

A Review of Influenza

Tippavan Nagachinta, M.D., M.Sc., MPH, DrPH*,
Narumol Sawanpanyalert, M.D., MPH**,
Puangpen Chanprasert, RN, MPH, MS, Ph.D.***

ABSTRACT

In March 2009, Mexico reported a large outbreak of influenza with multiple deaths. The first case of novel swine-origin influenza A (H1N1) virus (S-OIV) was later identified on April 15, 2009 in the United States. Since the World Health Organization declared a pandemic of S-OIV on June 11, 2009, the virus has continued to spread rapidly. At that time, about 70 countries had reported cases of S-OIV infection, and there were ongoing outbreaks in multiple parts of the world. At present, this number has nearly doubled. Thailand reported the first case of S-OIV infection on May 12, 2009. In responding to the pandemic, Thailand is committed to control the spread of S-OIV infection and to reduce the morbidity and mortality. This article provides background knowledge, recent updates, and practical aspects of influenza for clinicians who may be involved in patients who develop illnesses compatible with S-OIV infection. (*J Infect Dis Antimicrob Agents* 2009;26:115-32.)

INTRODUCTION

Influenza virus infections are the most important causes of medically attended acute respiratory illness.^{1,2} Their impact is universal, affecting individuals of all age groups in all parts of the world, including both temperate and tropical climates.³ Epidemics of influenza occur annually. Although they vary considerably in severity and intensity, the peak of acute respiratory illness causing individuals to seek medical care always coincides with the peak of influenza activity. The major

factor responsible for the recurring nature of influenza epidemics is antigenic variation of the surface glycoprotein of the influenza viruses.

On April 15 and 17, 2009, novel swine-origin influenza A (H1N1) virus (S-OIV) was identified in the specimens obtained from two epidemiologically unlinked patients in the United States. The same strain of the virus was identified in Mexico, Canada, and elsewhere.⁴⁻⁶ The virus spreaded very rapidly from human-to-human, causing the World Health

*Coordinating Office for Global Health, Centers for Disease Control and Prevention; 1600 Clifton Road, NE, Mailstop E-93, Atlanta, Georgia 30333, U.S.A.

**Department of Medical Services, Ministry of Public Health, Tivanon Road, Nonthaburi 11000, Thailand.

***Office of the Permanent Secretary, Ministry of Public Health, Tivanon Road, Nonthaburi 11000, Thailand.

Received for publication: September 29, 2009.

Reprint request: Dr. Tippavan Nagachinta, Division of Global Public Health Capacity Development, Coordinating Office for Global Health, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mailstop E93, Atlanta, GA 30333, U.S.A.

Keywords: Influenza, H1N1, S-OIV, pandemic, swine influenza, reassortment, antigenic shift, antigenic drift

Organization (WHO) to declare a worldwide pandemic, indicating uncontained community-level transmission of the S-OIV in multiple parts of the world and raise its pandemic alert to level 5 of 6.⁷ This virus has been notified as the first influenza pandemic of the twenty-first century. Worldwide transmission is likely to persist and might increase in the northern hemisphere during the fall and winter.⁸

Virology of influenza

Influenza is a contagious disease caused by an RNA virus. It poses both a global infectious disease threat and an annual public health problem. Influenza virus primarily infects the respiratory system (nose, throat, and lungs). The disease can cause severe illness leading to life-threatening complications including pneumonia in many individuals.

While there are three types of influenza viruses including types A, B, and C, only influenza types A and B are the two types of influenza viruses that cause epidemic human disease. Types B and C viruses are limited to humans, whereas type A viruses can cause severe disease in humans and affect more species.

Influenza A viruses are categorized into subtypes on the basis of the two surface antigens including

hemagglutinin (HA) and neuraminidase (NA). Hemagglutinin allows the virus to attach to host cells, while neuraminidase allows the virus to bud from the infected cells, and then to continually infect more cells. There are 16 known hemagglutinin and 9 known neuraminidase subtypes of influenza A. Each hemagglutinin subtype is named using an “H” plus a number, such as type H1, H2, and so on. In the same way, each neuraminidase subtype is named with an “N” plus a number, such as type N1, N2, and so on. Many different combinations of HA and NA proteins are possible.

Influenza viruses infect a number of animal species, including birds and mammals. An individual can be infected by influenza A and B viruses. Therefore, a combination of influenza A and B viruses is included in the annual influenza vaccine. Influenza A viruses circulate in wild birds and infect both wild and domesticated birds. Wild birds are primary source for influenza A viruses that infect other species.

The influenza virus undergoes mutations, or changes, frequently, so the virus is constantly changing. There are two main mechanisms for these changes to occur including antigenic drift and shift (Table 1).

Table 1. A comparison of two types of influenza virus mutation.

Antigenic DRIFT	Antigenic SHIFT
1. Minor change in HA/NA	1. Major change in HA/NA
2. Point mutations during replication, but subtype remains the same	2. New subtype introduced into human population
3. Continuous changes, so the virus avoids immunity	3. Caused by genetic reassortment when 2 subtypes infect a host simultaneously or
4. Results in the need to update vaccines annually	4. Caused by direct transmission from birds or other animals to humans; virus adapts to new host

HA: hemagglutinin, NA: neuraminidase

Antigenic “DRIFT” is a process of small changes, called point mutations, that occur during the normal virus replication process. These changes do not change the virus subtype but are just enough so that people do not have immunity to it after the changes occur. Antigenic drift results in a minor change to the shape of the virus surface proteins which allow influenza viruses to change slightly and re-infect people repeatedly. It is the reason why virus strains in the vaccine must be updated each year.

Much more rarely, the H and N proteins change altogether. When this major change occurs it is called antigenic “SHIFT” and it results in the introduction of a new influenza subtype into the human population. Antigenic shift can occur by two possible mechanisms including:

1. In the first possible mechanism, influenza A viruses of the two different subtypes simultaneously infect the same host cell, allowing “reassortment” or exchange of viral RNA segments in the host cell, resulting in a virus that contains genes from both subtypes. For example, pigs have been considered effective “mixing vessels” because they contain receptors for both avian and human viruses. Pigs may be infected by human and avian influenza A viruses at the same time, and a new “reassorted” virus may emerge and contains genetic material from both original viruses. If such a virus was to infect people and to spread easily, the human population would be completely susceptible and therefore unprotected against the new subtype. This mechanism likely created the viruses that were responsible for the pandemics of 1957 (H2N2) and 1968 (H3N2).⁹
2. The second possible mechanism for antigenic shift is a direct transmission of influenza virus from birds or other animal species to humans. The virus

then evolves to adapt to the new human host. Direct infections of humans with avian influenza viruses of A subtypes have occurred in the past decade (such as the recent infections associated with H5N1), but no sustained human-to-human transmission of avian influenza A viruses has occurred. There is also strong evidence that the 1918 pandemic resulted from direct transmission of influenza virus from avian species.⁹

Triple-reassortant swine influenza viruses, which contained genes from human, swine, and avian influenza A viruses, have been identified in swine in the United States since 1998.¹⁰ A sporadic infection with triple-reassortant swine influenza A (H1) virus was reported in individuals with exposure to pigs in the United States since 2005.¹¹ It may be plausible that the pandemic S-0IV influenza currently circulated is due to a triple-reassortant swine influenza A (H1) virus.

