

Avian influenza

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ABSTRACT

A rapidly spreading, highly pathogenic avian influenza virus A (H5N1) in the domestic poultry population has crossed the species barrier to humans and other mammalian species, thus, posing an increasing pandemic threat. The World Health Organization, other agencies, and countries worldwide are closely monitoring the prevalent influenza viruses and their related illnesses to detect any increased virulence or transmissibility that might signal the beginnings of any future pandemic. So far, the H5N1 virus has infected birds in more than 30 countries in Asia, Europe and Africa, while further geographical spread remains likely. Human infections are still rare and the virus does not spread easily from birds to humans or readily from person to person. Although antiviral drugs and vaccination are among the most important measures to be used in case of an influenza pandemic, a timely supply of sufficient quantities will not be possible. This review describes various aspects of avian influenza in birds and in humans; epidemiology, transmission, diagnosis and clinical manifestations. Also presented are the global preparedness, the anti-influenza drugs and vaccines.

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Influenza viruses, which belong to the family *Orthomyxoviridae*, are classified as A, B, and C on the basis of antigenic differences in their nucleoprotein (NP) and matrix (M1) protein.¹ All avian influenza viruses are classified as type A, and are further sub-typed based on the antigenicity of 2 surface glycoproteins, hemagglutinin (HA or H) and neuraminidase (NA or N). Sixteen HA subtypes and 9 NA subtypes are currently known.² The combination of these surface proteins describes the viral subtypes. This is why influenza A viruses have names like H1N1 or H5N1. Each subtype, in turn, is composed of numerous strains or isolates whose nomenclature requires connotation of the subtype, host of origin (other than human), geographic location, serial number of isolate and year of isolation. For example, the strain known as A/Teal/HK/W312/97 (H5N1) denotes a strain of subtype H5N1 that has been isolated from the teal in Hong Kong (HK) in 1997 and given serial number W312.

Avian influenza affects virtually all species of birds, domestic and wild. It was first described in 1878 following severe outbreaks in chicken in Italy.³ The etiologic agent was unknown at that time and the disease was coined "fowl plague" in reference to its marked severity and high mortality. Another early name of avian influenza was "Lombardian disease".⁴ The causative agent of "fowl plague" was isolated since 1902, marking the first documented isolation of an influenza virus.⁵ However, it was not until 1955 that the agent was identified as one of the influenza viruses.⁶ It was also found that avian influenza exhibits different forms of severity, ranging from mild or even asymptomatic infection to an acute and rapidly fatal disease.

Practically, all known subtypes of influenza A virus are found in wild birds, the latest being the H16 subtype recently reported in black-headed gulls in Sweden and the Netherlands.² This leaves little doubt that the virus originated in wild birds and subsequently

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made its way to domestic poultry and mammals, including man. Certain subtypes of the virus have become adapted to specific hosts, to the extent that at least 4 distinct entities of influenza A are currently recognized on the basis of host specificity: avian, human, swine and equine.⁷ The viruses commonly circulating in poultry are genetically different from those commonly circulating in humans. Under normal conditions, therefore, those viruses rarely produce cross-infections between man and bird. The swine, on the other hand, is susceptible to infection with subtypes prevailing in human and avian hosts, as well as its own subtypes, and for that reason it might play a role in some parts of the world as a "vessel" where these different viruses re-assort, namely, exchange some of their genetic material during replication within the same host cell.

Epidemiological and phylogenetic studies have indicated that although the influenza A virus occurs in numerous avian species in the wild, its primary reservoirs in nature are water fowls, such as wild ducks, geese and swans, which carry the virus asymptotically in their guts and excrete it in water through their feces.^{5,8}

The virus. Influenza A virus has a multipartite genome composed of 8 RNA gene segments that code for 10 genes: 2 surface glycoproteins (hemagglutinin and neuraminidase), 3 transcriptases (PB2, PB1, and PA), 2 matrix proteins (M1 and M2), one nucleocapsid protein (NP), and 2 nonstructural proteins (NS1 and NS2). The virus is encapsidated by a host-derived lipid envelope from which some 500 spikes protrude. Eighty percent of the spikes consist of HA and the rest are composed of NA. The HA is essential for endocytosis by host cells while the NA for exocytosis and variations in either of these glycoproteins are important, though not the sole causes for enhanced virulence, a polygenic trait in which other gene products might be implicated as well.⁹

