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EPIDEMIOLOGY AND CONTROL OF AVIAN INFLUENZA IN SOUTHEAST ASIA

Abstract

Avian influenza (AI) or bird flu, a zoonotic viral infection, has received an increased attention since the late 1990s. The causative agents, influenza A viruses of the subtypes H2N1, H7N7 and H9N2, generally pathogenic only to avian species, sometimes cross species barriers and infect a variety of animals, including humans, sea mammals. Migratory wild-birds are the natural reservoirs of these viruses. The first human outbreak, reported from Hong Kong in 1997, affected 18 people six of whom died. The outbreak was successfully controlled following mass culling of birds. Another wave of outbreak appeared in the late 2003 and is ongoing in a number of countries in the Southeast Asia. Vietnam, Thailand, Indonesia, the Netherlands, Canada China, South-Korea, Malaysia, Cambodia, Philippines, Hong Kong, Laos, Russia, Japan, Kazakhstan and Mongolia, are the 16 countries from where recent outbreaks have been reported. Few countries like India and Bangladesh are considered at risk. As of August 31 2005, total numbers of confirmed human cases are 112 with 57 deaths. More than 150 million domestic poultry has been destroyed or died during this episode of events accounting for loss of more than 10-15 billion US\$. Limited human-to-human transmission has been reported in some instances. It is feared that coinfection with human and avian strains of the virus may facilitate reassortment of genetic material resulting in an entirely new virus. This virus will be readily transmitted among humans and since the people will have no immunity against this entirely new strain, pandemic influenza is very likely to occur in the near future. Unavailability for an effective vaccine will even worsen such pandemics. Since poultry is the main source of infection for humans, the virus should be eradicated from poultry population. However, eradication in the near future is very unlikely to be achieved. Mass culling of infected birds, vaccination, implementation of strict biosecurity measures, are the options available for eradication of infection in poultry production. In the long term, the objective of control methods should be to eliminate the infection from as many production systems as possible, recognising that it will not be possible to eliminate AI viruses from wild birds. This essay summarizes the current knowledge on avian influenza, including the virology, epidemiology, diagnosis, and management of this emerging disease.

1 General Introduction

Avian influenza (AI) or bird flu is a zoonotic infection caused by Influenza A virus. These viruses infect a variety of animals, including humans, sea mammals, and different bird species. Among various bird species, domestic poultry flocks are particularly susceptible to infection that can rapidly reach epidemic proportions. Among the Influenza A virus, the H5, H7 and H9 subtypes are of especial concern in poultry production as they can cause up to 100% mortality in birds (Anon, 2005e). These genotypes are also highly pathogenic to humans and mortality may be as high as 70% or even more (Hien *et al.*, 2004; Trampuz *et al.*, 2004; Kaye, 2005; WHO, 2005a).

The disease, now occurring worldwide, was first detected in Italy in the early 1900. There have been three pandemics of influenza in the 20th century- “Spanish flu” in 1918, “Asian flu” in 1957, and “Hong Kong flu” in 1968- which killed more than 100 million people (Perez *et al.*, 2005). Interestingly, all the epidemics were caused by a new type of Influenza A virus of avian origin (Monto, 2005). The disease reemerged in humans in Southeast Asia in 1997 and then again in 2003. Since then, several major outbreaks with devastating outcomes have occurred.

The first outbreak of highly pathogenic influenza A H5N1 virus in humans occurred in 18 people in Hong Kong in 1997 (Saw *et al.*, 1997). In February 2003, H5N1 infection was seen in 2 persons from China, and in early in 2004, numerous cases of human infection occurred in Vietnam and Thailand (Chotpitayasunondh *et al.*, 2004; Hien *et al.*, 2004). As of August 31 2005, there have been 135 documented cases of H5N1 infection in humans in Asia, with 64 deaths. It is assumed that, because of economic and political reasons, a significant number of human cases are not reported (Trampuz *et al.*, 2004; CIDRAP, 2005a). Although the number of human infections and death are not very high, major concern is towards the ability AI virus to undergo genetic reassortment and mutate into an entirely new strain capable of person-to-person transmission (Shortridge *et al.*, 2003; Trampuz *et al.*, 2004; Broor, 2005; Monto, 2005; Stohr, 2005). As there will be no natural immunity among the world population against this entirely new subtype outbreaks may take global pandemic form. As the AI viruses are capable of undergoing constant genetic changes (genetic shift or drift), no vaccine can be developed before an outbreak due to that particular strain occurs. At least six months may be needed (Anon, 2005a). The World Health Organization has estimated that pandemic bird flu, if occurs, may kill as many as 50 million people (Kaye, 2005). Fortunately, as yet, human-to-human transmissions have been reported to have lower efficiency (Karcher, 2004; Gottlieb, 2005; Kaye, 2005; Kaye

and Pringle, 2005; Anon, 2005d). The infections are occurring only sporadically, mostly among poultry workers or those exposed to birds (Saw *et al.*, 1997; Bridges *et al.*, 2002; Fouchier *et al.*, 2004; FAO, 2005a). However, the economic losses incurred are quite huge. Since the early 2004, more than 150 million birds have been culled or died in these outbreaks, accounting for loss of more than 10-15 billion US\$ (FAO, 2005a). Wild birds and other wildlife have also been affected.

Avian influenza outbreaks in animals and humans have been reported from several countries of the world (Table 2). Although the disease is largely confined to Southeast Asian countries-Hong Kong, Japan, Korea, China, Thailand, Indonesia, Malaysia, Vietnam, Cambodia, Laos-both the developing and the developed countries are at risk. Beyond Southeast Asia, a few outbreaks have been reported from Canada (Tweed *et al.*, 2004) and the Netherlands (Fouchier *et al.*, 2004). Even within Southeast Asia, the infection is spreading in a larger area. Recently, the H5N1 has been reported from Magnolia (Anon, 2005f), Tibet (CIDRAP, 2005b) and Russia (Nepoklonov, 2005). As the domestic poultry are highly susceptible to AI virus and are the main source of human infection, all the countries keeping poultry are considered at risk.

This essay summarizes the current knowledge on avian influenza, with main focus on virology, epidemiology, pathogenesis, diagnosis, and prevention and control of this potentially pandemic disease.

2 The influenza virus

Avian influenza is caused by influenza A virus of the genus *influenzavirus A* and family *Orthomyxoviridae*. Orthomyxoviruses are enveloped virus having spherical, filamentous or pleomorphic shape, and measure 80-120 nm in diameter (Quinn *et al.*, 2002). The envelope derived from host cell membrane is made up of glycoprotein antigen which form “spike” like projections at the surface (figure 1), and are about 500 in number (Perez *et al.*, 2005). These antigens are of two types; hemagglutinin (HA), and neuraminidase (NA), based on which subtyping of the virus is done. Currently 15 HA antigens and 9 NA antigens are recognized which may yield several different HA and NA combination subtypes ((Anon, 2005c). The antigenic subtypes of influenza A virus occurring in human and animal diseases are given in table 1. Another classification categorizes the influenza A virus into two pathotypes as: highly (HPAI) and low (LPAI) pathogenic based on their severity of virulence to live poultry. HPAI causes up to 100% mortality in poultry whereas LPAI infections are generally mild, however, they may mutate into HPAI strain after circulating for certain duration in poultry population

(Capua *et al.*, 2003; Villarreal-Chávez and Rivera-Cruz, 2003; Anon, 2005e). Each subtype has LPAI and HPAI strains (Kaye and Pringle, 2005). The OIE definition of HPAI virus is “any influenza virus that is lethal for six, seven or eight of eight 4- to 8- week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of bacteria-free, infective allantoic fluid”(OIE, 2005a).

Table 1 Antigenic subtypes of influenza A virus isolated from human and animals (from Kaye and Pringle, 2005).

Subtype	Waterfowl	Human	Swine	Equines	Other mammals
H subtype					
H1	Yes	Yes	Yes	No	No
H2	Yes	Yes	No	No	No
H3	Yes	Yes	Yes	Yes	No
H4	Yes	No	No	No	Yes (seal)
H5	Yes	Yes	No	No	No
H6	Yes	No	No	Yes	Yes (seal)
H7	Yes	Yes	No	No	No
H8	Yes	No	No	No	No
H9	Yes	Yes	No	No	No
H10	Yes	No	No	No	Yes (mink)
H11	Yes	No	No	No	No
H12	Yes	No	No	No	No
H13	Yes	No	No	No	Yes (whale)
H14	Yes	No	No	No	No
H15	Yes	No	No	No	No
N subtype					
N1	Yes	Yes	Yes	No	No
N2	Yes	Yes	Yes	No	Yes (whale)
N3	Yes	No	No	No	No
N4	Yes	No	No	No	Yes (mink)
N5	Yes	No	No	No	No
N6	Yes	No	No	Yes	Yes (seal)
N7	Yes	Yes	No	Yes	No
N8	Yes	No	No	No	No
N9	Yes	No	No	No	Yes (whale)

Viral genome: The genome of influenza A virus consists of eight segments of linear single stranded negative-sense RNA (figure 1). These eight segments encode for ten proteins; three polymerase complex proteins (PA, PB1 and PB2), two surface glycoproteins (HA and NA), two matrix proteins (M1 and M2), two non-structural proteins (NS1 and NS2) and one nucleoprotein (NP) (Webster and Hulse, 2004). Matrix protein M1 lines the inside of the lipid bilayer while M2 serves the function of ion channel. During the infection process, hemagglutinin attaches the

viruses to sialic acid residue on the respiratory tract of the host whereas neuraminidase (NA) facilitates the release of the virus from infected cells (Perez *et al.*, 2005).

