Smith G.J.D., Chen H.L., Zhang L.J., Leung Y.H.C., Xu K.M., Lim W. *et al.* (2005). – Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. *J. clin. Microbiol.*, **43**, 5760-5767.
9. Cameron C.M., Cameron M.J., Bermejo-Martin J.F., Ran L., Xu L., Turner P.V., Ran R., Danesh A. *et al.* (2008). – Gene expression analysis of host innate immune responses during lethal H5N1 infection in ferrets. *J. Virol.*, **82**, 11308-11317.
10. Campbell C.H., Webster R.G. & Breese S.S. Jr. (1970). – Fowl plague virus from man. *J. infect. Dis.*, **122**, 513-516.
11. Centers for Disease Control (CDC) (2004). – Update: influenza activity – United States and worldwide, 2003-04 season, and composition of the 2004-05 influenza vaccine. *MMWR*, **53**, 547-552.
12. Chan M.C.W., Cheung C.Y., Chui W.H., Tsao S.W., Nicholls J.M., Chan Y.O., Chan R.W.Y., Long H.T. *et al.* (2005). – Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. *Respir. Res.*, **6**, 135.
13. Cheung C.Y., Poon L.L.M., Lau A.S., Luk W., Lau Y.L., Shortridge K.F., Gordon S., Guan Y. & Peiris J.S.M. (2002). – Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet*, **360**, 1831-1837.
14. De Jong M.D., Simmons C.P., Thanh T.T., Hien V.M., Smith G.J.D., Chau T.N.B., Hoang D.M., Chau N.V.V. *et al.* (2006). – Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nature Med.*, **12**, 1203-1207.
15. DeLay P.D., Casey H.L. & Tubiash H.S. (1967). – Comparative study of fowl plague virus and a virus isolated from man. *Public Hlth Rep.*, **82**, 615-620.
16. Donatelli I., Campitelli L., Di Trani L., Puzelli S., Selli L., Fioretti A., Alexander D.J., Tollis M., Krauss S. & Webster R.G. (2001). – Characterization of H5N2 influenza viruses from Italian poultry. *J. gen. Virol.*, **82**, 623-630.
17. Editorial team (2007). – Avian influenza A/(H7N2) outbreak in the United Kingdom. *Eurosurveillance*, **12** (22), pii=3206.
18. Fouchier R.A., Schneeberger P.M., Rozendaal F.W., Broekman J.M., Kemink S.A., Munster V., Kuiken T., Rimmelzwaan G.F. *et al.* (2004). – Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc. natl Acad. Sci. USA*, **101**, 1356-1361.
19. Govorkova E.A., Rehg J.E., Krauss S., Yen H.L., Guan Y., Peiris M., Nguyen T.D., Hanh T.H. *et al.* (2005). – Lethality to ferrets of H5N1 influenza viruses isolated from humans and poultry in 2004. *J. Virol.*, **79**, 2191-2198.
20. Gu J., Xie Z., Gao Z., Liu J., Korteweg C., Ye J., Lau L.T., Lu J. *et al.* (2007). – H5N1 infection of the respiratory tract and beyond: a molecular pathology study. *Lancet*, **370**, 1137-1145.
21. Guan Y., Peiris J.S.M., Lipatov A.S., Ellis T.M., Dyrting K.C., Krauss S., Zhang L.J., Webster R.G. & Shortridge K.F. (2002). – Emergence of multiple genotypes of H5N1 avian influenza viruses in Hong Kong SAR. *Proc. natl Acad. Sci. USA*, **99**, 8950-8955.
22. Guan Y., Shortridge K.F., Krauss S., Chin P.S., Dyrting K.C., Ellis T.M., Webster R.G. & Peiris M. (2000). – H9N2 influenza viruses possessing H5N1-like internal genomes continue to circulate in poultry in southeastern China. *J. Virol.*, **74**, 9372-9380.
23. Guo Y., Li J., Cheng X., Wang M., Zhou Y., Li C., Chai F., Liao H. *et al.* (1999). – Discovery of men infected by avian influenza A (H9N2) virus. *Chin. J. experim. clin. Virol.*, **13**, 105-108.
24. Guo Y., Xie J., Wang M., Dang J., Guo J., Zhang Y. & Wu K. (2000). – A strain of influenza A H9N2 virus repeatedly isolated from human population in China. *Chin. J. experim. clin. Virol.*, **14**, 209-212.
25. Guo Y., Xie J., Wu K., Dong J., Wang M., Zhang Y., Guo J., Chen J. *et al.* (2002). – Characterization of genome of A/Guangzhou/333/99 (H9N2) virus. *Chin. J. experim. clin. Virol.*, **16**, 142-145.
26. Hatta M., Gao P., Halfmann P. & Kawaoka Y. (2001). – Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science*, **293**, 1840-1842.
