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Review Article

Biological and epidemiological aspects of influenza virus H5N1 in context of India

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Since 1997, highly pathogenic avian influenza (HPAI) H5N1 virus crossed the species barriers from birds to humans and caused fatal disease, leading to great speculation about a possible influenza pandemic. This subtype is characterized by its pathogenicity in a large number of animal species and resistance to older class of antiviral drugs. At present, two out of three general conditions for the onset of pandemic have been met, emergence of new virus; and its ability to replicate in humans causing serious illness. Next influenza pandemic might be due to human to human transmission. This review addresses the biological and epidemiological aspects of influenza in context of India.

Keywords: Avian influenza, H5N1 virus, Bird flu, Human flu, Pandemic flu

"Pandemic (1918-1919) rank in respect not only of absolute but even of relative mortality not lower than third and perhaps even second upon the roll of great pestilences. No epidemic of smallpox or cholera, not even the typhus periods of the earlier nineteenth century, can vie with the influenza of 1918-19 as agents of destruction"[1].

Infectious diseases are major cause of health concern claiming large number of lives worldwide every year[2]. Although the impact of a number of infectious diseases has been lessened with the advent of antibiotics and vaccines, but emerging infectious diseases continue to be a major health problem. Emerging infectious diseases (EIDS) may be kept into three categories: (a) new emergent infections, (b) rare infections, which may re-emerge occasionally; and (c) common infections, which may increase in significance owing to issues such as social instability or resistance development[3]. Among EIDS, influenza deserves particular attention. An influenza pandemic is a global outbreak of disease that occurs when a new type of influenza strain emerges in the human population, causing serious illness, and then spreading easily from person to person worldwide. Pandemics are different from seasonal outbreaks of influenza, as the latter are caused by subtypes of influenza viruses that are already present among people, whereas pandemic outbreaks are caused by new subtypes or by subtypes that have not spread among people for a long time. Pandemics are caused by a highly contagious virus to which population has little or no immunity, and in the absence of ready preventive or therapeutic interventions, they have a devastating effect. Avian influenza virus (AIV) involving at least three subtypes, H5, H7 and H9 has emerged as an important pathogen in the poultry industry and is of major current global health concern[4]. Influenza is caused by viruses that undergo continuous antigenic change and have animal reservoir. Genome of influenza A viruses consists of eight single-stranded RNA segments, and the viral particle has two major glycoproteins [hemagglutinin (HA) and neuraminidase (NA)] on its surface (Figs 1, 2).

With at least 15 different hemagglutinins, each one able to combine with any of the 9 different neuraminidase subtypes, there is much antigenic variation among influenza viruses. Influenza viruses are grouped into three types, designated A, B, and C. Viruses of C type are common that usually cause no symptoms, or mild respiratory illness. They are not considered for public health concern. Type B viruses cause sporadic outbreaks of a more severe respiratory disease, particularly among young children. Both B and C viruses are human viruses; C viruses are stable, but A and B viruses are prone to mutation. Influenza A viruses are of great concern. These viruses mutate more rapidly than type B viruses that provide them antigenic flexibility. In addition to human, they infect

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pigs, horses, sea mammals, and birds. Influenza A viruses have many subtypes, all of which are maintained in their natural reservoir, the aquatic birds, which provides a perpetual source of viruses and a huge pool of genetic diversity[5]. The principal site of influenza virus replication in aquatic birds is the gastrointestinal tract resulting in high fecal viral titers and viral transmission in migratory feeding areas. Influenza A viruses are identified with the characteristics having two sets of protein spikes that protrude from the outer surface of the virus. The hemagglutinin (HA) spike is responsible for the virus binding and entry into cells, where copies of the virus are produced. There are 15 HA subtypes, designated H1–H15. The neuraminidase (NA) spike regulates the release of newly formed virus from infected cells into the host. There are nine NA subtypes, designated N1–N9. All of these HA and NA subtypes have been detected in free-flying birds. They provide a huge and highly flexible pool of genetic diversity. An individual virus strain is identified by the subtypes of HA and NA protein spikes on its surface. It is named by the letters H and N, each followed by the number of the subtype. For the onset of pandemics, emergence of a novel HA subtype is determinant, as it defines population susceptibility. To date, only subtypes H1, H2, and H3 are known to have circulated in humans for at least a century. As a virus from H5 subtype will be foreign entity to the immune system of everyone alive today, vulnerability to an H5N1-like pandemic virus would be universal.

Previous pandemics in humans have occurred as a result of changes in the surface glycoproteins of the virus. During the last decade, highly pathogenic strains of avian influenza virus, including H5N1 subtype, crossed the species barriers from birds to humans and caused fatal disease[6].

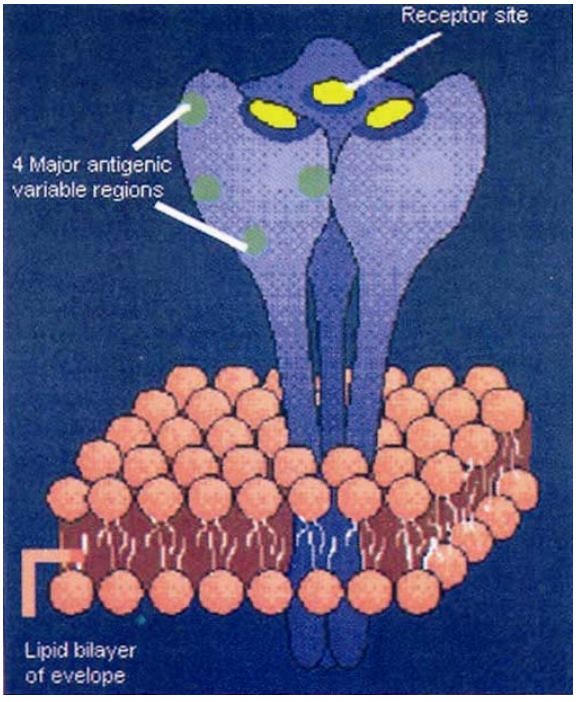

Fig. 1—Hemagglutinin (HA) is a viral protein anchored in the lipid bilayer that recognizes and binds to host cell's sialic acid (Source: www.arches.ug.edu, Julius Baer)

