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<b>Author(s)</b>	<b>Yuen, KY; Wong, SSY</b>
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KY Yuen 袁國勇  
SSY Wong 黃世賢

# Human infection by avian influenza A H5N1

## H5N1甲型禽流感之人類感染

The Southeast Asian outbreak of the highly lethal avian influenza A H5N1 infection in humans is unlikely to abate because of the enormous number of backyard farms providing poultry as the main source of food protein in developing countries. This increases the risk of the emergence of a reassortant pandemic influenza virus with improved human-to-human transmissibility. Currently triage of suspected cases by epidemiological risk factors remains the only practical way of case identification for laboratory investigation and infection control. The clinical usefulness of rapid diagnostic laboratory tests requires more vigorous evaluation. The lethality of this disease may reflect systemic viral dissemination, cytokine storm, or alveolar flooding due to inhibition of cellular sodium channels. The present circulating genotype Z is intrinsically resistant to amantadine and rimantadine. Prognosis may be improved by early treatment with a neuraminidase inhibitor with good systemic drug levels, and post-exposure prophylaxis for health care workers is recommended. The role of immunomodulators and other modalities of therapy requires evaluation in randomised controlled trials, with prospective monitoring of the viral load and cytokine profiles in various clinical specimens. In view of the high fatality of the disease, a combination of contact, droplet, and airborne precautions are recommended as long as resources allow despite the fact that the relative importance of these three modes in nosocomial transmission of avian influenza is still unknown.

### Key words:

*Hemagglutinins;*  
*Humans;*  
*Influenza A virus, avian;*  
*Influenza A virus, human*

### 關鍵詞：

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Department of Microbiology, University of  
Hong Kong, Queen Mary Hospital,  
Pokfulam Road, Hong Kong  
KY Yuen, FRCP, FRCPATH  
SSY Wong, MRCPATH, DTM&H

Correspondence to: Prof KY Yuen  
(e-mail: hkumicro@hkucc.hku.hk)

發展中國家無數以飼養家禽作為主要食糧的後院農場，令控制東南亞地區H5N1甲型禽流感的疫情一直困難重重，亦令這種人傳人的全球性重組流感病毒出現的風險增加。利用流行病學危機因素把懷疑個案分流，仍是目前唯一可以斷症以進行實驗室檢查和控制病毒傳染的方法。而快速斷症測試，仍需嚴格評估才可應用。這種導致死亡的病毒可以抑制細胞鈉道管的活動，導致全身病毒散播、細胞因子風暴或肺泡泛濫。現存的Z循環基因，本質上可抑制鹽酸金剛胺和金剛乙胺這兩種物質。神經氨基酶抑制劑以及適量系統性藥物的早期治療可改善預後；而醫務人員的事後預防亦非常重要。免疫調節劑和其他治療方法的可行性，需要透過隨機對照研究評估，以及對病毒讀數和不同臨床樣本細胞因子狀況進行預測研究。縱使在醫院內透過接觸、近距離飛沫及空氣感染到禽流感的機會仍然未能確定，但由於這種病高度致命，資源足夠的話，都應盡量防止病毒藉着這些途徑傳播。

## Introduction

Despite international efforts to contain the avian influenza epidemic that is rampant in Southeast Asia since late 2004, sporadic cases of human influenza A H5N1 infection are still being reported from time to time in

the endemic countries. Failure to control avian influenza infections in the domestic animals may increase the risk of the emergence of a reassortant pandemic influenza virus with improved human-to-human transmissibility. The World Health Organization (WHO) has advised all nations on preparedness for an influenza pandemic in view of the growing threat of avian influenza A H5N1 virus in Southeast Asia. This review presents current knowledge on avian influenza A H5N1, including optimum clinical management and public health measures.

## Virology

Influenza A virus is a negative-sense, single-stranded RNA virus, with an eight-segment genome encoding 10 proteins. It belongs to the family Orthomyxoviridae which includes the genera of influenzavirus A, B, and C as defined by the antigenicity of the nucleocapsid and matrix proteins. Generally, influenza A virus is associated with more severe disease in humans. Influenza A virus is further subtyped by two surface proteins—haemagglutinin (H) which attaches the virion to the host cell for cell entry, and neuraminidase (N) which facilitates the spread of the progeny virus by cleaving the host sialic acid receptors attaching the progeny virus.

### *Nomenclature and antigenic drift*

There are 16 H subtypes<sup>1</sup> and 9 N subtypes which make up all subtypes of the influenza A virus by various combinations of H and N. Infidelity of the RNA polymerase and the selective pressure of host immunity lead to the accumulation of mutations and change in surface antigenicity of these surface proteins. This antigenic change is called antigenic drift. Every year in February, the WHO decides the strains of virus to be used for the annual influenza vaccination in humans by following this antigenic drift. The nomenclature of the viral strain is written in the following order: type/place of isolation/strain number/year of isolation (subtype). Using the year 2005 as an example,<sup>2</sup> the WHO has recommended changing from influenza A/Fujian/411/2003(H3N2) to A/California/7/2004(H3N2) for vaccination purposes. Such changes are necessary because the neutralising antibody titre of the general population induced by the older vaccine strain will not be sufficient to give good protection against the new strain.