27. Hatta M., Hatta Y., Kim J.H., Watanabe S., Shinya K., Nguyen T., Lien P.S., Le Q.M. & Kawaoka Y. (2007). – Growth of H5N1 influenza A viruses in the upper respiratory tracts of mice. *PLoS Pathog.*, **3**, e133.
28. Hinshaw V.S., Webster R.G., Easterday B.C. & Bean W.J. (1981). – Replication of avian influenza A viruses in mammals. *Infect. Immun.*, **34**, 354-361.
29. Kandun I.N., Tresnaningsih E., Purba W.H., Lee V., Samaan G., Harun S., Soni E., Septiawati C. *et al.* (2008). – Factors associated with case fatality of human H5N1 virus infections in Indonesia: a case series. *Lancet*, **372**, 744-749.

30. Kandun I.N., Wibisono H., Sedyaningsih E.R., Yusharmen W., Hadisoedarsuno W., Purba H., Santoso C. *et al.* (2006). – Three Indonesian clusters of H5N1 virus infection in 2005. *N. Engl. J. Med.*, **355**, 2186-2194.
31. Katz J.M., Lim W., Bridges C.B., Rowe T., Hu-Primmer J., Lu X., Abernathy R.A., Clarke M. *et al.* (1999). – Antibody response in individuals infected with avian influenza A (H5N1) viruses and detection of anti-H5 antibody among household and social contacts. *J. infect. Dis.*, **180**, 1763-1770.
32. Koopmans M., Wilbrink B., Conyn M., Natrop G., van der Nat H., Vennema H., Meijer A., van Steenberghe J. *et al.* (2004). – Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet*, **363**, 587-593.
33. Kuiken T., Rimmelzwaan G.F., Van Amerongen G. & Osterhaus A.D. (2003). – Pathology of human influenza A (H5N1) virus infection in cynomolgus macaques (*Macaca fascicularis*). *Vet. Pathol.*, **40**, 304-310.
34. Kurtz J., Manvell R.J. & Banks J. (1996). – Avian influenza virus isolated from a woman with conjunctivitis. *Lancet*, **348**, 901-902.
35. Lee S.M., Cheung C.Y., Nicholls J.M., Hui K.P., Leung C.Y., Uiprasertkul M., Tipoe G.L., Lau Y.L. *et al.* (2008). – Hyperinduction of cyclooxygenase-2-mediated proinflammatory cascade: a mechanism for the pathogenesis of avian influenza H5N1 infection. *J. infect. Dis.*, **198**, 525-535.
36. Lin Y.P., Shaw M., Gregory V., Cameron K., Lim W., Klimov A., Subbarao K., Guan Y. *et al.* (2000). – Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates. *Proc. natl Acad. Sci. USA*, **97**, 9654-9658.
37. Ly S., Van Kerkhove M., Holl D., Froehlich Y. & Vong S. (2007). – Interaction between humans and poultry, rural Cambodia. *Emerg. infect. Dis.*, **13**, 130-132.
38. Maines T.R., Chen L.M., Matsuoka Y., Chen H., Rowe T., Ortin J., Falcon A., Hien N.T. *et al.* (2006). – Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model. *Proc. natl Acad. Sci. USA*, **103**, 12121-12126.
39. Matrosovich M.N., Krauss S. & Webster R.G. (2001). – H9N2 influenza A viruses from poultry in Asia have human virus-like receptor specificity. *Virology*, **281**, 156-162.
40. Matrosovich M.N., Stech J. & Klenk H.D. (2009). – Influenza receptors, polymerase and host range. In Avian influenza (T.C. Mettenleiter, ed.). *Rev. sci. tech. Off. int. Epiz.*, **28** (1), 203-217.
41. Meijer A., Bosman A., van de Kamp E.E., Wilbrink B., Du Ry van Beest Holle R. & Koopmans M. (2006). – Measurement of antibodies to avian influenza virus A(H7N7) in humans by hemagglutination inhibition test. *J. virol. Meth.*, **132**, 113-120.
42. Munster V.J., de Wit E., van Riel D., Beyer W.E., Rimmelzwaan G.F., Osterhaus A.D., Kuiken T. & Fouchier R.A. (2007). – The molecular basis of the pathogenicity of the Dutch highly pathogenic human influenza A H7N7 viruses. *J. infect. Dis.*, **196**, 258-265.
43. Nguyen-Van-Tam J.S., Nair P., Acheson P., Baker A., Barker M., Bracebridge S., Croft J., Ellis J. *et al.* (2006). – Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. *Eurosurveillance.*, **11** (18), pii=2952.