A major feature that distinguishes influenza A virus from the B and C viruses is its much wider host range. This might be partly related to the fact that numerous genotypes of influenza A virus exist in nature, depending on their gene combinations. Therefore, apart from birds, humans, equines and pigs, this virus has been reported in seals, whales, mustelids (such as weasels, ferrets, badgers, and others), silver fox, domestic cats, leopards, tiger, dogs, civet, New Zealand white rabbit, rats and monkeys.¹⁰⁻¹² By contrast, influenza B and C viruses are primarily human pathogens that have been only occasionally reported naturally in non-human mammals. Furthermore, Influenza C virus is a rare

and stable virus that causes only mild symptoms in humans; unlike A and B viruses, which have 8 RNA segments each, influenza C virus has only 7 segments.

Another important feature of influenza A virus is that it is genetically adaptable and mutates easily. This allows it not only to change its antigenic identity in order to evade host immunity but also to change its virulence and adapt to new hosts. A third and equally significant feature is the wide range of pathogenicity that this virus exhibits.

Influenza in birds. In its natural reservoirs, influenza A virus causes only mild disease that often passes unnoticed. Similarly, most infections in domestic poultry are mild or silent; however, some strains produce a moderate disease and some cause overwhelming outbreaks with dire economic losses.

In view of the marked variation in the pathogenicity of different subtypes of avian influenza, the disease in birds has been divided into 2 distinct pathotypes: low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI). The HPAI is far less common than LPAI and usually arises as a result of mutations of originally LPAI progenitors. Although known since 1959, it was only from 1997 onward that the current H5N1 has evolved into a vicious killer, causing the most devastating outbreaks of poultry disease in history. Those outbreaks so far claimed up to 150 million birds either as a result of disease or condemnation, with an estimated cost to the poultry industry in South East Asia alone of approximately US\$10-15 billion.

Virtually all currently known HPAI viruses belong to H7 and H5 subtypes but not all H7 and H5 subtypes are necessarily HPAI viruses. Certain criteria are therefore used to distinguish HPAI from LPAI strains, which primarily involve the inoculation of 1-2 month old chickens with the virus (in a biosecure laboratory) and determining mortality rate and a special marker known as the intravenous pathogenicity index (IVPI). Any virus is regarded as highly pathogenic if it killed 75% of the chickens within 10 days or had an IVPI greater than 1.2. Molecular techniques are also employed in determining the virulence of the virus. The amino acid sequences of the HA cleavage region, which is responsible for HA antigenicity, differ from subtype to subtype by 30% or more.¹³ Therefore, any H7 or H5 virus isolate is considered to be highly pathogenic, regardless of mortality or IVPI tests, if it showed an amino acid sequence at its HA cleavage site similar to that which has been previously observed in other HPAI viruses.

Transmission and spread. Influenza A virus is highly contagious in chickens and particularly turkeys, which are regarded as the “magnet” of the virus. One gram of manure of an infected chicken is believed to be sufficient to transmit disease to 1-10 million birds in contact.¹⁴ The virus is also shed in great amount in the saliva and nasal discharge of these birds. Other poultry like geese, pheasants, partridges and quail are also quite susceptible. Pigeons are more resistant¹⁵ although doves are not.¹⁶ However, of particular interest in the transmission of the disease is the domestic duck since it often survives the infection and may sometimes act as an asymptomatic carrier, even for H5N1. Furthermore, it excretes the virus into the environment in greater amount and for a longer time than either chickens or turkeys, and recent studies in Asia have indicated that its ability to survive and excrete H5N1 has increased with time resulting in the amplification of the spread of this virus among other poultry.¹⁷

