INTRODUCTION

Avian influenza has emerged as the primary public health concern of the 21st century ***(Juckett, 2006).***

There are many different subtypes of type A influenza viruses. These subtypes differ because of changes in certain proteins on the surface of the influenza A virus (hemagglutinin [HA] and neuraminidase [NA] proteins). There are 16 known HA subtypes and 9 known NA subtypes of influenza A viruses. Many different combinations of HA and NA proteins are possible. Each combination represents a different subtype. All known subtypes of influenza A viruses can be found in birds ***(CDC, 2007a).***

Usually, "avian influenza virus" refers to influenza A viruses found chiefly in birds, but infections with these viruses can occur in humans. The risk from avian influenza is generally low to most people, because the viruses do not usually infect humans ***(CDC, 2007a).***

"Human influenza virus" usually refers to those subtypes that spread widely among humans. There are only three known A subtypes of influenza viruses (H1N1, H1N2, and H3N2) currently circulating among humans. It is likely that some genetic parts of current human influenza A viruses came from birds originally. Influenza A viruses are constantly changing, and they might adapt over time to infect and spread among humans ***(CDC, 2007a).***

Influenza viruses are dynamic and are continuously evolving. Influenza viruses can change in two different ways: antigenic drift and antigenic shift. Influenza viruses are changing by antigenic drift all the time, but antigenic shift happens only occasionally. Influenza type A viruses undergo both kinds of changes ***(Thomas and Noppenberger, 2007)***.

Antigenic drift refers to small, gradual changes that occur through point mutations in the two genes that contain the genetic material to produce the main surface proteins, hemagglutinin, and neuraminidase. These point mutations occur unpredictably and result in minor changes to these surface proteins. Antigenic drift produces new virus strains that may not be recognized by antibodies to earlier influenza strains. This is one of the main reasons why people can become infected with influenza viruses more than one time and why global surveillance is critical in order to monitor the evolution of human influenza virus stains for selection of which strains should be included in the annual production of influenza vaccine ***(Thomas and Noppenberger, 2007)***.

Antigenic shift refers to an abrupt, major change to produce a novel influenza A virus subtype in humans that was not currently circulating among people. Antigenic shift can occur either through direct animal (poultry)-to-human transmission or through mixing of human influenza A and animal influenza A virus genes to create a new human influenza A subtype virus through a process called genetic reassortment.

Antigenic shift results in a new human influenza A subtype. A global influenza pandemic (worldwide spread) may occur if three conditions are met ***(Thomas and Noppenberger, 2007)***.

Although various strains of avian influenza have been recognized for decades, the scope, lethality, and mutability of the H5N1 subtype make it a likely source of the next human influenza pandemic-an event that could kill millions. H5N1 no longer is confined to waterfowl and poultry and appears to be expanding its host and geographic ranges ***(Juckett, 2006).***

Laboratory testing is the only way to confirm that infection with H5N1 influenza . Among these tests, polymerase chain reaction (PCR) is the most common approach used by most laboratories to confirm H5N1 infection ***(WHO, 2007b)***.

All countries are urged to share all H5N1 positive specimens/virus isolates from humans so that analyses of pandemic risk, development of H5N1 vaccines, development and updating of diagnostic protocols and reagents, monitoring of antiviral susceptibility patterns and other important risk assessment and response activities can proceed ***(WHO, 2007b)***.

Standardization of clinical care and antiviral management is fundamental to improve understanding of the disease course and to identify the appropriate therapy ***(Schünemann, 2007).***

AIM OF THE WORK

The current assay reviews structure and pathogencity of "avian influenza virus". It focuses on the key steps that should be undertaken in investigations and commonly used pharmacological and supportive treatment modalities on case management. It also reviews framework and approach for public health authorities and investigators at all levels to plan for and conduct investigations of human cases of avian influenza A virus (H5N1) (or other novel influenza viruses of pandemic potential) & prevention of infection for health care personnel.

