Background: Influenza virus is the major cause of respiratory illness, especially in young and older age groups. Since 1918, many subtypes, defined by hemagglutinin (HA) and neuraminidase (NA), have caused global infections or pandemics. The recently isolated swine origin influenza virus (S-OIV) subtype H1N1 has been defined by the World Health Organization (WHO) as the cause of the present influenza pandemic.

Objective: To describe and attempt to predict the epidemiology of the novel H1N1 2009 in Bangkok and to evaluate the effects of school closures during the outbreaks.

Materials and methods: The first two human cases infected by this S-OIV subtype H1N1 or H1N1 2009 in Thailand have been reported in May 12, 2009 by the Ministry of Public Health. Between May 12 and July 30, 2009, 1212 nasopharyngeal (NP) swabs from four private hospitals and Chulalongkorn Hospital, Bangkok have been sent to a laboratory for Influenza virus diagnosis. The diagnosis was based on real time RT-PCR for seasonal influenza (H1, H3) and H1N1 2009.

Results: One thousand two hundreds and twelve specimens of patients with influenza like illness were tested using real time RT-PCR detection. Between mid June and early July, the number of H1N1 2009 increased rapidly with a high prevalence among the 6- to 20-year olds. School closure policy, long public holiday, and additional preventive measures have led to a rapid reduction in the number of H1N1 2009 positive patients.

Conclusion: Preventive measures including school closures are important to slow down the outbreak and thus enable health care centers to cope with the large number of patients. Everyone should play a role in delaying the spread of this pandemic

Keywords: H1N1, influenza virus, swine origin, school closure, Thailand.

The Centers for Disease Control and Prevention, United States (US-CDC) have confirmed the first case of novel H1N1 2009 influenza virus infection in a child in southern California, United States on April 21, 2009 [1]. Upon virus isolation from this patient, scientists discovered a novel triple re-assorted virus that had never infected humans [2]. The initial outbreak had occurred in Mexico in mid March 2009, and by end of April when the virus had been identified, the disease had spread out of control in the United States and Canada. Subsequently, the pandemic has quickly spread throughout every continent, including southeast Asia [3]. Although the origin of this novel virus remains to be explained, research has shown that the viral genome is closely related to swine influenza virus [4-7]. Therefore, the virus has been named swine-origin influenza virus (S-OIV).

Since the initial outbreak in Mexico, the novel virus infection has expanded continuously. Until now, there have been many patients with symptoms of influenza-like illness. In this critical situation, the World Health Organization (WHO) had to increase the world pandemic alert from level five to six on June 11, 2009, indicating a global pandemic of novel H1N1 2009 influenza virus infection. To this day (July 8, 2009), 94,512 confirmed cases of human H1N1 influenza infection, including 429 deaths, from more than 120 countries have been reported to WHO [8]. This number has been increasing over the past few weeks.
However, many of these cases are reportedly linked to travel without community spread [9]. Moreover, this virus has been confirmed capable of person-to-person transmission. Therefore, as with seasonal influenza, it can be transmitted by droplet infection [10].

Research conducted in ferrets and mice comparing between the novel H1N1 and the contemporary seasonal H1N1 as to their respective pathogenic and transmission potential has shown that either virus can be transmitted by droplet infection from infected to immunologically naïve ferrets but the symptoms observed in ferrets infected with 2009 H1N1 virus trend to be more severe [11, 12]. Post mortem examination of the internal organs (lung, intestine and brain) revealed the novel pandemic virus present in lung tissue at a higher titer than the contemporary H1N1 virus [11, 12]. In addition, the novel virus can be recovered from the intestinal tract tissue of intra-nasally inoculated ferrets [11]. The novel virus appears to replicate in tissue more efficiently than the seasonal H1N1 and hence, cause increased morbidity. Comparison between the novel H1N1 and the 1918 Spanish flu virus as to the HA protein’s binding affinity to target cells has shown that although the binding patterns are similar, which may account for the novel virus’s more efficient tissue penetration, the 2009 H1N1’s binding efficiency is significantly lower than that of the 1918 pandemic strain [11].

In infected patients, the symptoms caused by this novel virus are not more severe than those found in seasonal influenza infection. Individuals infected will display fever and respiratory symptoms as with a common cold. More severe symptoms may include pneumonia or lung swelling [13]. In patients with underlying complications such as heart disease, chronic lung disease, or diabetes the symptoms are usually more severe [14]. According to data from Michigan Hospital, obesity presents a new risk factor for severe influenza. The fatality risk will increase in obese patients with BMI by more than 30. It has been suggested to increase the oseltamivir dosage for obese patients [15].

