Emerging influenza virus: A global threat

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Since 1918, influenza virus has been one of the major causes of morbidity and mortality, especially among young children. Though the commonly circulating strain of the virus is not virulent enough to cause mortality, the ability of the virus genome to mutate at a very high rate may lead to the emergence of a highly virulent strain that may become the cause of the next pandemic. Apart from the influenza virus strain circulating in humans (H1N1 and H3N2), the avian influenza H5N1 H7 and H9 virus strains have also been reported to have caused human infections, H5N1 H7 and H9 have shown their ability to cross the species barrier from birds to humans and further replicate in humans. This review addresses the biological and epidemiological aspects of influenza virus and efforts to have a control on the virus globally.

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1. Introduction

The major cause of health concern that claims a large number of lives worldwide every year are the infectious diseases (Ministry of Health Report 1920). The advent of antibiotics and vaccines has lessened the impact of a number of infectious diseases but they are still the number one cause of mortality. These emerging infectious diseases can either be new emergent infections or rare infections that may re-emerge occasionally or they may be common infections which increase owing to issues like social instability or resistance development (Ridley 2004). Of all the infectious diseases, influenza deserves the particular attention as it undergoes a high rate of antigenic change giving rise to a new type of influenza strain for which there is no immunity in the population. Moreover in the absence of ready or preventive therapeutic interventions, it poses a great threat. Human influenza viruses of at least three haemagglutinin subtypes, H1, H2 and H3 have emerged as important pathogens and are of major global health concern. Recently, the influenza virus with subtype H5, mutated from H7, has also emerged as a human pathogen and they are more lethal than the earlier strains (Webby and Webster 2003).

Of influenza A, B and C viruses, influenza A viruses mutate more rapidly thus showing more antigenic flexibility and hence are more virulent than the other two types (Eccles 2005) (table 1). They have a range of hosts that include humans, horses, pigs, sea mammals and birds. Each of the haemagglutinin sub-type can combine with all the subtypes of the neuraminidase, resulting in huge and highly flexible pool of genetic diversity. The emergence of any novel HA subtype to which a population does not have any immunity could lead to a pandemic.

Table 1. Comparison of the structure, virulence and efficacy of treatment of influenza virus A, B and C

Comparison of influenza A, B and C			
	Type a	Type b	Type c
Severity of illness	++++	++	+
Animal reservoir	yes	no	no
Human pandemics	yes	no	no
Human epidemics	yes	yes	no (sporadic)
Antigenic changes	shift, drift	drift	drift
Segmented genome	yes	yes	yes
Amantadine, rimantidine	sensitive	no effect	no effect
Zanamivir (Relenza)	sensitive	sensitive	
Surface glycoproteins	2	2	(1)

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2. Epidemiology

As influenza is caused by a variety of species and strains of viruses, in any given year some strains can die out while others create epidemics with a potential to cause a pandemic. The incubation period of this virus is generally 1-4 days, with an average of 2 days (Khanna *et al* 2002). The adults transmit influenza one day prior to onset of symptoms and up to 5 days after the symptoms begin. The children usually transmit it for 10 or more days (Elveback *et al* 1976). The groups of people that are at a high risk for contracting influenza and influenza related complications include (Mathew 2006):

- All individuals 50 years and older
- Children 6–23 months of age
- Women who are pregnant during influenza season
- Residents of long term care facilities
- Children 6 months to 18 years of age and who are receiving aspirin therapy for extended period of time
- Persons 6 months and older with any chronic illness