### *Antigenic shift*

All combinations of the 16 H and 9 N viral subtypes are found in water fowl, whereas only H1 to 3 and N1 to 2 viral subtypes are commonly found in humans with influenza. As a result of its segmented genome,

shuffling of gene segments can occur if two different subtypes of influenza A virus infect the same cell. For example, if a human H3N2 virus and an avian H5N1 virus co-infect a human or other member of a mammalian species, such an event can produce a novel H5N2 virus. This novel virus can then be efficiently transmitted from human to human because all or most of the gene segments apart from H5 come from the human virus. Such genetic reassortment would lead to a major antigenic change, a so-called antigenic shift, which would mean that most of the global population would not have any effective neutralising antibodies against the reassortant virus. Such a situation, coupled with the high mortality of influenza A H5N1 pneumonia,<sup>3</sup> is one of the most feared scenarios in the field of public health.

Phylogenetic studies have shown that the 1957 pandemic H2N2 virus came from the circulating H1N1, which acquired the H2, N2, and PB1 genes from avian species. Similarly, the 1968 pandemic H3N2 virus possibly came from the circulating H2N2 virus, which acquired H3 and PB1 genes from avian species. It is also possible that avian influenza A viruses undergo significant genetic mutations inside human or mammalian cells and adapt sufficiently to be transmitted from human to human. Alternatively, a human H3N2 virus that has undergone antigenic drift may acquire some internal gene segments of the H5N1 virus (such as NS1 or PB2) which may be associated with higher virulence. Such reassortant viruses can be generated in nature or in the laboratory by bioterrorists.<sup>4</sup>

## Influenza pandemics

Ten influenza pandemics defined by clinical and epidemiological records have occurred in the last 300 years, with an average of one per 33 years.<sup>5</sup> Viral and seroprevalence studies show that these pandemics were caused by H1N1 (1977), H3N2 (1968), H2N2 (1957), H1N1 (1918), possibly H3N8 (1900) and H2N2 (1889). The 1918 influenza pandemic was particularly devastating, with an estimated global fatality rate of 20 to 40 million. Unlike the usual pattern of fatality involving the very young and the old in the annual influenza seasons or the recent pandemics, those aged between 20 to 40 years were also badly affected.

Influenza epidemics are generally defined in terms of increased deaths from influenza-like illness (ILI) and pneumonia which is in excess of a pre-determined norm for all seasons of the year. The excess deaths in the United States were estimated to be 500 000 in 1918, approximately 69 800 in 1957, and 33 800 in 1968.

**Table 1. Impact of avian influenza on humans and poultry in the Hong Kong Special Administrative Region\***

Year	H5N1			H9N2	
	No. of documented human cases	No. of deaths	No. of poultry affected or destroyed	No. of documented human cases	No. of deaths
1997	18	6	1.544 million	-	-
1999	-	-	-	2	0
2001	-	-	1.372 million	-	-
2002	-	-	0.919 million	-	-
2003	2 (imported)	1	-	1	0
Total	20	7	3.835 million	3	0

\* Data accessed from Department of Health, Hong Kong Special Administrative Region

Influenza-associated deaths in the inter-pandemic years have been cited as 21 000 per year.<sup>6</sup> It is obvious that resources must be allocated for preparedness against such pandemics due to their enormous potential medical and socio-economic impact.

### Worrying aspects of the H5N1 virus

Most subtypes of influenza virus, such as H9, cause very mild disease in poultry but the H5 and H7 subtypes can inflict severe economic loss by causing outbreaks involving massive deaths in domestic poultry. Sporadic cases or outbreaks of zoonotically transmitted avian influenza A virus to humans have been reported. The majority of these cases were of acute conjunctivitis or ILI caused by H7N7 or H9N2.<sup>7-10</sup> There has been only one fatal case of pneumonia caused by H7N7.<sup>7</sup> The 1997 outbreak of H5N1 influenza in Hong Kong Special Administrative Region (HKSAR) involved 18 human cases, with six fatalities,<sup>11,12</sup> raising significant concern about the threat posed by the virus. Though the outbreak was terminated by the massive culling of 1.5 million poultry in the HKSAR, the H5N1 virus has since been isolated from poultry in an increasing number of geographical locations, from southern China to all of Southeast Asia and Japan. Table 1 summarises the impact of avian influenza on humans and poultry in the HKSAR.

Heralded by two imported human cases of H5N1 in the HKSAR in 2003,<sup>13</sup> three waves of human disease, affecting at least 88 people with 51 deaths have been reported since 2004 in Cambodia, Thailand, and Vietnam, giving an overall case-fatality rate of 58%.<sup>14</sup> Moreover, many genotypes of avian H5N1 have been found in a 4-year prospective surveillance study in southern China.<sup>15,16</sup> Over 20% of the poultry in southern China were shown to be affected during the winter time. At present the genotype Z is the dominant H5N1 virus. This is associated with high pathogenicity in zoo and

laboratory mammals including tigers, leopards, domestic cats, mice, cynomolgus monkeys, and ferrets.<sup>17-20</sup> This genotype of the H5N1 virus appears to be quite stable in the environment for up to 6 days and is resistant to amantadine and rimantadine. Like the 1997 outbreak, there is epidemiological or serological evidence of human-to-human transmission, though in a relatively inefficient manner.<sup>21,22</sup> It is notable that none of the primary H5N1 cases in Thailand contracted the virus in urban areas, where central slaughtering has been practised for more than 30 years. An epidemiological study in 1997 showed that the majority of HKSAR cases had a history of contact with poultry.<sup>23,24</sup> The present outbreak of H5N1 infections in poultry and similarly in human is unlikely to cease in the near future because of the huge number of backyard farms in Southeast Asia. The proximity of humans to poultry in these countries perpetuates the risk of human infection and that of the emergence of a reassortant or mutant virus, with good transmissibility between humans.