AVIAN FLU VIRUSES

Influenza viruses are sophisticated organisms with highly mutagenic genomes and wide antigenic diversity ***(Skeik and Jabr, 2008).***

Influenza viruses that infect birds are called avian influenza viruses that cause avian influenza which is an important infectious disease of birds ***(Somvanshi et al., 2008).***

Taxonomy

Avian influenza virus is type A influenza virus that infect birds and belongs to the Orthomyxovirus family which consists of five genera: Influenza virus A, Influenza virus B, Influenza virus C, Isavirus, and Thogotovirus ***(Palese and Shaw, 2007).***

Morphology & Ultrastructure

Influenza viruses A, B and C are the three most important genera of Orthomyxoviridae which are a group of enveloped viruses with helical capsid and a segmented genome made of eight single-stranded negative RNA segments of 890 to 2,341 nucleotides each. Influenza A virions are spherical (100 nm) in diameter ***(Indian Journal of Microbiology, 2009).*** The virus is covered with a lipid envelope which is derived from the cell surrounding the virus particle ***(Trampuz et al., 2004).*** The lipid envelope is covered with about 500 projecting spikes, 10 nm long on the surface of the particle, 'which can be seen easily under the electron microscope' **(Fig. 1)** ***(Cox and Zeigler, 2003).***

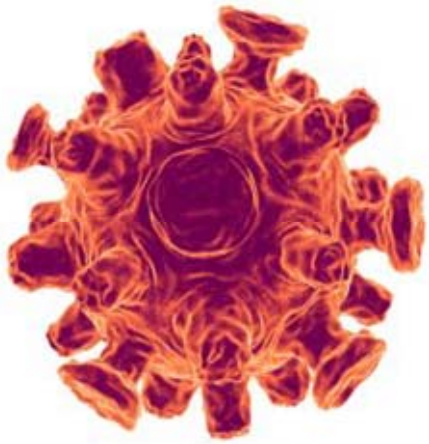

Figure (1): 3D illustration of influenza virus.
(www.abatement.com/.../pure_air_healthcare.htm, 2008).

Viral Genome

The eight RNA segments of the influenza A virus genome have eleven genes which encode 11 viral proteins. These include the polymerase proteins (PB1, PB2, PA, PB1-F2), nucleocapsid protein (NP), hemagglutinin (HA), neuraminidase (NA), matrix proteins (M1, M2), and nonstructural proteins (NS1, NS2) **(Fig. 2)** ***(Palese and Shaw, 2007).***

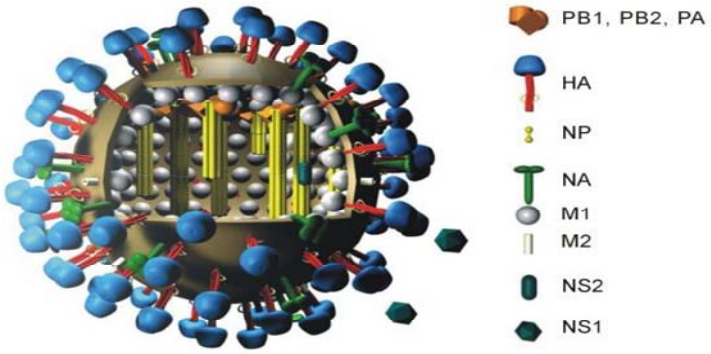

Figure (2): The molecular structure of the H5N1 virus's RNA***(Wikipedia,2008).***

This segmentation of the influenza genome facilitates genetic recombination by segment reassortment in hosts who are infected with two different influenza viruses at the same time ***(WHO, 2005a).***

1) Surface encoding gene segments

There are two surface antigen encoding gene segments which are hemagglutinin (HA) & neuraminidase (NA) ***(Greninger, 2007).***

HA gene segment codes for hemagglutinin which is an antigenic glycoprotein found on the surface of the influenza viruses that is responsible for binding of the virus to the cell that is being infected. This binding is required for efficient transfer of flu virus genes into cells. This process can be blocked by antibodies that bind to the hemagglutinin proteins ***(Greninger, 2007).***

Hemagglutinin mediates attachment to and entry of the virus into host cells by binding to sialic acid receptors at the cell surface. The binding affinity of hemagglutinin to the sialic acid residues partly accounts for the host specificity of the various influenza A virus subtype ***(Kuiken et al., 2006).*** Human viruses preferentially bind to sialic acid linked to galactose by α-2,6 linkages that are the main type found on the epithelial cells of the human respiratory tract, while avian viruses tend to bind to α-2,3 linkages that are found on duck intestinal epithelium ***(Wright et al., 2007).***

The change of one amino acid of the H5 protein is sufficient to change the receptor binding specificity of A/H5N1

viruses**.** Thus, the barriers to interspecies infection can be overcome easily ***(Gambaryan et al., 2005).*** Hemagglutinin is the main viral target of protective humoral immunity by neutralizing antibody ***(Kuiken et al., 2006).***

