Influenza pandemic : an overview

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Chapter 1

Influenza Pandemic: An Overview

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Introduction

Influenza A, B, and C viruses are negative sense single stranded RNA viruses belonging to the Orthomyxoviridae family. Influenza B viruses predominately infect humans, but have been isolated from seals (Osterhaus et al., 2000) and have been shown able to experimentally infect ferrets (Jakeman et al., 1994) and mice (Chen et al., 2001). Influenza C viruses have a range of described hosts including humans, dogs, and pigs. In comparison to influenza B and C viruses, influenza A viruses have a broader range of avian and mammalian hosts and they are further subdivided into subtypes based upon the antigenicity of their neuraminidase (NA) or hemagglutinin (HA) surface glycoproteins. Currently there are 9 NA and 16 HA subtypes indentified, but only N1 and N2 and H1, H2, and H3 have been documented as forming stable lineages in humans. Because of the fact that it is only influenza A viruses that have shown the capacity to emerge as pandemic viruses in humans, the remaining sections of this review will concentrate solely on these viruses.

Influenza A virus particles consist of 8 segments of RNA encoding up to 11 proteins: HA, NA, nucleoprotein (NP), matrix 1 and 2 (M1 and M2), polymerase acidic (PA), polymerase basic 1, 1-F2, and 2 (PB1, PB1-F2, PB2), non structural 1 and 2 (NS1 and NS2- also known as nuclear export protein or NEP). The virus particle is approximately 80-120 nanometers in diameter and is mainly spherical but can sometimes exist in a filamentous form (Lamb and Choppin, 1983). The particle is made up of a M1 protein-lined host-derived virus envelope in which the HA, NA, and M2 proteins are embedded and which encloses the ribonucleoprotein containing core.

In influenza terms, a pandemic is a disease caused by a novel virus that rapidly spreads through humans populations across large geographical areas. In contrast, an epidemic is a disease outbreak that affects more people than normal but is localized to one region. In humans, seasonal influenza typically reaches epidemic proportions during most winters in temperate climates while pandemics which are caused by interspecies transmission of animal viruses to humans are less frequent but of greater concern. The World Health Organization (WHO) has created a stage categorization system (stages 1 to 6 followed by "post-peak" and "post pandemic" stages) that defines epidemiologic features of an emerging outbreak. Stage 6 is characterized by global spread and marks the official declaration of a pandemic.

Previous influenza pandemics

The first reliable account of an influenza pandemic was in the summer 1580, where influenza-like illness spread from Asia to Africa and on to Europe (Potter, 2001). The second suspected influenza pandemic began in Russia during the spring of 1729, spread around the globe in about 3 years and became more severe in subsequent waves of infection (Patterson, 1986; Potter, 2001). The last influenza pandemic of the 18 century started in China in the fall of 1781 and quickly spread throughout Europe. Following the 1781 pandemic, there were three influenza pandemics in 1830-1831, 1833-1834, and a more severe Russian Flu from 1889 to 1890. The Russian flu originated in Central Asia in 1889 and rapidly spread along the rail networks into Europe (Tognotti, 2009). By December of 1889, it had already spread to North America and extended on to Asia, New Zealand, and Australia by March of 1890 (Cunha, 2004). Although the Russian flu was severe, historical records indicate the mortality was around 1% with an estimated 1 million deaths (Cunha, 2004).

In the past 100 years, there have been a total of four influenza pandemics (Figure 1). The first and most severe of these, the "Spanish flu", occurred from 1918-1919 with an estimated 2.5% mortality worldwide (Tognotti, 2009). Although disputed, the Spanish flu likely started in North America in March of 1918 (Potter, 2001) and rapidly spread across the U.S. among military troops and later into Europe during World War I. With the large number of movement of troops across Europe the causative H1N1 virus rapidly spread across Europe and had reached China and the Philippines by July of 1918 (Potter, 2001). The Spanish flu spread in three waves of increasing virulence.