### Public health control

The basic reproductive number for influenza—the number of secondary cases produced by one primary case—varies from 1.68 to 20.<sup>25</sup> In explosive outbreaks with high basic reproductive numbers, airborne transmission by respiratory droplet nuclei of less than 5 µm in diameter is likely, whereas in outbreaks with a low reproductive number, infection control by droplet precautions alone may be sufficient since the large droplet will settle within 1 m from the coughing or sneezing patient. At the initial or indeterminate stage, when the severity of illness and reproductive number of the impending pandemic are still unknown, and when resources are not yet overstretched, any patient with suspected avian influenza infection should be nursed with airborne precautions. If the basic reproductive number is around 3, as in the case of severe acute respiratory syndrome (SARS), there is

still a possibility of stopping a pandemic by isolation and quarantine. Unlike SARS patients whose viral shedding and infectious status peaks at around day 10, substantial transmission of pandemic influenza virus may occur before the onset of symptoms. Viral shedding by patients with influenza starts within 24 hours before the onset of symptoms and peaks within 48 hours afterwards. Therefore, community-wide measures that reduce contacts between persons irrespective of symptom status may be the most important preventive measure before the availability of sufficient and effective antiviral agents or vaccine.

## Transmission

Infected droplets may settle on conjunctival, nasopharyngeal, or other respiratory mucosal epithelium. The haemagglutinin of human influenza A virus will adhere to the alpha-2,6-linked sialic acid receptor which is the predominant type of sialic acid receptor on the surface of human respiratory epithelium. Attachment is followed by endocytosis and fusion of the viral and cell membrane, leading to entry of the virus into the cytoplasm. The H subtypes of avian influenza A virus (such as the H5N1, H9N2, or H7N7 subtypes) will preferentially attach to the alpha-2,3-linked sialic acid receptor present on the respiratory and alimentary epithelium of birds, but also on human conjunctiva and the ciliated portion of human respiratory pseudostratified columnar epithelium.<sup>26</sup> This finding may partly explain why these avian viruses can overcome the species barrier and cause human infections. Moreover, during the late phase of infection, ciliated human respiratory epithelial cells are permissive to both human and avian virus infection, which allows the potential for genetic reassortment of avian and human viruses.

As the respiratory epithelium of pigs contains both the alpha-2,6 and alpha-2,3 sialic acid receptors, pigs can be infected by both human and avian influenza viruses. Since pigs live in close proximity to humans and poultry in family backyards in Southeast Asia, they can theoretically serve as the 'mixing vessel' for gene reassortment between avian influenza virus and human influenza virus. Therefore, rearing of pigs in close proximity to poultry is to be avoided in order to minimise this risk.<sup>27</sup> It is important to remember that H1 and H3 viral subtypes are prevalent in pigs.<sup>28</sup>

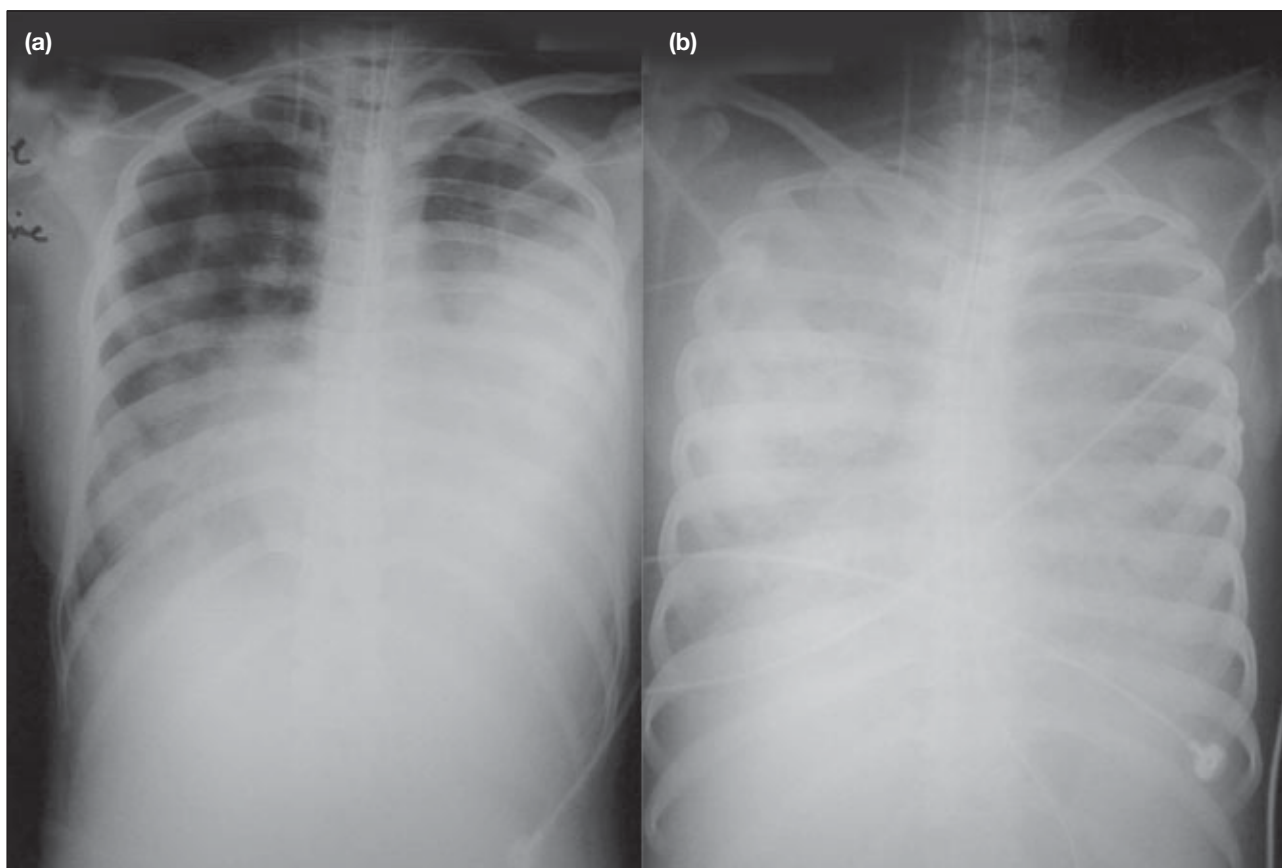
## Cellular pathogenesis and viral virulence