Avian influenza is not an air-borne infection for birds; it spreads between farms, regions and countries primarily through movement of sick birds and contaminated poultry products and fomites such as cages, egg trays, trucks, and others used on farms. While wild birds remain to be a major reservoir of infection, the role of migratory wild birds in the spread of the current H5N1 virus has not been established unequivocally. One argument is that H5N1 currently isolated from migratory birds is highly pathogenic to them, being isolated only from dead or dying birds that are incapable of migration. In China, for instance, more than 6000 wild aquatic birds died from infection with H5N1 in the Qinghai Lake wildlife sanctuary.^{18,19} Another observation that argues against a major role of migratory birds in the spread of H5N1 is that the patterns of spread of that virus in Asia did not seem to be consistent with the timing and routes of migratory birds, and that all investigated Asian outbreaks have been traced to movement of infected poultry and poultry products and materials. In many cases, the spread of the virus from one country to another has occurred along land and railroads, rather than migratory bird routes, and many outbreaks occurred in summer, the season for moulting rather than migration of most birds. Some outbreaks in Europe did occur along bird migratory routes but there are other explanations for their occurrence such as revival and increased activity of the virus with drop in temperature during winter.

Mechanisms of change, and the potential for human pandemics. Phylogenetic studies of influenza A viruses isolated

from different avian and mammalian hosts have revealed species-specific lineages of viral genes. These virus lineages have become significantly adapted to their respective hosts, while incapable of replicating easily in other hosts.⁵ Accordingly, it is unusual for viruses of avian lineages to undergo efficient replication in human cells and vice versa. However, the same studies have also revealed genes that have crossed species barriers, and when that occurred in humans, it caused major epidemics or even pandemics. Such events are relatively rare but can occur at any time. During the past century only 3 human flu pandemics have been recorded. The most devastating of those was the Spanish flu of 1918, in which an entire virus (H1N1) moved from birds to humans^{20,21} culminating in the death of over 20 million people worldwide. The remaining 2 pandemics, the Asian flu of 1957 and the Hong Kong flu of 1968 were caused by the reassortant viruses H2N2 and H3N2 respectively, which contained a combination of avian and human viral genes.²² Avian influenza viruses are therefore key contributors to the emergence of human influenza pandemics.

Change in the virulence and host range of these viruses is brought about by genetic drift and antigenic shift. Genetic drift refers to small genetic mutations (point mutations) that occur slowly but continuously in the virus. It explains the absence of solid immunity to influenza viruses in man and animals and the occurrence of seasonal influenza due to the appearance of new strains. Antigenic shift, on the other hand, consists of sudden, big genetic change. One way by which this change is brought about involves re-assortment of HA, and NA and other genes from 2 or more viruses replicating within one cell, resulting in a major change in antigenic structure. Another mechanism is adaptive mutation to a new host without prior re-assortment. In such cases, stepwise changes occur as the virus mutates during infection of the new host thereby gradually improving its transmissibility.²³ Re-assortment is known only for influenza A viruses and is responsible for the sudden appearance of new variants with different HA and NA combinations that cause epidemics or pandemics of flu in humans, birds or other animals. The intriguing similarity in a number of changes in the polymerase proteins of both the highly pathogenic H1N1 virus of 1918 and in the recently circulating, highly pathogenic strains of H5N1 avian viruses,²⁰ is reason for concern.

The tendency of influenza A viruses to undergo genetic changes is facilitated by the fact that the viral genome is a multipartite one. Theoretically, any of these viruses can genetically change at any point in time. Therefore, the potential for spontaneous

emergence of new strains of avian influenza viruses is always there. Some of these strains may change virulence or cross species barriers or both into new immunologically 'naïve' hosts. In "mixed" human infections with avian and human influenza subtypes, the potential also exists for emergence of a recombinant virus capable of spreading directly and rapidly from person to person, which could lead to epidemic or pandemic disease, which we may totally fail to resist. Crossing the species barrier has other unpleasant consequences that might complicate the epidemiological pattern of disease. When an agent crosses the species barrier into a new host, it remains unstable for some time. It infects additional new host species, moves to new areas and changes further during this process. For instance, when the scrapie agent crossed from sheep to cattle, it quickly infected domestic cats, wild Felidae, wild ungulates, sheep²⁴⁻²⁸ and humans (nvCJD). Similarly, after infecting humans, the H5N1 virus was reported in tigers and leopards fed on meats of infected poultry²⁹ and could also be established experimentally in non-human primates.¹⁰

The H5N1 in humans. Unlike pandemic flu, sporadic cases of human infection with avian influenza viruses are common. In recent times, human infections with H9N2, H7N3, H5N1 and H7N7 have been reported; most of these infections were acquired from poultry (**Table 1**).

