

Unraveling the Mystery of Swine Influenza Virus

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Influenza virus outbreaks occur with regularity, but the severity of outbreaks is not consistent. The recent flu epidemic caused by an H1N1 swine influenza virus presents an opportunity to examine what is known about virulence factors and the spread of infection to better prepare for major influenza outbreaks in the future.

The announcement on April 21, 2009 by the US Centers for Disease Control and Prevention (CDC) regarding two patients in California infected with a new swine influenza virus (H1N1) prompted tremendous media coverage and the initiation of pandemic flu preparedness plans by state and federal officials. According to World Health Organization guidelines, the current influenza outbreak is of pandemic proportion as it has caused “widespread human infection.” In response to the outbreak, hundreds of schools in the US and elsewhere have closed, including nearly 2000 schools in Japan. Officials in Mexico, at great economic and public sacrifice, have closed schools and many commercial establishments in an effort to stop the spread of H1N1 infection. Actions such as these reflect the threat that influenza viruses pose as pathogens and the uncertainty around how a particular influenza virus strain will evolve to become more or less virulent.

In the 4 weeks following initial case reports in the US, 41 countries reported swine flu infections, with Mexico and the US bearing the majority of disease burden. A total of 11,034 cases and 85 deaths were reported worldwide during that time (http://www.who.int/csr/don/2009_05_21/en/index.html). By comparison, ~80,000 children die from malaria and more than twice that number die of diarrheal diseases worldwide in any 4 week period (http://www.who.int/topics/infectious_diseases/en/). On a scale of global health crises, the current H1N1 swine influenza outbreak would seem to rank low on the list. Why, then, has this outbreak caused such alarm?

Fear around influenza viruses stems from the occurrence of three major pandemics over the last century. Indeed, influenza viruses have the potential to be among the deadliest of all known pathogens. In an average 4 week period during the 1918 Spanish flu pandemic caused by an H1N1 strain, 4,000,000 people died from influenza and secondary bacterial infections worldwide (Ahmed et al., 2007). This is equivalent to twice the number of people who died from AIDS in 2007 worldwide. In a 4 week period, there were ~150,000 deaths worldwide during the 1957 Asian flu pandemic caused by an H2N2 strain, and ~80,000 deaths worldwide during the 1968 Hong Kong flu pandemic caused by an H3N2 strain. The history of influenza disease reveals a simple truth: all pandemic strains are not created equal.

Swine Flu at Fort Dix

The swine influenza virus outbreak of 2009 is not the first of its kind. In 1976, swine virus infections were observed at an army training base in Fort Dix, New Jersey. Scientific evidence demonstrated that the virus causing infections was similar to strains that had been circulating in pigs for several years (Palese and Schulman, 1976), suggesting insufficient virulence to affect a human population of average density. Nonetheless, fear of the worst prevailed. Many officials felt that the causative virus was related to the 1918 pandemic virus and that the time was ripe for a new human pandemic. What followed the Fort Dix outbreak was a major panic, resulting in the appropriation of \$135 million to support

the first mass influenza virus vaccination. The mass vaccination program exposed a rare, but serious, vaccine side effect called Guillain-Barré syndrome, an acute inflammatory demyelinating disorder that can result in paralysis and death.

The pandemic never came, but events surrounding the swine flu episode brought to light the complete lack of preparedness for a disease outbreak in the US. In the wake of the debacle, the director of the CDC was replaced and President Ford, despite having made legitimate efforts to consult a variety of experts, was accused by some of acting with excessive force to bolster his re-election campaign. Lessons to be gleaned from the 1976 episode and recommendations for assessing potential pandemic situations are delineated in a chronicle of events by Richard Neustadt and Harvey Fineberg (Neustadt et al., 1978). They describe that, in 1976, deciding on a course of action in the face of a potential swine flu pandemic was “like placing a bet without knowing the odds.”