Once the virus enters the cell, the synthesis of the host protein is shut off by several mechanisms, such

as the degradation of host mRNA by the viral cap endonuclease. The loss of critical host cell proteins leads to cell death by necrosis. Cell death by apoptosis may also occur due to Fas antigen induced by double-stranded RNA produced during viral replication. The unusual severity of H5N1 infection in humans was initially attributed to the presence of multiple basic amino acids around the cleavage site of the haemagglutinin.<sup>29</sup> The cleavage process is essential for fusion between the viral envelope and the host cell membrane during entry of the virus into the host cytoplasm. The presence of these basic amino acids renders the protein susceptible to proteases from many different types of tissues and allows extrapulmonary dissemination due to broadened tissue tropism. An initial animal study suggested that amino acid substitution at position 627 of PB2 may affect replication efficiency or the severity of infection in a mice model.<sup>30</sup> However, when different animals and H5N1 strains were used, it became apparent that the virulence of the H5N1 virus depends on a constellation of genetic changes rather than a change in a single gene product.<sup>18</sup> For human isolates of H5N1 in particular, the non-structural protein NS1 was shown to antagonise the antiviral effect of interferon and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) produced by infected cells as an innate defence against viral infection.<sup>31</sup> Moreover, human H5N1 isolates are more potent than human H3N2 or H1N1 subtypes in the induction of pro-inflammatory cytokines, such as interferon- $\gamma$ -inducible protein-10 (IP10) and TNF- $\alpha$ , in primary human macrophage cells.<sup>32</sup> This may lead to a cytokine storm and death without extrapulmonary viral dissemination.<sup>13</sup> Recent studies have also shown that attachment by the haemagglutinin of the influenza virus onto the respiratory epithelium can cause rapid inhibition of epithelial sodium channels, leading to oedema. This may exacerbate influenza-mediated alveolar flooding and early acute respiratory failure.<sup>33,34</sup>

## Clinical disease

Asymptomatic or mildly symptomatic infection by the H5N1 virus is possible as shown by the seroprevalence study undertaken in the HKSAR after the 1997 outbreak.<sup>35,36</sup> It is likely that the 18 HKSAR patients with documented H5N1 disease represented only the more severe cases requiring hospitalisation. The main presenting complaint was high or persistent fever in most reported cases, while those with severe underlying medical illness had an exacerbation of their general debility. The initial clinical syndromes seen included: acute community-acquired pneumonia; ILI; upper



**Fig 1. Chest radiographs of a patient with influenza A H5N1 infection**

(a) bilateral primary influenza pneumonitis was seen on presentation; (b) the radiographical appearance 5 days later showed acute respiratory distress syndrome, with complete involvement of both lungs

Radiographs courtesy to Dr Edward Ho, Intensive Care Unit, Yan Chai Hospital

respiratory infections, such as acute rhinitis, conjunctivitis (though this presentation appears to be less commonly seen than in infections caused by influenza A H7N7),<sup>12</sup> and pharyngitis; an acute gastroenteritis-like syndrome with diarrhoea, vomiting, and abdominal pain<sup>11,37</sup>; and acute encephalitis with impaired consciousness and seizures.<sup>38</sup> Subsequently, many of those presenting with community-acquired pneumonia progressed to acute respiratory distress syndrome (Fig 1), or multiple organ dysfunction syndrome (MODS), and Reye's syndrome was seen in a young child who had received aspirin. The HKSAR experience in 1997 suggested that patients aged below 6 years usually had self-limiting, acute respiratory disease associated with fever, rhinorrhoea, and sore throat, with or without gastrointestinal symptoms. The children responded to supportive care and usually became afebrile within 48 hours of admission. The exception was the 3-year-old boy who had received aspirin, the first patient infected by the H5N1 virus in 1997, who subsequently died. Of the 28 Southeast Asian cases confirmed by WHO with demographic

information available on the web, only three were aged 6 years or less.<sup>39</sup>