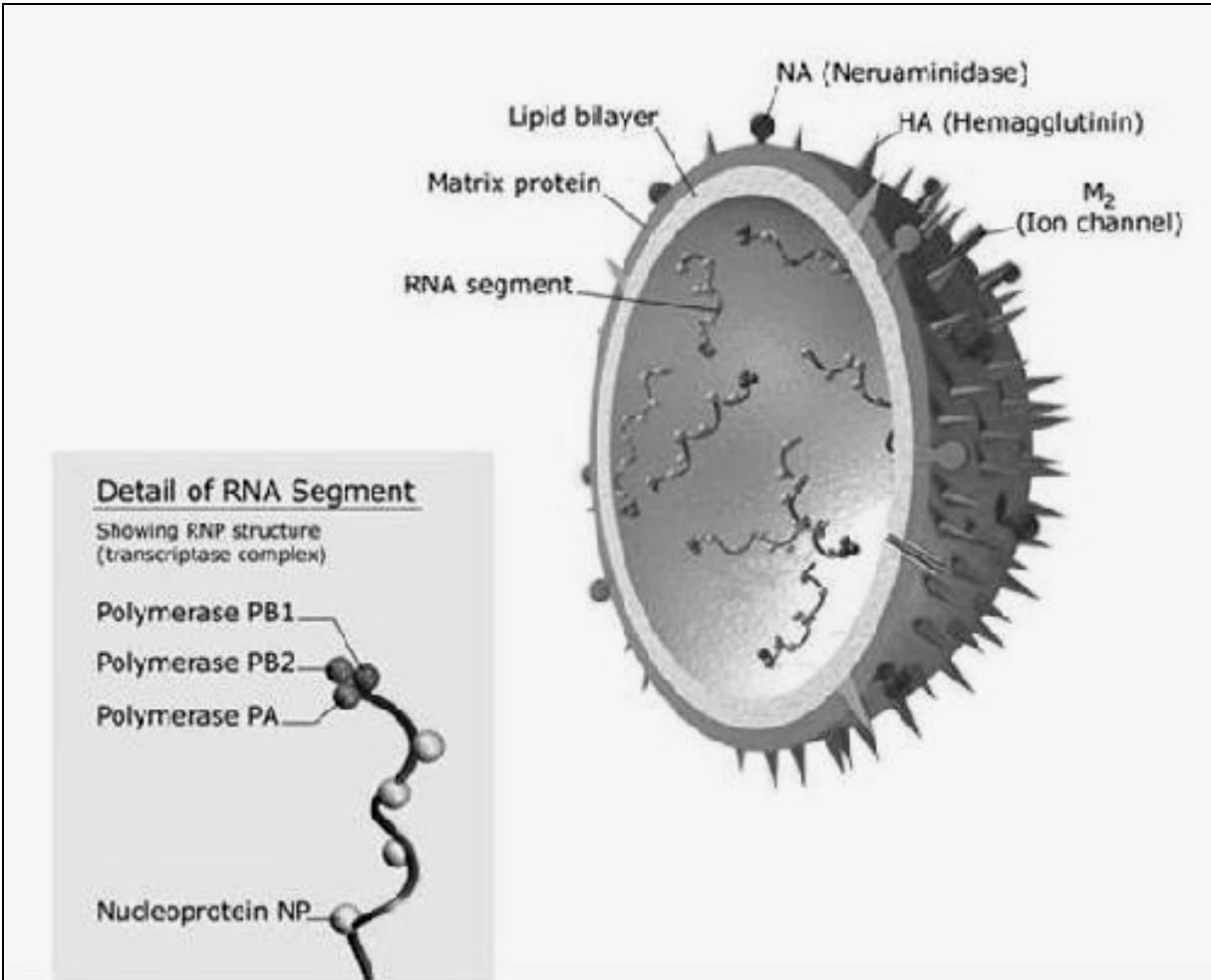


Figure 1 Diagram of the influenza virus (from Perez *et al.*, 2005).

Unique mechanism of replication: Influenza viruses are unique in that they are capable of undergoing constant changes in their genome (Trampuz *et al.*, 2004). These genetic changes occur in two ways; either by point mutation resulting in antigenic drift, or by genetic reassortment, resulting in antigenic shift (Quinn *et al.*, 2004). As their genetic make-up is changing continuously, vaccines for that particular subtype can't be made before an influenza outbreak occurs.

Antigenic drift:

Antigenic drift is the mechanism of acquiring new mutations. As influenza viruses lack “proof-reading” mechanisms, they are unable to repair the errors occurred during replication, and undergo mutation (Anon, 2005d). There will be alteration in the composition of the antigens

(HA and NA) produced by newly acquired genes against which the hosts will not have antibodies. It has been reported that antigenic drift is less evident in avian influenza viruses in their original aquatic bird reservoirs, in comparison to those invading domestic poultry (Webster and Hulse, 2004). As antigenic drift is regulated by antibody pressure, immune pressure in domestic poultry may induce antigenic variation (Webster and Hulse, 2004).

Antigenic shift:

The genetic change that enables a flu strain to jump from one animal species to another, including human, is called antigenic shift. Due to the segmented nature of their genome, it is likely that AI virus undergoes genetic reassortment, yielding up to 256 gene combinations, with a different genotype of influenza virus when both are coinfecting a cell (Nicholson *et al.*, 2003; Webster and Hulse, 2004). This may result in highly pathogenic and highly contagious virus subtype. Later, this virus may mutate to the point where it can be transmitted between species. It is feared that reassortment of AI virus with human influenza virus may evolve a virus subtype capable of human-to-human transmission ultimately resulting in influenza pandemic (Anon, 2005c). Reassortment has been reported between influenza viruses of different domestic avian species in a live poultry market in China (Liu *et al.*, 2003b). Since the 1997 outbreak of AI in Hong Kong, the subtypes H5N1, H7N2, H7N3, H7N7, and H9N2 have been found to cross species barrier and infect humans (Anon, 2005c). The mechanism of genetic reassortment in influenza virus is shown in figure 2. It is believed that novel subtypes of influenza virus cause major pandemics which occur at about 20 year intervals (Quinn *et al.*, 2002).

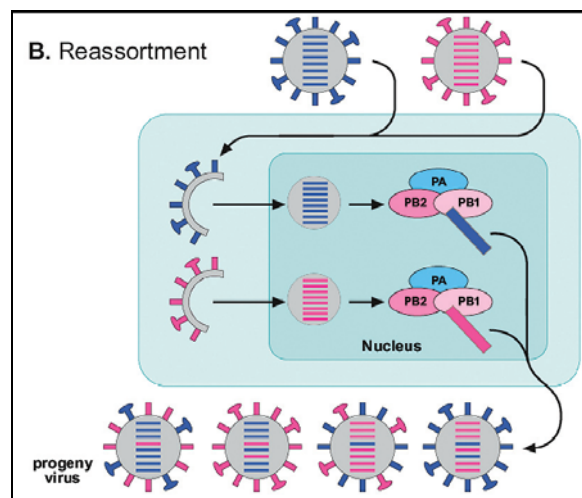


Figure 2 Reassortment or antigenic shift of influenza virus to adopt their host changes.

There is exchange of RNA segments between two genotypically different influenza viruses infecting a single cell, which can result in the generation of a novel strain and/or subtype (from Webster and Hulse, 2004).

Theoretically, all the combinations of H and N types have the potential to cause disease (Perez *et al.*, 2005). To date, HPAI viruses have been restricted to these two H5 and H7 subtypes, however all H5 and H7 subtypes are not always highly pathogenic (Kaye and Pringle, 2005; Perez *et al.*, 2005; Anon, 2005e). Available evidence suggests that most virulent subtype of influenza A virus is H5N1, followed by H3N1 (Table 1).