All influenza A viruses contain eight genes that encode the following proteins including polymerases PA, PB1, PB2; HA; NA; nuclear protein (NP); matrix proteins (M); and nonstructural proteins (NSs). The genes of the 1918 human and swine H1N1 and the 1979 H1N1 influenza A viruses were all recently descended from avian influenza A genes, and some genes have been presented to the pandemic human H1N1 strain. The novel S-0IV virus associated with the current S-0IV pandemic is a fourth-generation descendant of the 1918 virus.¹² All pandemic S-0IV analyzed to date are antigenetically and genetically similar.

Epidemiology of influenza

The influenza virus is transmitted easily from human-to-human via droplets and small particles produced when infected people cough or sneeze. Influenza A viruses can cause both epidemic and

pandemic. An epidemic is an outbreak of influenza confined to one location, such as a city, town, or country. In a given community, epidemics of influenza A virus infection have a characteristic pattern. In temperate climates in either the northern or southern hemisphere, epidemics occur almost exclusively in the winter months (generally October to April in the northern hemisphere, and May to September in the southern hemisphere), whereas influenza may be observed all year round in the tropics.

Pandemics are severe outbreaks that rapidly progress to involve all parts of the world, and associated with the emergence of a new virus to which the overall population possesses no immunity. The characteristics of pandemic include an extremely rapid transmission with concurrent outbreaks throughout the globe, the occurrence of disease outside the usual seasonality including during the summer months, high attack rates in all age groups with high levels of mortality particularly in healthy young adults. The interval between both pandemics is quite variable and unpredictable.¹³ In the 2009 revision of the pandemic phase descriptions, the WHO has retained the use of six-leveled approach for easy incorporation of new recommendations and approaches into existing national preparedness and response plans. The grouping and description have been revised to make them easier to understand, more precise, and based upon observable phenomena. The levels 1, 2, and 3 correlate with preparedness, including capacity development and response planning activities, while the levels 4, 5, and 6 clearly signal the need for response and mitigation efforts. Furthermore, periods after the first pandemic wave are elaborated to facilitate post pandemic recovery activities. The current WHO level of S-0IV pandemic alert is the level 6.

While seasonal influenza occurs every year, pandemic influenza rarely occurs. Previous pandemics have caused a great number of deaths worldwide. The 1918-1919 pandemic, often referred to as the Spanish influenza was caused by the H1N1 strain. It caused approximately 40 million deaths worldwide. The 1957-1958 pandemic, or Asian influenza was caused by the H2N2 strain and caused about 1-2 million deaths worldwide. In 1968-1969, Hong Kong influenza caused by the H3N2 strain, caused about 700,000 deaths worldwide. While the population usually has some immunity built up from previous exposures to seasonal influenza, the human population lacks any immunity to pandemic influenza strains.

The new influenza A subtypes have the potential to cause a pandemic when they are able to cause human illness and demonstrate efficient human-to-human transmission and little or no previously existing immunity has been identified among humans.¹⁴ Novel S-0IV is not a new subtype, but because the large majority of humans appear to have no preexisting antibody to key novel S-0IV hemagglutinin epitopes, substantial potential exists for widespread infection.¹⁵

Influenza circulates worldwide and can affect anybody in any age group. In seasonal influenza epidemics, infants and the elderly are most at risk of illness and complications. But during an influenza pandemic, even healthy, young people are at increased risk for serious complications.

Laboratory surveillance of influenza is maintained by a network of laboratories coordinated by WHO.^{16,17} Member laboratories submit recent influenza isolates to the three Collaborating Centers for influenza including one located at the Centers for Disease Control and Prevention (CDC) in Atlanta that is designated as the WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza. The other two

in London and Melbourne are designated as the WHO Collaborating Centers for Reference and Research on Influenza, where important work of systematically testing isolates for antigenic novelty is conducted. All laboratory-confirmed cases of current S-0IV pandemic as officially reported to the WHO by States Parties are shown in Table 2 and Figure 1.

Clinical manifestations of influenza (Table 3)

Influenza viral infection affects mainly the respiratory system including the nose, throat, bronchus, and occasionally the lungs. Infection usually lasts for about a week. The incubation period, the time period from exposure to the onset of symptoms, for influenza

is fairly short, between 1 and 5 days. The short incubation period makes influenza outbreaks difficult to control. The spectrum of illness associated with influenza virus infection ranges from inapparent infection to fulminant, fatal pneumonia. The severity of illness may depend on the previous experience with antigenically related variants. When a large proportion of the population has at least partial immunity, about 20 percent of infections will be inapparent, and about 30 percent will be manifested only by signs and symptoms of the upper respiratory tract involvement without fever.¹⁸

Typical uncomplicated influenza often begins with an abrupt onset of symptoms after an incubation