The currently circulating H5N1 has been responsible for massive HPAI outbreaks in Asian poultry for almost a decade. During the first outbreak that occurred in Hong Kong in 1997, 18 people became infected and 6 died. The entire poultry population was destroyed in an effort to stop the outbreak, which it did for a while. Starting from late 2003, new cases in poultry are now re-appearing in Hong Kong and outbreaks have been occurring in several parts of Asia. They involved different poultry species with massive losses to poultry producers. More recently, the virus has also appeared in birds in some parts of Europe, Africa and the Middle East (**Table 2**). Up until 4th April 2006, the number of confirmed human infections with H5N1 in different countries reached 191 cases and 108 (56.5%) of those patients died (**Table 3**). Transmission to humans, however, required

Table 1 - Documented human infections with avian influenza viruses.

Date	Country/Area	Strain	Cases	Deaths	Symptoms	Source
1959	USA	H7N7 ^a	1 (46-year-old man)	0	respiratory	overseas travel
1995	United Kingdom	H7N7	1 (43-year-old woman)	0	conjunctivitis	pet ducks (shared lake with migratory birds)
1997	Hong Kong SAR	H5N1 ^a	18	6	respiratory, pneumonia	poultry
1998	China (Guangdong)	H9N2	5	0	unknown	unknown
1999	Hong Kong SAR	H9N2	2 girls (4 years, 13 months)	0	respiratory	poultry for 4-year-old; unknown for 13-month-old
2003 (Feb.)	Hong Kong SAR ^b	H5N1 ^a	2 (9-year-old boy, 33-year-old father)	1	respiratory	unknown
2003 (Mar.)	Netherlands	H7N7 ^a	89	1 (57-year-old veterinarian)	conjunctivitis (pneumonia, respiratory insufficiency in fatal case)	poultry
2003 (Dec.)	Hong Kong SAR	H9N2	1 boy (5-year-old)	0	respiratory	unknown
2004	Vietnam	H5N1 ^a	33	25	respiratory	poultry
2004	Thailand	H5N1 ^a	17	12	respiratory	poultry
2004	Canada	H7N3 ^a	2	0	conjunctivitis	poultry

^a highly pathogenic for poultry
^b possibly acquired in mainland China
Source: World Health Organization (WHO). Avian Influenza: Assessing the Pandemic Threat.³⁶ 2005;
<http://www.who.int.csr/disease/influenza/H5N1-9reduit.pdf>

Table 2 - Countries affected with H5N1 in poultry.

East Asia	Europe, Siberia, Central Asia	Africa
Cambodia	Croatia	Nigeria
China	Kazakhstan	Egypt
Hong Kong	Romania	Niger
Indonesia	Russia (Siberia and European Russia)	Cameroon
Japan	Turkey	Burkina Faso
Laos	Iraq	
Malaysia	Cyprus	
Mongolia	Greece	
South Korea	Italy	
Thailand	Bulgaria	
Vietnam	Austria	
India	Germany	
	Slovenia	
	Azerbaijan	
	Ukraine	
	France	
	Hungary	
	Georgia (former Soviet Republic)	
	Slovakia	
	Bosnia-Herzegovina	
	Servia	
	Poland	
	Albania	
	Israel	
	Jordan	
	Czech Republic	
	Denmark	

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very close contact with birds or their environment. Infection is through inhalation of infectious droplets and droplet nuclei, by direct contact, and perhaps, by indirect (fomite) contact, with self-inoculation onto the upper respiratory tract or conjunctival mucosa.^{30,31}

Clinical presentation. A number of avian influenza strains have been recognized as a cause of human illness, mainly respiratory symptoms (Table 1). Occasionally, conjunctivitis was prominent, for example in the recent H7N7 outbreak in the Netherlands, while several patients had mild influenza-like illness and only one patient died of acute pneumonia.⁸ On the other hand, H5N1 repeatedly caused severe respiratory illness as well as other manifestations since it was first diagnosed in Hong Kong in 1997. To date, the vast majority of cases have been detected following hospitalization for respiratory illness. Table 4 summarizes the main clinical features and outcome of the reported cases in 5 Asian countries where the cases have been laboratory confirmed.³² The incubation period was typically 2-4 days but could extend up to 8 days, much longer than for other known human influenza. In addition to the classical influenza presentation, watery diarrhea and shortness of breath were also prominent. Of interest was the primary viral pneumonia, usually without secondary bacterial infection, at the time of hospitalization. Among the laboratory findings, lymphopenia and raised aminotransferase levels were remarkable features. In the cases from Thailand, the risk of death was particularly associated with lymphopenia.