In Thailand, the first two cases were confirmed by the Thai Ministry of Public Health on May 12, 2009. It was reported that the patients who had traveled to Mexico had been treated and allowed to return home. The virus has not been transmitted to anyone in contact with these two patients. Nothing was heard of this virus until 12 June, 2009 when outbreaks of H1N1 2009 influenza virus were reported in several schools in the Bangkok Metropolitan area and the eastern seaside resort town of Pattaya where due to overcrowding, the population is in close contact. Initially, the outbreak could be limited by strict control measures implemented by the government, but the virus has subsequently expanded rapidly and uncontrollably. At present (July 31, 2009), more than 8,500 cases have
been confirmed infected by this novel virus, including 65 fatalities ranging in age from four months to 91 years (average 38 years). Most of them (70%) had chronic underlying illness including obesity [16].

According to research conducted on human influenza in Srakaew and Nakorn Panom provinces, Thailand, between 64 and 91 cases of human influenza per 100,000 persons have been reported per year during the period from 1993 to 2002, and they were evenly distributed among all age groups [17]. Influenza appears to peak between June and September and again, slightly increase during January and February. This result indicates that the seasonal influenza in Thailand usually spreads from the start of the rainy season to the end of winter. Additional supportive information can be gleaned from the curve depicting the proportion of positive samples of influenza virus infection throughout the years 2001-2003, with the sharp peaks indicating the high proportion of positive isolates occurring typically between June and October. Moreover, when the age distribution of influenza-like illness was considered, it could be demonstrated that influenza is more likely to affect the older age groups [17].

Here, we are going to describe the epidemiology of influenza virus infection in Thailand during the outbreak of the novel H1N1 2009, age distribution and effects of school closures in Bangkok.

**Materials and methods**

**Specimen collection**

Our group has been involved in the investigation of the H1N1 2009 outbreak and service for influenza diagnosis since May 2009. Nasopharyngeal swabs were collected from patients with influenza-like illness, who attended the Bangpakok Hospital Network (Bangpakok 1, 3, 8, and 9 Hospital) and Chulalongkorn Memorial Hospital, Bangkok, Thailand between May and July 2009. These samples were received as part of service and the national public health surveillance for H1N1 2009 in Thailand. The samples were collected in 2 mL of virus transport medium with antibiotics (Penicillin G 2x10^6 U/liter and Streptomycin 200 mg/liter) and stored in a biohazard icebox for transportation to the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University. The collected specimens were kept at 4°C, and processed within 24 hours.

**RNA extraction**

The specimens were processed immediately upon arrival. RNA extraction was performed using the Viral Nucleic Acid Extraction Kit (RBC Bioscience Co, Taiwan) according to the manufacturer’s protocol. All experiments were performed in a Bio-safety Level 2 plus environment.

**Detection and subtyping of influenza virus by real-time RT-PCR**

To detect and subtype influenza virus, our group performed two separate reactions of single step multiplex real-time RT-PCR based on specific TaqMan probes as described previously [18]. The first reaction was aimed at detecting the GAPDH gene (internal control), matrix gene of influenza A virus and matrix gene of influenza B virus. This was to discriminate between influenza A and B and to indicate the quality of the extracted RNA. The second reaction was designed for detecting seasonal human influenza A virus (subtypes H1 and H3) and avian influenza A virus (subtype H5). The HA primers and probes used for the detection of novel 2009 H1N1 influenza A virus were modified from those previously described by CDC [19].

Real-time RT-PCR was performed using the SuperScript III Platinum One-Step RT-PCR system (Invitrogen, Carlsbad, USA). The reaction comprised 2.0 μL of RNA sample combined with a reaction mixture containing 5 μL of 2 X Reaction Mix, 0.2 μL of SuperScript III RT Platinum® Taq Mix (Invitrogen, Carlsbad, USA). They were each primed and probed at a final concentration of 0.25 μM and 0.125 μM, respectively and RNase-free water in a final volume of 10 μL. One-step multiplex real-time RT-PCR was performed by a Rotor-Gene 3000 (Corbett Research, New South Wales, Australia). Thermal cycling conditions included a reverse transcription step at 50°C for 30 minutes. After an initial denaturation step at 95°C for 10 minutes, amplification was performed during 50 cycles including denaturation (95°C for 15 seconds) and annealing together with extension (60°C for 30 seconds). Multiple fluorescent signals were obtained in each cycle upon completion of the extension step. Data acquisition and analysis of the real-time PCR assay were accomplished using the Rotor-Gene data analysis software, Version 6.0 (Corbett research, New South Wales, Australia).
Results