Most patients who presented with community-acquired pneumonia had a more severe clinical course requiring oxygen therapy or intensive care as compared with patients who presented with ILI. The case-fatality rate for H5N1 infection in HKSAR in 1997 is 33% for all cases and 55% for those with pneumonia. These patients were generally adults. The major radiographic abnormalities seen included extensive infiltration bilaterally, lobar collapse, focal consolidation, and air bronchograms.<sup>3</sup> A radiographic pattern of interstitial lung infiltrates was also reported but less commonly.<sup>40</sup> Radiological and clinical deterioration was rapid. Comparing the seven cases of uncomplicated ILI in the HKSAR in 1997 with the 11 cases of community-acquired pneumonia seen, patients with community-acquired pneumonia were significantly older, had lower total peripheral white blood cell counts, were more often lymphopenic, and more often had a fatal outcome. Over 60% of patients with

**Table 2. Diagnostic tests for avian influenza infection in humans\***

Viral detection methods on respiratory secretions and other clinically indicated specimens	Retrospective detection of host antibody response on acute and convalescent sera
(1) Viral culture on Madin-Darby canine kidney cell line or chick embryo (>48 hours) (2) Rapid antigen detection for influenza A nucleoprotein antigen using immunochromatographic or membrane enzyme immunoassay (0.5 hour) (3) Rapid antigen detection by immunofluorescent assay for influenza A nucleoprotein (2 hours) and if positive, specific detection for H1, H3, and H5 antigens (2 hours) (4) Reverse transcription-polymerase chain reaction (RT-PCR) for the M gene (for all influenza A subtypes) and if positive, specific diagnosis by RT-PCR for H1, H3, and H5 (>24 hours)	(1) Microneutralisation test—needs P3 level biosafety laboratory using the outbreak strain (96 hours) (2) Complement fixation test—for all influenza A subtypes, not specific for H5 (24-48 hours) (3) Western blot by baculovirus-expressed recombinant H5 for confirming that the neutralising antibody is against the H5 component (24 hours)

\* Approximate turnaround time is given in parentheses

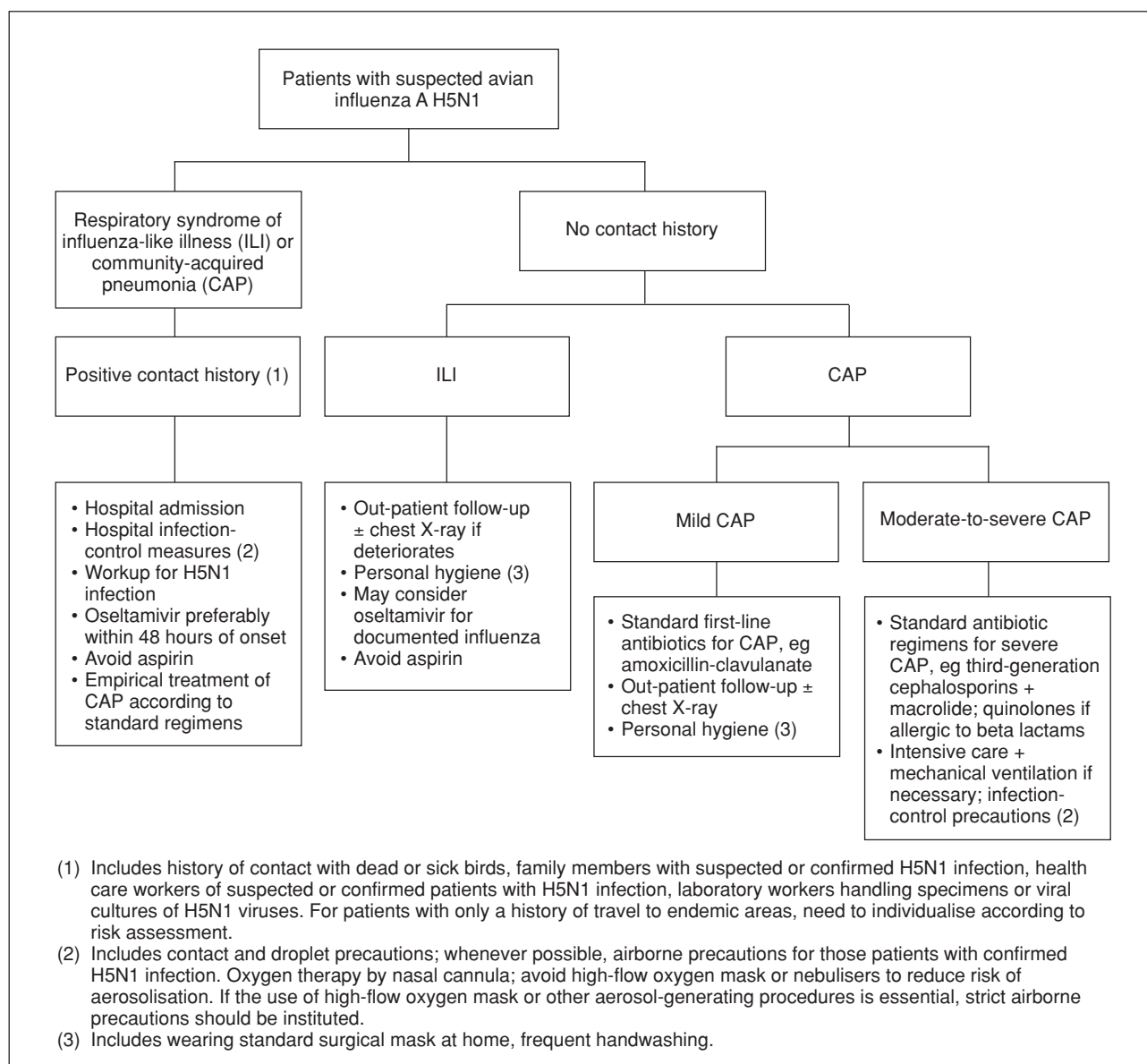
pneumonia had deranged liver function tests or gastrointestinal symptoms such as vomiting, diarrhoea, or abdominal pain, and one third had impaired renal function. Lymphopenia and liver dysfunction were present on initial presentation but renal dysfunction was seen as a late event. Some patients also had thrombocytopenia and prolonged clotting times. In a report of 10 cases from Vietnam, a reversed CD4<sup>+</sup>/CD8<sup>+</sup> ratio was observed in all five tested patients.<sup>3</sup> Impaired glycaemic control was reported in six of these patients but methylprednisolone had been given to seven patients. None of the HKSAR patients had evidence of rhabdomyolysis, myocarditis, or encephalitis but the recent Southeast Asian outbreak had one case of virologically confirmed encephalitis.<sup>38</sup> All six HKSAR patients of 1997 who died had MODS and acute respiratory distress syndrome. Three had evidence of haemophagocytosis on bone marrow examination. Medical co-morbidities were not significantly more common in those with more severe disease in the HKSAR outbreak. Two patients with mild ILI had a ventricular septal defect and glucose-6-phosphate dehydrogenase deficiency respectively. Four patients with severe disease had the following respective co-morbidities: active systemic lupus with immunosuppressive therapy, histiocytosis X, electrocardiographic evidence of an old myocardial infarct, and malignant thymoma treated with radiotherapy 10 years previously.