Table 3 - Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to WHO up until 4 April 2006

Country	2003		2004		2005		2006		Total	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Azerbaijan	0	0	0	0	0	0	7	5	7	5
Cambodia	0	0	0	0	4	4	1	1	5	5
China	0	0	0	0	8	5	8	6	16	11
Egypt	0	0	0	0	0	0	4	2	4	2
Indonesia	0	0	0	0	17	11	13	12	30	23
Iraq	0	0	0	0	0	0	2	2	2	2
Thailand	0	0	17	12	5	2	0	0	22	14
Turkey	0	0	0	0	0	0	12	4	12	4
Vietnam	3	3	29	20	61	19	0	0	93	42
Total	3	3	46	32	95	41	47	32	191	108

Total number of cases includes number of deaths.
 World Health Organization (WHO) reports only laboratory-confirmed cases.
 Source: Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO as of 4 April 2006.⁶⁰
 WHO available from URL: http://www.who.int/csr/disease/avian_influenza/country/cases_table_2006_04_04/en/index.html

Table 4 - Clinical presentation for different groups of patients in Asia.

Clinical presentation	Hong Kong	Thailand 2004	Vietnam 2004	Ho Chi Minh City 2005	Cambodia 2005
	(N=18)	(N=17)	(N=10)	(N=10)	(N=4)
No. of patients (%)					
Fever (>38°C)	17/18 (94)	17/17 (100)	10/10 (100)	10/10 (100)	4/4 (100)
Headache	4/18 (22)	NS**	NS**	1/10 (10)	4/4 (100)
Myalgia	2/18 (11)	9/17 (53)	0	2/10 (20)	NS**
Diarrhea	3/18 (17)	7/17 (41)	7/10 (70)	NS**	2/4 (50)
Abdominal pain	3/18 (17)	4/17 (24)	NS**	NS**	2/4 (50)
Vomiting	6/18 (33)	4/17 (24)	NS**	1/10 (10)	0
Cough	12/18 (67)	16/17 (94)	10/10 (70)	10/10 (100)	4/4 (100)
Sputum	NS**	13/17 (76)	5/10 (50)	3/10 (30)	NS**
Sore throat	4/12 (33)	12/17 (71)	0	0	<(25)
Rhinorrhea	7/12 (58)	9/17 (53)	0	0	NS**
Shortness of breath	1/18 (6)	13/17 (76)	10/10 (100)	10/10 (100)	NS**
Pulmonary infiltrates	11/18 (61)	17/17 (100)	10/10 (100)	10/10 (100)	4/4 (100)
Lymphopenia	11/18 (61)	7/12 (58)	NS**	8/10 (80)	= (50)
Thrombocytopenia	NS**	4/12 (33)	NS**	8/10 (80)	= (50)
Increased aminotransferase	11/18 (61)	8/12 (67)	5/6 (83)	7/10 (70)	NS**
Development of respiratory failure (usually with ARDS)*	8/18 (44)	13/17 (76)	9/10 (90)	7/10 (70)	4/4 (100)
Hospital Course – no. (%)					
Respiratory failure	8 (44)	13 (76)	9 (90)	7 (70)	4 (100)
Cardiac failure	NS**	7 (41)	NS**	0	NS**
Renal dysfunction	4 (22)	5 (29)	1 (10)	2 (20)	NS**
Deaths – no. (%)	6 (33)	12 (71)	8 (80)	8 (80)	4 (100)
ARDS -acute respiratory distress syndrome, *High levels of inflammatory mediators may contribute to ARDS and multiorgan failure, **NS - not stated, Table adapted from: WHO Writing Committee of WHO Consultation on Human Influenza A/H5. Avian influenza A (H5N1) infection in humans. ³²					