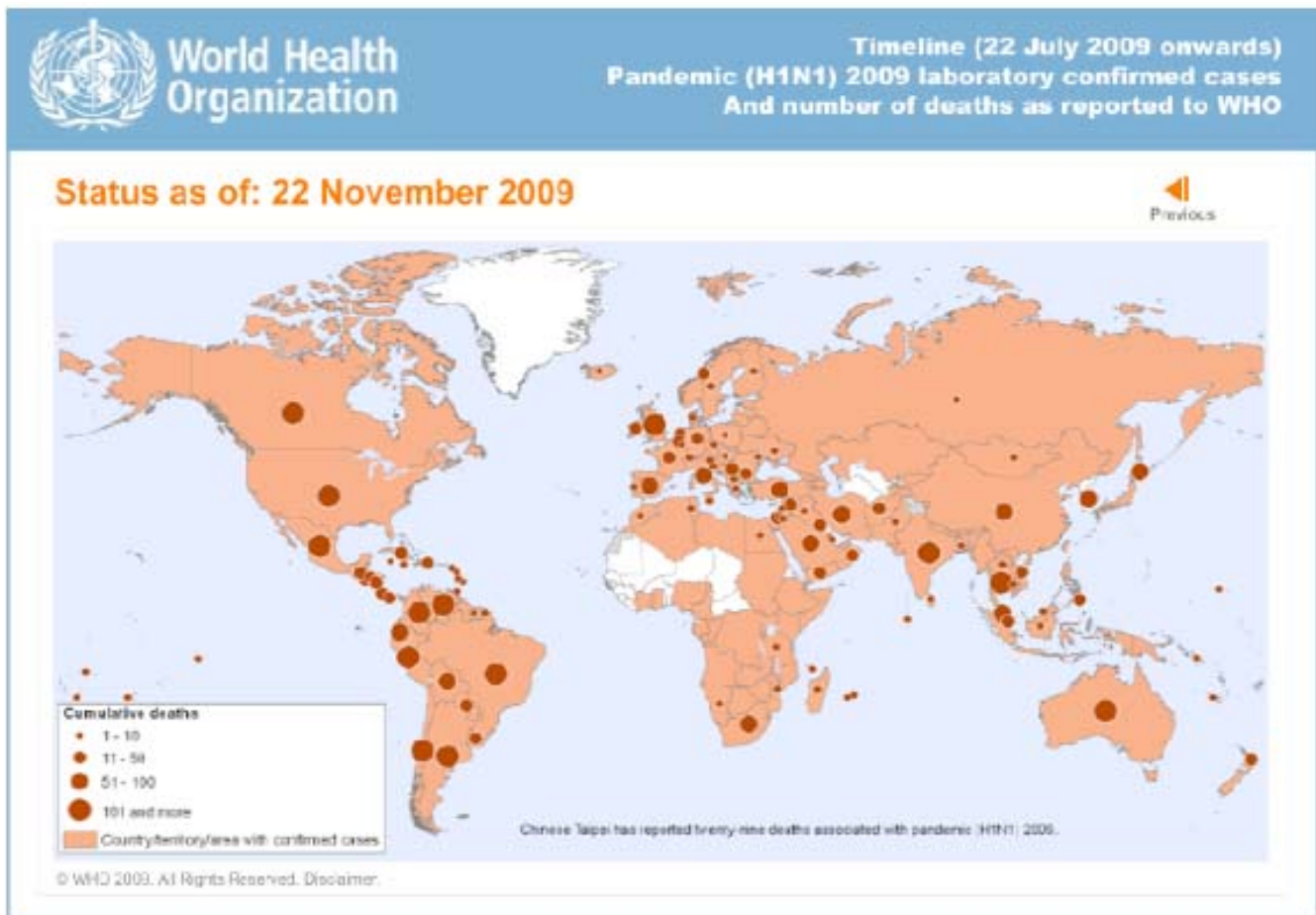
Table 2. Laboratory-confirmed cases of 2009 S-0IV pandemic as officially reported to the World Health Organization by States Parties to the IHR (2005) as of November 22, 2009.

Region	Cumulative total	
	As of 22 November 2009	
	Cases*	Deaths
WHO Regional Office for Africa (AFRO)	15,503	104
WHO Regional Office for the Americas (AMRO) **	190,765	5,360
WHO Regional Office for the Eastern Mediterranean (EMRO)	38,359	330
WHO Regional Office for Europe (EURO)**	Over 154,000	At least 650
WHO Regional Office for South-East Asia (SEARO)	47,059	738
WHO Regional Office for the Western Pacific (WPRO)	176,796	644
Thailand	26,016	187
Total	Over 622,482	At least 7,826

*Given that countries are no longer required to test and report individual cases, the number of cases reported actually understates the real number of cases.

**The total number of cases are no longer reported from these regions.

Figure 1. Laboratory-confirmed accumulative cases and number of deaths of 2009 S-OIV pandemic as officially reported to WHO by States Parties to the IHR (2005) since July 22, 2009. (http://gamapserver.who.int/h1n1/cases-deaths/h1n1_casesdeaths.html)



period of 1-2 days. Many patients can pinpoint the hour of onset.^{19,20} Initially, the systemic symptoms including feverishness, chilliness or frank shaking chills, headache, myalgia, malaise, and anorexia, predominate. Myalgia or headache is usually the most troublesome. Respiratory symptoms particularly a dry cough, severe pharyngeal pain, and nasal obstruction and discharge are usually present at the onset of the illness but are overshadowed by the systemic symptoms. The predominance of systemic symptoms is a major feature distinguishing influenza from other viral upper respiratory infections. Hoarseness, sore throat, and cough may also be present, but these symptoms tend

to appear as systemic symptoms diminish, and thus become more prominent as the disease progresses, persisting 3-4 days after the fever subsides. Older adults may simply present with high fever, lassitude, and confusion without the characteristic respiratory complaints, which may not occur at all. Fever is the most important physical finding. Typically, the duration of fever is 3 days, but it may last 4-8 days. Influenza attack rates are higher in children than in adults.^{21,22}

Regarding complications of seasonal influenza, two manifestations of pneumonia associated with influenza are well recognized: primary influenza viral pneumonia and secondary bacterial infection. The

Table 3. Age, hospitalization rate, clinical features, oseltamivir treatment, and fatality of cases reported to the World Health Organization from the United States, Canada, and the United Kingdom.⁷

Factors	United States (N=642)	Canada (N=173)	United Kingdom (N=53)
Age (year)	60% < 18	Median 22-24 (1-61)	10-29 (58%)
Comorbidities	41% of hospitalized	7% of 54	0
Hospitalization	36/399 (9%)	6/173 (3%)	1 (2%)
Fever	94%	87%	94%
Cough	92%	87%	NA
Sore throat	66%	48%	82%
Rhinorrhea	NA	27%	NA
Dypnea	NA	14%	NA
Malaise	NA	35%	80%
Chills	NA	28%	80%
Myalgia	NA	35%	NA
Arthalgia	NA	13%	56%
Headache	NA	38%	81%
Diarrhoea	25%	23%	28%
Vomiting	25%	15%	NA
Oseltamivir treatment	14/19 (74%) of hospitalized patients	6% of 54	98%
Fatality	2 (0.3%)	0%	0%

NA: not applicable

syndrome of primary influenza pneumonia was first well documented in the 1957-1958 influenza outbreak.^{23,24} However, it is clear that many deaths of young healthy adults in the 1918-1919 outbreak were the result of this syndrome. In outbreaks since 1918, primary influenza viral pneumonia has occurred predominantly among individuals with cardiovascular disease, especially

rheumatic heart disease with mitral stenosis, and to a lesser extent in others with chronic cardiovascular and pulmonary diseases. The illness begins with a typical onset of influenza, followed by a rapid progression of fever, cough, dyspnea, and cyanosis. Physical examination and chest radiogram reveal bilateral abnormalities consistent with the adult respiratory

disease syndrome but usually without consolidation signs. Blood gas studies show marked hypoxia. Gram's stain of the sputum fails to reveal significant bacteria, and bacteria culture yields sparse growth of normal flora, whereas viral cultures yield high titers of influenza A virus. Such patients do not respond to antibiotics, and the mortality is high.