## Pathology

Three postmortem examinations have been reported on patients in the HKSAR. Two were performed 1 month after the onset of illness,<sup>41</sup> and one in 2003 at day 10 after the onset of illness.<sup>13</sup> Histopathological examination in the latter patient showed alveolar

oedema, haemorrhage, fibrin exudation, alveolar infiltration with CD68<sup>+</sup> macrophages, interstitial infiltration of CD3<sup>+</sup> lymphocytes, and type-2 pneumocyte hyperplasia with increased expression of TNF- $\alpha$ . Reactive haemophagocytosis was also observed in the hyperplastic bone marrow and in the parafollicular areas of the bronchial and hilar lymph nodes.<sup>13</sup> The histopathological changes seen in the postmortems carried out 1 month after onset of illness included organising, diffuse alveolar damage, with interstitial fibrosis; hepatic central lobular necrosis; acute renal tubular necrosis; and lymphoid depletion. Viral antigen detection or reverse transcription-polymerase chain reaction (RT-PCR) did not suggest extrapulmonary viral disease.<sup>41</sup>

A prospective study of the serial serum cytokine profiles was not completed but a higher serum concentration of IP10 and monokine induced by interferon- $\gamma$  (MIG) was noted between day 6 and day 8 of the illness in one patient with a fatal outcome, whereas these markers decreased by day 5 of the illness in a patient who survived the disease.<sup>13</sup> The two postmortem cases in the HKSAR in 1997 had elevated serum interleukin-6, interferon- $\gamma$ , and soluble interleukin-2 receptor levels.<sup>41</sup> The initial working hypothesis was that H5N1 viral infection in the respiratory tract induced interferons and TNF- $\alpha$  as early response cytokines that triggered a cascade of other cytokines, including IP10 (a macrophage chemo-attractant), MIG (induced by interferon- $\gamma$  secreted by activated T lymphocytes), interleukin-6, and others. This was thought to explain the local and systemic inflammatory response syndromes in the absence of systemic viral dissemination. However, a recent case report has suggested that the virus can be cultured from the serum, cerebrospinal fluid, and stool in addition to



**Fig 2. Management of patients with suspected avian influenza A H5N1**

the respiratory secretions.<sup>38</sup> This was not an altogether unexpected finding as comprehensive virological screening by culture and RT-PCR from various body sites was not performed in the two 1997 postmortem cases and postmortem tissues were only available at a late stage of the illness. In infected poultry, laboratory and zoo animals, such as mice, ferrets, tigers and leopards, evidence of viral infection has been found in extrapulmonary sites, including the central nervous system.<sup>17,42</sup> The relative importance of virus-induced cytolysis and host immunopathological damage can only be determined by a prospective longitudinal study. This would evaluate the viral load and cytokines in various clinical specimens serially collected throughout the course of the illness, in the absence of intervention with

antivirals or immunomodulators. Such a study would also assist in ascertaining possible modes of transmission and the period of communicability of this disease.