Influenza virus pandemics

Influenza pandemics in past have led to high levels of illness, death, social disruption, and economic loss. Three episodes have occurred in the last century. In 1918, the 'Spanish' influenza, a highly contagious and deadly disease, had a major global impact. In fact, this pandemic, caused by H1N1 influenza virus led to an estimated death toll of 40 million people in less than a year[7]. Spanish flu caused most deaths in young and healthy persons in the age group of 15–35 years. In a complete reversal of previous patterns, 99% of deaths occurred in people younger than 65 years. Many of the deaths in 1918 were from pneumonia caused by secondary bacterial infections. But Spanish flu also caused a form of primary viral pneumonia. Antibiotics, which could have prevented many deaths from bacterial pneumonia, had not yet been discovered, and an effective vaccine was not available.

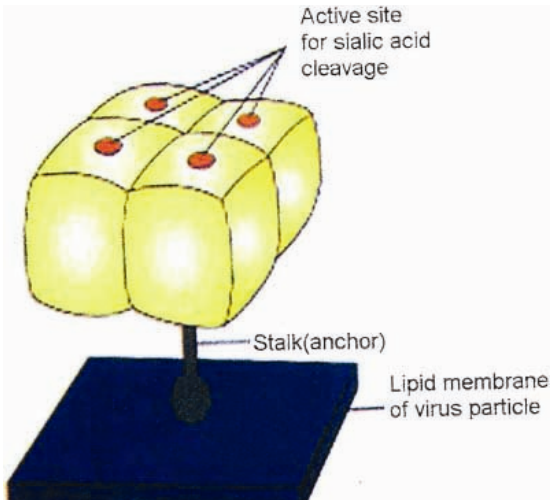

Fig. 2—Neuraminidase (NA) is a viral protein anchored in the lipid bilayer that after infection, cleaves off sialic acid from the host cell to prevent "recapturing" of the newly created virions (Source: www.arches.ug.edu, Julius Baer)

During the course of the pandemic, an estimated 25–30% of the world population fell ill. Life expectancy dropped by 10 years or more[8]. In 1957, a new influenza virus with two unknown surface proteins (H2N2) appeared. The hemagglutinin glycoprotein (H2 subtype) of the 1957 'Asian' influenza virus showed only 66% of amino-acid sequence identity with the hemagglutinin H1 subtype. The N2 subtype neuraminidase of the 1957 virus shared an overall sequence identity of only 37% with the N1 subtype neuraminidase. Thus, in 1957, after 39 years of H1N1 viruses, there was little or no pre-existing immune protection in human population against this new influenza virus[7]. The pandemic that began in 1957 was caused by a milder virus than the one responsible for the 1918 pandemic. In addition, the world was much better prepared to cope. Vaccines for seasonal epidemics had been developed and had already proven their value as the most effective method for prevention. Antibiotics were available to treat complications, including bacterial pneumonia. Nevertheless, estimates suggest that 2 million people died in the world during the Asian influenza pandemic caused by this new H2N2 virus[8]. Eleven years later, in 1968, another change in the surface glycoproteins again caused the virus to become pandemic, resulting in high morbidity and mortality rates worldwide. In this 1968 virus, only the gene that encodes hemagglutinin, *HA*, and *PB1* gene re-assorted to yield an H3N2 subtype[9]. H3 and H2 hemagglutinins differed in more than 60% of their amino acids. Conservation of neuraminidase in 1968, H3N2 virus conferred partial protection to the population who had been previously exposed to H2N2. The pandemic that began in 1968 was even milder than that in 1957. The virus spread from a focal point within mainland China. Although accurate mortality estimates are not available, global excess mortality was probably around 1 million[8].

There was still a fourth influenza pandemic in the last century, caused by an H1N1 strain that appeared in 1977. It caused disease mostly in people born after 1950, because the older population had protective immunity resulting from prior contact with H1N1 strains. This H1N1 strain and its descendants have been circulating ever since, and at present both H3N2 and H1N1 influenza viruses continue to be present in the human population[7].

Highly pathogenic avian influenza (HPAI) H5N1 virus

The HPAI (H5N1) virus represents an increasing global concern. Antigenic changes in the virus resulted in the generation of novel strain which spread to at least nine countries in East Asia and Southeast Asia (Cambodia, China, Indonesia, Japan, Laos, South Korea, Thailand, Hong Kong and Vietnam). This viral strain is characterized by pathogenicity to a larger number of animal species and by resistance to the older class of antiviral drugs represented by amantadine and rimantadine[10]. Prior to 1997, the H5N1 strain of avian influenza virus began circulating in poultry populations of certain regions of Asia. H5N1 initially caused only mild disease with symptoms, but after months of circulation in chicken, the virus mutated to a highly pathogenic form that could kill chicken within 48 h, with a mortality approaching 100%[8]. Since the first avian influenza outbreak in 1997, the main concern has been that highly pathogenic influenza A (H5N1) virus might either mutate and adapt to allow efficient transmission during the infection of mammals or reassort its gene segments with human influenza viruses during the co-infection of a single host, resulting in a new virus that would be both highly lethal and transmissible from person to person. Such events are believed to have preceded the influenza pandemics of 1918, 1957, and 1968[11]. HPAI (H5N1) outbreak in birds prompted health workers to slaughter almost 1.4 million birds in 1997. Outbreaks of highly pathogenic avian influenza A (H5N1) occurred among poultry in eight countries in Asia (Cambodia, China, Indonesia, Japan, Laos, South Korea, Thailand and Vietnam) during late 2003 and early 2004. At that time, more than 100 million birds either died from the disease or were culled. The first case report of bird-to-human transmission was in Hong Kong in 1997[12]. From December 30, 2003 to March 17, 2004, 12 confirmed human cases of avian influenza A (H5N1) were reported in Thailand and 23 in Vietnam, resulting in a total of 35 deaths[5]. As reported by the World Health Organization (WHO) in May 2005, the total number of deaths in Asia was 52[13]. Mortality associated with human H5N1 infection is high (72%) as compared with an estimated 2.5% for Spanish influenza[14] which took a toll of 20-40 million deaths globally and 10-20 million deaths in India alone. Most vulnerable population is rural farmers and their families living in poor hygienic conditions at resource-constrained places rearing chicken in their backyards.