NA gene segment codes for neuraminidase. Neuraminidase facilitates the spread of the virions in the host by its ability to bind plasminogen and cleaving the glycosidic linkages between the surface of the viral particles and sialic acid on host cells **(Fig. 3)** ***(Causey and Edwards, 2008).*** NA is the target of neuraminidase inhibitors ***(Wright et al., 2007).***

There are 16 HA and 9 NA subtypes are known, for a total of 144 possible different influenza subtypes, each with potentially different host susceptibility ***(Causey and Edwards, 2008).***

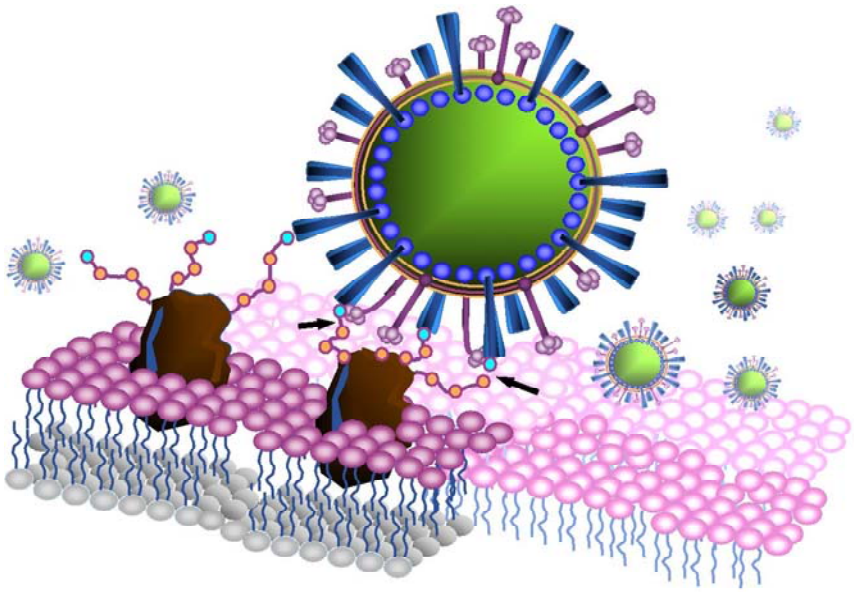

Figure (3): Neuraminidase help release of new virions ***(wisdom.eu-egee.fr/avianflu/pictures, 2008).***

2) Internal encoding gene segments

The Internal viral protein encoding gene segments on RNA molecule are: Matrix proteins (M), nucleocapsid protein (NP), nonstructural proteins (NS), polymerase proteins (PA, PB1, and PB2) ***(Squires et al., 2008).***

a) Matrix encoding gene segments

M gene codes for the matrix proteins (M1 and M2) that along with the two surface proteins (hemagglutinin and neuraminidase) make up the capsid (protective coat) of the virus. It encodes by using different reading frames from the same RNA segment ***(Squires et al., 2008).***

M1 is a protein that binds to the viral RNA. M2 is a protein that uncoats the virus exposing its contents (the eight RNA segments) to the cytoplasm of the host cell. The M2 transmembrane protein is an ion channel required for efficient infection by facilitating the pH-dependent dissociation of matrix proteins from the nucleocapsid during viral uncoating **(Fig. 4)** ***(National Academy of Sciences, 2004).*** It is also important for the trans-Golgi network during maturation of hemagglutinin molecules. M2 is the target of the adamantanes (amantadine and rimantadine). Mutation in the M2 from serine to asparagine at residue 31 invariably confers resistance to adamantanes ***(WHO, 2005a).***