Human H5N1 cases have been observed to increase towards the end of the year and during the early months of the subsequent year when the temperature and humidity is relatively lower and they occur in association with increases in H5N1 poultry outbreaks. A WHO review of 256 H5N1 cases found that the median age was 18 years (range 3 months-75 years), 89% of cases were more than 40 years old, the median duration from illness onset to hospitalization was 4 days, mortality was highest among cases aged 10–19 years, and the median duration from illness onset to death was 9 days (range 2–31 days) (WHO Report 2006). Investigations suggest that most human cases appear to have acquired H5N1 virus infection through avian-to-human transmission following direct or close contact with sick or dead poultry (e.g. slaughtering, culling, burying, preparing for food, de-feathering, etc.), well-appearing poultry (holding cock-fighting roosters, ducks or H5-vaccinated poultry) or wild birds (de-feathering dead wild swans) (Writing Committee, WHO 2008). Risk factors for H5N1 disease identified in Vietnam and Thailand include directly touching sick or dead poultry or preparing them for consumption, having sick or dead poultry in or around the home, and being within 1 meter of sick or dead birds (Areechokchai *et al* 2004; Dinh *et al* 2006). In urban areas, where poultry raising is uncommon, a risk factor for H5N1 disease appears to be visiting a live poultry market (Yu *et al* 2007). This suggests that environmental factors could play a role in H5N1 virus transmission to humans. In up to a quarter of cases, a source of H5N1 virus exposure could not be identified (Sedyaningsih *et al* 2007). Clusters of two or more epidemiologically linked H5N1 cases have been identified in several countries (Olsen *et al* 2005). Approximately 25% of all H5N1 cases reported to date have occurred in clusters. Most clusters have involved two to three cases; the largest to date was eight cases (seven confirmed one probable) with seven deaths. While most cluster cases probably acquired H5N1 virus infection through common poultry exposures, limited, non-sustained human to human H5N1 virus transmission through close, prolonged, unprotected contact with a severely ill H5N1 patient has probably occurred or could not be excluded in some clusters (Ungchusak *et al* 2005; Kandun *et al* 2006). Probable, limited, human-to-human transmission in health care settings has been reported in health care workers and family members (Ungchusak *et al* 2005). Nearly all H5N1 cluster cases have occurred among blood-related family members, suggesting a possible role of genetic susceptibility. The occurrence and frequency of clinically mild and asymptomatic H5N1 virus infection are unknown. A sero-prevalence study among 1525 Hong Kong poultry workers in 1997 found that 10% had H5N1 neutralizing antibodies (Bridges *et al* 2002). Of the 11 pediatric H5N1 cases identified in 1997, seven had relatively mild disease, but four had severe or fatal illness. Limited data from case investigations and sero-surveys since 2003 suggest that clinically mild disease and asymptomatic H5N1 virus infection is uncommon. A small number of clinically mild H5N1 cases have been identified among children (Kandun *et al* 2005). Sero-surveys among rural villagers in Cambodia, poultry workers in Chinaand Nigeria, and health care workers in Vietnam and Thailand suggest that human H5N1 virus infection has been very rare to date. Therefore, the high proportion of fatal cases among reported H5N1 cases may be accurate, although further studies are needed on the spectrum of H5N1 virus infection. At admission, most H5N1 cases have presented with a history of high fever, cough, shortness of breath, dyspnoea, and evidence of pneumonia on chest X-ray (Writing Committee, WHO 2008). About one-third of cases have had diarrhoea. Common laboratory findings at hospitalization include leucopoenia, lymphopenia and mild to moderately decreased platelet counts (Writing Committee, WHO 2008). Clinical complications include progression to respiratory failure, acute respiration distress syndrome (ARDS), multi-organ failure, sepsis, shock and reactive haemophagocytosis.

3. Pandemics due to human influenza virus

The pandemics caused by the influenza A viruses in the past have led to a high level of illness, death, social disruption and economic loss (Kawaoka *et al* 1989; Palese *et al* 2004; WHO, Geneva 2005) (table 2). Today, the H1N1 and H3N2 strains are the commonly circulating strain in the human population (Palese *et al* 2004).

Since 1997, a highly pathogenic avian strain, H5N1, have been found to cross the species barrier from birds to humans leading to 100% mortality in humans. The earlier less

virulent avian strain H9N2 along with H5N1 strain which is far more virulent and has crossed the species barrier to humans and deaths in humans due to this strain has been reported from many countries (table 3). The persons who are in regular contact with fowls and their products or excreta, have a chance to be infected with the deadly strain provided the fowl has been infected with influenza virus. The human to human transmission of this virus can lead to a great pandemic (Stephenson *et al* 2004).