On 4 May 2005, few birds were found dead with H5N1 infection among water fowl in lake Qinghai, Gangcha County, Qinghai Province in Western China, and by the end of June more than a thousand birds were affected. This lake is one of the most important breeding locations for migratory birds congregating from Southeast Asia, Siberia, Australia and New Zealand that over winter in Southeast Asia, Tibet and India. Until recently, migratory water fowl seemed to be exempted from wide spread infection, although sporadic cases were recorded[15], the occurrence of HPAI H5N1 infection in them indicates that this virus has the potential to be a global threat. A number of species were found infected, including bar-headed goose (*Anser indicus*), great black-headed gull (*Larus ichthyaetus*) and brown-headed gull (*Larus brunnicephalus*). Several H5N1 viruses isolated from viscera, brain and swabs of oropharynx and cloaca of sick and dead birds have been completely sequenced. None of the GenBank sequence data for known H5N1 AIV genomes completely match with the sequences, implying the viruses are reassortants[16], and emerging from reassortants[17]. Three peculiar characteristics have been found in HPAI H5N1 isolates—(i) The sequence PQGERRRKKR/G, denoting multiple basic amino acids at the cleavage site of the hemagglutinin; (ii) A virulence island in the PB2 gene, E627K, first seen in in Hong Kong in 1997[18]; and (iii) A deletion of 20 amino acids in neuraminidase (amino acid positions 49 to 69) associated with high virulence. Evidently H5 viruses are becoming more capable of causing disease (pathogenic) for mammals than earlier H5 viruses and are becoming more widespread in birds in the region. Ducks infected with H5N1 are now shedding more virus for longer periods of time without showing any symptoms of illness. This has implications for the role of ducks in transmitting disease to other birds and, possibly, to humans as well. Bird-to-human transmission has continued, and it has been associated with the cause of 61 deaths in 117 patients with confirmed cases[13] (Table 1).

A series of genetic reassortment events traceable to the precursor of H5N1 viruses that caused the initial human outbreak in Hong Kong in 1997 and subsequent avian outbreaks in 2001 and 2002 were recently demonstrated. These events gave rise to the dominant H5N1 genotype in chicken and ducks that was responsible for the regional outbreak in 2003–2004. Domestic ducks in southern China had a central role in the generation and maintenance of this virus, and wild birds may have contributed to the increasingly wide spread of the virus in Asia[19]. The HA molecules of this genotype virus isolated since late 2002 in Asia acquired a potential N-linked glycosylation site at positions 154–156. Glycosylation at this site, adjacent to the receptor binding, is capable of altering the receptor-binding profile and may help the virus to evade the host antibody response[19]. The genetic stability of this new gene constellation indicates that viruses of this genotype have not yet fully adapted to poultry, and this raises the possibility that they may continue to evolve through mutation or reassortment to achieve greater viral fitness[20]. Viral isolates from human as well as avian shows high level

Table 1—Cumulative number of virologically confirmed cases of avian influenza A (H5N1) in humans reported to WHO since 2003

Date of Onset	Vietnam		Thailand		Cambodia		Indonesia		Total	
	A	B	A	B	A	B	A	B	A	B
Dec. 26, 2003 to March 10, 2004	23	16	12	8	0	0	0	0	35	24
July 19, 2004 to Oct. 8, 2004	4	4	5	4	0	0	0	0	9	8
Dec. 16, 2004 to Aug. 5, 2005	63	20	0	0	4	4	1	1	68	25
Total	90	40	17	12	4	4	1	1	112	57

*Additional details are available at www.who.int/csr/disease/avian_influenza/country/cases_table_2005_08_05/en/print.

† Cases continue to occur. The total number of cases includes fatal ones. This list does not include the 18 patients, 6 of whom died, identified in Hong Kong in 1997 or the 2 patients, 1 of whom died, identified in Fujian Province, China, in 2003.

A—No. of cases; B—No. of deaths

of antigenic differences, exemplified by a difference of 8 amino acids between a pair of viruses isolated from the same patient[21]. Another relevant mutation, also found in this strain, is Lys 627 in the PB2 protein. This mutation has been associated with increased virulence and lethality of H5N1 viruses in mice[22] and with H7N7 viruses in humans[23].

Certain other studies have documented H5 infection among pigs in China and H5 infection in felines (experimental infection in house cats in The Netherlands and isolation of H5N1 viruses from infected tigers and leopards in Thailand), suggesting that cats could host or transmit the infection. These findings emphasize that reassortment of avian influenza genomes is most likely to occur when these viruses demonstrate a capacity to infect multiple species[5]. Recent report from Gir reserve documents the death of an adult lion and python with some unknown infection, although the exact cause of the death remains to be established. So far, no evidence of efficient person-to-person transmission has yet been reported, though possible person-to-person transmission has been suspected[24]. Hemagglutinin of human influenza viruses preferentially binds to sialic acid receptors containing α2, 6-galactose linkages, whereas avian influenza viruses preferentially bind to those containing α2, 3-galactose linkages. Although the molecular mechanisms responsible for receptor-binding specificity are not well defined, it is believed that hemagglutinin of avian origin must acquire human receptor-binding specificity to generate influenza strains capable of sustained human-to-human transmission[25]. Site-directed mutagenesis studies have shown that only one or two aminoacid mutations are required for this change[26]. In human beings, the limited passage of a virus possessing an avian hemagglutinin, such as occurring in Asia, may be sufficient to generate such a change[27].