Secondary bacterial pneumonia often produces a syndrome that is clinically indistinguishable from that occurring in the absence of influenza.^{25,26} The patients (most often older adults or those with chronic pulmonary, cardiac, and metabolic or other diseases) have a classic influenza illness, followed by a period of improvement lasting usually 4-14 days. Recrudescence of fever is associated with symptoms and signs of bacterial pneumonia including cough, sputum production, consolidation signs, and alveolar infiltration on chest radiogram. Gram's stain and culture of the sputum reveal a predominance of bacterial pathogen most often *Streptococcus pneumoniae* or *Haemophilus influenzae* and notably, an increased frequency of *Staphylococcus aureus*. These patients usually respond to specific antibiotic therapy.^{27,28}

In children, pneumonitis may occur, but it is less common than adults. Bronchitis may also occur as a result of influenza A or B virus infection, but respiratory syncytial virus and parainfluenza virus type 3 are more important causes of bronchitis. Influenza has been noted to cause severe disease with an increased incidence of pneumonia in immunosuppressed children with cancer, compared to age-matched individuals without immunosuppression.²⁹ In patients with HIV infection, influenza has not been recognized as a major clinical problem, although disease of greater severity has been noted in some patients.³⁰

The study finding of pneumonia patients confirmed with S-OIV infection revealed that S-OIV

infection can cause severe illness, the acute respiratory distress syndrome, and death in previously healthy individuals who are young to mid-aged. Although risk factors for severe S-OIV illness are still unknown, one possible contributing factor to death could be due to delayed hospitalization and delayed initiation of antiviral.³¹

Most patients infected with influenza virus recover within one to two weeks without requiring medical treatment. However, in the very young, the elderly, and those with other serious medical conditions, infection can lead to severe complications of the underlying condition, pneumonia, and death.

Among 187 mortality cases reported by the Bureau of Epidemiology (BOE), Ministry of Public Health, Thailand, 35 percent were classified as "healthy individuals who had no underlying diseases prior to hospitalization, 19 percent were obese, 10 percent had diabetes, 6 percent were pregnant women, and 6 percent had chronic lung diseases.

Diagnosis of influenza

The WHO recommended that uncomplicated influenza can be diagnosed based on signs and symptoms presented by suspected patients when influenza virus is known to be circulating in a community. All patients should be advised to return to their health-care provider for follow-up if they develop signs or symptoms of progressive disease.

Signs of progressive illness can include:

1. Persistent high fever beyond 3 days
2. Shortness of breath or difficulty in breathing, or turning blue
3. Bloody or colored sputum, chest pain or low blood pressure
4. Fast or labored breathing (in paediatric patients)
5. Drowsiness, confusion, or severe weakness

6. Dehydration, which can cause dizziness, decreased urine output or lethargy.

Diagnostic testing to confirm the pandemic virus should be prioritized for patients at higher risk for severe illness. However, clinicians should not delay the treatment of a patient with symptoms of influenza-like illness before obtaining the laboratory results.

Virus isolation or detection of viral antigen in respiratory secretions is the technique of greatest utility in the setting of acute illness. Virus can be isolated from the nasal swab specimens, throat swab specimens, nasal washes, or the combined nose and throat swab specimens. Virus can also be isolated from the sputum specimens, if they are being produced. Specimens for influenza are inoculated onto the rhesus monkey kidney, cynomolgus monkey kidney, or Madin-Darby canine kidney cell cultures, where virus is detected by cytopathic effect or hemadsorption test. Less commonly, embryonated egg can be used for virus isolation. Over 90 percent of positive cultures can be detected within 3 days of inoculation³² and the remainder by 5 to 7 days.

A variety of techniques have been employed to speed up diagnosis using rapid testing kits. The most widely used tests are based on immunologic detection of viral antigen in the respiratory secretions. For influenza, such tests include the Directigen Flu A+B (Becton-Dickenson), FLU OIA (BioStar), and QuickVue Influenza A+B test (Quidel Corporation). All of these tests are designed to detect both influenza A and B viruses, are relatively simple to perform, and can provide results within 30 minutes. The reported sensitivities of each test in comparison to cell culture have ranged from 40 to 80 percent, depending on the nature of the specimens tested and the patients whom they were derived.³³⁻³⁶ Recently, an evaluation of rapid influenza diagnostic tests for detection of novel S-OIV

for their ability to detect viral antigens in the respiratory clinical specimens indicated the overall low sensitivity (ranged from 40 to 69%) and declined substantially as the amount of virus decreased.³⁷ Reported specificities have ranged from 85 percent to 100 percent. Therefore, patients with illnesses compatible with novel S-OIV infection but with negative rapid influenza diagnostic test results should be treated empirically based on the level of clinical suspicion, underlying medical conditions, severity of illness, and risk for complications.

A diagnosis can also be made on epidemiologic grounds. That is, when the presence of influenza virus is confirmed in a region or community, healthy adults with acute influenza-like illness most commonly have influenza. Several studies have shown that the accuracy of a clinical diagnosis in healthy adults in the setting of an influenza outbreak is as high as 80 to 90 percent.^{38,39}

Since the first confirmed case of S-OIV was reported to Ministry of Public Health of Thailand in May 2009, the ministry has set up a public health team to review all reported cases and deaths. Another medical expert team has been created to review and revise the guidelines for treatment of patients who developed influenza-like illnesses. The reports obtained from the Ministry of Public Health indicated that the majority of clinical cases have uncomplicated influenza-like illnesses which were resolved without antiviral treatment. Among hospitalized patients admitted with influenza-like illnesses, about two to eight percent of them were confirmed to be positive for influenza A H1N1, with a case fatality rate of 0.4 percent. Among laboratory-confirmed cases, the median age was 15 years old. However, 58 percent of the fatal cases were found to be between the ages of 21 and 50 years. Regarding the underlying conditions, 70 percent of hospitalized patients were reported of having the

following conditions including pregnant, obesity, diabetes, chronic obstructive pulmonary disease, asthma, chronic renal failure, coronary heart diseases, and carcinoma. About 72 percent of 130 fatal cases had comorbidity with one of those underlying conditions.