Two pandemics occurred in the 1950's and 1960's; the "Asian" and "Hong Kong" influenza pandemics. The Asian influenza pandemic appeared in February of 1957 in China and was caused by a virus of the H2N2 subtype. This virus transmitted readily among the human population that was immunologically naive to this new subtype and it displaced the circulating H1N1 virus, although the latter reemerged in 1977 (Potter, 2001). Within 6 months the "Asian" flu had circulated worldwide and by 1958 an estimated 1-2 million people had died (Tognotti, 2009). The next pandemic began in 1968 in Hong Kong and was caused by a virus of the H3N2 subtype. As in 1957, the new virus rapidly dis-
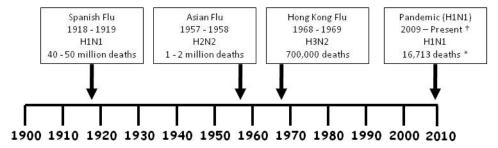

Figure 1:   **Timeline of modern influenza pandemics.** † As of 19 March 2010, the H1N1 pandemic status remains at phase 6. * As of 19 March 2010 there have been 16,713 reported deaths attributed to the 2009 H1N1 pandemic. This value is an underestimate because many deaths are not confirmed cases of influenza and may be the result of other conditions.

placed the circulating 1957 pandemic virus. By mid 1969 the new pandemic virus had spread to Africa, North and South America (Tognotti, 2009).

The current influenza pandemic

The pandemic of the new millennium was detected in March of 2009, when nasopharyngeal swabs were collected from two epidemiologically unrelated children in California who were experiencing respiratory infections. The swabs tested positive for influenza but could not be subtyped. By mid April, a novel H1N1 virus (pH1N1) was identified by the U.S. CDC (Dawood et al., 2009). Retrospectively it was shown the virus had already been circulating for some time in Mexico. By May 6th 2009, the virus had spread beyond North America to South America and Europe with 1882 laboratory confirmed cases (Centers for Disease Control and Prevention, 2009a).

In a little more than a month, June 11th 2009, the number of laboratory confirmed cases had jumped to 28,774 as reported to the WHO by 74 countries with 144 deaths giving a conservative mortality rate of about 0.5% which is slightly higher than seasonal influenza (World Health Organization, 2009). At this time, the WHO raised the pandemic alert to phase 6 indicating sustained human-to-human transmissions in multiple regions with no possibility of geographic containment. It took previous pandemics strains several months to spread across the globe while pH1N1 virus took a little more than a month. This can likely be attributed to more accessible modes of transportation and increased affordability of international.

Although pH1N1 continued to circulate widely and spread to additional countries, the number of new cases detected began to decline in July suggesting the initial wave of the pandemic was on the decline (Figure 2). By late September and early October, both the CDC and WHO reported increasing flu activity in temperate climates signaling the start of the second wave of the pandemic which peaked by late October and early November of 2009 in the Northern Hemisphere and was at near baseline levels by March 2010 (Figure 2).

A remarkable response to the 2009 pandemic was the near real-time characterization of the virus and the dissemination of this information. The pandemic virus originates from a reassortment event between a "triple reassortant" North American swine virus, and an Eurasian swine strain (Dawood et al., 2009; Garten et al., 2009). The North American triple reassortant lineage established itself in the US swine population in 1998 (Zhou et al., 1999) and has been identified in humans in the US from 2005 to 2009 (Shinde et al., 2009). The Eurasian swine viruses transmitted from birds to swine in Europe in the late 1970's. Antigenically, and as suggested by the genetic data, the pandemic virus shares more antigenic sites with North American swine viruses than with the seasonal human H1N1 strains (Garten et al., 2009).

Smith et al. have used evolutionary reconstructions to confirm that each gene segment of pH1N1 had swine ancestry, although the pandemic virus was not detected in swine before its emergence in humans (Smith et al., 2009a). These investigators also hypothesized that pH1N1 had infected humans several months before the first human case was identified and that this was also likely the case with the three pandemic viruses of the 20th century (Smith et al., 2009a; Smith et al., 2009b).
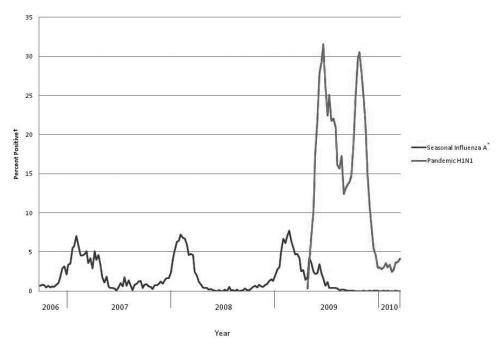
Figure 2:   **Influenza activity in the United States** (adapted from CDC data). † The percent
positive includes only subtyped laboratory confirmed cases divided by the total number
of samples tested.* Seasonal Influenza values include both H1N1 and H3N2 subtyped
viruses.