Recent reports from many European countries *viz* Romania, Turkey, United Kingdom (a parrot death in quarantine with infection of H5N1) and Tula province of Russia indicate the presence of H5N1 virus, which have prompted the authorities for culling and adopting preventive measures. In Eastern Siberia, Russia 600,000 domestic poultry have been culled since July 2005 to check the spread of HPAI H5N1. According to a report of 1st November'2005, authorities plans to kill 82,000 chickens in Ibaraki prefecture in Northern Japan after detecting signs of bird flu. More than 1.5 million of birds have already been culled in the same area because of H5N1 fears. The German government, on a suggestion of Federal Research Institute for Animal Health (FLI), has ordered that all domestic poultry be kept indoors from October 22 through December 15 to avoid contact with migratory birds or face a fine of 25000 euros. The 25-nation EU including Austria and Poland, has also enacted similar emergency ruling based on new bio-security steps. The expanding geographic distribution of avian influenza A (H5N1) infections, with recent outbreaks in Kazakstan, Mongolia, and Russia, indicates that more human populations are at risk[28,29].

Clinical characteristics of influenza H5N1 infection

HPAI H5N1 infection in birds is characterized by abnormal signs (tremors and opisthotonus) and diarrhoea. Histology reveals pancreatic necrosis[19], glial cell infiltration in brain sections, perivascular cuffing and congestion in blood vessels[28]. Seasonal outbreaks of low pathogenic subtypes of influenza in human cause mainly respiratory distress and conjunctivitis, whereas highly pathogenic subtypes cause diverse symptoms[30]. The clinical spectrum of influenza A (H5N1) in humans is based on descriptions of hospitalized patients. The frequencies of milder illnesses, subclinical infections, and atypical presentations (e.g., encephalopathy and gastroenteritis) have not been determined, but case reports[31–33] indicate that the patients have different symptoms, although the (Table 2) patients have been previously healthy young children or adults.

It is pertinent to note that clinical signs and symptoms of avian influenza H5N1 may be more complex than originally described. In southern Vietnam, a 4-year-old boy presented with severe diarrhoea, followed by seizures, coma, and death. The diagnosis of avian influenza A (H5N1) was established by isolation of the virus from cerebrospinal fluid, fecal, throat, and serum specimens. The patient's 9-year-old sister had died from a similar syndrome 2 weeks earlier. In both siblings, the clinical diagnosis was acute encephalitis. Neither patient had respiratory symptoms at presentation[38]. Based on these cases, it has been stressed that avian influenza H5N1 should be included in the differential diagnosis of a much wider clinical spectrum of diseases than previously considered and that clinical surveillance of influenza H5N1 should focus not only on respiratory illnesses, but unexplained deaths or severe illnesses of any kind be

Table 2—Presentation and outcome among patients with confirmed avian influenza (H5N1)

Outcome or Measure	Hong Kong, 1997 (N=18)	Thailand, 2004 (N=17)	Vietnam, 2004 (N=10)	Ho Chi Minh City, 2005 (N=10)	Cambodia, 2005 (N=4)
Age — yr	1-60	2-58	5-24	6-35	8-28
Male sex — no. (%)	8(44)	9(53)	6(60)	3(30)	1(25)
Time from last presumed exposure to onset of illness — days	NA	2-8	2-4	NA	NA
No. of family clusters	NA	1	2	1	1
Patients with exposure to ill poultry — No. /total no. (%)	11/16 (70) visited poultry markets	14/17 (82)	8/9 (89)	6/6 (100)	3/4 (75)
Time from onset of illness to presentation or hospitalization — days		1-7	3-8	4-7	5-8
Clinical presentation — no./total no. (%)					
Fever (temperature >38°C)	17/18 (94)	17/17 (100)	10/10 (100)	10/10 (100)	4/4 (100)
Headache	4/18 (22)	NA	NA	1/10 (10)	4/4 (100)
Myalgia	2/18 (11)	9/17 (53)	0	2/10 (20)	NA
Diarrhea	3/18 (17)	7/17 (41)	7/10 (70)	NA	2/4 (50)
Abdominal pain	3/18 (17)	4/17 (24)	NA	NA	2/4 (50)
Vomiting	6/18 (33)	4/17 (24)	NA	1/10 (10)	0
Cough§	12/18 (67)	16/17 (94)	10/10 (100)	10/10 (100)	4/4 (100)
Sputum	NA	13/17 (76)	5/10 (50)	3/10 (30)	NA
Sore throat	4/12 (33)	12/17 (71)	0	0	1/4 (25)
Rhinorrhea	7/12 (58)	9/17 (53)	0	0	NA
Shortness of breath§	1/18 (6)	13/17 (76)	10/10 (100)	10/10 (100)	NA
Pulmonary infiltrates	11/18 (61)	17/17 (100)	10/10 (100)	10/10 (100)	4/4 (100)
Lymphopenia¶	11/18 (61)	7/12 (58)	NA	8/10 (80)	1/2 (50)
Thrombocytopenia	NA	4/12 (33)	NA	8/10 (80)	1/2 (50)
Increased aminotransferase levels	11/18 (61)	8/12 (67)	5/6 (83)	7/10 (70)	NA
Hospital course — no. (%)					
Respiratory failure	8 (44)	13 (76)	9 (90)	7 (70)	4 (100)
Cardiac failure	NA	7 (41)	NA	0	NA
Renal dysfunction	4 (22)	5 (29)	1 (10)	2 (20)	NA
Antiviral therapy**					
Amantadine	10 (56)	0	0	0	NA
Ribavirin	1 (6)	0	2 (20)	0	-
Oseltamivir	0	10 (59)	5 (50)	10 (100)	-
Corticosteroids***	5 (28)	8 (47)	7 (70)	5 (50)	NA
Inotropic agents	NA	8 (47)	2 (20)	NA	-
Time from onset of illness to death— days	8-29	9-30	4-17	4-21	6-10
Deaths — no. (%)	6 (33)	12 (71)	8 (80)	8 (80)	4 (100)