Although it has just been only 8 months after the first case of novel S-OIV was identified, it seems unlikely that this pandemic will lead to widespread of severe illness and deaths. However, this may be just the first wave of the pandemic that will need to be monitored carefully. The H1N1 Influenza Center has been recently established to provide the most up-to-date information on the outbreak, which includes an interactive map from HealthMap (<http://healthmap.org/nejm>). This information will be useful to health professionals as they participate in the control of this pandemic.⁴⁰

Impact of influenza

Since influenza is an acute viral infection that spreads easily from human-to-human, it poses a serious public health problem that causes severe illnesses and deaths for higher risk populations. Influenza epidemics are regularly associated with excess morbidity and mortality,⁴¹ usually expressed in the form of excess rates of pneumonia and influenza-associated hospitalizations and deaths during epidemics.⁴²

Pneumonia and influenza deaths fluctuate annually in a predictable fashion with peaks in the winter and troughs in the summer. Because not all influenza-related deaths are manifested as pneumonia, the pneumonia and influenza mortality statistics probably underestimate the true impact of seasonal influenza on the population. Influenza is usually associated with a U-shape epidemic curve. An attack rate is usually highest in the young, whereas the mortality is generally highest among older adults.²⁰

Seasonal influenza epidemics impose a substantial health burden on all age groups, but the highest risk of complications occur among children of less than 2 years, adults of older than 64 years, and persons of any age with certain medical conditions including chronic cardiovascular, pulmonary, renal, hepatic, hematologic, or metabolic diseases; immunosuppression; pregnancy; and neurologic/neuromuscular conditions causing compromise respiratory function. Secondary complications of influenza include bacterial pneumonia, including coinfection with methicillin-resistant and methicillin-susceptible *S. aureus* pneumonia, viral pneumonia, worsening of underlying conditions, febrile seizures, encephalitis/encephalopathy, myositis, multiple organ failure, pulmonary emboli and adult respiratory distress syndrome, and Reye syndrome in association with use of salicylates in children.⁴³

Influenza pandemic can take an economic toll through lost of workforce productivities and strain health care services.

Prevention and control of influenza

Protecting yourself and others against infection by seasonal influenza is important to help everyone stay as healthy as possible and to reduce high rates of illness and mortality that occur annually because of influenza. Some basic steps to take are 1) get vaccinated, 2) practice good personal hygiene, especially frequent hand washing and wearing mask, 3) stay home if you are sick, and 4) keep informed about influenza outbreaks. The United States CDC recommends that people with influenza-like illness remain at home until at least 24 hours after they are free of fever (100°F or 37.80°C), or signs of a fever without the use of fever-reducing medications. (<http://www.cdc.gov/h1n1flu/guidance/exclusion.htm>)

At present, S-OIV influenza is now so wide-

spread that the WHO has stopped counting individual cases. Health experts are afraid it could worsen, especially when the northern hemisphere's influenza season starts in the autumn. The total number of individuals infected with H1N1 flu is not known, and countries are no longer testing and reporting each individual case of a person falling ill. The total number of laboratory-confirmed cases is really only a subset of the total number of cases. The WHO has suggested that more emphasis should be placed on preventing infection and treating the most serious cases to avoid unnecessary death.

Influenza vaccine

The most effective measure available for the prevention and control of influenza and influenza-related complications is the annual administration of inactivated or live-attenuated influenza vaccine. The efficiency of vaccine production has been improved through the development of techniques to create high-yield reassortant strains adapted to grow in high yield from hens' eggs.⁴⁴ The current each vaccine is generally formulated as a trivalent preparation containing one strain of influenza A (H1N1) virus, influenza A (H3N2) virus, and influenza B virus thought to be most likely to cause disease in the upcoming season on the basis of epidemiologic and antigenic analysis of currently circulating strains. The viruses in the vaccine change each year based on international surveillance and scientists' estimations about which types and strains of viruses will circulate in a given year. About two weeks after vaccination, antibodies that provide protection against influenza virus infection develop in the body.

Influenza vaccine is generally well tolerated in adults. Systemic reactions including malaise, flulike

illness, and fever are relatively uncommon. Severe, life-threatening, immediate hypersensitivity reactions to parenteral inactivated vaccine are rarely reported. However, hypersensitivity to hens' eggs, in which the vaccine virus is grown, is a contraindication to vaccination. During the 1976 National Immunization Program against swine influenza, 45 million individuals received influenza vaccine. In the first 4 to 6 weeks after vaccination, the incidence of Guillain-Barrel syndrome (GBS) among vaccinees exceeded those persons who did not receive the vaccine. The estimated risk of acquiring GBS during that vaccination program was 1 in 100,000 vaccinations; the mortality for those with GBS was 5 percent, and another 5 percent to 10 percent had some residual neurologic abnormalities.⁴⁵

Increases in hemagglutination inhibition (HAI) antibody are noted in about 90 percent of healthy adult recipients of seasonal influenza vaccine. Only single dose of vaccine is required in individuals who were previously vaccinated or who experienced prior infection with a related subtype, but a two-dose schedule may be required in unprimed individuals.^{46,47} Serum antibodies peak between 2 and 4 months after vaccination but fall quickly, reaching near baseline before the next influenza season.⁴⁸ Groups of adults with potentially decreased responses to inactivated influenza vaccine include older adults, individuals on immunosuppressive therapy, those with renal disease, and some transplant recipients.

Live-attenuated influenza vaccine for use in humans, the cold-adapted influenza vaccine-trivalent, was first licensed for use in the United States. The use of live-attenuated viruses as influenza vaccines offers several potential advantages over parenteral inactivated vaccines, including induction of a mucosal immune response that closely mimics the response induced by natural influenza virus infection.⁴⁹ In

addition, the use of nasal, rather than the parenteral, route of administration might be more acceptable to patients, particularly in certain age groups.

Trivalent inactivated influenza vaccine (TIV) can be used for individuals of older than 6 months, including those with high-risk conditions. Live-attenuated influenza vaccine (LAIV) may be used in healthy, non-pregnant individuals aged between 2 to 49 years. No preference is indicated for live-attenuated influenza vaccine when considering vaccination of healthy, non-pregnant individuals aged 2 to 49 years. Each year, the Advisory Committee on Immunization Practices (ACIP) provides general, annual updates information regarding control and prevention of influenza.⁵⁰

Although influenza vaccination is the most effective strategy for preventing influenza and influenza-like complications, current seasonal influenza vaccines are not likely to provide protection against novel S-0IV.⁵¹ Specific vaccines against the novel S-0IV are being manufactured. Five manufacturers are producing novel S-0IV vaccine in the United States including Sanofi Pasteur, Novartis, GlaxoSmithKline, Medimmune, and CSL Biotherapies. Distribution of the first lot of licensed S-0IV vaccine in the United States became available in mid-October, although in a small volume. Licensed S-0IV vaccine is expected to be more available in early 2010.

The CDC has recommended pregnant women, individuals who live with or provide care for infants aged <6 months, health-care and emergency medical services personnel, children and young adults aged 6 months-24 years, and individuals aged 25-64 years who have medical conditions that put them at higher risk for influenza-related complications are at the front of the line to get vaccinated.⁵² The CDC's Influenza Division provides influenza surveillance and antiviral resistance

data. The most updated recommendations were presented to the full ACIP and approved in February 2009. A summary of vaccine recommendations for different groups of person is currently available. Further updates, if needed, will be posted at CDC influenza website (available at <http://www.cdc.gov/flu>).

