

REVIEW ARTICLE

Novel Swine-origin Influenza Virus A (H1N1): The First Pandemic of the 21st Century

Luan-Yin Chang,^{1†} Shin-Ru Shih,^{2,3,4†} Pei-Lan Shao,¹ Daniel Tsung-Ning Huang,⁵ Li-Min Huang^{1,6*}

An influenza epidemic was detected in April 2009 at the border between the United States and Mexico. The virus was identified soon after to be a swine-origin influenza virus A (S-OIV A) (H1N1). This virus has an HA gene that is derived from the 1918 swine influenza virus and other genes from human, avian, and Eurasian swine influenza viruses. Clinically, it behaves similarly to seasonal influenza. The only differentiating characteristics are vomiting and diarrhea in a quarter of infected patients, which are rare in seasonal influenza. On June 11, 2009, the World Health Organization declared the first pandemic of the 21st century, caused by S-OIV A (H1N1). Vaccination is the only way to dampen this pandemic. Many questions await answers, including the clinical impact of the pandemic, optimal doses of vaccine, and the future destiny of the virus. A breakthrough in vaccinology against influenza is needed to address the recurring influenza pandemic. [*J Formos Med Assoc* 2009;108(7):526–532]

Key Words: influenza vaccine, influenza, pandemic, reassortment, swine influenza

Early in April 2009, several patients infected with novel H1N1 swine-origin influenza virus A (S-OIV A) were found in the United States and Mexico. Through rapid and frequent international travel, it has spread to over 74 countries around the world and over 29,000 cases, including 145 deaths, have been reported up to June 12, 2009.¹ On June 11, 2009, the World Health Organization declared an influenza pandemic, caused by novel S-OIV A (H1N1).

The three previous influenza pandemics, A/H1N1 from 1918 to 1919, A/H2N2 from 1957 to 1963, and A/H3N2 from 1968 to 1970, were characterized by a shift in the virus subtype, a shift in the highest mortality to younger populations, successive pandemic waves, higher transmissibility

than seasonal influenza, and different impacts in different geographic regions.² The present novel H1N1 influenza has one of the most important characteristics, a shift in virus subtype, and it is very possible the other characteristics will develop.

Clinical Manifestations

According to a report of 642 confirmed cases of novel S-OIV A (H1N1) infection in the United States, patients ranged from 3 months to 81 years in age; 60% of patients were ≤ 18 years, 40% were 10–18 years, and only 5% were ≥ 51 years.³ Therefore, younger populations were much more susceptible than the elderly. The most common

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¹Department of Pediatrics, National Taiwan University Hospital, College of Medicine, National Taiwan University;

²Research Center for Emerging Viral Infections, ³Department of Medical Biotechnology and Laboratory Science, and

⁴Clinical Virology Laboratory, Chang-Gung Memorial Hospital; ⁵Department of Pediatrics, Mackay Memorial Hospital;

and ⁶Graduate Institute of Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan.



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*Correspondence to: Dr Li-Min Huang, Department of Pediatrics, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei 100, Taiwan.

E-mail: lmhuang@ntu.edu.tw

[†]Luan-Yin Chang and Shin-Ru Shih contributed equally to this work.

presenting symptoms were fever (94%), cough (92%), and sore throat (66%); 25% of patients had diarrhea, and 25% had vomiting. Of the 399 patients for whom hospitalization status was known, 36 (9%) required hospitalization.³ Of 22 hospitalized patients with available data, 12 had underlying characteristics that conferred an increased risk of severe seasonal influenza, and 11 had radiologically confirmed pneumonia. These included one (in each group) with pneumomediastinum, necrotizing pneumonia, and empyema that was surgically drained (no microbiological growth was detected in the fluid). Eight patients required admission to an intensive care unit, and four had respiratory failure that required mechanical ventilation. A 22-month-old child with neonatal myasthenia gravis and a 33-year-old pregnant woman have died.³

Therefore, most confirmed cases of novel S-OIV A (H1N1) infection have been characterized by self-limited, uncomplicated febrile respiratory illness and symptoms similar to those of seasonal influenza (a cough, a sore throat, rhinorrhea, headache, and myalgia). Approximately 38% of cases have also developed vomiting or diarrhea, neither of which is typical of seasonal influenza. Some patients have developed severe illness and required hospitalization, and two patients have died. The observation that 60% of patients were ≤ 18 years old suggests that children and young adults are more susceptible than older persons, or that because of differences in social networks, transmission to older persons has been delayed. It is also possible that elderly persons may have had some level of cross-protection from preexisting antibodies against other influenza A (H1N1) viruses—this requires further confirmation.