3 Reservoirs and species affected

Avian influenza virus can affect a variety of mammals and bird species. Migratory aquatic birds of the orders *Anseriformes* and *Charadriiformes* are the natural reservoirs of AI viruses which disseminate the virus across international borders (Quinn *et al.*, 2002; Kaye and Pringle, 2005; Anon, 2005e). All 15 HA and 9 NA subtypes of AI virus have been isolated from these birds (Alexander, 2000). Humans, equines, swine are the other common species of animals affected. The virus has been reported to be infective for cats, tigers, and leopards, (Keawcharoen *et al.*, 2004; Kuiken *et al.*, 2004; Thanawongnuwech *et al.*, 2005). It has been argued that cattle may too foster the virus (available from <http://www.nature.com/nsu/020107/020107.html>), and antibodies against the virus has been detected in cattle (cited in Quinn *et al.*, 2002). Sea mammals like whales, seals, and minks are also reported to be infected. Under normal conditions, AI virus doesn't affect species other than birds and pigs (Broor, 2005), and the most susceptible bird species are domestic chickens and turkeys (Anon, 2005e).

Chickens, ducks, geese, turkeys, waterfowl, pheasants, quail, pigeons, emu, guinea-fowl, ostrich, rhea, chukar partridge, eagles, gulls/pelicans are some bird species from which AI virus has been isolated (Senne, 2003; Borm *et al.*, 2005). Most of these migratory feral birds get only asymptomatic infection (Broor, 2005; Anon, 2005e). In sea mammals like minks, whales and seals, sporadic infection has been reported (Perez *et al.*, 2005). Humans are rarely affected by AI virus, and if they are, the infection is usually mild and short-lived (Anon, 2005e). However, infection with HPAI virus often has been lethal.

4 Transmission and risk factors

The influenza A virus multiplies in the intestine of reservoir water birds, and large quantities of viral particles are excreted with the feces into the water, initiating a cycle of oral-fecal transmission (Quinn *et al.*, 2002). It is well established fact that natural reservoirs like the wild aquatic birds introduce the infection into domestic poultry population (Anon, 2005e). The extent of transmission of AI virus in domestic birds is largely determined by the degree of contact with

reservoir birds in a particular area (Alexander, 2000). However, direct contact is not necessarily essential for the introduction of infection in poultry farms. Usually surface water contaminated by the wild birds used as drinking water in poultry farms serves as the source of infection (Anon, 2005e). Besides, the infection may be carried mechanically to these farms by the farm workers and attendants, animals or equipments. Airborne transmission may occur in birds that are in close proximity (Perez *et al.*, 2005). Spread of infection within farm or between farms are then mainly through drivers visiting farms, trucks delivering farm supplies, farmers etc.

As stated earlier, AI virus is capable of jumping species barrier and infecting other species of animals, including humans. Although the precise mechanism of transmission is not known, direct or indirect exposure to infect poultry is considered the most important risk factor as most of the human illness are occurring among the poultry workers (Saw *et al.*, 1997; Bridges *et al.*, 2002; Fouchier *et al.*, 2004; FAO, 2005a). Contact with infected birds or surfaces contaminated with their secretions are the most important source of infection (Trampuz *et al.*, 2004). Live bird markets are another ideal place of spread and transmission of viruses ((Senne *et al.*, 2003; Perez *et al.*, 2005). Cock-fighting, a popular game in Asia, is also implicated as an important factor for transmission of infection among humans (Anon, 2005d). Besides, smuggling of birds across international borders may introduce AI virus in a new location (Borm *et al.*, 2005). However, this risk factor (contact with infected birds) seems mysterious as among thousands of poultry workers in Asia that are involved in mass culling of infected birds, only a few are infected.

The influenza viruses show little genetic variation in the wild migratory bird reservoirs (Kaye and Pringle, 2005). The viruses replicate poorly in humans (Quinn *et al.*, 2002). Pigs are generally considered to be “mixing-vessels” producing genetic reassortment of influenza A virus (Peiris *et al.*, 2001; Anon, 2005d) because both human and avian subtypes replicate well in pigs (Quinn *et al.*, 2002). It may be because the respiratory tract of pigs contain both sialic acid receptors -[alpha]2,3-N-acetylneuraminic acid-galactose- for avian, and ([alpha]2,6-acetylneuraminic acid-galactose- for humans influenza viruses (Kaye and Pringle, 2005). However, in none of the past four major influenza pandemics in humans, pigs have been implicated as the reassortment host (Kaye and Pringle, 2005). Although pigs have been found to be infected by AI virus, transmission between pigs was not seen under experimental conditions (Choi *et al.*, 2005). Figure 3 shows the transmission dynamics of influenza A virus among birds, pigs and human populations.

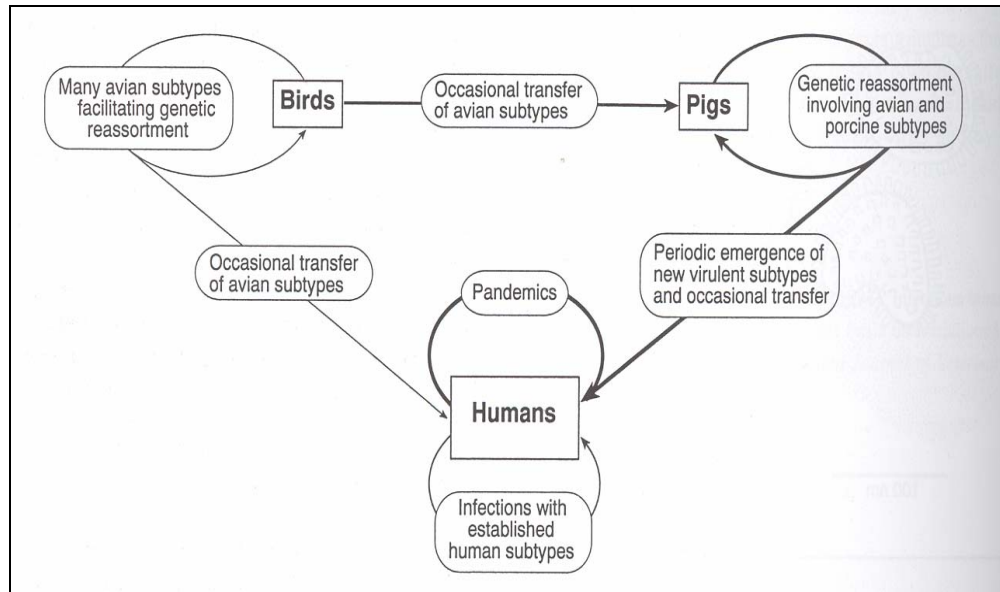


Figure 3 The circulation of subtypes of influenza A virus in bird and pig populations with the emergence of virulence subtypes which occasionally may be responsible for pandemics in the human population (from Quinn *et al.*, 2002)

Risk of via foodborne transmission is relatively low. Influenza viruses are susceptible to environmental exposure and are sensitive to heat, lipid solvents, detergents, extreme changes of pH, irradiation and oxidizing agents (Quinn *et al.*, 2002; Perez *et al.*, 2005). Although the AI viruses may be detected in chicken meat (Swayne and Beck, 2005; Anon, 2005d) or eggs (FAO, 2005c), normal cooking conditions (approximately 70° C) will kill the virus (OIE, 2005b). Therefore, AI virus in humans is not considered to be a foodborne infection (Embarek, 2005).

Major concern is about human-to-human transmission. Some H5N1 cases of probable human-to-human transmission have been documented recently (Gottlieb, 2005; Ungchusak *et al.*, 2005; WHO, 2005a). However, there has been neither genetic reassortment in the virus nor efficient human-to-human transmission to date (Monto, 2005; Wilson, 2005). Although, the risk of genetic reassortment of AI virus and the potential pandemic will always be there as long as the AI virus continues circulation in humans.

5 Epidemiology

The world has witnessed three flu pandemics in the 20th century; the 1918-1919 influenza pandemic known as “Spanish flu”, which killed approximately 40-50 million people, and the pandemics of 1957 “Asian flu” and the 1968 “Hong Kong flu”. In each of these pandemics, there was emergence new HA subtype of influenza virus (Perez *et al.*, 2005). The current wave (late 1990s to present) of AI infection in human and animals is summarized in Table 1.