Antiviral medications

Antiviral medications are adjunct to vaccination and are effective when administered as treatment and when used for chemoprophylaxis after an exposure to influenza virus. Four different influenza antiviral drugs including amantadine, rimantadine, oseltamivir, and zanamivir are approved by the United States Food and Drug Administration (FDA) for the treatment of influenza; three are approved for prophylaxis. All four agents have activity against influenza A viruses.

The matrix 2 protein (M2) inhibitors, amantadine and rimantadine, are active against all strains of influenza virus in a variety of cell culture systems and animal models.⁵³ The antiviral activity of these drugs is the result of inhibition of the M2 ion channel activity of susceptible viruses. Both amantadine and rimantadine are effective in the therapy of experimentally induced and naturally occurring influenza A. Amantadine treatment of H3N2 influenza A during 1968 pandemic within the first 48 hours of illness was associated with a decreased duration of fever by about 24 hours.⁵⁴ Rimantadine treatment results in a significantly more rapid improvement in small airways dysfunction in healthy adults with uncomplicated H3N2 influenza.⁵⁵ Rimantadine has also been evaluated in the treatment of influenza A in children, and shown to reduce the level of virus shedding early in infection when compared to acetaminophen.

The neuraminidase inhibitors, oseltamivir and zanamivir, act by inhibiting the functioning of the

influenza virus neuraminidase. This enzyme cleaves terminal sialic acid from sialic acid-containing glycoproteins that serve as host cell receptors for attachment of influenza viruses. The destruction of these receptors by neuraminidase is critical in allowing newly formed viruses to subsequently aggress from the cell and spread to other cells. Among influenza viruses susceptible to neuraminidase inhibitors are avian viruses with all nine known neuraminidase subtypes. In studies of naturally occurring, uncomplicated influenza in healthy adults, therapy with oseltamivir initiated within the first 36 hours of symptoms resulted in 30 to 40 percent reductions in the duration of symptoms and severity of illness and reduced rates of prolonged cough.^{56,57} Similarly, in healthy adults, early therapy of uncomplicated influenza A or B with inhaled zanamivir has been shown to result in a reduction of approximately 0.8 to 1.5 days in the duration of influenza symptoms, and an early return to normal activities. Early treatment of healthy adults with zanamivir may also reduce the frequency of complications, with reduction in the use of antibiotics and in hospitalization.^{58,59}

The WHO states that mild illness continues to characterize most cases, and basic supportive care (to relieve aches or fever) is sufficient for most people. However, healthcare providers should give all of their patients guidance on how to recognize signs of progressive illness, and when to seek medical attention.

For pregnant women, the WHO advises early antiviral treatment for suspected or confirmed pandemic influenza illness.

Infants and very young children (those under 2 years of age), especially those with underlying conditions, should also be treated with antiviral medication if warning symptoms arise.

In general, antiviral treatment recommendations are:

1. Patients who have severe or progressive illness should be treated with antiviral medication as soon as possible.
2. People with mild symptoms but who are at higher risk for severe illness (e.g. pregnant women, infants and young children, and those with chronic lung problems) should be treated with antiviral as soon as possible.
3. Antiviral treatment is not necessary for people have uncomplicated or mild illness and are not in a high risk group for severe illness.

Mothers who are breastfeeding can continue breastfeeding while ill and receiving antiviral treatment.

In hospital settings, healthcare providers should monitor the oxygen levels closely and supplement oxygen as needed, following guidelines. When pneumonia is present, each patient should be treated with both antiviral medication and antibiotics as early as possible.

In healthcare settings where resources are limited, clinical care should focus on early use of primary healthcare (by the family doctor or at health clinics, for example) to determine what type of care or treatment is necessary for a patient, and to set priorities for who needs hospital care most urgently. Healthcare decisions should be based on signs and symptoms of illness, and the level of influenza activity in the local area.

Decentralizing stocks of antiviral medications, even if supplies are limited, is important to reach at-risk groups and disadvantaged populations.

Drug resistance

Drug resistance has been a factor in limiting the more widespread use of antiviral agents. Although resistant viruses are noted in less than 1 percent of

unexposed individuals, they emerge fairly frequently in treated individuals, particularly children.^{60,61} The emergence since 2005 of resistance to one or more of the four licensed antiviral agents among circulating strains has complicated antiviral treatment and chemoprophylaxis recommendation. The CDC has issued the interim recommendations for antiviral treatment and chemoprophylaxis of influenza,⁶² and these guidelines should be consulted pending issuance of new recommendations.

Resistance to the M2 inhibitors, amantadine and rimantadine, is the result of single point mutations in the membrane-spanning region of the M2 protein, and it confers complete cross-resistance between amantadine and rimantadine. Resistant virus can be transmitted to, and can cause disease in, susceptible contacts.⁶³

Resistance to the neuraminidase inhibitors has been hypothesized to be relatively limited problem since these agents interact with highly conserved residues within the influenza virus neuraminidase. Unfortunately, resistant virus strains have been infrequently isolated from immunologically intact individuals treated with neuraminidase inhibitors in clinical trials.^{64,65} Analysis of these viruses has revealed two mechanisms of resistance including 1) mutations within the catalytic framework of the NA that abolish binding of the drugs and 2) mutations in the receptors binding region of the HA which is resulted in reducing the affinity of the HA for its receptors and allowing cell-to-cell spread of virus in the absence of NA activity.^{66,67}

The WHO announced that the first six oseltamivir-resistant S-0IV strains was detected in Denmark (1 isolate), Japan (3 isolates), Hong Kong (1 isolate), and Canada (1 isolate). All except one patient had previously received oseltamivir and have recovered well. All resistant viruses had the characteristic

mutation at position 274/275 associated with resistance. On August 6, 2009, the CDC detected resistance to oseltamivir by pyrosequencing viral RNA from clinical specimens obtained from two immunosuppressed patients. The pyrosequencing detected a mutation that results in a histidine-to-tyrosine substitution at position 275 (H275Y) in the neuraminidase gene, known to be associated with oseltamivir resistance. Oseltamivir-resistant viruses isolated from both patients were determined to be susceptible to zanamivir by neuraminidase inhibition assay. Sequence analysis showed that oseltamivir resistance was not the result of gene reassortment with seasonal influenza A (H1N1) virus.⁶⁸

Global surveillance of pandemic S-0IV infections in humans

The approach and methods for global surveillance vary at different levels of the pandemic. In countries with no or very few cases, the main aims of surveillance remain early detection of the introduction of the virus using laboratory confirmation of cases and initial risk assessment.

In countries where the pandemic S-0IV is established, the main aims of surveillance are continuous monitoring of the epidemiological, virological and clinical picture of the pandemic and its impact on the healthcare infrastructure. Timely sharing of information is needed throughout the pandemic to enable ongoing risk assessment to take place.