Since its detection in humans, pH1N1 viruses have shown a very low genetic diversity
suggesting a unique introduction into the human population as well as a relatively naïve
population limiting the selection pressure thus far (Garten et al., 2009).

Properties of the current pandemic influenza virus

pH1N1 viruses have been shown to be slightly more pathogenic than seasonal H1N1
viruses in mice and ferrets models (Itoh et al., 2009; Maines et al., 2009; Munster et al.,
2009). Unlike seasonal H1N1 strains, pH1N1 viruses replicate in the intestinal tract
(Maines et al., 2009) as well as in the trachea, bronchi, and bronchioles of ferrets (Mun-
ster et al., 2009).

In pigs, where pH1N1 infections have been acquired from humans, the resulting disease
is similar to that caused by endemic swine influenza viruses with similar clinical symptoms
as well as viral tropism (Brookes et al., 2010; Itoh et al., 2009). In guinea pigs, pH1N1
viruses replicate with similar efficiency to that of a seasonal H3N2 influenza virus (Steel et
al., 2010) and they have also been shown able to replicate in non-human primates (Itoh et
al., 2009). In adult humans, pH1N1 induced symptoms are generally similar to those of sea-
sonal influenza with clinical manifestations such as fever, cough and sore throat (Shimada
et al., 2009). In the young, however, disease progression can be somewhat more severe with
the pandemic strain and it is able to replicate lower in the respiratory tract.

The transmission of pH1N1viruses has been studied in several animal models. In ferrets, the gold-standard for modeling transmission in humans, the virus was efficiently transmitted to contact animals (Itoh et al., 2009; Maines et al., 2009; Munster et al., 2009). Comparing transmission of pH1N1 and seasonal H1N1 viruses in ferret was, however, not trivial: while Munster et al. observed equally efficient aerosol or respiratory droplets transmissions for the two viruses (Munster et al., 2009), pH1N1 had less efficient respiratory droplet transmission in a study by Maines et al. (Maines et al., 2009) highlighting the importance of experimental variations. pH1N1 viruses are also transmitted well in guinea pigs (Steel et al., 2010) and pigs (Brookes et al., 2010; Itoh et al., 2009; Lange et al., 2009), although chickens in contact with infected pigs did not contract the virus (Lange et al., 2009).

The ferret model has widely been used to evaluate co-infections and viral fitness between seasonal (H1N1 or H3N2) and pH1N1 viruses. In a recent study (Perez et al., 2009), all ferrets in contact with pandemic and seasonal virus infected ferrets contracted only the pandemic strain suggesting a biological advantage of pH1N1 over its seasonal counterparts.

In the influenza season of 2009, seasonal and pandemic H1N1 strains co-circulated in the Southern hemisphere raising the possibility of natural co-infections. In New Zealand, seasonal H1N1 was the dominant strain circulating during the early months and co-infections with pH1N1 were therefore of particular concern due to the risk of emergence of a pandemic virus containing a contemporary seasonal H1N1 neuraminidase gene; a gene which imparts oseltamivir resistance. The two viruses circulated in the country with varying ratios throughout the winter: in the week of June 8th, 14% of the influenza positive samples were confirmed as pH1N1, this number rising to 80% in the week of June 29th (Centers for Disease Control and Prevention, 2009b). Natural co-infection events involving pH1N1 and seasonal H1N1 viruses have been described but so far no natural reassortant have been identified (Scalera and Mossad, 2009). Another fear is that pH1N1 might reassort with highly pathogenic H5N1 strains eventually leading to a much higher case fatality rate.

Risk factors for infection with pandemic influenza viruses

Several factors are associated with increased susceptibility to pandemic influenza viruses. Underlying medical conditions such as hypertention, pulmonary diseases and immunocompromised status all contribute to increased susceptibility (Campbell et al., 2010). The mechanisms related to an increased risk for infection with zoonotic viruses such as H5N1 are not yet fully elucidated, however, reports suggest that exposure to wild birds (Bouscambert-Duchamp et al., 2009) or variations in host genes (Boon et al., 2009; Ocana-Macchi et al., 2009; Srivastava et al., 2009) are important. Pre-existing diseases also place individuals at higher risk to severe outcome with pH1N1 infection but it should be noted that factors such as age, obesity, secondary bacterial infections, and pregnancy have been the focus of research. With mechanisms not yet understood, young people make up the majority of pH1N1 cases which is in direct contrast to what is observed with seasonal influenza. Age stratified studies show that the elderly are at reduced risk of infection or developing complications (Dawood et al., 2009; Hancock et al., 2009), strongly suggesting age-related susceptibility. Likely, the presence of pre-existing antibodies that cross react with pH1N1 are associated with lowered risk in the elderly. Obesity is a more recent-