4. Clinical characteristics of human influenza infection

The disease is usually most severe in very young children (under 5 years of age) and the elderly. Many people are so ill that they are confined to bed for several days, with aches and pains throughout their bodies, which are worst in their backs and legs (Eccles 2005). Symptoms of influenza may include:

- Sudden onset of fever
- Dry cough
- Body aches, especially joints and throat
- Coughing and sneezing
- Chills and rigor
- Fatigue
- Headache

Table 2. Pandemics due to influenza virus and the causative strains

Pandemics caused by influenza A Major antigenic shifts associated with influenza A pandemics		
Year	Sub type	Prototype strain
1918	H1N1	A/FM1/47
1957 (Asian flu)	H2N2	A/Singapore/57
1968 (Hong Kong flu)	H3N2	A/Hong Kong/68
1977	H1N1	A/USSR/77
1987	H3N2	No pandemic Various strains circulated worldwide

Table 3. Cases and deaths caused due to the infection of human beings with avian influenza virus in different geographical areas of the world

Year	Total No. of cases	Deaths (due to avian flu)
2003	4	4
2004	46	32
2005	98	43
2006	115	79
2007	86	59
2008	20	17

- Irritated watering eyes
- Nasal congestion
- Reddened eyes, skin (especially face), mouth, throat and nose
- Abdominal pain (in children with influenza B) (Kerr *et al* 1975)

Common symptoms of the flu such as fever, headaches, and fatigue come from the huge amounts of proinflammatory cytokines and chemokines (such as interferon or tumour necrosis factor) (Eccles 2005; Schmitz *et al* 2005) produced from influenza-infected cells. Of the symptoms listed above, the combinations of findings including fever, cough, sore throat and nasal congestion can improve diagnostic accuracy (Call *et al* 2005).

5. Surveillance

A measure of the severity of influenza in any one year is the excess of deaths due to pneumonia or influenza compared to the seasonally adjusted norm. The influenza A subtypes currently circulating in humans, H1 and H3, continue to experience the antigenic changes. Although the potential of H1N1 and H3N2 strains are diminishing, as their ability to cause serious disease has become increasingly attenuated, the continual modification may lead to an increase in virulence.

The World Health Organization (WHO) maintains constant surveillance of influenza outbreaks world wide and has a series of 'sentinel' labs to look at what is happening in the circulating virus population. It has a network of 112 National Influenza Centers that monitor influenza activity and isolate influenza viruses in all continents. The Center for Disease Control and Prevention (CDC) does the same in the United States and co-operates with WHO. In India, Department of Health and Human Services (DHHS)-CDC in collaboration with Indian Council of Medical Research (ICMR) is involved in the intensive influenza surveillance programme. National Institute of Virology, Pune is the referral center for the ongoing influenza surveillance programme which controls all the regional centers. The main objectives of the surveillance programme, are establishment of epidemiological and virological influenza surveillance network in different geographical areas of India, development of human resource through training and strengthening of infrastructure, expansion and strengthening of the surveillance in a phased manner, timely dissemination of information generated and improvement of awareness and contribution of the influenza strains and information generated to the global influenza surveillance.

Large number of suspected influenza speciemens were collected from various centres .A total number of 364 isolates

were found positive for H3N2, H1N1 and B influenza virus, out of which 273 isolates were sequenced for HA gene and sent to CDC, US. The sequence analysis shown that most of the isolates were sensitive against Adamantane while some were resistant for the same.

5.1 *Laboratory diagnosis*

A number of tests help in the diagnosis of influenza (table 4). Specimens are preferably collected within the first 4 days of illness. Rapid influenza tests provide results within 30 min or less; viral culture provides results in 3-10 days.Routine serological testing for influenza requires paired acute and convalescent sera and does not provide results to help with clinical decision-making.