Finally, monitoring of avian influenza A viruses for resistance to influenza antiviral medications is important. Ongoing surveillance is essential for both seasonal influenza and identification of potential pandemic strains. In addition, ongoing collaborative planning among groups helps ensure readiness among all. Governments and other organizations around the

world have been working together to prepare for the influenza pandemic by early detection of emergence of novel influenza viruses; rapid intervention to limit the spread of the viruses; identify high-risk groups for early treatment; vaccination to prevent and control spreading of infection; and taking action to coordinate preparedness.

ACKNOWLEDGEMENTS

We would like to thank Dr. Amorn Leelarasamee for his encouragement and support and Dr. Henry Walke for providing suggestions and comments to improve this manuscript.

References

- Gill PW, Murphy AM. Naturally acquired immunity to influenza type A: a clinical and laboratory study. *Med J Aust* 1976;2:329-33.
- Glezen WP, Decker M, Perrotta DM. Survey of underlying conditions of persons hospitalized with acute respiratory disease during influenza epidemics in Houston, 1978-1981. *Am Rev Respir Dis* 1987; 136:550-5.
- Assaad F, Cockburn WC, Sundaresan TK. Use of excess mortality from respiratory diseases in the study of influenza. *Bull World Health Organ* 1973;49: 219-33.
- Centers for Disease Control and Prevention (CDC). Swine influenza A (H1N1) infection in two children-- Southern California, March-April 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:400-2.
- Dawood FS, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;360:2605-15.
- Centers for Disease Control and Prevention (CDC). Outbreak of swine-origin influenza A (H1N1) virus infection - Mexico, March-April 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:467-70.
- World Health Organization. New influenza A (H1N1) virus: global epidemiological situation, June 2009. *Wkly Epidemiol Rec* 2009;84:249-57.
- Fraser C, Donnelly CA, Cauchemez S, et al. Pandemic potential of a strain of influenza A (H1N1): early findings. *Science* 2009;324:1557-61.
- Vincent AL, Ma W, Lager KM, Janke BH, Richt JA. Swine influenza viruses a North American perspective. *Adv Virus Res* 2008;72:127-54.
- Treanor JJ. Influenza virus. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 6th ed. Philadelphia: Churchill Livingstone Inc., 2005:2060-85.
- Shinde V, Bridges CB, Uyeki TM, et al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. *N Engl J Med* 2009;360: 2616-25.
- Morens DM, Taubenberger JK, Fauci AS. The persistent legacy of the 1918 influenza virus. *N Engl J Med* 2009;361:225-9.
- Simonsen L, Clarke MJ, Schonberger LB, Arden NH, Cox NJ, Fukuda K. Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. *J Infect Dis* 1998;178:53-60.
- Cox NJ, Subbarao K. Influenza. *Lancet* 1999;354: 1277-82.
- Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A (H1N1) influenza viruses circulating in humans. *Science* 2009;325:197-201.
- Canil KA, Pratt D, Sungu MS, Phillips PA. Influenza surveillance: alternative laboratory techniques for a developing country. *Bull World Health Organ* 1985;63:79-82.
- Pereira M, Assaad FA, Delon PJ. Influenza surveillance. *Bull World Health Organ* 1978;56:192-203.

18. Centers for Disease Control and Prevention. Influenza surveillance summary--United States, 1982-1983 season. *MMWR Morb Mortal Wkly Rep* 1983;32:373-7.
19. Douglas RG Jr. Influenza in man. In: Kilbourne ED, ed. *The Influenza Viruses and Influenza*. New York: Academic Press, 1975:395-447.
20. Kilbourne ED, Loge JP. Influenza A prime: a clinical study of an epidemic caused by a new strain of virus. *Ann Intern Med* 1950;33:371-9.
21. Glezen WP, Keitel WA, Taber LH, Piedra PA, Clover RD, Couch RB. Age distribution of patients with medically-attended illnesses caused by sequential variants of influenza A/H1N1: comparison to age-specific infection rates, 1978-1989. *Am J Epidemiol* 1991;133:296-304.
22. McIntosh K, Halonen P, Ruuskanen O. Report of a workshop on respiratory viral infections: epidemiology, diagnosis, treatment, and prevention. *Clin Infect Dis* 1993;16:151-64.
23. Martin CM, Kunin CM, Gottlieb LS, Barnes MW, Liu C, Finland M. Asian influenza A in Boston, 1957-1958. I. Observations in thirty-two influenza-associated fatal cases. *AMA Arch Intern Med* 1959;103:515-31.
24. Luria DB, Blumenfeld HL, Ellis JT, Kilbourne ED, Rogers DE. Studies on influenza in the pandemic of 1957-1958. II. Pulmonary complications of influenza. *J Clin Invest* 1959;38:213-65.
25. Schwarzmans SW, Adler JL, Sullivan RJ Jr, Marine WM. Bacterial pneumonia during the Hong Kong influenza epidemic of 1968-1969. *Arch Intern Med* 1971;127:1037-41.
26. Bisno AL, Griffin JP, Van Epps KA, Niell HB, Rytel MW. Pneumonia and Hong Kong influenza: a prospective study of the 1968-1969 epidemic. *Am J Med Sci* 1971;261:251-63.
27. Fry J. Lung involvement in influenza. *Br Med J* 1951;2:1374-7.
28. Fry J. Influenza A (Asian) 1957; clinical and epidemiological features in a general practice. *Br Med J* 1958;1:259-61.
29. Kempe A, Hall CB, MacDonald NE, et al. Influenza in children with cancer. *J Pediatr* 1989;115:33-9.
30. Safrin S, Rush JD, Mills J. Influenza in patients with human immunodeficiency virus infection. *Chest* 1990;98:33-7.
31. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. *N Engl J Med* 2009;361:680-9.
32. Newton DW, Mellen CF, Baxter BD, Atmar RL, Menegus MA. Practical and sensitive screening strategy for detection of influenza virus. *J Clin Microbiol* 2002;40:4353-6.
33. Zambon M, Hays J, Webster A, Newman R, Keene O. Diagnosis of influenza in the community: relationship of clinical diagnosis to confirmed virological, serologic, or molecular detection of influenza. *Arch Intern Med* 2001;161:2116-22.
34. Covalciuc KA, Webb KH, Carlson CA. Comparison of four clinical specimen types for detection of influenza A and B viruses by optical immunoassay (FLU OIA test) and cell culture methods. *J Clin Microbiol* 1999;37:3971-4.
35. Boivin G, Hardy I, Kress A. Evaluation of a rapid optical immunoassay for influenza viruses (FLU OIA test) in comparison with cell culture and reverse transcription-PCR. *J Clin Microbiol* 2001;39:730-2.
36. Landry ML, Ferguson D. Suboptimal detection of influenza virus in adults by the Directigen Flu A+B enzyme immunoassay and correlation of results with the number of antigen-positive cells detected by cytospin immunofluorescence. *J Clin Microbiol*