ly identified risk factor. In one report, obesity was among the most frequently identified underlying conditions in fatal pH1N1 cases (Vaillant et al., 2009). Secondary bacterial infections are common in children and adults previously infected with influenza and it has been demonstrated that bacterial pneumonia commonly co-exists with pH1N1 (Centers for Disease Control and Prevention, 2009b) and is associated with death or hospitalization in the U.S. (Louie et al., 2009). Lastly, for reasons unknown, pregnant or post partum women (up to 2 weeks) have more severe disease with pH1N1 (Jamieson et al., 2009; Louie et al., 2010; Pratt, 2010). Animal studies are required to elucidate the mechanisms that occur during or immediately after pregnancy that increases disease severity to pH1N1.

Immunity to zoonotic and pandemic influenza viruses

Infection with all influenza viruses triggers both innate and adaptive immunological responses. Effector mechanisms include, but are not limited to, cytokines, macrophage and natural killer (NK) cells, all of which provide the first line of defense to influenza by limiting replication and spread of the virus in the respiratory tract. When viruses are not controlled by innate immune mechanisms, the host then relies upon adaptive responses which include T and B lymphocytes. Antibodies raised against hemagglutinin (HA) are especially important in the response to influenza virus because they neutralize virus particles and limit infection (Possee et al., 1982; Tamura et al., 1993; Bizebard et al., 1995). Taken together, innate and adaptive immune mechanisms cooperatively protect against influenza infection and disease. Although these factors play an important role in the defense against common influenza subtypes H1N1, H3N2 in humans and animals (Sladkova and Kostolansky, 2006; Svitek et al., 2008), little is known of their relevance with regards to putative zoonotic or pandemic strains, H5N1 and H1N1 respectively.

Despite limited knowledge of immune mechanisms associated with zoonotic and pandemic strains, emerging evidence point to the role of antibody and effector cells. In mice, neutrophils and macrophages are important (Tumpey et al., 2005). In contrast, elderly survivors of the 1918 H1N1 pandemic have sustained circulating B lymphocytes that secrete antibodies able to neutralize virus (Yu et al., 2008), demonstrating that neutralizing antibodies may have played a role in immunity to 1918 H1N1. Little is known about later pandemics although reports revealed that a proportion of humans have cross reactive antibodies to pandemic H2N2 or H3N2 viruses after exposure to a different subtype of virus (Pyhala, 1985; Viboud et al., 2005). Much of what is known about immune responses to avian H5N1 strains involves studies in animal models. In ferrets, molecules important for T and B lymphocyte signaling are down-regulated and the chemically-induced inhibition of chemokine CXCR3 signaling results in decreased disease severity of H5N1, thus pointing to important roles for innate and adaptive immune responses. In *in vitro* studies, H5N1 viruses attenuate type I interferon responses in human bronchial epithelial cultures seemingly as a means to maintain a high level of virulence (Zeng et al., 2007). Healthy naïve human subjects possess strong CD4^{+} T lymphocyte responses to HA, NA, M and NP proteins of H5N1 presumably because of the genetic conservation between these proteins and those of recent circulating H1N1, H2N2, H3N2 strains (Roti et al., 2008).

Studies that show reactivity in humans to ancient influenza strains show that [i] a great amount of antibodies to zoonotic or pandemic strains are naturally present in naïve populations and [ii] older humans possess a large degree of immunological memory. These

observations suggest a degree of conservation between epitopes of ancient and current pandemic viruses as exemplified in the elderly, where a large proportion of this age group have pre-existing serum antibodies with reactivity to pH1N1 (Hancock et al., 2009). The strength of such preexisiting immunity at the population level is likely a key factor to whether a zoonotic influenza virus has the capacity to spread widely in humans.