44. Nicholls J.M., Chan M.C.W., Chan W.Y., Wong H.K., Cheung C.Y., Kwong D.L.W., Wong M.P., Chui W.H. *et al.* (2007). – Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. *Nature Med.*, **13**, 147-149.
45. Oner A.F., Bay A., Arslan S., Akdeniz H., Sahin H.A., Cesur Y., Epcacan S., Yilmaz N. *et al.* (2006). – Avian influenza A (H5N1) infection in eastern Turkey in 2006. *N. Engl. J. Med.*, **355**, 2179-2185.
46. Peiris J.S., de Jong M.D. & Guan Y. (2007). – Avian influenza virus (H5N1): a threat to human health. *Clin. Microbiol. Rev.*, **20**, 243-267.
47. Peiris J.S., Guan Y., Markwell D., Ghose P., Webster R.G. & Shortridge K.F. (2001). – Cocirculation of avian H9N2 and contemporary 'human' H3N2 influenza A viruses in pigs in southeastern China: potential for genetic reassortment? *J. Virol.*, **75**, 9679-9686.
48. Peiris J.S., Yu W.C., Leung C.W., Cheung C.Y., Ng W.F., Nicholls J.M., Ng T.K., Chan K.H. *et al.* (2004). – Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet*, **363**, 617-619.
49. Peiris M., Yuen K.Y., Leung C.W., Chan K.H., Ip P.L.S., Lai R.W.M., Orr W.K. & Shortridge K.F. (1999). – Human infection with influenza H9N2. *Lancet*, **354**, 916-917.
50. Perrone L.A., Plowden J.K., Garcia-Sastre A., Katz J.M. & Tumpey T.M. (2008). – H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS Pathog.*, **4**, e1000115.
51. Profeta M.L. & Palladino G. (1986). – Serological evidence of human infections with avian influenza viruses. Brief report. *Arch. Virol.*, **90**, 355-360.
52. Puzelli S., Di Trani L., Fabiani C., Campitelli L., De Marco M.A., Capua I., Aguilera J.F., Zamboni M. & Donatelli I. (2005). – Serological analysis of serum samples from humans exposed to avian H7 influenza viruses in Italy between 1999 and 2003. *J. infect. Dis.*, **192**, 1318-1322.
53. Rowe T., Abernathy R.A., Hu-Primmer J., Thompson W.W., Lu X., Lim W., Fukuda K., Cox N.J. & Katz J.M. (1999). – Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J. clin. Microbiol.*, **37**, 937-943.

54. Salomon R., Franks J., Govorkova E.A., Ilyushina N.A., Yen H.L., Hulse-Post D.J., Humbert J., Trichet M. *et al.* (2006). – The polymerase complex genes contribute to the high virulence of the human H5N1 influenza virus isolate A/Vietnam/1203/04. *J. experim. Med.*, **203**, 689-697.
55. Salomon R., Hoffmann E. & Webster R.G. (2007). – Inhibition of the cytokine response does not protect against lethal H5N1 influenza infection. *Proc. natl Acad. Sci. USA*, **104**, 12479-12481.
56. Seo S.H., Hoffmann E. & Webster R.G. (2002). – Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. *Nature Med.*, **8**, 950-954.
57. Shortridge K.F. (1992). – Pandemic influenza: a zoonosis? *Semin. respir. Infect.*, **7**, 11-25.
58. Smith G.J., Naipospos T.S., Nguyen T.D., de Jong M.D., Vijaykrishna D., Usman T.B., Hassan S.S., Nguyen T.V. *et al.* (2006). – Evolution and adaptation of H5N1 influenza virus in avian and human hosts in Indonesia and Vietnam. *Virology*, **350**, 258-268.
59. Stephenson I., Nicholson K.G., Gluck R., Mischler R., Newman R.W., Palache A.M., Verlander N.Q., Warburton F. *et al.* (2003). – Safety and antigenicity of whole virus and subunit influenza A/Hong Kong/1073/99 (H9N2) vaccine in healthy adults: phase I randomised trial. *Lancet*, **362**, 1959-1966.
60. Stephenson I., Wood J.M., Nicholson K.G., Charlett A. & Zambon M.C. (2004). – Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/Duck/Singapore/97 vaccine. *Virus Res.*, **103**, 91-95.
61. Szretter K.J., Gangappa S., Lu X., Smith C., Shieh W.J., Zaki S., Sambhara S., Tumpey T.M. & Katz J. (2007). – Role of host cytokine responses in the pathogenesis of avian H5N1 influenza viruses in mice. *J. Virol.* (in press).
62. Tanaka H., Park C.H., Ninomiya A., Ozaki H., Takada A., Umemura T. & Kida H. (2003). – Neurotropism of the 1997 Hong Kong H5N1 influenza virus in mice. *Vet. Microbiol.*, **95**, 1-13.