- 2003;41:3407-9.
37. Centers for Disease Control and Prevention. Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) Virus - United States, 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:826-9.
 38. Boivin G, Hardy I, Tellier G, Maziade J. Predicting influenza infections during epidemics with use of a clinical case definition. *Clin Infect Dis* 2000;31:1166-9.
 39. Monto AS, Gravenstein S, Elliott M, Colopy M, Schweinle J. Clinical signs and symptoms predicting influenza infection. *Arch Intern Med* 2000;160:3243-7.
 40. Baden LR, Drazen JM, Kritek PA, Curfman GD, Morrissey S, Campion EW. H1N1 influenza A disease--information for health professionals. *N Engl J Med* 2009;360:2666-7.
 41. Glezen WP. Serious morbidity and mortality associated with influenza epidemics. *Epidemiol Rev* 1982;4:25-44.
 42. Perrotta DM, Decker M, Glezen WP. Acute respiratory disease hospitalizations as a measure of impact of epidemic influenza. *Am J Epidemiol* 1985;122:468-76.
 43. Heymann DL. *Control of Communicable Diseases Manual*. 19th ed. Washington DC: APHA, 2008.
 44. Kilbourne ED, Schulman JL, Schild GC, Schloer G, Swanson J, Bucher D. Related studies of a recombinant influenza-virus vaccine. I. Derivation and characterization of virus and vaccine. *J Infect Dis* 1971;124:449-62.
 45. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, et al. Guillain-Barre syndrome following vaccination in the National Influenza Immunization Program, United States, 1976-1977. *Am J Epidemiol* 1979;110:105-23.
 46. Wright PF, Thompson J, Vaughn WK, Folland DS, Sell SH, Karzon DT. Trials of influenza A/New Jersey/76 virus vaccine in normal children: an overview of age-related antigenicity and reactogenicity. *J Infect Dis* 1977;136 Suppl:S731-41.
 47. Wright PF, Cherry JD, Foy HM, et al. Antigenicity and reactogenicity of influenza A/USSR/77 virus vaccine in children--a multicentered evaluation of dosage and safety. *Rev Infect Dis* 1983;5:758-64.
 48. Lerman SJ, Wright PF, Patil KD. Antibody decline in children following A/New Jersey/76 influenza virus immunization. *J Pediatr* 1980;96:271-4.
 49. Johnson PR, Feldman S, Thompson JM, Mahoney JD, Wright PF. Immunity to influenza A virus infection in young children: a comparison of natural infection, live cold-adapted vaccine, and inactivated vaccine. *J Infect Dis* 1986;154:121-7.
 50. Fiore AE, Shay DK, Broder K, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep* 2009;58(RR-8):1-52.
 51. Centers for Disease Control and Prevention (CDC). Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. *MMWR Morb Mortal Wkly Rep* 2009;58:521-4.
 52. Centers for Disease Control and Prevention (CDC). Use of influenza A (H1N1) 2009 monovalent vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep* 2009;58(RR-10):1-8.
 53. Dolin R. Amantadine and rimantadine. In: Peterson PK, Verhoef J, eds. *The Antimicrobial Agents Annual*. Vol. 3. New York: Elsevier Science, 1988:361-70.
 54. Galbraith AW, Oxford JS, Schild GC, Potter CW, Watson GI. Therapeutic effect of 1-adamantanamine hydrochloride in naturally occurring influenza A 2 - Hong Kong infection. A controlled double-blind study. *Lancet* 1971;2:113-5.
 55. Little JW, Hall WJ, Douglas RG Jr, Mudholkar GS, Speers DM, Patel K. Airway hyperreactivity and

- peripheral airway dysfunction in influenza A infection. *Am Rev Respir Dis* 1978;118:295-303.
56. Treanor JJ, Hayden FG, Vrooman PS, et al. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. US Oral Neuraminidase Study Group. *JAMA* 2000;283:1016-24.
57. Nicholson KG, Aoki FY, Osterhaus AD, et al. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. *Lancet* 2000; 355:1845-50.
58. Hayden FG, Osterhaus AD, Treanor JJ, et al. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. GG167 Influenza Study Group. *N Engl J Med* 1997;337:874-80.
59. The MIST (Management of Influenza in the Southern Hemisphere Trialists) Study Group. Randomised trial of efficacy and safety of inhaled zanamivir in treatment of influenza A and B virus infections. *Lancet* 1998;352:1877-81.
60. Hayden FG, Belshe RB, Clover RD, Hay AJ, Oakes MG, Soo W. Emergence and apparent transmission of rimantadine-resistant influenza A virus in families. *N Engl J Med* 1989;321:1696-702.
61. Hayden FG, Sperber SJ, Belshe RB, Clover RD, Hay AJ, Pyke S. Recovery of drug-resistant influenza A virus during therapeutic use of rimantadine. *Antimicrob Agents Chemother* 1991;35:1741-7.
62. Centers for Disease Control and Prevention. CDC issues interim recommendations for the use of influenza antiviral medications in the setting of oseltamivir resistance among circulating influenza A (H1N1) viruses, 2008-09 influenza season. Atlanta, GA: US Department of Health and Human Services, CDC; 2008 [cited 2009 Dec 9]. Available from: <http://www2a.cdc.gov/HAN/ArchiveSys/ViewMsgV.asp?AlertNum=00279>
63. Hay AJ, Wolstenholme AJ, Skehel JJ, Smith MH. The molecular basis of the specific anti-influenza action of amantadine. *EMBO J* 1985;4:3021-4.
64. Covington E, Mendel DB, Escarpe P, Tai CY, Soberbarg K, Roberts NA. Phenotypic and genotypic assay of influenza virus neuraminidase indicates a low incidence of viral drug resistance during treatment with oseltamivir. *J Clin Virol* 2000;18:326.
65. Barnett JM, Cadman A, Gor D, et al. Zanamivir susceptibility monitoring and characterization of influenza virus clinical isolates obtained during phase II clinical efficacy studies. *Antimicrob Agents Chemother* 2000;44:78-87.
66. Gubareva LV, Bethell R, Hart GJ, Murti KG, Penn CR, Webster RG. Characterization of mutants of influenza A virus selected with the neuraminidase inhibitor 4-guanidino-Neu5Ac2en. *J Virol* 1996;70: 1818-27.
67. Gubareva LV, Robinson MJ, Bethell RC, Webster RG. Catalytic and framework mutations in the neuraminidase active site of influenza viruses that are resistant to 4-guanidino-Neu5Ac2en. *J Virol* 1997; 71:3385-90.
68. Centers for Disease Control and Prevention (CDC). Oseltamivir-resistant novel influenza A (H1N1) virus infection in two immunosuppressed patients - Seattle, Washington, 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:893-6.