Broadly the techniques are classified as direct and indirect diagnostic techniques. The Direct method involves detection of direct presence of viral particles, viral antigens, and viral genome through sirect immunoflourescence (Spada.*et al* 1991), Enzyme linked immunosorbent assay (Orskov and Orskov 1990), Antigen capture and staining of cells with monoclonal antibody (Brumback and Wade 1996). Other methods are indirect where the clinical samples may be inoculated in cell cultures, eggs, or animals for growth of the virus and its further typing. Hen's eggs and cell lines are used for virus growth. Many continuous cell lines are being employed for influenza virus growth like MDCK, LLC-MK2 etc. (Davies.*et al* 1978). The most common used cell line is MDCK. The presence of virus is detected by the cytopathic effect (CPE) like slow rounding and degeneration of cells. Further confirmation of influenza virus is done by by haemagglutination test and haemagglutination-inhibition test which are used for routine diagnosis (Hirst 1941).

In recent years molecular techniques are being increasingly used for diagnostics. The advantage of these techniques is its sensitivity and quick turnaround time. Some molecular techniques for influenza diagnosis include reverse transcriptase polymerase chain reaction (RT-PCR), multiplex PCR, real time RT-PCR, nucleic acid based amplification (NASBA) and loop mediated isothermal amplification (LAMP) (Khanna and Srivastava 2006). The most commonly used technique for rapid detection of influenza viruses is RT-PCR. It is extremely sensitive and rapid (Wright *et al* 1995). A reverse multiplex PCR is employed for typing and subtyping of number of different influenza strains together. The appropriate combination of primer sets and optimizations of PCR conditions allow formation of multiplex PCR for detection of influenza A (H1N1 and H3N2) and B from clinical samples (Stockton 1998). The advent of molecular methods such as real time PCR has allowed improvement of detection methods currently used in laboratories, although not all of these methods include an internal positive control (IPC) to monitor for false negative results. A one-step reverse transcription real time PCR (RRT-PCR) with a minor groove binder (MGB) probe, for the detection of different subtypes of AIVs (against the HA and NA gene) has also been (Lee and Suaraz 2004) designed. The PCR is performed and the corresponding graphs indicate the type, sub-types and amount of the viral strains without performing agarose gel analysis (Trani *et al* 2006). Most recent rapid, sensitive technique which is not found to be affected by other biological compounds in the clinical samples is called loop mediated isothermal amplification (LAMP) which is conducted under isothermal conditions of 60-65 ˚C by using enzyme called Bst DNA polymerase. Thus, it is able to quantify the amount of DNA accurately without being affected by presence of other inhibitory substances (Poon *et al* 2005; Kaneko 2007).Thus more and more sensitive techniques must be explored for the development of efficient and effective strategies against influenza.

Table 4. Various techniques used for the laboratory diagnosis of the influenza virus

Procedure	Influenza types detected	Acceptable specimens	Time for results
Viral culture	A and B	NP (Naso Pharyngeal) swab, throat swab, nasal wash, bronchial wash, nasal aspirate, sputum	3-10 days
Immunofluorescence	A and B	NP swab, nasal wash, bronchial wash, nasal aspirate, sputum	2-4 h
RT-PCR	A and B	NP swab, throat swab, nasal wash, bronchial wash, nasal aspirate, sputum	2-4 h
Serology	A and B	paired acute and convalescent serum samples	2 weeks or more
Enzyme Immuno Assay (EIA)	A and B	NP swab , throat swab, nasal wash, bronchial wash	2 h
Rapid detection tests (using kits)			
Directigen flu A (Becton-Dickinson)	A	NP (Naso Pharyngeal) wash and aspirate	Less than 30 min

6. Prevention and cure

Influenza causes significant morbidity and mortality and is responsible for considerable medical expenditures. Antiviral therapy and vaccination are important strategies for the control of human/ avian influenza, but the efficacy of these modalities is limited by the timings of administration and shortage of supply. Healthcare workers currently must apply strict standards, contact and droplet precautions when dealing with suspected cases, and upgrade to airborne precautions when performing aerosol-generating procedures. Non-pharmacological measures such as early case isolation, household quarantine, school/workplace closure, good community hygiene and restrictions on travel are useful measures in controlling a pandemic (Hui 2008; Tambyah 2008).