Is There Any Cross-protection of Seasonal Influenza Vaccine Against the Novel S-OIV A (H1N1)?

The United States Centers for Disease Control (CDC) has assessed the level of cross-reactive antibody to the novel influenza A (H1N1) virus in

cohorts of children and adults before and after vaccination with the 2005–2006, 2006–2007, 2007–2008, or 2008–2009 seasonal influenza vaccines.⁴ In children, before vaccination, there were no cross-reactive antibodies to S-OIV A (H1N1). Among adults, before vaccination, cross-reactive antibodies were detected in 6–9% of those aged 18–64 years, and in 33% of those aged > 60 years. Previous vaccination of children with any of the four seasonal trivalent, inactivated influenza vaccines (TIVs), or with live attenuated influenza vaccine, did not elicit a cross-reactive antibody response to S-OIV A (H1N1).⁴ In adults aged 18–64 years, vaccination with seasonal TIV resulted in a twofold increase in cross-reactive antibody response to S-OIV A (H1N1), compared with a 12- to 19-fold increase in response to the seasonal H1N1 strain. No increase in cross-reactive antibody response to the S-OIV A (H1N1) was observed among adults aged > 60 years.⁴ These data suggested that receipt of recent (2005–2009) seasonal influenza vaccines did not elicit a protective antibody response to the novel influenza A (H1N1) virus. In addition, the researchers suggested that about one third of those aged > 60 years may have had preexisting cross-reactive antibodies—this may explain why only 5% of S-OIV A (H1N1) patients were ≥ 51 years.³

Case-fatality Rate (CFR) and Reproduction Number (R0) of the Novel H1N1 Influenza

By analyzing the outbreak in Mexico, early data on international spread, and viral genetic diversity, Fraser et al made an early assessment of transmissibility and severity.⁵ Their estimates suggested that 23,000 (range, 6000–32,000) of individuals were infected in Mexico by late April, which gave an estimated CFR of 0.4% (range, 0.3–1.5%), based on confirmed and suspect deaths reported by that time. In a community outbreak in the small community of La Gloria, Veracruz, no deaths were attributed to infection, which gave an upper 95% bound CFR of 0.6%. Thus, while substantial

uncertainty remains, clinical severity appears less than that seen in 1918 but comparable with that in 1957.

Clinical attack rate in children aged < 15 years in La Gloria was 61%, which was more than twice that in adults aged \geq 15 years (29%). R_0 is defined as the average number of secondary cases generated by a primary case. Three different epidemiological analyses estimated R_0 to be 1.4–1.6, while a genetic analysis gave a central estimate of 1.2. This range of values was consistent with 14 to 73 generations of human-to-human transmission that occurred in Mexico by late April. Transmissibility was therefore substantially higher than for seasonal influenza, and comparable with lower estimates of R_0 obtained from previous influenza pandemics.

Risk Factors for Severe Cases or Mortality

To date, there is insufficient information about the clinical complications of S-OIV A (H1N1) infection. Deaths have been caused by previous variants of swine influenza viruses and the novel H1N1 virus. While data are being collected on the spectrum of illnesses and complication risk associated with infection, clinicians should expect that both this and seasonal influenza infections will share the same age and risk factors.

Groups at higher risk of seasonal influenza complications include: children aged < 5 years; persons aged \geq 65 years; children and adolescents aged < 18 years who are receiving long-term aspirin therapy, and who might be at risk for Reye's syndrome after influenza; pregnant women; adults and children who have chronic pulmonary, cardiovascular, hepatic, hematological, neurological, neuromuscular, or metabolic disorders; adults and children with immunosuppression caused by medication or human immunodeficiency virus; and residents of nursing homes and other chronic-care facilities.