2009 Swine Flu: A Serious Pandemic Threat?

Unlike that situation in 1976, we now have an understanding of some specific markers that make a particular influenza virus more or less deadly. Faced with a new influenza virus strain, we can evaluate the virus’s genome for the presence or absence of these markers in order to inform our expectations and preparations. The presence of a coding sequence for PB1-F2, the smallest protein in the influenza virus repertoire,

Table 1. Comparison of Avian and Swine Influenza Viruses

	2009 Swine H1N1	1997 Avian H5N1
Transmission between humans	Yes	No
Causes human disease	Yes (mild)	Yes (severe)
Degree of immunity in population	Likely	No
Known molecular markers of pathogenicity	No	PB1-F2; polybasic cleavage site in hemagglutinin

is one molecular marker of pathogenicity. PB1-F2 is not present in all human influenza viruses; however, it is consistently present in viruses known to be of increased virulence in humans, including the viruses that caused the 1918, 1957, and 1968 pandemics. Laboratory studies have confirmed it as a virulence factor in mice, and some mechanisms underlying its role in increased disease pathology have been elucidated (Conenello et al., 2007; Zamarin et al., 2005, 2006). A second marker of virulence that can be assessed by sequences alone is the degree of identity between the viral hemagglutinin molecules of the new strain and those of other human viruses. Low identity indicates antigenically distinct hemagglutinin structures, suggesting that transmission from human to human will not be blunted by a degree of "herd" immunity resulting from exposure to similar viruses. A third molecular marker known to be relevant in the patho-

genicity of avian influenza viruses is the polybasic cleavage site, a protease site in the viral hemagglutinin that enables an expanded array of host proteases to activate the hemagglutinin molecule, enabling virus fusion with a host cell.

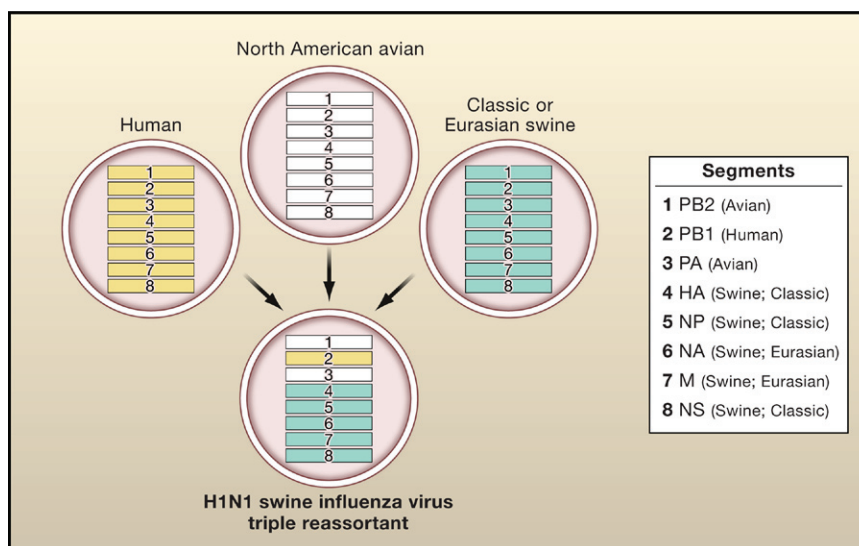
Some weeks out from the start of an influenza outbreak, the severity of the continuing situation can be assessed based on the following: the number of people who have become ill (measured by hospitalizations and newer monitoring systems such as Google search data, <http://www.google.org/flutrends/>) and the apparent mortality rate associated with the disease. The drug resistance profile of clinical isolates may also be worthy of consideration; however, in a fast-moving outbreak, it is unlikely that enough people could be treated prophylactically for the rate of transmission to be affected.

So how does the 2009 H1N1 swine influenza virus measure up? This virus demonstrates requisite characteristics

of a pandemic strain: it can be passed from human to human, it is of sufficient virulence to cause human disease, and to a certain degree, humans are immunologically naive to the virus such that exposure can result in a productive infection. Importantly, H1N1 viruses have circulated consistently in the human population since 1977 and are a component of annual influenza vaccines. Although infection of humans with this particular H1N1 swine influenza virus is a new event, prior exposure to H1N1 viruses would predict a degree of protective immunity in the human population (Table 1). The current circulating H1N1 swine influenza virus does not contain the known molecular marker of pathogenicity PB1-F2, suggesting that it causes a mild disease compared to the three major pandemic viruses of the last century. Clinical isolates demonstrate resistance to the class of flu drugs called adamantanes (M2 inhibitors), but fortunately the virus remains sensitive to neuraminidase inhibitors such as Tamiflu and Relenza.

The H5N1 avian influenza virus, in contrast, currently cannot be transmitted efficiently between humans. It is similarly resistant to adamantane flu drugs, but remains sensitive to neuraminidase inhibitors. Unlike the H1N1 swine virus, the H5N1 virus does contain known molecular markers of pathogenicity. Because of these molecular characteristics, the phenotype of disease caused by the H5N1 avian influenza virus is severe, with many cases resulting in acute lung injury and death. Nonetheless, because very few people become infected with this avian virus, the relative severity of threat it poses in its present form is low. If, however, the H5N1 avian influenza virus adapts so that it is transmitted readily from human to human, it could result in a devastating pandemic.