High Security Animal disease Laboratory (HSADL), Bhopal and National Institute of Virology, Pune have developed testing kits for avian influenza virus in bird droppings to detect the avian flu at the earliest. Special task forces are set up under the supervision of the Chief Medical Officers at each district to spread information about the disease and to monitor all suspicious cases of influenza. Indian Council of Agricultural Research (ICAR) has developed bird flu vaccine to control the spread of the virus and also for vaccination in anticipation of an outbreak.

6.1 *Chemotherapy*

Rimantadine and amantadine are the two well known antivirals to prevent and treat influenza A. Amatadine and rimantadine are M2 inhibitors and block virus entry across the endosome and also interfere with virus release (Wang *et al* 1993). They are good prophylactic agents for influenza A and may be given as protective agents during an outbreak, especially to those at severe risk and key personnel. They may also be given at the time of vaccination for a few weeks, until the humoral response has time to develop. (There is some evidence that these drugs can help prevent more serious complications and reduce the duration of influenza A, if given early.) However, in the 2005-2006 influenza seasons, 92% of H3N2 strains examined had mutations that would confer resistance to these drugs as did 25% of the H1N1 strains tested. Similar problems were seen in 2006-2007 and so these drugs are not recommended until the percent resistance in the major circulating type drops. The resistance to amantadine and rimantadine is detected by sequencing the M2 gene which detects the point mutations responsible for imparting the resistance (Belshe *et al* 1988; Hayden *et al* 1992; Abed *et al* 2005). Two neuraminidase inhibitors (Zanamivir [Relenza] and Oseltamivir [Tamiflu]) have also been approved by the Food and Drug Administration (FDA) for prophylaxis as well as treatment (Gubareva *et al* 1998; Monto *et al* 1999). They are active against both influenza A and influenza B and can reduce the duration of uncomplicated influenza (by approximately 1day in about 70–90% of adults) if taken within two days of the onset of illness.

Low-dose steroids may be considered in the treatment of refractory septic shock. Noninvasive positive pressure ventilation (NPPV) may play a limited supportive role for acute lung injury, but it is contra-indicated in critically ill patients with multi-organ failure and haemodynamic instability. Intravenous gammaglobulin should be used with caution for treatment of reactive haemophagocytosis due to its thrombogenic effects, whereas the role of etoposide needs evaluation with animal models (Hui 2008; Tambyah 2008).

6.2 *Vaccination*

Influenza vaccine is often recommended for high-risk groups, such as children and the elderly. Influenza vaccines can be produced in several ways; the most common method is to grow the virus in fertilized eggs of hens (Osterholm 2005). A new vaccine is formulated annually with the types and strains of influenza predicted to be the major problems for that year (predictions are based on worldwide monitoring of influenza). The vaccine is multivalent (trivalent) and the current one, recommended by WHO, is to two strains of influenza A (one for H1N1 and one for H3N2) and one of influenza B. It has a short lived protective effect and is usually given in the fall so that protection is high in December/January – the usual peak months for flu in the northern hemisphere. In India, the poor population especially children living in slums and other over crowded areas should be targeted for vaccination in the start of November. About two weeks after vaccination, the antibodies that provide protection against virus infection develop in the body. (Belshe *et al* 2000a, b; Dunnil 2006).

Types of flu vaccine:

- *Inactivated virus vaccine*: When the vaccine and the circulating strains are antigenically similar, inactivated vaccines prevent the illness among approximately 70–90 % of healthy adults with age less than 65 years (Demicheli *et al* 2000). Older persons and persons with chronic diseases might develop lower post-vaccination antibody titers and can remain succeptible to influenza infection and other respiratory tract infection (McElhaney *et al* 1990).
- *Live attenuated vnfluenza vaccine (LAIV)*: The virus can be grown in eggs until it loses virulence and the avirulent virus given as a live vaccine (Hilleman *et al* 2002). Attenuation is done by multiple changes in the various genome segments. Reassortment is used to generate viruses which have six gene segments

from the attenuated virus and the HA and NA coding segments from the virus which is likely to be a problem in the up-coming influenza season (Jin *et al* 2003; Hoffman *et al* 2005).