* Data from Hong Kong are from Yuen *et al.*[33] and Chan[34]. Data on Thailand are from Chotpitayasunondh *et al.* [35]. Data on Vietnam are from Hien *et al*. [36]

NA denotes not available.

‡ Some patients had multiple outpatient illness visits before hospitalization.

§ In Hong Kong, shortness of breath later developed in 11 of 18 patients (61 percent) during hospitalization. In Thailand, all patients had cough and shortness of breath at hospitalization.

¶ In Vietnam, the median lymphocyte count was 700 per cubic millimeter (range, 250 to 1100), and the median leukocyte count was 2100 per cubic millimeter (range, 1200 to 3400).16 In Thailand, the mean leukocyte count was 4900 per cubic millimeter (range, 1200 to 13,600),15 and the lymphocyte count was 1453 per cubic millimeter (range, 454 to 3400).

** In Thailand, 7 of 10 patients given oseltamivir died a mean of 11 days after the onset of symptoms (range, 5 to 22 days), as compared with 5 of 7 untreated patients. Oseltamivir was used in conventional doses (75 mg orally, twice daily for 5 to 10 days with a weight-based dose reduction in children) in the majority of recipients. In Vietnam, one of five recipients of oseltamivir recovered, as compared with one of five untreated patients.16 The use of relatively low doses of oral ribavirin in two patients was not associated with obvious effectiveness.

*** Initial patients in Vietnam received methylprednisolone (5 mg per kilogram of body weight per day or 1 to 2 mg per kilogram) for one to four days16; subsequent patients in Ho Chi Minh City received dexamethasone at 0.4 mg per kilogram per day for five days in a randomized trial. In Thailand, methylprednisolone (2 mg per kilogram per day) was administered for two to five days.

also investigated. The broad spectrum of clinical manifestations of influenza poses a big challenge for clinical diagnosis. The diagnosis of influenza A (H5N1) should be further confirmed by means of immunofluorescence (with antibodies specific for the virus), isolation of virus after the culture in susceptible cell line and reverse transcriptase–polymerase chain reaction with primers specific for H5 and N1. The whole process may further prove another bottleneck in India as the required resources and trained personnel are not available at places with a true risk of influenza outbreak. Apart from this, another limitation is that many of the surveillance centres are not capable of handling HPAI H5N1 virus. Therefore, training of personnel and resources procurement in the face of an imminent and perhaps inevitable influenza pandemic must be part of a National Pandemic Preparedness Plan.

Surveillance

Preliminary findings have identified H2, H5, H6, H7, and H9 subtypes of influenza A as those most likely to be transmitted to humans. The influenza A subtypes currently circulating in humans, H1 and H3, continue to experience antigenic changes. Although these continual modifications may lead to an increase in virulence, the mildness of the past three influenza seasons suggests that potential of H1N1 and H3N2 viruses is diminishing as their ability to cause serious disease becomes increasingly attenuated. H2 influenza viruses are included in the high-risk category because they are the causative agent of the 1957 "Asian flu" pandemic and are the only influenza A subtype circulating in humans between 1957 and 1968[4]. Not only are the H1, H2, and H3 influenza viruses of concern. The H7 and H5 viruses have a unique ability to evolve into a form highly virulent to chicken and turkeys by acquiring additional amino acids at the HA cleavage site (HA cleavage is required for viral infectivity)[39]. Highly pathogenic H7N7 influenza viruses that are lethal to poultry infected the eyes of more than 80 humans and killed one person[23]. The remaining two viral subtypes on the priority list, H6 and H9, have spread from a wild aquatic bird reservoir to domestic poultry over the past 10 years. H9N2 viruses have also been detected in humans and in pigs[40,41], and have acquired human-like receptor specificity[42]. In Egypt, in April 2004, two infants presenting with mild febrile respiratory symptoms had H10N7 influenza viruses isolated from respiratory samples [Avian influenza virus A (H10N7) circulating among humans in Egypt].

Options for controlling influenza

Primary option for reducing the effect of influenza and reducing its health consequences during pandemic is immunoprophylaxis with vaccine. No influenza A H5N1 vaccines are currently available for commercial use in humans. Earlier H5 vaccines were poorly immunogenic and required two doses of high hemagglutinin antigen content[43] or addition of MF59 adjuvant[44] to generate neutralizing antibody responses. A third injection of adjuvanted 1997 H5 vaccine variably induced cross-reacting antibodies to human isolates from 2004[45]. Reverse genetics has been used for the rapid generation of nonvirulent vaccine viruses from recent influenza A (H5) isolates[4,46] and several candidate vaccines are under study. One such inactivated vaccine with the use of a human H5N1 isolate from 2004 has been reported to be immunogenic at high hemagglutinin doses[47]. Studies with approved adjuvants like alum are urgently needed. Live attenuated, cold-adapted intranasal vaccines are also under development. These are protective against human influenza after a single dose in young children[48].

There are two approaches for the prevention of influenza—the live, attenuated, cold-adapted, intranasally administered influenza vaccine [CAIV-T (FluMist; Med-Immune Vaccines)] and traditional trivalent inactivated vaccine (TIV; Table 3).