The risk factors for complications of the present S-OIV A (H1N1) infection may be similar

to those of seasonal influenza. However, the 1918 epidemic and the early reports of the present S-OIV A (H1N1) outbreak have shown that younger rather than older people are more susceptible, and that infected patients of any age should be observed carefully for the occurrence of complications.

Transmission: Pandemic Threat and Infection Control

Pending clarification of transmission patterns for the S-OIV A (H1N1), the CDC recommends that personnel providing direct care for patients presenting with febrile respiratory illness (fever $> 37.8^\circ\text{C}$, plus one or more of the following: rhinorrhea or nasal congestion, sore throat, cough), in a community in which S-OIV A (H1N1) infection has been reported, should wear a disposable N95 respirator, a gown, gloves, and goggles when entering the patient's room. The patient should also wear a surgical mask and be placed in a private room, preferably an airborne infection isolation room. These are interim recommendations and subject to change at any time. Healthcare personnel entering the room of a patient in isolation should be limited to those performing direct patient care. It is vital to promote good hand washing and respiratory/cough etiquette for the prevention of all respiratory infections in the healthcare setting.

Antiviral Therapy and Post-exposure Antiviral Chemoprophylaxis

Either oseltamivir or zanamivir is recommended for treatment of S-OIV A (H1N1) infection, including all hospitalized patients with confirmed, probable, or suspected novel infection, and symptomatic patients who are at higher risk of seasonal influenza complications. Post-exposure antiviral chemoprophylaxis with oseltamivir or zanamivir should be considered for the following: close contacts of cases (confirmed, probable or suspected)

and healthcare personnel; public health workers; or those who have had recognized, unprotected, close-contact exposure to an infected person (confirmed, probable or suspected) during that person's infectious period.

Characteristics of Novel S-OIV A (H1N1) in Humans

Where did the swine influenza virus come from?

Influenza A virus can infect various host species, including birds, humans, and swine. Influenza A H1N1 virus was first isolated from swine in 1930⁶ and from humans in 1933.⁷ Swine influenza A viruses are antigenically very similar to the 1918 human influenza A virus, and they may all have originated from a common ancestor.^{8,9} From 1930 to the late 1990s, swine influenza A viruses were called "classical swine influenza" and they have remained relatively stable antigenically.^{10,11}

In around 1998, the classical swine influenza virus resorted with human influenza A H3N2 virus and a North American Lineage avian influenza virus (unknown subtype), which resulted in the emergence of a triple resorted H3N2 swine virus. This resorted virus has been circulating in the swine population throughout North America.¹²⁻¹⁴ Also in around 1998, the triple resorted H3N2 virus resorted again with the classical swine influenza virus. This generated two new subtypes of swine influenza A virus, the H1N1 and the H1N2 viruses,¹¹ which have been circulating in the Asian swine population. Although human and swine H1N1 viruses are all of avian origin, they have evolved in different host species. Antigenic drift has occurred amongst different lineages of H1N1 viruses; therefore, cross-protection antibodies against avian, swine, and human H1N1 viruses are not expected to exist. Indeed, a recent study has demonstrated that ferret post-infection antisera raised against the currently circulating, seasonal human H1N1 viruses did not react with the novel S-OIV, according to a hemagglutination inhibition assay.¹⁵

The newly emerged S-OIV A (H1N1) contains a combination of gene segments that have not been previously identified in swine or human influenza viruses. The PB2 and PA genes originated from an avian virus that was introduced into swine viruses around 1998. PB1 originated from the human H3N2 virus, which acquired the gene from an avian virus in 1968. HA, NP, and NS genes came from classical swine virus and these three genes are closely related to the 1918 human influenza A virus. The other two genes, NA and M, were from the Eurasian swine virus and were introduced to swine viruses in 1979.¹⁶ The Figure depicts the origins of each gene segment of S-OIV A (H1N1).