Overall, the case-fatality rate for H5N1 influenza is approximately 50%. This high rate suggests that the pathogenicity of H5N1 may be similar to the 1918 H1N1 pandemic strain. Researchers have hypothesized that cytokine storm (namely, overproduction of cytokines) may have played an important role in the pathogenesis of the 1918 pandemic strain. A laboratory-based study involving H5N1 strains taken from ill humans in Asia (during 1997 and 2004) and an ordinary current H1N1 strain (circulating in Asia in 1998) found that all the H5N1 viruses caused human alveolar cells and bronchial epithelial cells to secrete significantly higher levels of various cytokines and chemokines than did the ordinary virus.³³ These findings support the role of cytokine storm in the pathogenesis of H5N1, but further work is needed to clarify the clinical implications of these findings. Asymptomatic H5N1

infections have been documented serologically by detecting specific antibodies among household and social contacts³⁴ and healthcare workers exposed to patients with confirmed diagnosis.³⁵

It is important to stress that human H5N1 cases are still rare and sustained person-to-person transmission has not yet occurred. Almost all cases have been linked to close contact with diseased household flocks, often during culling, slaughtering, de-feathering, evisceration and preparation of poultry for consumption. No cases have been linked to the consumption of properly cooked poultry meat or eggs, even in households where disease was known to be present in flocks. In this connection, the World Health Organization (WHO) recommends thorough cooking of meat to 70°C, a temperature that inactivates influenza viruses, thus rendering safe

any contaminated poultry raw meat with the H5N1 virus.³⁶

Surveillance. Guidelines regarding reporting have been issued from WHO and Centers for Disease Control and Prevention (CDC).^{37,38} According to these recommendations, testing for H5N1 of patients hospitalized in the United States is indicated for patients who have both of the following conditions: 1) Radiographically confirmed pneumonia, acute respiratory distress syndrome (ARDS), or other severe respiratory illness for which an alternative diagnosis has not been established. 2) A history of travel within 10 days of symptom onset to a country with documented H5N1 avian influenza infection in poultry or humans.

Testing for influenza A (H5N1) should also be considered for patients with all of the following: 1) Documented temperature of over 100.4°F (38°C), sore throat, or shortness of breath. 2) History of contact with poultry or domestic birds (example, visited a poultry farm, a household raising poultry, or a bird market) or a known or suspected patient with influenza A (H5N1) in an H5N1-affected country within 10 days of symptom onset.

Laboratory testing. Diagnostic tests for influenza include viral culture, polymerase chain reaction (PCR), rapid antigen testing, and immunofluorescence. The sensitivity and specificity of these tests might differ between laboratories depending on the type of test used as well as the type of specimen examined. Nasopharyngeal aspirates are typically more effective among the types of routine respiratory specimens acceptable for use (throat swab, nasal wash, or nasal aspirate). Optimally, samples should be collected within the first 3 days of onset of illness and should be promptly delivered to the laboratory for processing.

Rapid diagnostic tests that detect the influenza antigen in respiratory specimens can give result in less than 30 minutes and can differentiate between types, namely, influenza A and B. However, the commercially available rapid chromatographic tests for H5N1 have a sensitivity of only 70% as compared with virus culture.³⁹ Another rapid assay, the immunofluorescence staining of respiratory epithelial cells fixed onto glass slides and using specific monoclonal antibodies and conjugates, can generally yield a diagnosis in 2-4 hours. Although the immunofluorescence assay allows for the rapid differentiation of human H5/influenza infection from other influenza types and subtypes, it cannot exclude H5N1 infection due to lack of sensitivity.