The second appears to mediate protection almost exclusively through induction of serum antibody. The degree of immunity induced by CAIV-T is predicted to be lower in adults than in children[49]. TIV vaccines are produced from virus grown in embryonated hen eggs and are of three types—whole-virus, split-product, or subunit (surface-antigen) formulations. Trivalent vaccines contain 15 mg each of two A subtypes (H1N1 and H3N2) and one B strain[50]. TIV elicit a relatively strain-specific humoral response, have reduced efficacy against antigenically drifted viruses, and are ineffective against unrelated strains. The influenza A virus components of annual influenza vaccines are typically derived from egg-grown reassortment viruses that have the relevant hemagglutinin and neuraminidase genes of antigenically relevant strain, and six remaining gene segments from A/Puerto Rico/8/34 (H1N1). This process requires large numbers of eggs and many

Table 3—Comparison of live attenuated influenza vaccine with inactivated influenza vaccine

Factor	LAIV	Inactivated influenza vaccine
Route of administration	Intranasal spray	Intramuscular injection
Type of vaccine	Live virus	Killed virus
No. of included virus strains	3 (2 influenza A, 1 influenza B)	Same as LAIV
Vaccine virus strain updated	Annually	Same as LAIV
Frequency of administration	Annually	Same as LAIV
Approved age and risk groups	Healthy persons aged 5-49 years	Persons aged ≥ 6 months
Can be administered to family members or close contacts of immunosuppressed persons not requiring a protected environment	Yes	Yes
Can be administered to family members or close contacts of immunosuppressed persons requiring a protected environment (e.g., hematopoietic stem cell transplant recipient)	Inactivated influenza vaccine preferred	Yes
Can be administered to family members or close contacts of persons at high risk but not severely immunosuppressed	Yes	Yes
Can be simultaneously administered with other vaccines	Yes*****	Yes[#]
If not simultaneously administered, can be administered within 4 weeks of another live vaccine	Prudent to space 4 weeks apart	Yes
If not simultaneously administered, can be administered within 4 weeks of another inactivated vaccine	Yes	Yes

***** No data available regarding effect on safety or efficacy

[#] inactivated influenza vaccine coadministration has been evaluated systematically only among adults with pneumococcal polysaccharide vaccine

companies lack the flexibility to respond rapidly to a pandemic event[51]. In addition, highly pathogenic H5 and H7 viruses cannot be grown in large quantities because they are lethal to chicken embryos[52].

Vaccines for highly pathogenic subtypes

The most promising method to accelerate the response to pandemic influenza is by means of plasmid-based reverse genetic systems to construct influenza virions and vaccines. As viable viruses can be generated from individually cloned cDNA copies of each of the eight viral RNA segments, reassortment can be prospectively defined and directed, and the extra amino acids at the HA cleavage site (which are associated with high virulence) can be removed to allow rapid generation of a vaccine seed strain in eggs. Plasmids encoding the internal genes of the base vaccine are already available. A strain-specific vaccine can thus be created by cloning the appropriate hemagglutinin and neuraminidase genes from the target virus, altering its HA connecting peptide if necessary, and transfecting an appropriate cell line[5]. The next step is to take these plasmid-derived influenza vaccines through clinical trials to address crucial questions such as number and quantity of doses and the role of adjuvants. Reverse genetic systems introduce new challenges. One of the most limiting of these is the need to use cell lines. Perhaps the only cell line that meets all criteria for international use at this time is the African green monkey kidney cell line Vero, and these cells must be of certified quality for human vaccine production. A second new challenge is the use of a genetically modified virus seed strain. Because the traditional vaccine strains are made by natural reassortment, they have escaped being labeled "genetically modified." This difference may affect the acceptance of the new vaccines[5] because of laws and regulations limiting the human use of genetically modified organisms. Safety and legal problems should be solved before reverse genetic systems are used for vaccine development. A risk to be considered by vaccine manufacturers is the occurrence of adverse reactions in a percentage of recipients. These reactions may be due to the vaccine, to the host, or to a unique combination of the vaccine and the host. Legislative measures can be taken to reduce the impact of liability exposure. In addition, intellectual property rights on reverse genetics technology are held, and licenses may need to be granted for commercial use of vaccines. Finally, viral evolution should be considered during the development of new vaccines.