NA and M are the targets of two classes of clinically used antivirals, oseltamivir (Tamiflu)/zanamivir (Relenza) and amantadine/rimantadine. Eurasian swine viruses are oseltamivir-sensitive and amantadine-resistant. The novel S-OIV A (H1N1) also has inherited sensitivity to oseltamivir and resistance to amantadine.¹⁶

Virulence factors of S-OIV A (H1N1)

The mortality rate for infection with S-OIV A (H1N1) appears not to be particularly high. However, virulence may change as the number of adaptive gene mutations increases, and the virus may have more opportunities to replicate in the new host species. Like other influenza A viruses, swine influenza virus enters host cells by binding to receptors that contain sialic acid. Swine are known to contain two types of receptors, 2,6-linked sialic acids that appear abundantly in the human respiratory tract, and 2,3-linked sialic acids that tend to be found in avian cells. The binding affinity of S-OIV A (H1N1) to different sialic acids is unclear. However, since the S-OIV A (H1N1) has been transmitted from human to human, this virus is expected to bind to human receptors. However, adaptive mutations may occur that promote the binding of S-OIV A (H1N1) to 2,6-linked sialic acids, if more humans become infected in the near future.

Adaptive mutations may occur in any other gene segments apart from the receptor binding

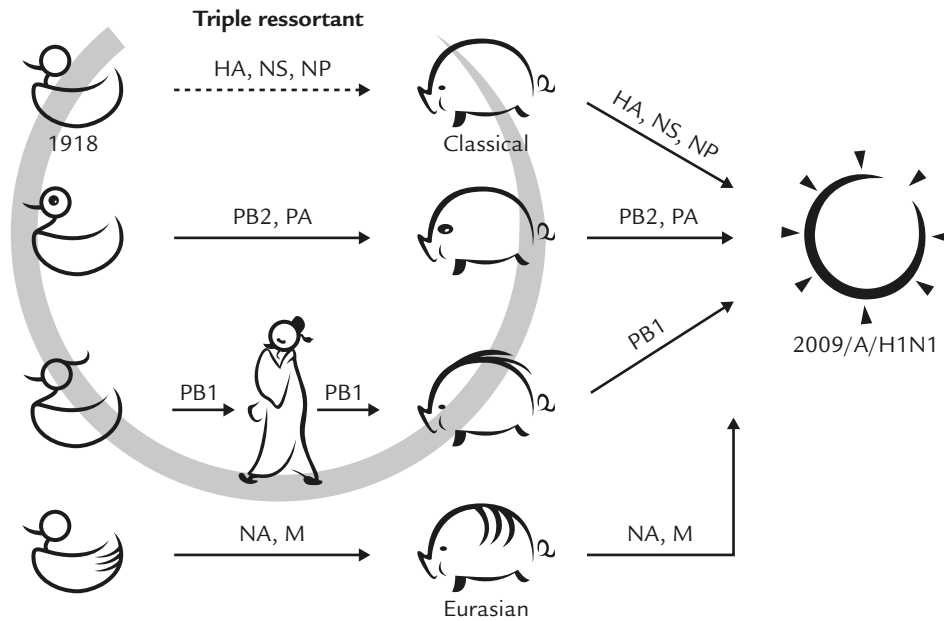


Figure. The origin of each gene segment of swine-origin influenza virus A (H1N1). NA and M were derived from the Eurasian swine virus that originated from the Eurasian avian influenza A virus. The remaining genes were derived from the triple reassorted swine virus that originated from different lineages of avian viruses.

site, and alter viral pathogenesis and virulence. Currently, predicting which adaptive mutations will increase or reduce the virulence of S-OIV A (H1N1) is difficult. However, the following genetic features may be of interest.

PB2 is a viral ribonucleoprotein subunit that is responsible for viral replication in cells infected with influenza virus, and is considered to be a genetic factor that is associated with host restriction. Almost all of the human influenza A viruses have lysine (K) at position 627 in the PB2 protein, and most of the avian viruses have glutamic acid (E) at this position.¹⁷ The E to K mutation in an avian virus has been shown to increase its virulence in mammalian experimental systems.¹⁸ The PB2 gene segment of S-OIV A (H1N1) has reassorted from an avian influenza A virus of an unknown subtype and has retained E at position 627. H7N7 avian influenza viruses have infected humans previously, and one human isolate from a fatal case has been found to have the E to K mutation.¹⁹ Therefore, monitoring changes in the amino acid sequence at position 627 of S-OIV A (H1N1) in humans is important for predicting a change in virulence.