Reports suggest that the degree of preexisiting humoral immunity to pH1N1 viruses associates positively with age (Hancock et al., 2009; Itoh et al., 2009). Children (6 months-9 years) and adults (18-64 years) had a low level of pre-existing serum antibodies to pH1N1, whereas, older adults (≥ 60 years) had a much greater level. It is believed that previous exposure to older strains of H1N1 (such as the 1918 pandemic) induced cross reactive antibody responses to antigenically similar pH1N1 in mainly older individuals. Although we are gaining some insights, we are yet to fully understand the factors required for immune responses to zoonotic and pandemic strains of influenza despite their importance.

Pandemic preparedness

In 2005, the WHO released an updated set of recommendations for preparation and response to future influenza pandemics. These recommendations included proper planning, surveillance, intervention, reduction and evaluation during the different pandemic phases (World Health Organization, 2009). During the first three phases of a potential pandemic, the recommendations are intended to increase communication between local and national health authorities as well as the WHO to minimize improper response plans. In pandemic phase 4, the focus shifts to containing the new influenza virus. This includes actions at the household, local, national and multi-national levels. The national and international responsibilities include continued surveillance and reporting to the WHO as well as the sharing of specimens and reagents with other countries for further virus characterization. National agencies are also recommended to begin releasing antivirals and vaccines, if available. These responsibilities continue through the remaining phases of the pandemic, but for phases 5 and 6 national agencies are recommended to increase vaccine and antiviral availability as well as help to provide resources to nations with limited resources as well as to countries which may have a heavier burden of disease. As the pandemic begins to subside, the WHO recommends countries remain vigilant for future waves of disease by continuing to monitor activity and to begin rebuilding inventories of therapeutics and vaccine. Assessing the effectiveness of measures taken during the present pandemic will help for future pandemic preparedness. Because these are merely suggested guidelines, many countries take different steps in an effort to prevent and minimize the effect of the pandemic, however, the steps taken during the current pandemic across many nations were very similar.

In Mexico, once a "sanitary emergency" was declared, local and national agencies established weekly meetings. Various forms of media, including news broadcasts, posters and brochures were disseminated instructing the general population to use masks, wash hands frequently and utilize alcohol gel sanitizers, as well as to avoid crowded events and to remain home as a form of self quarantine (Cordova-Villalobos et al., 2009): very similar non pharmaceutical interventions as recommended by WHO or the U.S. CDC (Jain and Goldman, 2009).

From late April to mid May, Australia attempted to prevent entry of pH1N1 by increasing surveillance of incoming travelers. Pilots were instructed to monitor the health of passengers (Appuhamy et al., 2010). Thermal scanners were also installed in airports to identify incoming passengers with fevers (Department of Health and Ageing, 2009). Passengers identified as sick were treated by border nurses and referred to health units (Appuhamy et al., 2010). Surveillance of the general population was also greatly increased by establishing temporary flu clinics to respond to clusters of disease (Appuhamy et al., 2010). These clinics helped to prevent overcrowding of hospitals. Once the virus had entered, Australian health officials attempted to contain the viral spread by closing schools in which a student was diagnosed with the novel virus strain (Waterer et al., 2010). School closures were also employed in many other countries, such as the U.S. and Mexico, to curb the spread of the pandemic (Balinska and Rizzo, 2009). After the virus could no longer be contained, the focus shifted to providing resources to those most at risk of severe complications (Appuhamy et al., 2010), as did most other countries. Similar responses were seen worldwide with various successes. The ultimate goal is to modify current pandemic preparedness responses in order create more effective plans.

Conclusion

Despite the huge public health consequence, even with its comparatively low mortality the World Bank estimated the cost burden of pH1N1 at 0.7 to 4.8% of the global gross domestic product, we know remarkably little about the factors modulating the emergence of pandemic influenza in humans. The latest pandemic is a great example of how much we do not know. The pandemic virus likely came from swine, not directly from birds; the virus emerged in the Americans, not Asia; the virus did not contain any of the genetic markers associated with viral pathogenicity; the virus was of a subtype already in humans; the hardest hit were the young, not the elderly; the disease severity was not unlike epidemic influenza in most age groups. Although in hindsight we can reconcile some of these observations, many we cannot. Despite some of the perceived deficiencies in the planning for and response to the latest pandemic, one thing is for sure, we have been offered our best opportunity to follow the emergence and spread of a pandemic in real time. The consequence of this is that whereas the past pandemics provided only minimal guidance, the data collected during the next few years will hopefully place us in a stronger position to deal with the next

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