Antiviral therapy

An additional important resource to face influenza is the existence of antiviral agents. Two classes of drugs are currently available for prophylaxis and treatment of influenza virus infection: M2 ion channel blockers (amantadine and its derivative rimantadine) and NA inhibitors (zanamivir and oseltamivir). Amantadine and rimantadine block the ion channel activity of the M2 protein of most influenza A viruses, and viral replication is inhibited by the blockage of hydrogen ion flow, principally when virus enters the host's cells[53]. NA inhibitors interrupt an established infection in its late stages by inhibiting the release of virions from infected cells, which results in aggregation of virions at the cell surface and the consequent inhibition of viral penetration of mucous secretions and spread to other cells[54]. Main disadvantages to the use of M2 blockers are that drug-resistant variants develop rapidly and that these agents are ineffective against influenza B virus[55]. By days 5-7 of therapy, 16-35% of isolates from treated patients may be resistant, and these drug-resistant variants are fully pathogenic and transmissible to close contacts[56]. Initial studies indicate that both H5N1 influenza viruses (2003 and 2004 human strains) currently being isolated from humans are naturally resistant to amantadine and rimantadine[57]. NA inhibitors are more costly, but they are active against both influenza A and B viruses and elicit fewer side effects; in addition, the emergence of drug-resistant variants has been reported in less than 1% of treated adults[58]. One pediatric study of oseltamivir treatment has reported that 5.5% of influenza isolates has evidence of neuraminidase resistance[59]. Patients with suspected influenza A (H5N1) should promptly receive a neuraminidase inhibitor pending the results of diagnostic laboratory testing. The optimal dose and duration of treatment with neuraminidase inhibitors are uncertain, and currently approved regimens likely represent the minimum required. These viruses are susceptible *in vitro* to oseltamivir and zanamivir[60,61]. Oral oseltamivir[60] and topical zanamivir are active in animal models of influenza A (H5N1)[62,63]. Recent murine studies indicate that as compared with an influenza A (H5N1) strain from 1997, the strain isolated in 2004 requires higher oseltamivir doses and more prolonged administration (eight days) to induce similar antiviral effects and survival rates[64]. Inhaled zanamivir has not been studied in cases of influenza A (H5N1) in humans. Early treatment will provide the greatest clinical benefit[36] although the use of therapy is reasonable when there is a likelihood of ongoing viral replication. Placebo-controlled clinical studies of oral oseltamivir[65,66] and inhaled zanamivir[67] comparing currently approved doses with doses that are twice as high, found that the two doses had similar tolerability, but no consistent difference in clinical or antiviral benefits in adults with uncomplicated human influenza. Although approved doses of oseltamivir (75 mg twice daily for five days in adults and weight-adjusted twice-daily doses for five days in children older than one year of age — twice-daily doses of 30 mg for those weighing 15 kg or less, 45 mg for those weighing 15 to 23 kg, 60 mg for those weighing 23 to 40 kg, and 75 mg for those weighing more than 40 kg) are reasonable for treating early, mild cases of influenza A (H5N1). Higher doses (150 mg twice daily in adults) and treatment for 7 to 10 days are considered in treating severe infections, but prospective studies are needed. High-level antiviral resistance to oseltamivir results from the substitution of a single amino acid in N1 neuraminidase (His-274-Tyr). Such variants have been detected in up to 16 per cent of children with human influenza A (H1N1) who have received oseltamivir[68]. Not surprisingly, this resistant variant has been detected recently in several patients with influenza A (H5N1) who were treated with oseltamivir[32]. Although less infectious (in cell culture and in animals) than susceptible parental virus[69], oseltamivir-resistant H1N1 variants are transmissible in ferrets[70]. Such variants retain full susceptibility to zanamivir and partial susceptibility to the investigational neuraminidase inhibitor peramivir *in vitro*[71,72]. In contrast to isolates from the 1997 outbreak, recent human influenza A (H5N1) isolates are highly resistant to M2 inhibitors amantadine and rimantadine, and consequently, these drugs do not have a therapeutic role. Agents of clinical investigational interest for treatment include zanamivir, peramivir, long-acting topical neuraminidase inhibitors, ribavirin[73,74] and possibly, interferon alpha[75].

Immunomodulators

Corticosteroids have been used frequently in treating patients with influenza A (H5N1), with uncertain effects. Among five patients given corticosteroids in 1997, two treated later in their course for the fibroproliferative phase of ARDS survived. In a randomized trial in Vietnam, all four

patients given dexamethasone died. Interferon alpha possesses both antiviral and immunomodulatory activities, but appropriately controlled trials of immunomodulatory interventions are needed before routine use is recommended. As described, antiviral drugs may be of benefit in protecting individuals in essential services while waiting for an effective vaccine to be prepared. However, supply and cost issues would limit their effect on the course of a pandemic[45]. These drawbacks should also be overcome if an effective response to a major influenza epidemic is desired.

Future course of action in India

Human influenza outbreak has the potential of triggering a pandemic[76], and HPAI H5N1 virus isolation from humans and death of more than 60% (33% fatality rate documented among human H5N1 cases in 1997) of the infected individuals have forced the Union Government of India to implement and practice pandemic preparedness plans, involving complete shut down of import of birds, vaccination of birds, stockpiling of antiviral drugs, instituting a rigorous check on entry points to country, and close monitoring of migratory birds for signs that they might be diseased or infected as well as to keep surveillance over poultry around areas where migratory birds gather. This is the key part of Union Government's strategy to protect country from HPAI H5N1 outbreak, which largely depends on knowledge of where the birds are coming from and the places to which they might travel in India. This gigantic task of bird monitoring is being carried out by the venerable 120-year-old Bombay Natural History Society (BNHS) in association with a large number of Government and Non-Government Organizations supported by volunteer organizations. It is clear that the present danger as the true risk group for the pandemic hit is densely populated areas which can be proved as slaughter ground for the deadly virus, should it mutate into a form easily transmissible from human to human. Epidemiological surveillance and viral monitoring in our country is very well in place. Five FluNet centers based at Chennai, Kolkata, Dibrugarh, Delhi and Pune are constantly conducting surveillance[77], clinical[78], pathogenesis[79] and immunomodulation[80,81] studies in an active collaboration with WHO and ICMR. India confirmed its first bird flu infections in poultry on 18 February 2006 after 50,000 birds died in Nandurbar district of western Maharashtra state and in some poultry farms near Surat in Gujarat. High Security Animal Disease Laboratory (HSADL), Bhopal, confirmed the H5N1 infection in dead birds. Over 31,045 people were tested in Navapur town and an additional 23,925 people in the infective zone village, but none was found positive for H5N1 infection. In Gujarat, about 33,799 people were tested for bird flu with no positive results. HSADL tested about 30,500 birds out of which 15 tested positive. As a preventive measure, all the birds within 10 km radius of the affected area were ordered to be culled, so as to prevent the spread of the virus to other areas and to stop any bird to human and human to human transmission. Also stockpiling of the drugs (oseltamivir) and the vaccines were ordered to combat any emergency situation. The H5N1 virus strain is extremely sensitive to heat. Therefore, in summer when the temperature will rise to 40ºC, the virus may perish. Also, the rising temperature would mean the return of migratory birds, major cause of this outbreak, from India to cooler countries. As a preventive measure, the people have been advised to eat eggs and chicken only after cooking them at 70ºC or above for about 30 min. But the real challenge is the close monitoring of migratory birds like gulls, bar-headed geese, cormorants etc, arriving at more than 173 bird areas throughout the vast country. Some of the bird areas are reservoir lakes of dams in north, Mumbai's creeks, salt pans of Kutch, Haigam and Hokasur in Kashmir, Pong dam in Himachal, Harike in Punjab, Keoladev National Park in Rajasthan, Kahar lake in Bihar, Bharatpur in UP and Chilike lake in Orissa. Recently, Kerala has become the first Indian state declaring red alert against avian influenza, although no report of H5N1 as yet but the state is a favourite destination among migratory birds which makes it vulnerable to viral attack. All the bird areas ranging from Aakulam and Veli in Thiruvananthapuram, Kandachira, Ashtamudi and Sasthamcotta in Kollam, Vembanad lake covering Alappuzha, Kottayam and Ernakulam, the Thattekad bird sanctuary in Ernakulam, Purathur in Malappuram, Kadalundi in Kozhikode to Thattampalli and Madakkara in Kannur, of the state will be covered under the provisions of red alert in cooperation with forest department. All the poultry farms in the public and private sector have been directed to undertake bio-security measures, including regular sprinkling of anti-viral solution comprising formalin and iodine. Pre-pandemic stockpiling of oseltamivir is cost-saving to the economy over a wide range of treatment strategies.