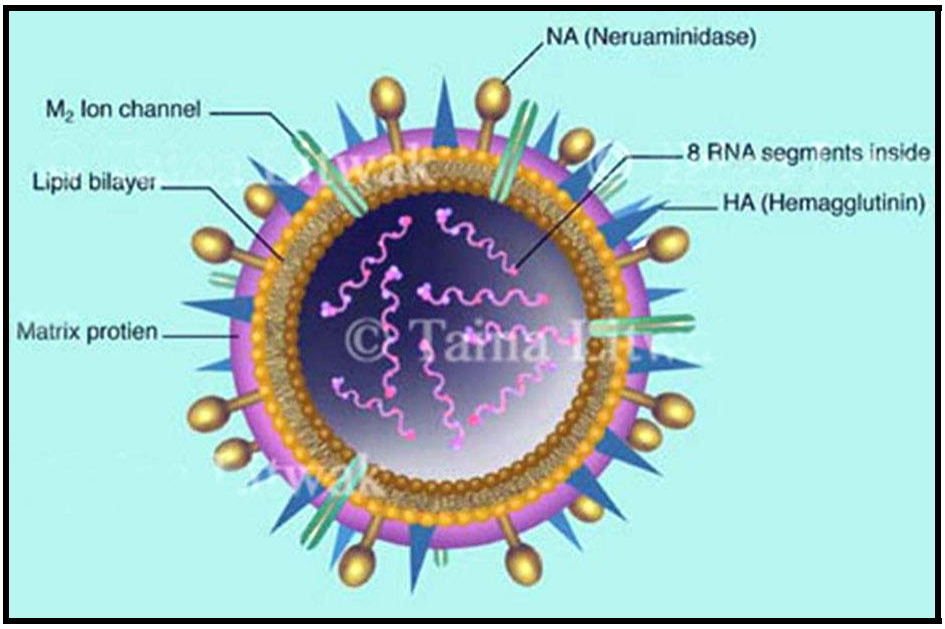

Figure (4): M2 ion channel
(science-art.com/gallery/31/31_1082005155557.jpg, 2008).

b) Nucleoprotein encoding gene segments

NP gene codes for neucleoprotein. NS gene codes for two nonstructural proteins [(NS1) and nuclear export protein (NEP) formerly known as (NS2)]. "The pathogenicity of influenza virus was related to the nonstructural (NS) gene of the H5N1/97 virus" ***(CDC, 2006a).***

Nonstructural protein1 (NS1) is multifunctional protein that is one determinant of virulence functions to defeat the cellular innate immune response by affecting cellular RNA transport, splicing, translation, thus controlling the temporal synthesis of viral-specific mRNA and viral genomic RNAs ***(Min et al., 2007).***

Its anti-interferon action which circumvent the host cell antiviral responses ***(Greninger, 2007)*** achieved in a number of ways, including:

i) Blocking 2'-5'-oligoadenylate synthetase (2'-5'-OAS) activation of RNase ***(Min and Krug, 2006).***

ii) Limiting the induction of IFN-β by preventing the activation of transcription factors ***(Ludwig, 2002).***

iii) Interacting with the cellular protein phosphatidylinositol-3-kinase (PI3-kinase) ***(Ehrhardt, 2007 and Shin et al., 2007),*** which may cause a delay in virus-induced apoptosis ***(Zhirnov and Klenk, 2007).*** It has also been shown that NS1 prevents the maturation of human primary dendritic cells, thereby limiting host T cell activation ***(Fernandez-Sesma, 2006).***

Recent large-scale genome sequence analysis of avian influenza virus isolates indicated that four C-terminal residues of the NS1 protein may represent a virulence determinant ***(Jackson et al., 2007).***

The NS1 of the highly pathogenic avian H5N1 viruses might be responsible for an enhanced proinflammatory cytokine response especially (TNFα) induced by these viruses in human macrophages ***(Gambaryan et al., 2006)***. H5N1 NS1 is characterized by a single amino acid change at position 92. By changing the amino acid from glutamic acid to aspartic acid, the researchers were able to abrogate the effect of the H5N1 NS1. This single amino acid change in the NS1 gene greatly increased the pathogenicity of the H5N1 influenza virus ***(Harder and Werner, 2006).***

The nuclear export protein (NEP), formerly referred to as the NS2 protein mediates the export of virus ribonucleoproteins (vRNPs) ***(Chen et al., 2004).***

c) Polymerase encoding gene segments:

The influenza virus RNA polymerase is a multifunctional complex composed of the three viral proteins [Polymerase protein B1(PB1), polymerase protein B2 (PB2) and polymerase protein A (PA)] which, together with the viral nucleoprotein (NP), form the minimum complement required for viral mRNA synthesis and replication ***(Michael et al., 2006).***

PA gene codes for the polymerase protein A which is a critical component of the viral polymerase ***(Causey and Edwards, 2008).***

PB1 gene codes for the polymerase protein B1 which is also a critical component of the viral polymerase and the polmeraseB1-F2 protein which is encoded by an alternative open reading frame of the PB1 RNA segment and interacts with 2 components of the mitochondrial permeability transition pore complex sensitizing cells to apoptosis ***(Chanturiya et al., 2004).*** PB1-F2 likely contributes to viral pathogenicity and might have an important role in determining the severity of pandemic influenza ***(Li et al., 2005).***

