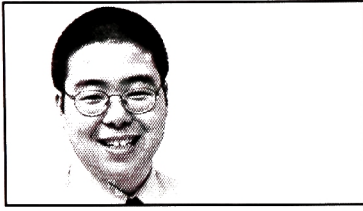# Avian Flu H5N1: The Edge of Pandemic

Jaron Chong

Entering the scene on May 21st, 1997, a human pathogenic H5N1 strain of Avian influenza was isolated from a clinic in Hong Kong (Nature, 2005). What had typically been known as a strain of influenza afflicting only birds had suddenly made the jump to being pathogenic in humans. That singular event has triggered a tremendous amount of research activity and H5N1 has since captured the attention of international health organizations, governments, and the media. Despite this overwhelming concern, as of October 24th, 2005, only a total of 62 deaths directly attributable to H5N1 have been reported to the World Health Organization since 2003 (WHO, 2005). This is in stark contrast to the 10-25% of Canadians that are expected to contract conventional flu annually, with these cases resulting in an estimated 500-1500 influenza-related fatalities (Health Canada, 2003). What is the basis of our distress with H5N1? Is it justified? Is it all hype? Not surprisingly, the truth lies somewhere in the middle.

INFLUENZA STRUCTURE AND FUNCTION

To understand H5N1, we must first establish the features common to all influenza subtypes. Influenza can be classified into three categories: A, B, and C. The distinctions are made from differences in nucleoprotein, matrix protein, visual attributes, and genomes. H5N1 itself is categorized as an influenza type A. (Horimoto, 2005)

Figure 1 is a graphical representation of a typical influenza virion (a virus particle). The two most striking features present are the glycoprotein spikes located on the surface of the virion envelope as well as the eight genome segments located within. The spikes generally consist of two proteins: rod-shaped hemagglutinin (HA) and mushroom-shaped neuraminidase (NA). Variants of HA and NA exist and are characteristic of an influenza subtype. Researchers label subtypes of influenza based upon these two proteins, combining the assigned HA number and NA number to form a name like H5N1. The primary function of these surface proteins is to facilitate the transport of the virus to and from the host cell during influenza's life cycle (Figure 2).

Hemagglutinin and neuraminidase are encoded by and translated from the 4th and 5th segments of influenza's RNA genome, appropriately named HA and NA. A high degree of genetic shift and reassortment will result in the various subtypes of influenza, a process greatly accelerated due to the modular nature of influenza's genome. Each surface protein variant has a unique amino acid sequence and will induce a specific response from the adaptive immune system. As the expressed variants of hemagglutinin and neuraminidase are in constant flux from one generation to the next, no single vaccine can permanently inoculate a population. Vaccines must be updated to reflect the predicted dominant influenza strain for the upcoming season of inoculation.