Table 2 Avian influenza outbreaks reported from different parts on the world: 1990s to 2005

Country	Period	Subtype	Animals affected	Humans cases (deaths)	Additional information	References
Vietnam	2004-5 ongoing	H5N1	Chickens, pigs	90 (40)	Limited human to human transmission, 2.9 M chicken culled, latest outbreak reported on August 5 2005	(Hien et al., 2004; Choi et al., 2005; WHO, 2005b)
Thailand	2004-5 ongoing	H5N1	Chickens	17 (12)	10.7 M birds destroyed, No human cases reported since December 2004	(Karcher, 2004; EUROPA, 2005)
Hong Kong	1997	H5N1	Chickens	18 (6)	Human to human transmission, 1.5 M birds destroyed	(Saw et al., 1997; Karcher, 2004; EUROPA, 2005)
	1999	H9N2	Chickens	2 (0)		(Karcher, 2004; EUROPA, 2005)
Cambodia	2003	H9N2	?	1 (0)		
	2003	H5N1	?	2 (1)	22 000 birds culled	
	2003-5 on going	H5N1	Chickens	4 (4)	No new cases since May 5 2005	(Fouchier et al., 2004; WHO, 2005b)
Netherlands	2003	H7N7	Chickens, pigs	89 (1)	One veterinarian died, 30 M birds culled	(Fouchier et al., 2004; EUROPA, 2005)
Indonesia	2004-5	H5N1	Chickens, Pigs	1(1)	Recent outbreaks reported in Chickens	(Cyranski, 2005; EUROPA, 2005)
Egypt	2004	H10N7	wild ducks	2 (0)	Two child affect, father of one was poultry worker	(NIAID, 2005; PAOH, 2005)
Canada	2004	H7N3	Chickens	2 (0)	High pathogenic strain, 19 M birds depopulated	(Tweed et al., 2004)
Laos	2004	H5	Chickens	0	40000 birds died or destroyed	(FAO, 2004a)
	2005	H5N1	Chickens	0		(Witt and Malone, 2005)
Malaysia	2005	H5N1	Chickens	0	Birds within 1 km radius culled, No recent outbreaks reported	(OIE, 2004a)
Japan	2004	H5N1	Chickens	0	Second outbreak	(FAO, 2004a)
	2005	H5N2	Chickens	0	Low pathogenic strain	(OIE, 2005d)
South Korea	2003	H5N1	Chickens	0	No outbreaks since February 2004	(FAO, 2004a)
China	2005	H5N1	Migratory birds	0	Reported from Qinghai , Tibet and Xinjiang province, no human cases	(EUROPA, 2005; Ng et al., 2005)
Taiwan	2004	H5N2	Chickens	0	Low pathogenic strain	(FAO, 2004a)
Kazakhstan	2005	H5N1	Geese, ducks	0	9000 birds affected, 2400 destroyed	(OIE, 2005f)
Pakistan	2005	H7 & H9	Chickens	0	Low risk to human health	(Ng et al., 2005)
Mongolia	2002	H9N2	Chickens	0	Low pathogenic strains	(Bano et al., 2003)
	2005	H5N1	Wild birds, Chickens?	0	Detected in wild birds near Russian border	(OIE, 2004b)
Russia	2005	H5N1	Chickens, wild ducks	0	120000 birds destroyed, Viral genome identical to the Chinese type	(Nepoklonov, 2005)
USA	2001-2	H7N2	Chickens	0	Low pathogenic strain,	(Dunn et al., 2003)
Central America	2000-1	H5N2	Chickens	0	Reported from Guatemala, Honduras, and El Salvador	(Senne, 2003)
Mexico	1992-1995	H5N2	Chickens	0	Virus evolved from LP to HP form, successfully eradicated by vaccination	(Villarreal-Chávez and Rivera-Cruz, 2003)
Italy	1997-2001	H5N2, H7N1	Chickens	0	Virus evolved from LP to HP form, killed 13 M chickens	(Capua et al., 2003)
Australia	1997	H7N4	Chickens	0	Highly pathogenic strain. 0.3 M chickens killed	(Senne, 2003; Selleck et al., 2003a)
Philippines	2005	H5	Chickens	0	Low pathogenic (LP) virus	(Anon, 2005b; OIE, 2005e)

First outbreak due to H5N1 was reported in May 1997 in Hong Kong. Eighteen people were infected, six of whom died (Saw *et al.*, 1997). The infections in humans overlapped with an epidemic of HPAI in birds in Hong Kong caused by the same virus subtype. Reassorted virus was detected that had H gene from an avian influenza virus present in geese and an N gene from waterfowl (teal). The remaining 6 internal genes originated either from the virus present in teal or from the virus present in quail (Kaye and Pringle, 2005). It was concluded that the virus spread mainly from birds to humans especially in live bird markets. This epidemic was controlled after culling 1.5 million birds. There were no outbreaks reported until 1999. In 1999 in China and Hong Kong infection due to subtype H9N2 was reported in two children, but both patients recovered. The evidence suggested that poultry was the source of infection and the main mode of transmission was from birds to humans (EUROPA, 2005).

The second wave of H5N1 AI virus outbreak was reported **2003** in two members of a family in Hong Kong who had recently traveled to southern China. One person recovered, the other died. How or where these two family members were infected was not determined (EUROPA, 2005). Similarly, an outbreak of H7N7 occurred in February **2003** in the Netherlands that led to the destruction of around 30 million birds and cost an estimated €150 million. Later, infections were reported among pigs too. A total of 89 human infections occurred, mostly among the poultry workers, including 1 resulting in the death of a Dutch veterinarian (van Kolfshoeten, 2003; Fouchier *et al.*, 2004).

In December **2003** **Korea** reported sudden death of large of number of chicken diagnosed with HPAI caused by H5N1 virus (FAO, 2004a). In January **2004** in Kyoto Japan also an epidemic of avian influenza due to H5N1 virus was reported (FAO, 2004a). However both of these epidemics were controlled and no human cases were reported. These two countries were able to contain the epidemic by culling more than one million birds.

In February 2004, an outbreak of highly pathogenic avian influenza H7N3 occurred in poultry in British Columbia, Canada (Tweed *et al.*, 2004). Surveillance identified two persons with confirmed avian influenza infection. No further spread was reported after depopulation 19 million birds.

In **January 2004**, the first human case of infection with avian influenza, caused by H5N1 strains of influenza virus A were reported from Thailand and Vietnam (Karcher, 2004). The cases in humans were directly linked to outbreaks of highly pathogenic H5N1 avian influenza outbreaks in poultry in these countries. By February 2004, for the first time, seven Asian countries

(Cambodia, China, Indonesia, Japan, Laos, Thailand, and Vietnam) were affected at once with the AI virus. The losses incurred by the poultry industries were enormous as more than 120 million birds died or well culled in three months period. There were gradual decline in the number of outbreaks by March 2004. However in August 2004, new outbreaks were reported in birds from Cambodia, China, Indonesia, Thailand, Vietnam, and for the first time from Malaysia (OIE, 2004a). New cases of human infection were also reported from Vietnam and Thailand, with probable human-to-human transmission (Chotpitayasunondh *et al.*, 2004; Hien *et al.*, 2004; Ungchusak *et al.*, 2005). Sporadic outbreaks human infections with AI virus are ongoing in Vietnam, Thailand, Cambodia, and Indonesia since December 2003 with 112 laboratory confirmed cases and 57 deaths (WHO, 2005b). No new human cases have been reported since August 5 2005.

The total tally of human cases since 1997 to date is 135 cases with 64 deaths; Vietnam accounting for 90 cases and 40 deaths, Thailand 17 cases and 12 deaths and Hong Kong 23 cases 7 deaths. Recent outbreaks in birds also follow the similar pattern. Figure 3 shows the latest updates on outbreaks of HPAI virus in poultry birds in Asian countries. Vietnam has reported the highest number of outbreaks followed by Thailand and Indonesia. However the actual number may be even higher as significant number of human cases and outbreaks in birds are not reported because of economic and political reasons (Trampuz *et al.*, 2004; CIDRAP, 2005a).

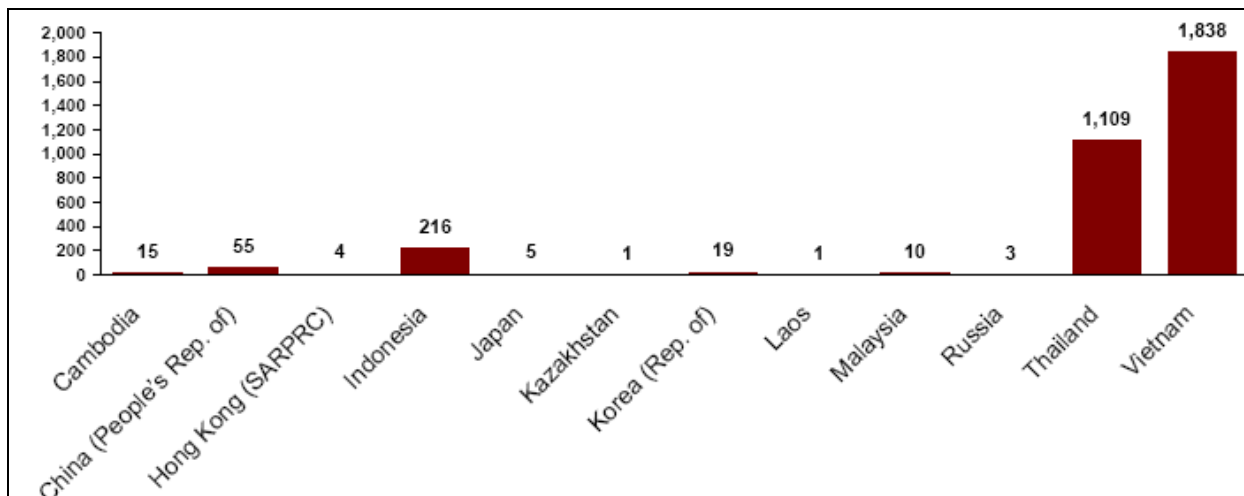


Figure 4 Outbreaks of highly pathogenic avian influenza (as of August 11 2005) in Asia (from (OIE, 2005c).