63. Taubenberger J.K. & Morens D.M. (2009). – Pandemic influenza – including risk assessment of H5N1. In *Avian influenza* (T.C. Mettenleiter, ed.). *Rev. sci. tech. Off. int. Epiz.*, **28** (1), 187-202.
64. Taylor H.R. & Turner A.J. (1977). – A case report of fowl plague keratoconjunctivitis. *Br. J. Ophthalmol.*, **61**, 86-88.
65. Tumpey T.M., Lu X., Morken T., Zaki S.R. & Katz J.M. (2000). – Depletion of lymphocytes and diminished cytokine production in mice infected with a highly virulent influenza A (H5N1) virus isolated from humans. *J. Virol.*, **74**, 6105-6116.
66. Tweed S.A., Skowronski D.M., David S.T., Larder A., Petric M., Lees W., Li Y., Katz J. *et al.* (2004). – Human illness from avian influenza H7N3, British Columbia. *Emerg. infect. Dis.*, **10**, 2196-2199.
67. Uiprasertkul M., Kitphati R., Puthavathana P., Kriwong R., Kongchanagul A., Ungchusak K., Angkasekwinai S., Chokephaibulkit K. *et al.* (2007). – Apoptosis and pathogenesis of avian influenza A (H5N1) virus in humans. *Emerg. infect. Dis.*, **13**, 708-712.
68. Uiprasertkul M., Puthavathana P., Sangsiriwut K., Pooruk P., Srisook K., Peiris M., Nicholls J.M., Chokephaibulkit K., Vanprapar N. & Auewarakul P. (2005). – Influenza A H5N1 replication sites in humans. *Emerg. infect. Dis.*, **11**, 1036-1041.
69. Ungchusak K., Auewarakul P., Dowell S.F., Kitphati R., Auwanit W., Puthavathana P., Uiprasertkul M., Boonnak K. *et al.* (2005). – Probable person-to-person transmission of avian influenza A (H5N1). *N. Engl. J. Med.*, **352**, 333-340.
70. Uyeki T.M., Chong Y.H., Katz J.M., Lim W., Ho Y.Y., Wang S.S., Tsang T.H., Au W.W. *et al.* (2002). – Lack of evidence for human-to-human transmission of avian influenza A (H9N2) viruses in Hong Kong, China 1999. *Emerg. infect. Dis.*, **8**, 154-159.
71. Wan H., Sorrell E.M., Song H., Hossain M.J., Ramirez-Nieto G., Monne I., Stevens J. *et al.* (2008). – Replication and transmission of H9N2 influenza viruses in ferrets: evaluation of pandemic potential. *PLoS ONE*, **3**, e2923.
72. Wang H., Feng Z., Shu Y., Yu H., Zhou L., Zu R., Huai Y., Dong J. *et al.* (2008). – Probable limited person-to-person transmission of highly pathogenic avian influenza A (H5N1) virus in China. *Lancet*, **371**, 1427-1434.
73. Webster R.G., Hinshaw V.S., Bean W.J., Vanwyke K.L., Geraci J.R., Staubin D.J. & Petursson G. (1981). – Characterization of an influenza-A virus from seals. *Virology*, **113**, 712-724.
74. Xu K.M., Smith G.J., Bahl J., Duan L., Tai H., Vijaykrishna D., Wang J., Zhang J.X. *et al.* (2007). – The genesis and evolution of H9N2 influenza viruses in poultry from southern China, 2000 to 2005. *J. Virol.*, **81**, 10389-10401.
75. Yuen K.Y., Chan P.K.S., Peiris M., Tsang D.N.C., Que T.L., Shortridge K.F., Cheung P.T., To W.K. *et al.* (1998). – Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. *Lancet*, **351**, 467-471.
76. Yuen K.Y., Woo P.C.Y., Liang R.H.S., Chiu E.K.W., Chen F.F.E., Wong S.S.Y., Lau Y.L., Ha S.Y. *et al.* (1998). – Clinical significance of alimentary tract microbes in bone marrow transplant recipients. *Diagn. Microbiol. infect. Dis.*, **30**, 75-81.

77. Zheng B.J., Chan K.W., Lin Y.P., Zhao G.Y., Chan C., Zhang H.J., Chen H.L., Wong S.S. *et al.* (2008). – Delayed antiviral plus immunomodulator treatment still reduces mortality in mice infected by high inoculum of influenza A/H5N1 virus. *Proc. natl Acad. Sci. USA*, **105**, 8091-8096.
78. Zhu Q.Y., Qin E.D., Wang W., Yu J., Liu B.H., Hu Y., Hu J.F. & Cao W.C. (2006). – Fatal infection with influenza A (H5N1) virus in China. *N. Engl. J. Med.*, **354**, 2731-2732.
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