Stockpiling is also directly cost-saving to the healthcare system, if oseltamivir use is limited to treating patients at high risk. Investment in stockpiling remains cost-saving to the economy as long as the estimated annual pandemic risk remains >1 pandemic every 80 years. In the last 400 years, at least 31 pandemics have been recorded[82], so that regardless of recent events in Southeast Asia, present investments in antiviral agents can be expected to yield a substantial economic return of >INR 157 per 45 (>$3.68 per $1) invested, while saving many lives. This favorable cost-benefit ratio can be achieved if stockpiled antiviral drugs are administered either solely as a therapeutic measure or as short-term prophylaxis for exposed contacts, a strategy termed "ring prophylaxis"[83] or "targeted prophylaxis"[84]. Drug prices are expected to change substantially as a result of contractual negotiations with manufacturers (although stockpiling may remain cost-saving even if drug costs are more than tripled, as would be the case if preprepared capsules are purchased). Powder-form antiviral drugs have considerable advantages in terms of cost and shelf life, but the logistical aspects of their preparation and distribution should be further assessed to confirm feasibility. Finally, it is assumed that strain-specific vaccine would not be available in sufficient quantities during the first stages of the pandemic. Efforts are currently being directed towards shortening this delay. Once available, strain-specific vaccines would likely be favoured intervention, with antiviral agents serving as adjunct treatment. Several countries have already begun active stockpiling efforts[85], sufficient in some cases to allow antiviral treatment of up to 25% of the population[86]. India lately started the stockpiling and after much talk with Roche (the only manufacturer of oseltamivir, generic name Tamiflu), Government of India decided to buy 1 million doses of antiviral medicines from Roche and oral inhaler, Relenza (Zanamivir) from GlaxoSmithKline. Tamiflu tablets as well as Relenza inhaler taken twice a day for five days blocks the virus movement from one cell to another by disabling neuraminidase. It is believed that antiviral stockpiling is a prudent investment that may help mitigate this impending global threat. Apart from these, Union Government of India and Ministry of Health must address five topics- surveillance and laboratory issues, communications, maintenance of community services, medical care and supply and delivery of vaccines and drugs, in the face of catastrophic event such as pandemic influenza. Such a plan shall be based on Pandemic Preparedness Plan laid down by WHO, the components of which may be as depicted in the following table (Table 4).

Table 4—Components of Pandemic preparedness plan

Likelihood	High
Warning	Days to months
Occurrence	Nationwide
Transmission/duration of exposure	Person-to-person, 6–8 wks
Casualties	Hundreds of thousands to millions
First responders susceptible?	Yes
Main site for preparedness, response, recovery, and mitigation	State and local areas
Essential preparedness components	
Surveillance	Yes
Investigation	Yes
Research	Yes
Liability programs	Yes
Communication systems	Yes
Medical triage and treatment plans	Yes
Vaccine supply issues	Yes
Drug supply issues	Yes
Training/tabletop exercises	Yes
Maintenance of essential community services	Yes
Essential response components	
Effective communications/media relations strategy	Yes
Vaccine delivery	Yes
Drug delivery	Yes
Hospital/public health coordination	Yes
Global assistance	Yes
Medical care	Yes
Mental health support	Yes
Mortuary services	Yes
Supplies and equipment	Yes
Enhanced surveillance	Yes
Vaccine stockpile	Prototype vaccines only
Drug stockpile	Yes
Pre-event vaccination	Vaccination of groups at medical high risk with available vaccines

Conclusion

Clinical, epidemiologic and lab evidence suggests that a pandemic caused by current H5N1 strain would be more likely to mimic the 1918 pandemic than those that occurred in 1957 and 1968[87]. Extrapolation of rate of death associated with 1918 pandemic to that of current population predicts 180-360 million human deaths globally[88] in addition to huge losses to poultry industry devastating several local economies. A

severe outbreak of avian influenza could cost the asia-pacific region between 250-290 billion US dollars in short term, according to preliminary estimates by Asian Development Bank due to the effects of reduced consumption, investment and trade. It is envisaged that government, people and public health care system must be geared up for the challenge of such magnitude. It is in the interests of all countries to contribute ensuring that resources, infrastructure and collaborative relationships are in place within the region most likely to be the source of pandemic. 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 endeavours are given the best possible chance of success. Apart from the international collaborations and cooperations, India must also enact a national policy based on pandemic preparedness plan. A strong political desire will be the deciding factor in tackling this imminent and probably inevitable menace.

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