PB1-F2 is translated from another reading frame of the PB1 gene segment because of an alternative translation initiation, and has also been reported to increase the pathogenicity of the 1918 virus and the highly pathogenic H5N1 virus.^{18,20} The PB1 gene of the novel S-OIV A (H1N1) has been found to have truncated forms of PB1-F2 because of the presence of a stop codon at position 12. Hence, a point mutation at position 12 may lead to production of a full-length PB1-F2 in the novel S-OIV to increase viral pathogenicity in humans. However, the mutation may not be favored in human hosts because human viruses have tended not to express PB1-F2 as they have evolved in humans.²¹

Another well-known virulence factor for the influenza virus is the NS1 protein. NS1 protein suppresses the antiviral mechanism in host cells upon viral infection.²² The C-terminal domain of the NS1 protein contains the ESEV signal in many avian influenza A viruses; this signal interacts with cellular modulators that contain the PDZ domain. This interaction may increase viral pathogenicity. Although the NS gene segment of S-OIV A (H1N1) originated from an avian virus, it is truncated by

a stop codon at position 220. Hence, NS1 protein in S-OIV does not have the PDZ ligand domain. It is difficult to predict whether a further mutation in humans will change the sequence at position 220 and thereby alter the virulence of S-OIV A (H1N1).

How Did S-OIV A (H1N1) Overcome Host Restriction and Pass from Swine to Humans?

The crossing of host species by the novel S-OIV A (H1N1) is very important and interesting. Given the known virulence factors discussed above, the causes of human infection and its spread among humans remain unknown. Clearly, other previously unrecognized molecular determinants are responsible for the ability of S-OIV A (H1N1) to replicate and be transmitted in humans. The so-called species-specific signatures of avian and human influenza A viruses have been reported.²³ We examined the amino acid sequences of S-OIV A (H1N1) at those species-specific positions and found that most of the sequences were avian-like signatures. However, some of them had changed from avian- to human-like signatures. For example, at position 271 of the PB2 gene, the avian-like signature is T (threonine); whereas the human-like signature is A (alanine). Most swine viruses contain T at this position, whereas S-OIV in humans has A at position 271 of PB2. More studies should be conducted to identify the unrecognized molecular markers and thus help to determine the mechanism by which an animal influenza A virus crossed the species barrier to infect humans. Additionally, these molecular determinants will be used to predict viral virulence and pathogenicity for diagnosis.

Combating the Pandemic: Vaccines

As the S-OIV A (H1N1) infection has become a pandemic, the most critical question is how to contain it. From the experience so far, it is impossible

to prevent the virus from spreading further because the first wave of the epidemic hit many developed countries and containment has been a failure. Although this virus remains sensitive to oseltamivir, the medication is for treatment and short-term prophylaxis rather than epidemic control. The only way to control this pandemic is through large-scale immunization. The production of vaccines against S-OIV A (H1N1) is feasible; however, several questions remain unanswered. First, how many doses are needed to induce effective protection? For seasonal influenza vaccine, one dose is sufficient for those aged > 8 years. For pandemic vaccines such as H5N1, two doses are needed.²⁴ Second, what is the optimal antigen content in the vaccine? Seasonal influenza vaccine contains 15 µg per strain and 45 µg in total. Without adjuvant, even at 90 µg, H5N1 vaccine is not sufficiently immunogenic. It is not known whether adjuvant is needed, or the optimal amount of antigen in the vaccine. Finally, there is a historic precedent of rushed production of influenza vaccine to contain swine influenza (as demonstrated in 1976). Unfortunately, an increase in the incidence of Guillain-Barré syndrome was demonstrated in the same year and vaccination had to be stopped.²⁵ The mechanism remains uncertain, although the antiganglioside antibody was raised as a possible explanation.²⁶ How to prevent this from being repeated in 2009 is an area of concern.

Conclusion

Forty-one years after the last influenza pandemic, we have witnessed the first pandemic caused by a novel S-OIV A (H1N1) in the 21st century. With our knowledge and experience about influenza viruses, we should be able to cope with this pandemic with the least possible morbidity and mortality. Vaccination is the only effective way to stop this pandemic and will be available in late 2009. More understanding of influenza viruses and continuous development of broad-spectrum influenza vaccines are of critical importance.

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