Available reports since late July 2005 suggests that AI virus is expanding its geographical range (figure 4). An H5N1 outbreak has been reported in wild migratory birds from Qinghai, Xinjiang and Tibet province of China ((Ng *et al.*, 2005; CIDRAP, 2005b) and Kazakhstan (OIE, 2005f)).

Similar outbreaks of H5N1 in wild birds and chickens are reported from Mongolia, near the Russian border (Anon, 2005f) and Russia (Nepoklonov, 2005). Viral genome analysis by sequencing revealed the virus isolated in Russia is related to those found in outbreaks in China. In these recent waves of outbreaks, no new human cases have been reported.

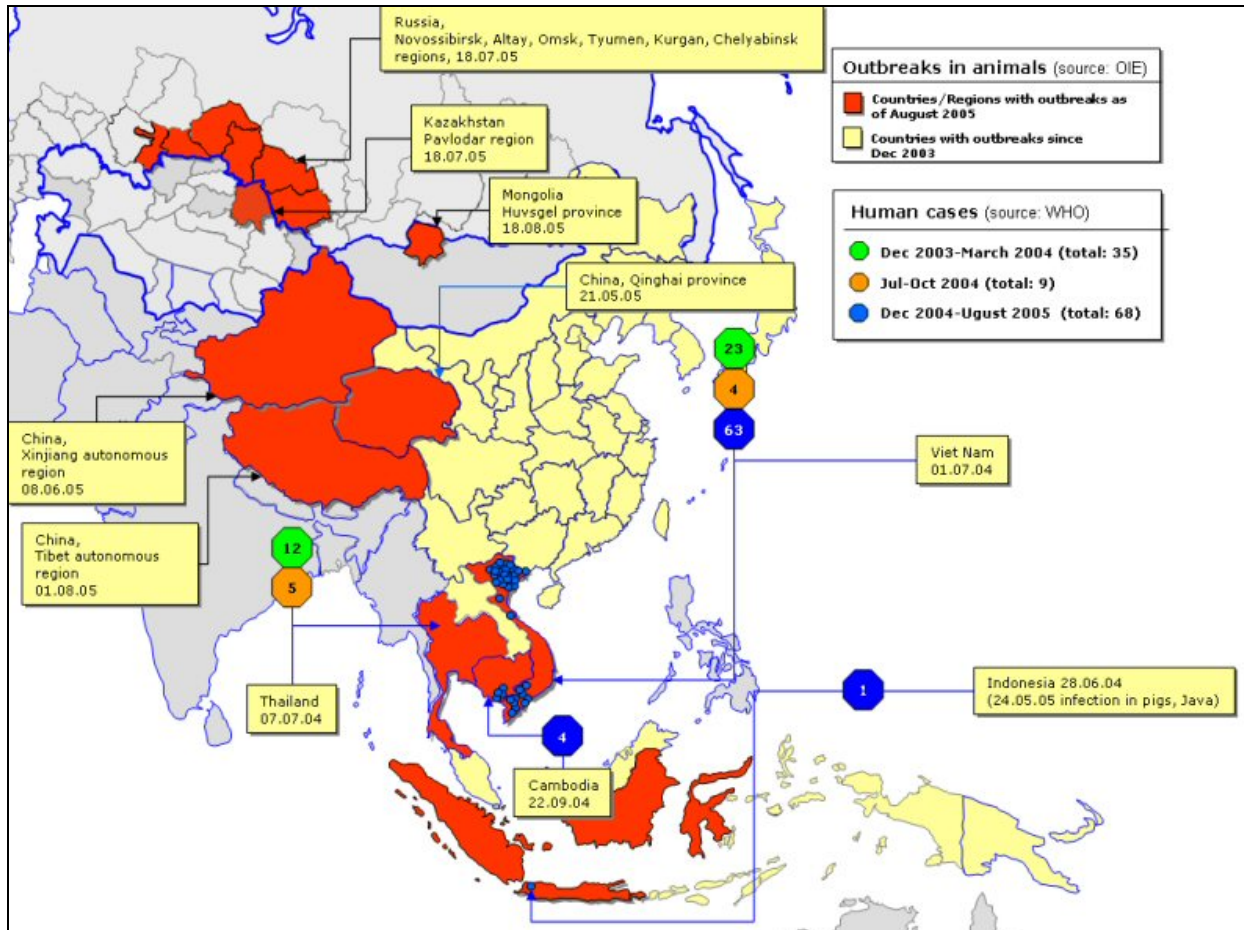


Figure 5 Countries and regions that have reported confirmed and suspected H5 outbreaks. (22.09.04 = date of first report of outbreaks in animals) (from (EUROPA, 2005).

6 The disease

6.1 Pathogenesis

Limited information is available on the pathogenesis of avian influenza virus infection in humans. Severity of pathogenesis depends on subtypes of the species involved (Zambon, 2001). The H5N1 subtype causes severe disease in humans by inducing proinflammatory cytokines like the tumor necrosis factor TNF α (TNF- α) (Cheung *et al.*, 2002). Death is usually due to multiple organ dysfunction resulting from burst of cytokine production in an attempt to limit to viral replication (Julkunen *et al.*, 2000; To *et al.*, 2001; Trampuz *et al.*, 2004). Respiratory and

digestive tract are the major organs for infection of influenza A virus in humans as well as birds (Julkunen *et al.*, 2000; Quinn *et al.*, 2002; Rimmelzwaan *et al.*, 2003; Uiprasertkul *et al.*, 2005). Epithelium of these organs contains sialic acid receptors which are the binding sites for the virus particles. Entry of virus into the cell requires breakdown of viral haemagglutinin by proteases. Therefore dissemination of virus in a given tissue is determined by the protease present and composition of viral hemagglutinin molecule (Quinn *et al.*, 2002). Once the virus enters the cell and replication starts (Figure 6) the synthesis of the host protein is shut off by several mechanisms, such as the degradation of host mRNA by the viral cap endonuclease. The loss of critical host cell proteins leads to cell death by necrosis or apoptosis (Yuen and Wong, 2005).

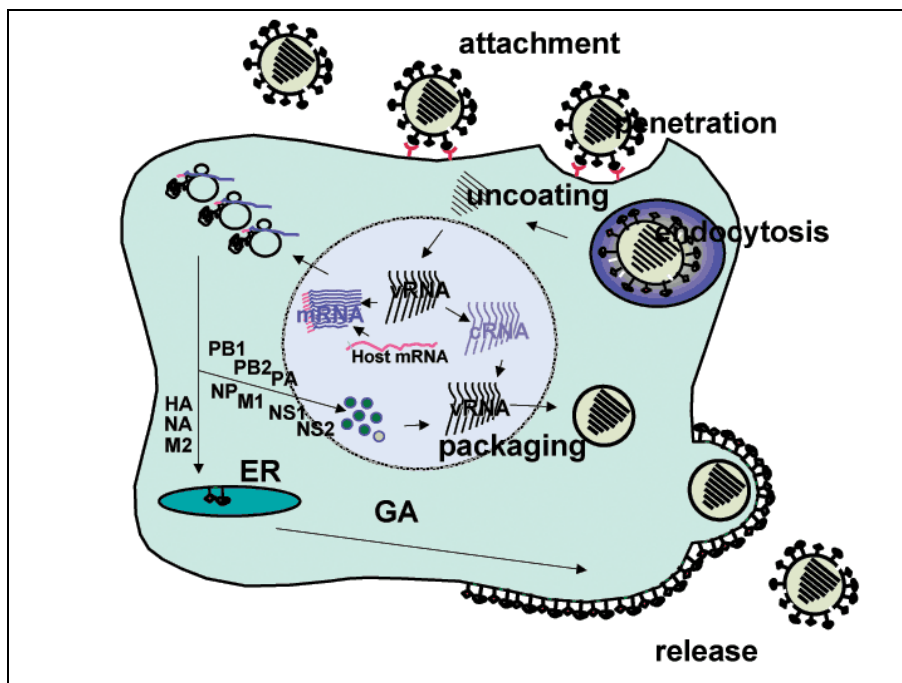


Figure 6 Replication of Influenza A virus inside a host cell (from Perez *et al.*, 2005)

6.2 Clinical manifestation

6.2.1 In Humans

The common symptoms are that of classical influenza like: fever, cough, runny-nose, sore-throat, and muscle aches (Yuen *et al.*, 1998). Conjunctivitis might be seen in some cases. Infection with highly pathogenic strains like the H5N1- may lead to viral pneumonia and acute respiratory distress, and is often fatal. Similar to human influenza, incubation period of AI virus infection in humans varies between 2-5 days (Kaye and Pringle, 2005; Ungchusak *et al.*, 2005; Anon, 2005d). The main clinical features of H5N1 outbreak in 10 people in Vietnam in January 2004

were fever, shortness of breath, cough and diarrhoea (Hien *et al.*, 2004). Mean incubation period was 3 days. However, common symptoms like sore-throat and conjunctivitis were not observed. Pathological findings observed were marked lymphopenia, chest radiographic abnormalities, liver dysfunction, and kidney failure. Eight out of 10 patients died accounting for 80% mortality. Conjunctivitis was reported in 78 out of 89 patients infected with H7N7 strain of influenza virus in the Netherlands (Fouchier *et al.*, 2004), and in two patients infected with H7N3 strains in Canada (Tweed *et al.*, 2004).