7. Threat to humans due to avian influenza virus (H5N1)

Human infections with highly pathogenic avian influenza A (H5N1) viruses resulted in rare, sporadic, severe and fatal cases among persons in 14 countries in Asia, the Middle East, Eastern Europe and Africa from 1997 through 2007. Of 369 reported human H5N1 cases that occurred from 1997 through 2007, overall mortality was 60%. However, H5N1 viruses continue to circulate and evolve among poultry in many countries, and there are many unanswered questions about human infection with H5N1 viruses (Uyeki 2008).

7.1 *Avian flu in India*

The outbreak of avian flu in India dates back to 18 February, 2006 when agricultural authorities in India confirmed the country's first outbreak of highly pathogenic H5N1 avian influenza in poultry. The disease was detected at several commercial farms in the Navapur sub-district in the western state of Maharashtra and Gujarat. Further outbreak was reported in Jalgaon district of Maharashtra and it spilled over to the adjoining areas of Madhya Pradesh. India declared a fresh outbreak of avian influenza among poultry on 25 July 2007. Samples tested at the High Security Animal Disease Laboratory in Bhopal and the National Institutes of Virology in Pune confirm that the samples are positive for H5 strain of avian influenza in Chingmeirong Village, East Imphal District, Manipur in North East India. Around 350,000 birds had been culled, 30,000 eggs and 23,000 bags of chicken feed were also destroyed. The outbreak had affected poultry but there were no reported human cases. Department of Animal Husbandry, Dairying and Fisheries notified outbreak of avian influenza among poultry in various district of West Bengal from 15th January 2008 to 16th May 2008. In the earlier notified area of English Bazar block of Malda district, populations of 217999 were covered. Fifteen cases of fever/ URI were detected with no history of handling of dead/ sick birds. H5N1 virus poses great risk of pandemic and epidemic in India because of limited influenza awareness and surveillance activity,

8. Discussion

Human influenza outbreak has the potential of triggering a pandemic when a new influenza virus appears against which the human population has no immunity. With the increase in global transport and communications, as well as urbanization and overcrowded conditions, epidemics due to the new influenza virus are likely to quickly take hold around the world leading to enormous numbers of deaths and illness. Outbreaks of influenza in animals, especially when happening simultaneously with annual outbreaks in humans, increase the chances of a pandemic through the merging of animal and human influenza viruses. During the last few years, the world has faced several threats with pandemic potential, making the occurrence of the next pandemic just a matter of time. A surveillance network has been developed by WHO for rapid detection of unusual influenza outbreaks, isolation of possible pandemic viruses and immediate alert to the WHO system by national authorities for mounting a timely and efficient response to pandemics. Ensuring an adequate system for alert, response and disaster management should be the basis of every national pandemic preparedness plan.

In India, the Ministry of Health and Family Welfare has developed well coordinated strategies for Influenza Pandemic Preparedness which aims at reducing the morbidity and mortality due to influenza and decreasing social disruption and economic loss. The phasing of pandemic preparedness, action plan and response has been done in accordance with the WHO classification system (2005) in which the Inter Pandemic Period, Pandemic Alert Period and Pandemic Period has been divided into six phases to tackle the tough future pandemic situation. The action plan includes (i) developing plan with co-ordination at international, national, state and district level for preparedness and response, (ii) identifying the roles and responsibilities of all stake holders, strong virological surveillance for early detection of novel virus, (iii) institutionalizing mechanism for developing sufficient quantity of vaccines, (iv) ensuring availability of adequate quantity of anti viral drugs, (v) strengthening hospital systems and planning for optimum utilization of services institute public health measures including infection control practices, (vi) establishing effective communication with community health care providers and the media, and (vii) establishing synergies with other existing programmes / schemes for optimal utilization of resources. A major component of pandemic preparedness is to strengthen the capacity to respond to yearly epidemics of influenza, investment in *pandemic* vaccine research and promoting domestic production of influenza vaccines The challenges are great but the costs of failure are potentially so catastrophic that it is imperative for the international community to ensure that the efforts and endeavors are given the best possible chance of success.

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