Influenza A virus
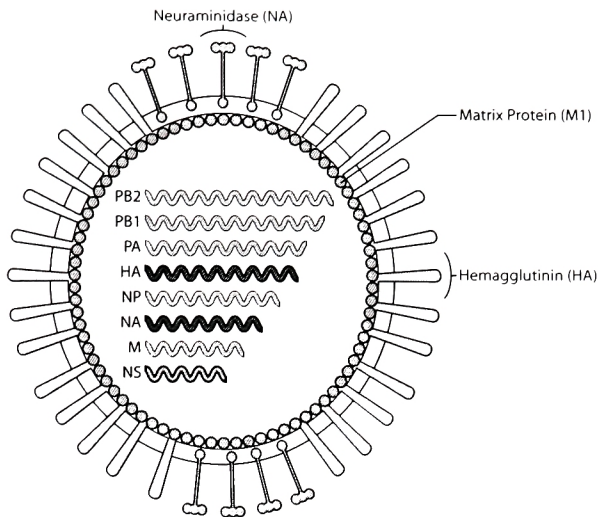
Figure 1: Representation of an Influenza virion (a virus particle). Notable features include the surface glycoprotein spikes hemagglutinin (HA) and neuraminidase (NA). The eight genome segments promote accelerated genetic recombination of influenza (Murphy & Webster, 1996).
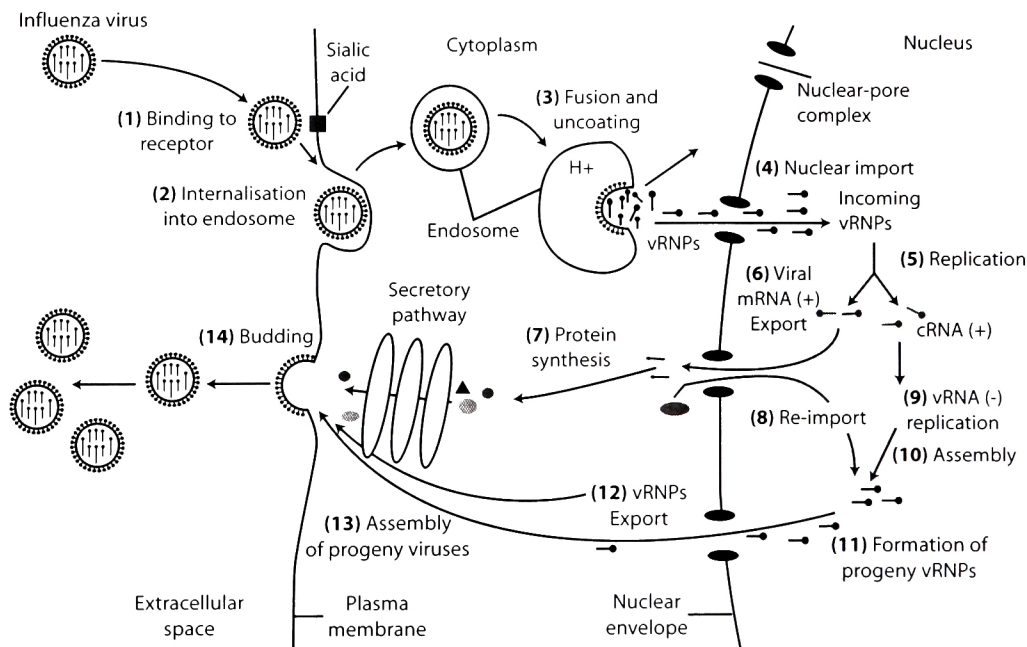
After fusion of the virion with the host cell takes place, endosome uncoating results in the release of the viral genome (viral RiboNucleoProteins; vRNPs) into the cytoplasm. vRNPs are then imported into the nucleus where they are replicated. Positive-sense mRNA is exported out of the nucleus back into the cytoplasm to synthesize influenza proteins. Some of these proteins are re-introduced back into the nucleus to assist with viral RNA replication and vRNP assembly. Towards the end of the replication cycle, vRNPs form and leave the nucleus. The final stage then involves the progeny virion assembling and budding from the host cell's plasma membrane completing the cycle of infection and replication (Whittaker, 2001; Knipe & Howley, 2001).

INCREASED H5N1 VIRULENCE

H5N1 has been shown to exhibit much greater virulence than common strains of influenza. As of October 26, 2005, H5N1 has displayed a fatality rate of just over 50%, with the previously mentioned fatality count of 62 being out of a total 112 cases (WHO, 2005). While this figure may be inflated due to incomplete H5N1 surveillance and sub-clinical infections (i.e. infections of H5N1 that do not result in symptoms), it still remains far in excess of the usual 0.06% to 0.25% fatality rate of typical influenza (CDC, 2005a).

## Replication Cycle of an Influenza Virus

Figure 2: Replication cycle of an influenza virus. (1) The influenza virion binds to sialic-acid containing receptors on the surface of the host cell and (2) fuses to form endosomes. (3) Endosome uncoating results in the release of the viral genome (viral RiboNucleoProteins; vRNPs) into the cytoplasm. vRNPs are then (4) imported into nucleus where they are (5) replicated. (6) Positive-sense mRNA is exported out of the nucleus back into the cytoplasm (7) to synthesize influenza proteins. Some of these proteins are (8) re-introduced back into the nucleus to assist with (9) viral RNA replication and (10) vRNP assembly. Towards the end of the replication cycle, (11) vRNPs form and (12) leave the nucleus. The final stage then involves the (13) progeny virion assembling and (14) budding from the host cell's plasma membrane (Whittaker, 2001).

What is it about H5N1 that causes this increased virulence?

The answer is by no means simple and the question is still being actively pursued by researchers. There are, however, several theories explaining this increased virulence.

Human H5N1 Results from a Zoonotic (Animal-To-Human) Transmission

Aquatic birds form the primary reservoir for influenza (Figure 3) and yet with most strains of waterfowl, influenza is non-lethal (Horimoto, 2001). This interesting observation raises a key concept in the gene-centred view of evolution: given sufficient time and generations of replication, an organism is expected to evolve to maximize the number of copies of its genome globally, also known as its inclusive fitness. This is the very concept that Richard Dawkins proposes in his influential but controversial book, *The Selfish Gene* (Dawkins, 1976).