In terms of clinical manifestation, the spectrum of influenza H5N1 infection is wider than previously seen. A case of atypical H5N1 AI virus infection in a 39 years old woman of Thailand was described as having no respiratory signs (Apisarnthanarak *et al.*, 2004). The patient had only fever and diarrhoea. Similarly, in one Vietnamese patient infected with H5N1 influenza virus, none of the characteristic symptoms were observed. Rather, that patient died from encephalitis of unknown origin five days after being hospitalized (de Jong *et al.*, 2005; Parry, 2005a).

6.2.2 In Poultry

The severity clinical manifestation of AI virus infection in poultry depends on the pathogenicity of the infecting virus subtype (OIE, 2005a). Incubation period varies from 3-7 days depending on the virus isolate, age and species of bird (Quinn *et al.*, 2002). LPAI infection may remain almost asymptomatic. Some flocks with LPAI may experience mild respiratory symptoms like coughing and sneezing, and reduced egg production (Mutinelli *et al.*, 2003a; Anon, 2005e). Secondary bacterial infection may worsen the clinical conditions of bird that were asymptomatic while having only LPAI infection (Bano *et al.*, 2003). Commonly observed clinical symptoms in HPAI infection are; marked depression and loss of appetite, ruffled feathers, excessive thirst, and watery diarrhea, swelling and cyanosis of wattles and combs, lacrimation, and rapid decline in egg production or production of soft-shelled and deformed eggs (Campbell and Butterfield, 1981; Mutinelli *et al.*, 2003a; OIE, 2005a). Neurological manifestations like torticollis and ataxia may also be seen (Perez *et al.*, 2005). Death may occur as within 48-72 hours of infection, and mortality rate may reach up to 100% (Mutinelli *et al.*, 2003a). The disease in turkeys, ducks, geese, and quail resembles to that seen in chickens, but it may last 2 to 3 days longer (Perez *et al.*, 2005).

Gross pathological lesions include areas of diffuse hemorrhage on the legs, hocks and feet, petechial to ecchymotic hemorrhage in the peritoneum, serosal surfaces on the intestines and

proventriculus, necrotic changes in the skin, comb and wattles, mucus accumulation on the organs of respiratory system, urate deposition on the kidneys, air-sacculitis, peritonitis, and hemorrhages or degeneration of ovary in layers (Campbell and Butterfield, 1981; Perez et al., 2005). Some of these gross lesions resemble with velogenic viscerotropic Newcastle disease (NDV), and thus, they cannot be used for differential diagnosis of HPAI (Perez *et al.*, 2005).

Pathology: The common pathological findings of avian influenza A infection in birds are: edema, hyperemia, hemorrhage, and foci of perivascular cuffing mainly in the myocardium, spleen, lungs, brains, wattles, and to a lesser extent liver and kidneys. Parenchymal degeneration and necrosis are presenting spleen, liver, and kidneys. There may be foci of necrosis in liver, lungs, intestines, pancreas, and brain. Signs of encephalitis may occur due to advanced central nervous system lesions (Campbell and Butterfield, 1981).

7 Laboratory diagnosis

Although diagnosis is AI in humans and animals is sometimes done based on symptoms and clinical pathology, laboratory tests are needed to provide confirmatory diagnosis (OIE, 2005a). As the laboratory procedures for the isolation and characterization of AI viruses from humans and lower animals are essentially the same, they are discussed together in this section. Most of these tests are technically demanding and can be performed only at well-equipped laboratory with high level of expertise. The commonly used tests (table 3) involve virus isolation and characterization, analysis of the host's immune status or degree of susceptibility to the virus.

Table 3 Commonly used tests for the diagnosis of influenza A virus from human or animals samples

Tests	Sensitivity	Specificity	Comments	Reference
Virus isolation (VI) methods	100%	100%	expensive and time consuming (3-21 days), detects only viable viruses, requires infectious virus	(Cram <i>et al.</i> , 1999)
Reverse transcriptase polymerase chain reaction (RT-PCR) based methods	~100%	~100%	Results available rapidly, allows further molecular analysis, less sensitive than VI methods gives no indication of viability	(Cram <i>et al.</i> , 1999; Spackman <i>et al.</i> , 2003b)
Nucleic acid sequence-based amplification (NASBA)	100 %	100%	Allows differentiation of high- and low- pathogenicity strains	(Collins <i>et al.</i> , 2003)
Enzyme immunoassay (ELISA)s	98.1%	95.7%	Allows testing of antibody against H7 antigen, 99% concordance of results with HI test	(Sala <i>et al.</i> , 2003)
Reverse transcriptase PCR coupled with ELISA (RT-PCR-ELISA)	91%	97%	Sensitivity comparable to virus isolation in embryonated eggs, 100 times more sensitive than conventional PCR methods	(Munch <i>et al.</i> , 2001; Dybkær <i>et al.</i> , 2003)
Enzyme immunoassay	62-100%	63-100%	Ease of use, rapid test, moderate	(Nicholson <i>et al.</i> ,

(EIA) based methods			cost, unable to detect in early course of infection	2003; Cattoli <i>et al.</i> , 2004)
PCR coupled with EIA (PCR-EIA)	92%	98%	Sensitivity of the test depends on the course of infection	(Leonardi <i>et al.</i> , 1994; Ellis and Zambon, 2002)
Direct immunofluorescence (DIF)	70%	90%	Rapid and cheap test, technical expertise necessary, not used widely	(Cram <i>et al.</i> , 1999)
Haemagglutination and Haemagglutination inhibition (HAI)			Test of choice for WHO influenza surveillance	(WHO, 2002)

7.1 Viral isolation (VI) methods

The presence of live virus in clinical specimens is confirmed by isolation of the virus, which is done by characterization of the viral genome or viral antigen. Isolation of influenza virus can be done by inoculation of pathological samples in cell cultures, embryonated eggs or in live chicken of specific age.

7.1.1 Viral isolation in cell cultures

Traditional diagnosis of influenza virus infections involves viral isolation in tissue culture on primary monkey kidney cells or Madin-Darby canine kidney (MDCK) cells (Yuen *et al.*, 1998; Rowe *et al.*, 1999; Apisarnthanarak *et al.*, 2004). MDKC cell line exhibit typical epithelial morphology and are susceptible to number of viruses including the influenza A virus (Anon, 2005g). These cell lines are inoculated with the virus are examined after 1-3 days for the signs of viral infection. This technique is considered to be of gold standard (100% sensitive and specific) for isolation and identification if influenza viruses and sensitivity of other methods are compared against it (Cram *et al.*, 1999). However, there are some drawbacks. This technique requires well-equipped laboratory and high level technical expertise. Also, results are available only after several days (up to 21), so is not suitable for rapid diagnostic purposes. Nonetheless, as only culture yielded viruses can be further characterized, this technique remains crucial in diagnosis of influenza virus infection.

7.1.2 Viral isolation in embryonated eggs

In this method, influenza viruses are grown by the inoculation of 9-10 days old embryonated specific pathogen free (SPF) hen eggs, or specific antibody negative (SAN) eggs (Rowe *et al.*, 1999; OIE, 2005a). At least five eggs are inoculated from one sample and are incubated at 35-37°C for 4-7 days. The allantoic fluid is then screened for haemagglutination (HA) activity for the presence and concentration of the virus. Like cell culture method, in spite of being quite

reliable, this method is also a laborious and time-consuming (Cattoli *et al.*, 2004). Since some human and porcine virus subtypes grow poorly in eggs, they should be inoculated on MDCK cells (WHO, 2002).

7.1.3 Intravenous pathogenicity test (IVP)

This method involves inoculation of infective material into live birds, mainly to determine the pathogenicity of the infecting virus. Minimum of eight susceptible 4- to 8- weeks old chickens are inoculated with the infective virus. The subtype is considered to be highly pathogenic (HPAI) if they cause more than 75% mortality within 8 days (WHO, 2002).