We can now make a cautious assessment of the relative severity of the flu outbreak caused by the 2009 H1N1 swine influenza virus. The number of people known to be infected is not growing rapidly, although the geographic breadth of cases reported is large. The mortality rate associated with infection appears to be low. Under these circumstances, the spread and impact of this swine influenza virus are similar to those of viruses during an average flu season. Mutations

**Figure 1. Lineage of the 2009 Swine Influenza Virus**

Influenza A viruses have a segmented negative-sense RNA genome that encodes up to 11 proteins including the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) and the virulence factors NS1 (host interferon antagonist) and PB1-F2 (proapoptotic factor). The 2009 H1N1 swine virus is a triple reassortant; it includes segments from swine, avian, and human influenza viruses. Figure modified from Butler (2009).

enabling the expression of PB1-F2, however, could affect this status (circulating strains currently include two stop codons preventing expression of this protein). Alternatively, a reassortment event involving the shuffling of genes with other influenza viruses could lead to incorporation of a gene that would result in PB1-F2 production.

The Virus and the Swine

Influenza A viruses have segmented, negative-sense RNA genomes that encode up to 11 proteins. These include the surface glycoproteins hemagglutinin and neuraminidase and the virulence factors NS1 (host interferon antagonist) and PB1-F2 (proapoptotic factor). The viruses are grouped according to the expression of 1 of 16 hemagglutinin subtypes and 1 of 9 neuraminidase subtypes (Palese and Shaw, 2007).

The elusive nature of influenza viruses as targets of adaptive immunity is mostly a function of the hemagglutinin molecule, the major surface antigen eliciting protective antibodies. The hemagglutinin is a remarkable structure, accommodating significant morphological changes while maintaining its crucial functions in attaching the virus to the host cell and mediating fusion of the viral and host membranes.

Entry of influenza viruses into cells depends on the presence of a functional hemagglutinin molecule and expression of the host cell receptor for hemagglutinin, sialic acid. Humans and avian species differ in the expression of sialyltransferases in mucosal and respiratory tissues resulting in N-glycans with either α 2,6 (human) or α 2,3 (avian) linked sialic acids. The ability of a particular virus to bind to either of these two conformations of terminal sialic acid is thought to be the basis for the host restriction that is characteristic of influenza viruses. An avian virus that acquires the ability, via mutation or reassortment, to bind to α 2,6-linked sialic acids may cross the species barrier and become infectious in humans. Infections in swine

are thought to mediate virus reassortment because swine tissues express both forms of sialic acid, enabling cells to be coinfecting with avian and human viruses (Olsen, 2002). Viruses adapted to swine can also combine with human and avian viruses to produce triple reassortants that may have the ability to infect humans. The 2009 H1N1 swine influenza virus is an example of a triple reassortant (Figure 1).

Influenza viruses demonstrate variations in their ability to be transmitted between humans. Molecular markers predicting the ability of a virus to be transmitted have not yet been determined. However, low temperature and low humidity are known to be environmental conditions that favor aerosol transmission, explaining the seasonal nature of normal influenza (Lowen et al., 2007).

Moving Forward

Only through research can we improve our ability to predict which influenza viruses have the potential to cause serious pandemic disease. Similarly, the informed development of preventative medicines and vaccines depends on progress in basic research. It is critical that regulations governing our work on influenza viruses in the laboratory be rooted in prudence and reason over fear. Arbitrary rules serve only to limit the questions that can be asked at the bench; this translates into unrealized options for preventing disease.

The threat posed by atypical influenza viruses in the human population is not new and is difficult to overstate. The success of research programs around the world, however, has contributed to a new degree of understanding such that, when faced with a new circulating virus, we may (cautiously) make judgments about the likely severity of a pandemic and move forward accordingly.

In all likelihood, a vaccine providing protective immunity to the 2009 swine H1N1 virus will be developed in the com-

ing months. Additionally, in contrast to 1918, when secondary bacterial pneumonias caused the majority of deaths associated with influenza infection (Ahmed et al., 2007), we now live in an age of antibiotics and antiviral medications that can prevent the onset of pneumonia. For these reasons, and considering the molecular characteristics of the circulating virus, we feel confident that the 2009 H1N1 swine influenza virus will not cause a pandemic on the scale of those seen during the 20th century.

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