Habitats of influenza A viruses
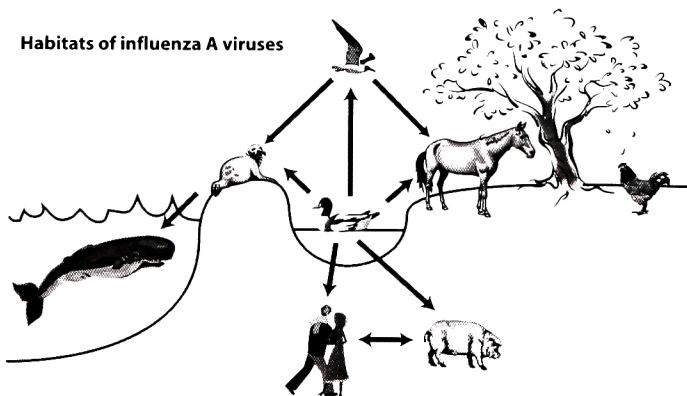
Figure 3: Waterfowl are the predominant reservoirs for influenza A viruses such as H5N1. Contact between species can result in viral transmission and adaptation to a new host population. Zoonotic (Animal-To-Human) transmissions are lethal as humans most likely lack pre-existing immunity (Horimoto and Kawaoka, 2001).

Assuming the validity of the gene-centred view of evolution, we arrive at an important conclusion: lethality is not in the best interest of any virus for it destroys the host the virus lives within. Without a host, a virus can neither replicate nor propagate. Evolutionarily, natural selection would favour strains of viruses that possess high transmission and infectivity but low case-fatality (Dawkins, 1976). This natural selection takes time as a particular virus becomes adapted to a new host. However, in the short term, zoonotic viral transmission can render any previously acquired immune modulating adaptations ineffective and virulence can dramatically increase to the detriment of the new host species. This viral novelty in conjunction with a lack of any pre-existing immunity in the host culminates in a highly virulent pathogenic transmission, suspected to be the case with H5N1 (Lederberg, 1997).

Potential Mechanisms of Increased Virulence

Several mechanisms have been proposed to explain the workings of this increased virulence.

Increased Hemagglutinin Cleavage

One proposed mechanism considers genetic variability of the hemagglutinin surface glycoprotein. As previously mentioned, hemagglutinin (HA) is critical in fusing influenza with host cells. It does this by attaching to sialic-acid receptors on the surface of a host cell and undergoing a conformational change. This fuses the viral envelope and the host cell together, initiating the complex viral infection process leading to the production of more virion (Whittaker, 2001). In the late stages of influenza replication, the HA expressed on the newly created virion will bind to its host cell while being released and prevent immediate dispersion of the infection. Surrounding proteases (protein-degrading enzymes) cleave the HA, releasing the newly created virion and leaving it free to infect neighbouring cells. These proteases are oftentimes body region-specific, meaning viruses will adapt their HA glycoproteins to cleave in the presence of certain enzymes, most commonly, those present near respiratory endothelial cells (Goto & Kawaoka, 1998). Research conducted by Goto and Kawaoka describes how a critical adaptation of neuraminidase (NA) protein is able to bind and increase the amount of surrounding plasminogen proteases and thereby enhance HA cleavage. As a result, the variety of tissues which are susceptible to infection is increased (Goto & Kawaoka, 1998). As this mechanism was discovered to apply to a murine (mouse) strain of influenza derived

from H1N1 (the 1918 Spanish Flu strain), there is a remote possibility that a similar mechanism occurs with Avian Flu H5N1. At the moment, this mechanism has only been observed in laboratory strains and not in wild-type H5N1 (Katz et al., 2000). However, should the connection be made between enhanced HA cleavage and H5N1, it would be consistent with the increased virulence and systemic (body-wide) symptoms being observed in patients such as multi-organ failure and non-bacterial sepsis syndrome (Beigel et al., 2005). Investigations into this possibility are ongoing.

H5N1 Escapes Anti-Viral Cytokine Responses

Another recent theory for increased virulence relates to findings that describe lethal H5N1 influenza virus having the ability to escape host anti-viral cytokine responses. The key genome segment implicated in this immune system evasion is NS1, the only nonstructural protein of the influenza A virus. NS1's primary function is "the inhibition of cellular, anti-viral interferon (IFN) response" (Garcia-Sastre et al., 1998; Cox et al., 2005, pg. 658). Despite these viral strains inducing a strong immune response, they remain resistant to any antiviral effects. In a study conducted by Seo, Hoffman, and Webster, it was demonstrated that the 1997 Hong Kong strain of H5N1 is unaffected by the activity of interferons and tumour necrosis factor-alpha, two cytokines released by the body's innate immune system and the first line of defence in the response against an influenza infection (2002). In this theory, a mutation in the NS1 molecule of H5N1's genome that encodes two non-structural proteins seems to confer resistance to H5N1, a conclusion drawn from the unaffected viral titers (concentrations) after treatment with a variety of cytokines. Their hypothesis is that the NS genome segment both resists degradation and encodes proteins that inactivate anti-viral proteins produced by the host's defence system. Further specifics and mechanisms are currently unknown (Seo, Hoffman, & Webster, 2002).

LETHALITY IS NOT IN THE BEST INTERESTS OF ANY VIRUS FOR IT DESTROYS THE HOST THE VIRUS LIVES WITHIN. WITHOUT A HOST, A VIRUS CAN NEITHER REPLICATE NOR PROPAGATE.