### Laboratory diagnostics

A summary of the reported diagnostic tests for avian influenza infection in humans is presented in Table 2. A positive culture for H5N1 virus in Madin-Darby canine kidney cell line or chick embryo allantoic sac inoculated with a properly collected clinical specimen remains the gold standard for the definitive diagnosis of this disease. The low number of cases and lack of systematic collection of clinical specimens has precluded proper evaluation of culture



and various rapid diagnostic tests, such as RT-PCR and antigen detection by enzyme immunoassay or immunofluorescent tests. Our experience in 1997 suggested that RT-PCR is superior to antigen detection in both sensitivity and specificity. If the direct antigen detection test is for influenza A, a positive detection of H5 antigen or RNA and a negative detection of H1 and H3 by either specific monoclonal antibodies or RT-PCR would strongly suggest a diagnosis of H5 infection. It is important to remember that the commercial immunochromatographic membrane enzyme immunoassay tests are not specific for H5 and only has a sensitivity of 70% when compared with viral culture. Therefore, a negative test does not exclude influenza A infection and a positive test does not confirm the diagnosis of H5 infection. There was an impression that the viral load in H5N1 infection was relatively low—in the order of 31.6 copies of the M gene per  $\mu\text{L}$  in a nasopharyngeal specimen collected between day 5 to 10 of illness—compared with that of human influenza. However, in a recent case report, the viral load was estimated at 85 (serum), 64 (cerebrospinal fluid), 180 (throat swab), and 98 (rectal swab) copies of M gene per  $\mu\text{L}$ .<sup>38</sup> Nasopharyngeal aspirate or bronchoalveolar lavage followed by nasopharyngeal swab or throat swab placed in viral transport medium should be collected with airborne precautions (see below) in patients suspected to have this infection. A stool or rectal swab placed in viral transport medium should also be considered in suspected cases presenting with diarrhoea. Serum and cerebrospinal fluid should be collected in those with encephalitis. If the patient survives the infection, a 4-fold rise in neutralising antibody titre between the acute and convalescent sera is also diagnostic of the infection.<sup>43</sup> Unfortunately, the microneutralisation test requires the use of a virus of similar antigenic lineage, often the circulating virulent genotype, which necessitates the test being performed in biosafety level 3 laboratories. The results of the microneutralisation test can also be confirmed by a Western blot assay using baculovirus-expressed recombinant H5 antigen.<sup>44</sup>

### Clinical management

During the initial stage of the illness, clinical examination cannot accurately differentiate H5N1 infection from other causes of community-acquired pneumonia, ILI, acute gastroenteritis, and acute encephalitis. Epidemiological markers such as TOCC (Travelling history to endemic areas; Occupation: poultry, laboratory or health care workers; Contact history with poultry, dead or sick bird or H5N1 patients; and Clustering of infection) should be used to triage patients for isolation, early empirical treatment

with oseltamivir and laboratory investigations (Fig 2).

Good evidence from randomised placebo-controlled trials is not available for the use of antivirals or immunomodulators in H5N1 infections. Aspirin must be avoided to prevent Reye's syndrome, especially in those younger than 16 years but this complication has also been reported rarely in adults.<sup>45</sup> Respiratory support and intensive care remain the most important aspects of clinical management during the acute stage of the pneumonic illness. Pneumothorax has been reported when patients were placed on mechanical ventilation.<sup>40</sup> To avoid barotrauma and further lung injury, a lung-protected ventilation strategy should be considered.

Data from gene sequencing of genotype Z of the H5N1 virus has identified an asparagine residue at position 31 of the M2 protein, which is invariably associated with resistance to amantadine and rimantadine.<sup>16</sup> Both neutralisation tests in cell culture and a mice model have shown that the neuraminidase inhibitors, oseltamivir and zanamivir, appear effective against the virus. However, the mortality in infected mice was seen to increase with the duration of delay in administering the neuraminidase inhibitor.<sup>46</sup> Five of six reported patients died despite the use of oseltamivir at or after 6 days of illness.<sup>3,13</sup> The high mortality may reflect the lack of in-vivo efficacy, or delayed treatment. Therefore, antiviral therapy must be given as early as possible, as is the case with human influenza where clinical efficacy is maximal if given within 48 hours of onset of illness. Oseltamivir is usually given at a dosage of 75 mg orally twice daily for 5 days, and zanamivir given at 10 mg inhaled twice daily for 5 days. A higher dose of oseltamivir of 150 mg orally twice daily has been suggested and used in some clinical trials.<sup>47</sup> The use of a higher dose of oseltamivir is associated with a larger reduction in viral load in respiratory secretions, as well as a shorter duration of illness in human influenza infections. However, there is currently no evidence that this higher dose of oseltamivir equates with a significant clinical advantage over the standard dosage.<sup>48-50</sup> Whether higher doses of oseltamivir might be of benefit in avian influenza requires further investigation. A higher dose of oseltamivir should be considered in the group of patients with very severe disease and especially those having diarrhoea (which may impair drug absorption) or immunosuppressed patients (including the very young and very old) who may have a high initial viral load. The duration of treatment should also be extended to 2 weeks since neutralising antibodies appear much later in patients infected with H5N1 than human influenza viruses. This is because most humans

do not have prior exposure to H5N1 and therefore immunological memory against cross-reacting antigens is absent. Zanamivir has little systemic absorption and may not be useful if extrapulmonary dissemination has occurred but can still be considered for post-exposure prophylaxis or early treatment in compliant patients with no underlying pulmonary disease.<sup>51</sup> Although zanamivir has not been FDA-approved for prophylaxis against influenza, meta-analyses of clinical trials have shown that it is highly effective as a prophylactic agent for human influenza.<sup>52</sup> Further clinical trials may serve to confirm the role of zanamivir as a prophylactic agent against human and avian influenza infections.