After comparing viruses from the Hong Kong 1997 H5N1 outbreak, one amino acid change (N66S) was found in the PB1-F2 sequence at position 66 that correlated with

pathogenicity. This same amino acid change (N66S) was also found in the PB1-F2 protein of the 1918 pandemic A/Brevig Mission/18 virus ***(Robert et al., 2006).***

PB2 gene codes for the polymerase B2 protein which is a component of the ribonucleoprotein (RNP) complex, transported between nucleus and cytosol during viral infection and is therefore likely to require host adaptation ***(Chen, 2007).***

Indeed, specific mutations of the PB2 protein are known to participate in human-to-human transmission adaptation ***(Chen, 2007).*** Several studies have reported amino acid sites thought to be involved in this adaptation and Genomic signatures of human versus avian influenza A viruses ***(Wright et al., 2007).***

As of 2005, 75% of H5N1 human virus isolates had a mutation consisting of Lysine at residue 627 in the PB2 protein; which is believed to cause high levels of virulence ***(WHO, 2006a).*** Until H5N1, all known avian influenza viruses had a Glutamine at position 627, while all human influenza viruses had a lysine ***(Govorkova et al., 2005 and Amonsin et al., 2005).***

As of 2007, "The emergence of 3 (or more) substrains from the EMA [EMA=**E**urope, **M**iddle East, **A**frica] clade represents multiple new opportunities for avian influenza (H5N1) to evolve into a human pandemic strain. In contrast to strains circulating in Southeast Asia, EMA viruses are derived from a progenitor that has the PB2 627K mutation. These

viruses are expected to have enhanced replication characteristics in mammals. Indeed the spread of EMA has coincided with the rapid appearance of cases in mammals**.** Lysine at PB2–627 is believed to confer to avian H5N1 viruses the advantage of efficient growth in the upper and lower respiratory tracts of mammals ***(CDC, 2007a).***

Types of Influenza Viruses

The influenza viruses are three types A, B and C. Influenza A viruses are classified into subtypes based on the two surface proteins hemagglutinin (HA) and neuraminidase (NA), while influenza B and C are not classified into subtypes. Influenza A subtypes and B viruses are further classified into strains. New strains of influenza viruses appear and replace older strains. This process occurs through antigenic drift ***(Palese and Show, 2007).***

1) Influenza A subtypes

Influenza type A viruses can infect people, birds, pigs, horses and other animals, but wild birds are the natural hosts for these viruses. Only influenza A viruses and all its known subtypes can infect birds. However, there are substantial genetic differences between the subtypes that typically infect both people and birds. Within subtypes of avian influenza A viruses there are also different strains ***(CDC, 2007a).***

Influenza type A viruses are divided into subtypes and named on the basis of two proteins on the surface of the virus: hemagglutinin (HA) and neuraminidase (NA). For example, an

"H7N2 virus" designates influenza A subtype that has an HA 7 protein and a NA 2 protein. Similarly an "H5N1" virus has an HA 5 protein and an NA 1 protein. There are 16 known HA subtypes and 9 known NA subtypes. Many different combinations of HA and NA proteins are possible. Only some influenza A subtypes (i.e., H1N1, H1N2, and H3N2) are currently in general circulation among people. Other subtypes are found most commonly in other animal species. For example, H7N7 and H3N8 viruses cause illness in horses, and H3N8 also has recently been shown to cause illness in dogs ***(CDC, 2007a).***

However, there are substantial genetic differences between the influenza A subtypes that typically infect birds and those that infect both people and birds Three prominent subtypes of the avian influenza A viruses that are known to infect both birds and people are: Influenza A H5, Influenza A H7 and influenza A H9 ***(CDC, 2007b)***.

Avian Influenza A H5 and H7 viruses are potentially nine different subtypes. They can be distinguished as "low pathogenic" and "high pathogenic" forms on the basis of genetic features of the virus and the severity of illness they cause in poultry. H5 infections have been documented among humans, sometimes causing severe illness and death ***(CDC, 2007a)***.

Although, the majority of H7 infections have resulted in self-limiting conjunctivitis and probable human-to-human transmission has been rare, glycan microarray technology determine the receptor-binding preference of H7 influenza