PREREQUISITES FOR A PANDEMIC

Put simply, there are three prerequisites in order for a pandemic to emerge.

1. Novel: A novel pathogen emerges – one that humans have no immunity against.
2. Virulent: It infects humans causing serious illness.
3. Contagious: It spreads easily and sustainably among humans.

As amply demonstrated, H5N1 is both novel and virulent. It is novel in the sense that the human population has not been exposed in any significant manner to H5N1 and therefore any hosts it encounters will lack immunity. H5N1 has definitely shown to be a highly virulent strain of influenza with a large case fatality rate (CDC, 2005b).

All that remains is for H5N1 to be contagious. Therein lies the lynchpin to the entire sound, fury, and commotion - for without "the establishment of efficient and sustained human-to-human transmission of the virus" (CDC, 2005b, para. 27) all of our fears remain hypothetical.

ASSESSING THE PANDEMIC POTENTIAL

Imagine a highly contagious airborne pathogen with a 50% fatality rate and a mean time from onset of illness to death ranging from 9 to 23 days (WHO, 2005; Beigel et al., 2005). That scenario is exactly what faces the international health community should H5N1 obtain the final pandemic prerequisite of human-to-human transmission. As of October 26, 2005, reports have been inconclusive as to whether efficient human-to-human transfer has occurred. Transmissions reported have ranged from household infections to a single case of child-to-mother transmission (Beigel et al., 2005; Hien et al., 2004; Ungchasuk et al., 2005). However, the major criticism of these reports has been that primary infection from avian sources or poultry sources were the likely vector of infection and in the case of the child-to-mother transmission, close

contact between child and mother was involved. All current reports indicate that transmission through regular contact is very inefficient and has a low likelihood of transmission.

Further underscoring how the current strain of H5N1 in south-east Asia is currently unable to transmit very effectively is a cross-sectional survey conducted on hospital workers working with H5N1 case-patients in Hanoi, Vietnam. While more than 95% reported exposure to more than one H5N1 case-patient, 72% reported symptoms, and 2.4% fulfilled criteria for a secondary infection, not one employee had detectable antibodies for H5N1 Influenza A (Liem, 2004).

How will H5N1 acquire the ability to efficiently transmit from human-to-human?

Influenza ordinarily acquires mutations through two processes: antigenic drift and antigenic shift. Of the two processes, antigenic drift is the more gradual whereby natural selection pressures applied to influenza strains select for advantageous hemagglutinin (HA) and neuraminidase (NA) genes. A single point mutation can result in sufficient drift to confer a benefit to the influenza virus. However, it remains unlikely that efficient human-to-human transmission will evolve from such gradual processes (Cox et al, 2005).

Antigenic shift is a change of a significantly greater order whereby RNA segments from two different viral strains are reassorted. As previously mentioned, the eight genetic segments in which influenza A's genome is composed allow for exchanges between viral strains. Multiple scenarios have been proposed whereby reassortment might take place between conventional human influenza, which has efficient human-to-human transmission and Avian Bird Flu H5N1, which does not. There are two ideal hosts for this exchange. An H5N1 infected human could also simultaneously be infected with conventional influenza. With both viruses in a common organism, reassortment can occur. Alternatively, reassortment could take place in an intermediary organism such as swine under conditions of dual infection. In either exchange of genetic components, should a combination be created that retains the

virulence of H5N1 with the transmission abilities of conventional influenza, all three prerequisites for a pandemic would be fulfilled (Horimoto & Kawaoka, 2001). This reassortment has not yet been reported and is the critical event that international health organizations are anxiously awaiting. It is the last remaining prerequisite for pandemic Avian Flu.

Two Down, One To Go

From the epidemiological information present, it is clear that a grave threat exists should H5N1 acquire pandemic status. Many comparisons have been made between the Avian Bird Flu and the 1918 Spanish Flu pandemic that claimed countless lives (Horimoto & Kawaoka, 2001; Seo et al., 2002; Unchusak et al., 2005). In popular media, it is often stated that we are "overdue" for a pandemic (Piller, 2005). Such claims imply disasters follow some preordained schedule – a misleading impression. All that is occurring is simply viral microevolution, following no set timeline and having very few certainties.

It is at this unique point in time that we can be both calm and anxious. We can take solace in the knowledge that on the global stage, H5N1 still has a minor effect on loss of life in comparison to conventional influenza. At the same time, the excellent surveillance efforts and cooperation of the World Health Organization, the Centers for Disease Control and Prevention, and national health agencies throughout the world must continue and be adequately funded. Public attention may wax and wane with the whims of popular media, but it is ultimately the long-term efforts of scientists, epidemiologists, and health policy makers that will determine our preparedness and response. 𝕄