7.2 Molecular methods

Hybridization, polymerase chain reaction (PCR) based tests, microarray analysis, and nucleic acid sequence-based amplification (NASBA), are the molecular methods suitable for detection of influenza viruses (Ellis and Zambon, 2002). PCR-based tests are powerful tools for the identification of AI viruses as very low amount of DNA is needed for these tests. Since the genome of influenza virus is negative-sense RNA, a DNA copy (cDNA) complementary to the RNA is needed to be synthesized in **reverse transcriptase-PCR (RT-PCR)** before the start of PCR reaction (WHO, 2002). The **real-time RT-PCR** uses a fluorescent probe for the simultaneous amplification and detection of the PCR products (Hindiyeh *et al.*, 2005). **Multiplex PCR** involves the use of more than one set of primers for the detection of more than one gene or genome in a single pathogen or in more than one pathogen (Ellis and Zambon, 2002). In **Nested PCR** two internal primers are used to amplify PCR products of two external primers.

Several RT-PCR based protocols have been used for the detection of AI virus. (Payungporn *et al.*, 2004) developed a **multiplex RT-PCR** targeting M, H5 and N1 genes corresponding to the fragments 276-, 189- and 131-bp, respectively. This method was recommended for the detection of influenza A virus subtype H5N1, both from human and avian cases. (Spackman *et al.*, 2003b) developed a multiplex real-time reverse-transcriptase PCR (**RRT-PCR**) for the simultaneous detection of H5 and H7 virus subtypes. It was claimed that this technique is mainly useful for screening in the live bird markets. Dybkær *et al.* (2003) developed a RT-PCR method coupled with ELISA. The diagnostic sensitivity and specificity of this **RT-PCR-ELISA** was reported to be 91% and 97%, respectively, and superior than virus inoculation methods. Recently Ng *et al.* (2005) reported that they developed a **real-time multiplex RT-PCR** to detect the H5N1 virus subtype. The primers were targeted to amplify two different regions of the HA gene. These

workers claimed that this test was more sensitive than nested RT-PCR and even more sensitive than real-time RT-PCR. **Nested PCR** was used to diagnose AI virus infection in 10 patients in Vietnam (Hien *et al.*, 2004).

The main advantages of PCR-based test are they are highly sensitive and specific. So, co-infection of a host with a number of AI virus subtypes can precisely identified by the use of appropriate primers targeting the specific genes. In addition, PCR-based diagnostic test are usually rapid, compared to the culture or serological methods (Ng *et al.*, 2005). Thus, preventive measures can be started in the early stages. Also, PCR-based methods are capable of early detection of the virus compared EIAs (Cattoli *et al.*, 2004). However, there are some disadvantages as well. One of the disadvantages is inability of these methods to detect the viability of the virus. It has also been suggested that rapid diagnostic tests like the real-time RT-PCR have low sensitivity in small number of cases (Apisarnthanarak *et al.*, 2004; Hien *et al.*, 2004). Another disadvantage is that these methods are technically demanding and prone to contamination (Hindiyeh *et al.*, 2005).

7.3 Serological methods

Serological methods are aimed at detecting the binding of virus antigen and the antisera specific to it. These tests are important when clinical samples unavailable or facilities for virus isolation don't exist. Some of the common serological tests used for the diagnosis of avian influenza infection are discussed briefly in this section.

7.3.1 Haemagglutination (HA) and haemagglutination inhibition (HAI) assay

These tests are based on the principle of agglutination of erythrocytes or their inhibition by the haemagglutinin (HA) protein of the influenza virus (WHO, 2002). HA is the most abundant (of 15 different subtypes) glycoprotein on the surface of influenza viruses. In HA test, standardized quantity of antigen is added to serially diluted antisera, whereas in HAI test, erythrocytes are added, in addition, to the antigen-antibody complex. Specific binding or inhibition of that is then observed. The HAI test is considered extremely reliable and the World Health Organization is using this test for the WHO global influenza surveillance program (WHO, 2002).

7.3.2 Direct immunofluorescence (DIF)

This test involves the detection of viral antigen directly on the infected epithelial cells (Cram *et al.*, 1999). The presence of virus is confirmed by demonstration of viral antigen in the epithelial

cells stained with fluorescent antibody. This is a rapid test as result can be obtained on the same day. Sensitivity and specificity are reported to be 60-80% and 90%, respectively (Cram *et al.*, 1999). Main demerits of this and other rapid methods (e.g. optical immunoassays, ELISAS), is having lower sensitivity (50-80%) (Hindiye *et al.*, 2005). Besides, immunofluorescence assays are labor intensive and require a high degree of technical expertise (Leonardi *et al.*, 1994).

7.3.3 Virus neutralization test

The virus neutralization test is done for the identification of virus-specific antibody in animals and humans. The test consists of two steps. Firstly, specific amounts of antibody and the virus are mixed and incubated. The complex is then inoculated into a suitable susceptible host to detect the residual viral infectivity. The absence of infectivity indicates a positive neutralization reaction and the presence of virus-specific antibodies in human or animal sera (WHO, 2002). ELISA based microneutralization test, in combination with Western-blotting has been used widely for the identification of H5N1 virus outbreaks in humans (Rowe *et al.*, 1999; Liem and Lim, 2004). Sensitivity and specificity of this combined test ranges from 80-100% and 96-100%, respectively.

7.3.4 Agar-gel immunodiffusion test (AGID)/ Agar-gel precipitation test (AGPT)

In this method, the presence of virus is confirmed by demonstrating the nucleocapsid and matrix protein antigen common to all influenza viruses (Quinn *et al.*, 2002). Inoculation of embryonated egg is done as mentioned in section 7.1.2. Antigens are then prepared by concentrating the virus from the infected allantoic fluid or chorioallantoic membrane. Serum from suspected animals is then mixed with the prepared antigen on the wells made in 1% agarose gel. This test is based on the principle of simultaneous migration of antigen and antibody towards each other through an agar gel (WHO, 2002). AIGD tests have been widely used for the mass screening AI virus infection in domestic poultry (OIE, 2005a).

7.3.5 Enzyme-immunoassays (EIA) s

In this method, presence of virus is confirmed by detection of free viral antigen in the nasopharyngeal secretions (Cram *et al.*, 1999). These tests allow the results to be obtained in hours. Varieties of EIA kits are available commercially. These tests, thus, can be performed

even where well-equipped laboratory or technical expertise is lacking. Sensitivity and specificity are documented to be 85% and 100%, respectively (Cram *et al.*, 1999).

A variety of EIA based methods are available. Some of them have been coupled with molecular methods to overcome their lower sensitivity. In **PCR-EIA** methods, a single round RT-PCR is performed and the resulting PCR products identified by hybridization in solution to a biotinylated RNA probe and detected in EIA (Ellis and Zamon, 2002). Reliability of **antigen capture enzyme immunoassay (AC-EIA)** are comparable with virus isolation methods as they have excellent agreement ($k=0.82$) with those tests (Cattoli *et al.*, 2004).

Besides the above mentioned tests, characterization of the isolated virus has been done by genome sequencing (Nepoklonov, 2005; Ungchusak *et al.*, 2005) or electron microscopy (Quinn *et al.*, 2002).

8 Prevention and Control

Since poultry is the main source of infection for humans, the virus should be eradicated from poultry population. However, eradication in the near future is very unlikely to be achieved. Mass culling of infected birds, vaccination, implementation of strict biosecurity measures, are the options available for eradication of infection in poultry production (Villarreal-Chávez and Rivera-Cruz, 2003).

8.1 Mass culling

Available evidence suggest that mass culling of infected or exposed domestic poultry largely helped to prevent further spread of AI infection among people in Southeast Asia. Since 1997, when the first human cases of bird flu appeared, more than 150 millions of sick or exposed birds, primarily chickens, have been culled (FAO, 2005a). These birds were destroyed either by burning or by burying. This method is considered by the WHO as the first-line of defense against avian viruses. This method is however, difficult to implement as there will be huge economic loss and is usually opposed by the general public.

8.2 Vaccination

In poultry: Vaccination of poultry has been successful in controlling AI outbreaks in the recent years and is thus considered to be an important control strategy by FAO and OIE (FAO, 2005a). Majority of the AI vaccines available for immunization in chickens are inactivated whole virus antigen in an oil based emulsion adjuvant or recombinant DNA vaccines with H5 AI gene insert

(FAO, 2004b). These vaccines are administered via parenteral route. Whole virus vaccines are recommended to use once or twice at the interval of 3-4 weeks, depending on the category of chicken (Trani *et al.*, 2003). Birds should be vaccinated against the circulating HA subtype to offer maximum protection. As these vaccines can only be administered by injection to each single bird, emergency vaccinations may not be carried out efficiently. Investigations are underway to develop vaccines that can be administered by spray or drinking water route so that mass vaccination programs can be made more effective (Swayne *et al.*, 2003).