Therefore, despite the availability of these 2 rapid diagnostic assays, the conventional viral culture that takes 5-10 days remains important especially for reference laboratories since it is cheap, sensitive and is critical for providing specific identification of circulating strains and subtypes of influenza viruses. Molecular diagnostic techniques, the PCR and Real-time PCR (RT-PCR) assays that are now available for the detection of viral RNA in respiratory samples or infected cell cultures can give results in a few hours. The PCR sensitivity depends heavily on the type of primers used to bind specifically to the targeted part of the influenza virus genome.^{37,40} The RT-PCR with its superior sensitivity,⁴¹ has already gained prominence in influenza pandemic preparedness since it will enable laboratories to make a rapid and accurate diagnosis of human cases of avian influenza. For confirmation and typing of influenza viruses, the WHO recommends forwarding all isolates positive for H5, H7 or H9 to a designated influenza reference laboratory. These special laboratories are listed by the WHO.⁴²

Serologic testing can be used for retrospective diagnosis of recent influenza infection and therefore it is rarely useful for patient management. It entails taking an acute phase serum sample followed by a convalescent specimen 2-4 weeks later. Detection of a significant increase - more than fourfold rise in antibody titers - would be consistent with recent infection. However, the main value of influenza serology lies in epidemiological investigations of yearly epidemics, avian to human transmissions and drug and vaccines trials.

In view of its high pathogenicity, H5N1 virus or suspected specimens can be handled safely only by specially trained staff working in specially equipped laboratories operating at high level of biosecurity. These facilities do not presently exist in the majority of affected countries, thus, an infrastructure needs to be developed to complement national testing with rapid international verification in WHO certified laboratories, especially as each confirmed human case yields information essential to risk assessment. Culture of specimens from suspected avian influenza cases requires special containment facilities and should only be conducted under enhanced biosafety level-3 (BSL-3) conditions. This includes controlled access to a specially designed negative pressure facility, double-door entry with changing room, use of respirators, and proper decontamination of all waste. These diagnostic activities must be kept separate from routine influenza diagnostic activities (example, probable H1 or H3 isolates) to prevent recombination.^{43,44} Other diagnostic tests require

BSL-2 containment, which still ensures a high degree of protection and safety within the laboratory.

Antiviral agents. Two groups of antiviral agents are available for treatment and prophylaxis of influenza: the M2 inhibitors (amantadine and rimantadine), and the neuraminidase inhibitors [oseltamivir (Tamiflu) and zanamivir (Relenza)]. Patients suspected of having influenza A (H5N1) should promptly receive a neuraminidase inhibitor pending the results of the diagnostic laboratory testing, since early treatment, within 48 hours after onset, can reduce the duration of illness. It should be noted, however, that the optimal dose of neuraminidase inhibitors and the duration of treatment are uncertain and their effectiveness has not been adequately assessed. Currently approved regimens are likely to represent the minimum required dose.³² Oseltamivir is approved for treatment of influenza in adults and children more than 1 year of age and zanamivir is approved for treatment of adults and children older than 7.⁴⁵ Oseltamivir also is approved for prevention of influenza in adults and children more than 1 year of age.⁴⁶

The H5N1 influenza viruses isolated from poultry and humans in Southeast Asia in 2004 have shown resistance to amantadine and rimantadine.⁴⁷ Similar resistance was more recently demonstrated in the 2005 - 2006 seasonal influenza A (H3N2) isolates in the United States and therefore these agents are not recommended for treatment.⁴⁸ Oseltamivir-resistant H5N1 strains have been recently isolated from several patients in Vietnam. One was a Vietnamese child who received prophylactic treatment with the drug⁴⁹ and another report involves 2 additional patients, both of whom died of H5N1 influenza.⁵⁰

If H5N1 avian influenza escalates into a pandemic, antiviral agents will be the only major medical counter measures available. They will be used at the start of the pandemic in an attempt to curtail spread particularly as vaccines might not be available in a timely manner. Once a potential pandemic strain of influenza virus is identified, it takes at least 6 months to develop a vaccine and manufacture it on a large scale⁵¹ and then availability will depend on production rates. At the same time, international demand for the vaccine will be high.

Several countries are developing their own stockpiles of oseltamivir for domestic use during the lead-time before a new vaccine becomes available. The WHO also has a dedicated stockpile of the antiviral drugs sufficient for 3 million treatment courses by early 2006. These drugs will be strictly reserved for use in the first areas affected by an

emerging pandemic virus. Recent studies, based on mathematical modeling, suggest that these drugs could be used prophylactically near the start of a pandemic to reduce the risk that a fully transmissible virus will emerge or at least to delay its international spread, thus gaining time to augment vaccine supplies. However, there are limitations to their use and their effectiveness in a pandemic situation has yet to be tested. The drugs will be stored centrally as WHO has considerable experience in the rapid dispatch of medical supplies during emergencies. Recently, Roche company (producer of oseltamivir) pledged to donate an additional 3 million treatment courses to WHO⁵² significantly increasing the WHO stockpile.