Chemoprophylaxis has been recommended for health care workers or other personnel who might have been exposed to the highly pathogenic avian influenza viruses. In view of the widespread resistance to amantadine, neuraminidase inhibitors are currently the drugs of choice for this purpose. The present WHO recommendation suggests giving oseltamivir 75 mg orally once a day for at least 7 days, starting as soon as possible after exposure.<sup>53</sup> In addition to post-exposure prophylaxis, vaccination against human influenza viruses is also recommended for at-risk personnel, such as health care workers, field investigators, and poultry workers who might come into contact with avian influenza viruses.<sup>53,54</sup> Although this does not protect the workers against avian influenza, this may theoretically reduce the possibility of genetic reassortment between human and avian influenza viruses in the same human host.

The use of RNA interference is a novel approach to antiviral therapy.<sup>55</sup> This technique involves the use of small interfering RNA (siRNA) molecules, which mediate degradation of viral mRNA by cellular enzymes. RNA interference has been studied for a number of viruses including influenza viruses.<sup>56,57</sup> In-vivo efficacy of this approach in prevention and treatment of influenza has been demonstrated in animal models.<sup>57,58</sup> Optimisation of target sequences and delivery systems may allow future clinical trials of siRNA in humans.

At the stage of acute respiratory distress syndrome and MODS, it is tempting to try immunomodulators. However, six of the seven reported patients treated with the immunomodulator methylprednisolone died.<sup>3</sup> This again suggests that the indication, choice, and dose of immunomodulators should be investigated in the setting of a randomised controlled trial. If the viral load could be effectively reduced by early antiviral therapy, the systemic inflammatory response due to cytokines may corre-

spondingly be reduced. It is important to remember that interferons and TNF- $\alpha$  produced by patients are important suppressors of viral replication. It would be counter-productive if the immune defence against viral replication is impaired by immunomodulators, while antiviral therapy is not optimised. Concomitant monitoring of the cytokine profile and viral load would be an important component of clinical trials.

### Hospital infection control

The most important route of acquisition of H5N1 infection is through contact with infected birds or their excreta. However, hospital-acquired infection by H5N1 virus was evident in a retrospective cohort study comparing H5N1 seropositivity in health care workers exposed to patients with H5N1 disease with unexposed controls.<sup>35</sup> Health care workers exposed to patients with H5N1 infection were more likely to be seropositive to the H5N1 virus and this difference could not be attributed to differences in exposure to poultry. Two health care workers with unprotected exposure to virologically documented cases were seropositive. One developed a mild flu-like illness but no virus could be cultured.

The route of infection from avian influenza-infected patients to health care workers is uncertain, although it would be reasonable to presume that inhalation of infective respiratory secretions (including large droplets and droplet nuclei) and/or contact with virus-laden secretions could be responsible. This is in line with the most common routes of infection for human influenza viruses, namely via droplets and contact with mucous membranes.<sup>59-61</sup> Therefore, in addition to standard precautions, droplet and contact precautions are the cornerstones in hospital infection control for avian influenza-infected patients. Nevertheless, epidemiological and animal studies have shown that airborne transmission is possible for human influenza,<sup>59-61</sup> although there is much less conclusive evidence compared to other diseases, such as tuberculosis. It has been suggested that airborne transmission might explain the sometimes explosive nature of human influenza epidemics. Avian influenza to date has not been shown to be efficiently transmitted from person to person, and it would be impossible to predict when the avian influenza viruses would acquire the potential for efficient interpersonal spread. Presumably because of this consideration, and the high case-fatality rate of the disease, the WHO and Centers for Disease Control and Prevention both have recommended airborne precautions, in addition to standard, contact, and droplet precautions, in the care of patients infected by avian influenza viruses.<sup>53,62</sup> This may necessitate

resources not always available in developing countries. However, it is still advisable to care for in-patients with suspected or documented avian influenza A H5N1 infections with airborne precautions as far as practicable. The period of communicability of H5N1 infection is not well studied but can last for 3 weeks in young children.<sup>53</sup> Laboratory-acquired infections by avian H7N7 but not H5N1 virus have been reported.<sup>63</sup> Therefore, all health care and laboratory workers should comply strictly with infection control and laboratory biosafety procedures.

## Conclusion

The high case-fatality rate of the present avian influenza epidemic in Southeast Asia is alarming. This, together with the propensity of influenza viruses to undergo genetic reassortment and mutation, makes the avian influenza virus a likely candidate for the next human influenza pandemic. The prospect of control appears to be somewhat grim in the endemic rural parts of Southeast Asia where modern biosecurity measures are not easily implemented to control the transmission of the avian viruses among and between different animal species. Human vaccination at this stage is not a viable way to minimise or control the threat. The neuraminidase inhibitors remain the only group of compounds effective for the treatment of avian influenza infections. Although avian influenza is a treatable disease from the antiviral point of view, the therapeutic efficacy of currently available antivirals appears to rely on their use early in the course of the illness. This is a significant impediment in developing countries because patients are unlikely to present to hospital within the first 48 hours if the disease initially manifests as an ILI. Even if the patient presents to health care facilities, the ability of facilities to provide specific and rapid diagnosis for H5N1 infection remains limited in rural areas. Therefore, apart from research on vaccine and antiviral development, more effort should also be put into the development of rapid diagnostic tests that are sensitive, specific, and above all, affordable for developing countries.<sup>64</sup>

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