To date, no effective vaccines are available against avian influenza virus infection in humans. Recently (in August 2005), a vaccine for use in humans has been developed in France. The US government is purchasing millions of doses of this experimental vaccine as the vaccine was found to stimulate immune response against the avian strain in healthy adults below 65 years of age (Anon, 2005d). Further tests of this vaccine are ongoing. Other currently available influenza vaccines provide no protection against the avian strains H5, H7, or H9 (Matsuoka *et al.*, 2003). However people are advised to be vaccinated to minimize the risk of coinfection with avian and human subtypes. Coinfection may facilitate the reassortment genome and evolution of a novel virus which may spread efficiently between humans and lead to global pandemic (Trampuz *et al.*, 2004).

The problem in development of human vaccine lies at unsuitability of traditional chicken embryo method as HPAI strains are pathogenic to the chicken embryo and will cause their death. Alternative options for the production of vaccine against H5, H7 and H9 are being investigated using the plasmid-based reverse genetic technology (Cherbonnel *et al.*, 2003). It has been stated that a vaccine strain virus can be created in the laboratory by transferring the HA and NA or matrix genes from a target virus to the laboratory virus (Chen *et al.*, 2003). Such viruses will be non-pathogenic but immunogenic to humans. Safety of such vaccines for use in humans is being evaluated (Matsuoka *et al.*, 2003).

8.3 Surveillance and biosecurity

Constant monitoring and surveillance of influenza activity in poultry or human will facilitate early detection and characterization of the virus and take control actions accordingly. Surveillances should be carried out on regular basis using some reliable serological tests in an adequate number samples. Owing to the highly contagious nature of influenza virus, proper sanitation and biosecurity plays a vital role in preventing the spread of infection. Biosecurity can be implemented as two different elements- bio-containment; prevention of spread of virus

from infected premises, and bio-exclusion; stopping introduction of infection on new premises (FAO, 2004b). Proper cleaning and disinfection of poultry sheds or live bird markets promotes quick eradication of the virus (Senne *et al.*, 2003). As wild birds are the main source of introduction of infection in poultry farms, their contacts with each other should be minimized. Surface water should not be given for drinking to poultry. Farm worker should be made aware about transmission patterns of avian influenza virus. Quarantine should be imposed on import of birds from influenza outbreak places. Individuals should avoid contact with birds in an area affected by avian influenza. Maintenance of high level of sanitation and disinfection is important for the eradication of viruses from farm premises. The virus is easily killed by common disinfectants such as alcohol, bleach, formalin or iodine compounds. Well cooked poultry products and eggs are not considered a source of infection as the virus is susceptible to heat (56°C for 3 h, 60°C for 30 min, or 70°C for 1 min).

8.4 Antiviral treatment

Neuraminidase inhibitor preparations have been used successfully for the prophylaxis and treatment of avian influenza virus infection in humans (Monto, 2003). The antiviral agents limit shedding of viruses from infected cell during replication (figure 7) by inhibiting budding or by inducing clumping of virus particles. Oseltamivir, zanamivir, amantadine and rimantadine are the available antiviral preparations. Owing to small number of human cases, no efficacy trial has been done for these drugs. However, it has been reported that the influenza virus develops resistance fairly quickly (Anon, 2005d). The current H5N1 strain is already resistant to amantadine and rimantadine, however, but still susceptible to oseltamivir and zanamivir (Trampuz *et al.*, 2004). Tamiflu (oseltamivir) is currently the choice of antiviral drug for treatment and prophylaxis of avian influenza infection in humans (Anon, 2005d), and should be administered in individuals within 48 hours of exposure to H5N1 virus (Trampuz *et al.*, 2004). There are several drawbacks; Tamiflu treatment should be started within very short period of exposure to the virus. Also, it is very expensive and is in short supply. Thus global influenza pandemic control strategy of the WHO has been emphasizing on stockpiling of these antiviral preparations to make them easily available.

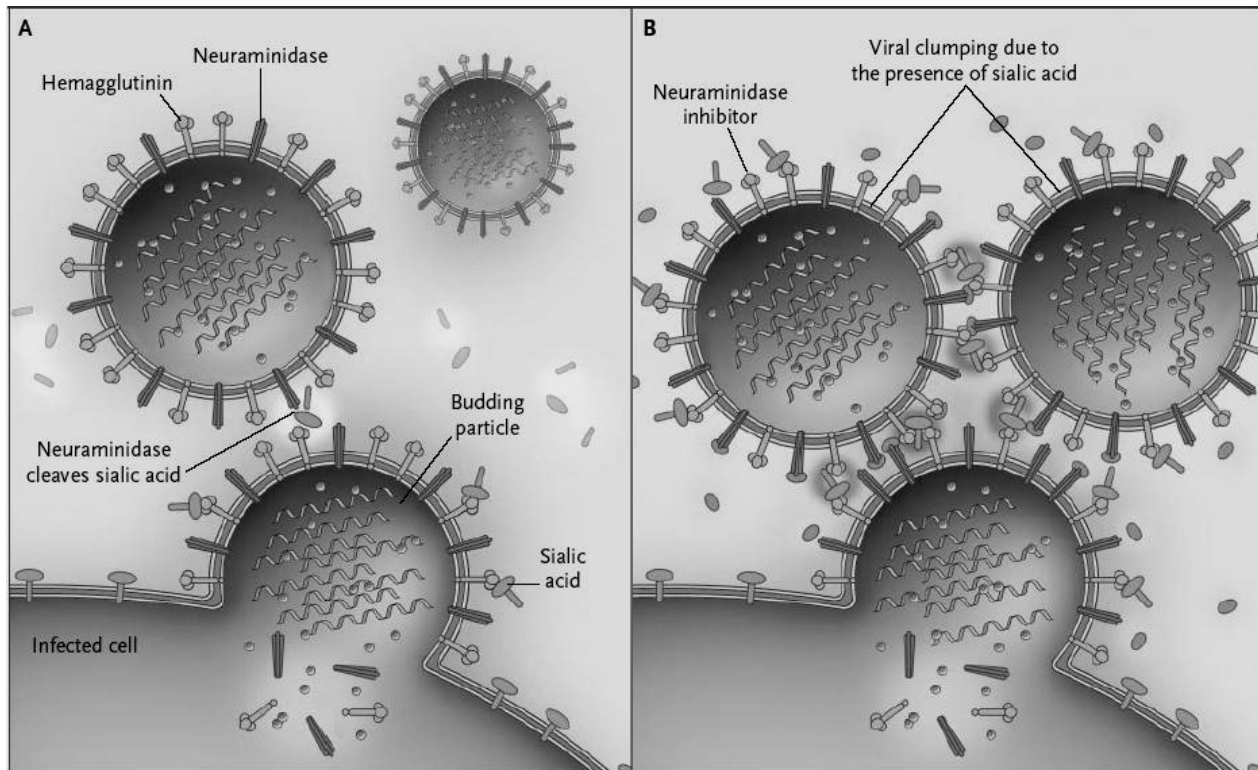


Figure 7 Normal budding and release of influenza virus from an infected cell (Panel A) and release restricted by a neuraminidase inhibitor (Panel B) (from Monto, 2005).

9 Discussion and conclusions

Although avian influenza outbreaks in domestic poultry caused huge economic losses, major concern is emergence of new viral strain that spreads easily from human-to-human. Since the first outbreak of avian influenza in humans in Hong Kong in 1997, scientists have been warning about the evolution of new virus subtype. As there will be no natural immunity among the world population against this entirely new subtype global pandemic is inevitable (Anon, 2005a). In recent years, some probable human-to-human transmission has been documented, however all the virus subtype detected in these outbreaks were of avian origin (Chotpitayasunondh *et al.*, 2004; Hien *et al.*, 2004; Gottlieb, 2005; Ungchusak *et al.*, 2005). However, to date, there has been neither genetic reassortment in the virus nor efficient human-to-human transmission (Monto, 2005; Wilson, 2005). Scientists have been wondering why H5N1 has not reassorted with human subtype even when they were circulating simultaneously in humans (Stohr, 2005). It has been argued that if avian strain could reassort with human strain, it should have done so by now. One explanation is that reassortment might have resulted into a low pathogenic or non-viable virus (Stohr, 2005).

The risk of genetic reassortment of AI virus and the potential pandemic will always be there as long as the AI virus continues circulation in humans. Experience from past influenza pandemics suggest that they start abruptly and explosively causing very high fatalities. The relatively few outbreaks in Southeast Asian countries is giving the world plenty of time to become prepared to combat the possible pandemic. Keeping this point in view, the World Health Organization (WHO) has formulated some strategies as preparedness for global influenza pandemic. As eradication of avian influenza virus from the world is unlikely in near future, main focuses should be development of an effective strain-specific vaccine for human use, and stockpiling of antiviral preparations. In long term, eradication of the virus may be achieved by delivery of efficient veterinary and public health services to the affected countries and sharing of disease information among them. For this, the World Health Organization (WHO), Food and Agriculture Organization (FAO) and the World Association for Animal Health (OIE) shall work in a coordinated manner.

10 References

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