Vaccines. Influenza vaccination is the cornerstone for the control of influenza. By acquiring immunity to the surface antigens, particularly the HA, the likelihood of infection and severity of disease, if infection occurs, are greatly reduced. Antibody against one influenza virus type or subtype confers limited or no protection against another type or subtype. Furthermore, antibody to one antigenic variant of influenza virus might not completely protect against a new antigenic variant of the same type or subtype.⁵³ Frequent development of antigen variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual incorporation of one or more new strains in each year's influenza vaccine.

The WHO is recommending targeted use of seasonal influenza vaccine among persons at risk of H5N1 infection to reduce the potential for humans to be infected with H5N1 at the same time that they are harboring a human influenza strain.⁵⁴ This will decrease the opportunity for genetic re-assortment of the avian H5N1 strain with genes from a human (H1 or H3) strain and thereby reduce the likelihood that a novel pandemic strain will emerge from the current situation. Groups recommended for vaccination include (a) all persons expected to be in contact with poultry or poultry farms suspected or known to be affected with avian influenza (H5N1), especially cullers involved in destruction of poultry and people living and working on poultry farms where H5N1 has been reported or is suspected or where culling takes place (b) healthcare workers involved in the daily care of known or confirmed human cases of influenza H5N1, and (c) healthcare workers in emergency care facilities in areas where there is confirmed occurrence of influenza H5N1 in birds (provided that sufficient vaccine supplies are available).

No vaccines are commercially available for protecting humans against H5N1, a likely candidate

for starting a pandemic. However, an active biomedical research program is underway to develop candidate vaccines such as inactivated H5N1 as well as live attenuated H5N1 and H9N2 cold-adapted vaccine formats that are undergoing clinical trials.⁵⁵ The WHO has been working with laboratories in the WHO influenza network to develop vaccines against H5N1⁵⁶ so that once a pandemic is declared, all manufacturers will switch from production of seasonal vaccines to the newly developed and matching pandemic strain. However, finite capacity to produce the necessary antigen content of vaccines and the time needed to produce sufficient quantities of the vaccine itself are critical limiting factors.

Pandemic preparedness. The spread of H5N1 virus, which has so far been limited to poultry, to migrating birds, mammals and human population, represents a distinct threat of a pandemic. According to WHO, at this time the pandemic alert level for H5N1 influenza is at phase 3: a new viral subtype is causing disease in humans but is not yet spreading efficiently and sustainably.⁵⁷ As a result, the WHO, other organizations and countries worldwide are making preparations to deal with this serious challenge.

While neither the timing nor the severity of the next pandemic can be predicted, history shows that these events consistently bring an explosive surge in the number of illnesses and deaths sufficient to temporarily paralyze public services and economic productivity. All governments therefore, need to be prepared to convert all health services to cope with a sudden and large increase in demand.

Hospital infection control. Although avian influenza to date has not been shown to transmit efficiently from person to person, limited transmission to health care workers occurred, but did not cause severe disease. Serological evidence of acquiring the infection was evident in 2 health care workers with unprotected exposure to patients and one of them had a mild flu-like illness.³⁵ Therefore all health care and laboratory workers should strictly comply with infection control and laboratory biosafety procedures.

Infection control guidance is important for protection of health care workers involved in the care of patients with known or suspected avian influenza. In addition to standard precautions, contact and airborne precautions (including use of high-efficiency masks and negative-pressure rooms when available) should be implemented.^{58,59} Health care workers should also be vaccinated with the most recent seasonal human influenza vaccine, as well as being monitored for

development of fever or any respiratory symptoms. Such monitoring allows early diagnosis of infection, appropriate advice on reducing further transmission and starting antiviral therapy at the earliest possible stage.

In conclusion, a concerted global effort is required for the containment of avian viruses with pandemic potential. This will present extraordinary challenges for developing appropriate responses and strategies for influenza surveillance, prevention and treatment.

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