In the course of this study, 1,212 specimens were collected from patients with symptoms of influenza-like illness between May and July 2009. These patients comprised 617 females and 595 males with their age ranging from 3 months to 93 years. After RNA extraction, all the clinical samples were subjected to multiplex one-step real-time RT-PCR using primers specific for several kinds of influenza viruses, specifically, H1N1 and H3N2 seasonal influenza A viruses, influenza B, and the novel swine origin H1N1 2009 influenza A virus. The proportional distribution of these viruses is shown in Fig. 2. After the first case of H1N1 2009 infection was confirmed in Thailand, samples were sent to our laboratory for influenza confirmation. The result shows that the number of H1N1 2009 positive samples was slightly increasing during late June until it sharply peaked at the beginning of July. Subsequently, it rapidly decreased during the next few days due to the long public holiday and the school closure policy of Bangkok Metropolitan Administration as shown in Fig. 2.

By late June to mid July 2009, nearly 90% of Influenza A was caused by H1N1 2009. The seasonal influenza A viruses detected within the chosen timeframe amounted to 7.92% of all specimens. The prevalent seasonal influenza A virus circulating in this season was H3N2. The age distribution among reported cases of novel H1N1 2009 influenza infection in Thailand is shown in Fig. 3.

Most patients infected by H1N1 2009 were between 7 and 20 years of age whereas the seasonal influenza A was found among all age groups. After school closures from early July, the incidence of affected children (age 6-20 years) with H1N1 2009 infection had declined, whereas it had increased among children below the age of 6 years and middle aged (20-40 years) individuals (Fig. 4).

Fig. 2 Proportion of specimens positive for novel H1N1 2009 influenza A virus relative to seasonal H1N1 and H3N2 influenza A viruses during the periods specimen retrieval (from May, 2009 to July, 2009). a: The public holiday (4-9/7/2009), b: School closed as Bangkok Metropolitan Administration policy (15-19/7/2009), c: Tutorial schools closed as Bangkok Metropolitan Administration policy (13-28/7/2009).
Comparison between the number of H1N1 infected people in Thailand and the total number of H1N1 infected people on a global scale as confirmed by WHO (Fig. 1) has shown the confirmed cases in Thailand to amount to 2.8%. However, as shown in Fig. 2, about 70% of influenza patients have been infected by the novel H1N1 strain, which appears to have successfully replaced the contemporary seasonal influenza virus. Previously, surveillance data have arrived at a high death ratio for novel influenza infection in Mexico. This report has caused global concern with people assuming the infection will elicit severe disease [20]. In fact, the death ratio of all patients with laboratory-confirmed novel influenza A infection residing outside of Mexico amounts to approximately 0.1-0.2% [21]. Moreover, reports of fatalities have rapidly decreased.
The novel H1N1 2009 represents an emerging disease, implying that due to lack of acquired immunity everyone is susceptible to the virus. The new influenza virus can be transmitted by contaminated droplets with the basic reproductive number equal to 1.4-1.6 in Mexico [22], 1.96 in New Zealand [23] and 2-2.6 in Japan [24]. From an epidemiological point of view, the disease will cease to flare up as an outbreak and mature to seasonal influenza once the herd immunity to the new H1N1 amounts to approximately 30% (herd immunity = 1-1/R₀) [25]. For Thailand, the expected number of infections or immunity from vaccination could be 20 millions of the population (based on \( R₀ = 1.5 \)).

Since the first outbreak of H1N1 2009 occurred in a school within the Bangkok Metropolitan area, Bangkok Metropolitan Administration has opted for a long public holiday and school closure policy to effectively limit the expansion of this virus. Since then, cases positive for H1N1 2009 have rapidly decreased. However, it should be noted that this incidence rate of H1N1 2009 does not mirror the trend of this virus in all provinces of Thailand for the samples we received originated exclusively from Bangkok province. However, the school closures to slow down the expansion of seasonal flu in Hong Kong may not lead to a significant reduction in influenza-like illness when compared to the rate of influenza-like illness in areas where no holiday had been introduced after the peak and class dismissal policy will have social and economic implications [26]. According to the present study though, the class dismissal policy in schools and tutorial schools represents one of the effective pandemic mitigation strategies (including administration of antiviral drugs, and informing people about appropriate preventive measures). The differences between both studies may be due to the transmissibility and age distribution of the novel pandemic H1N1 virus. The seasonal H1N1 virus differ from the novel H1N1 virus as it can be transmitted more effectively [11]. Therefore, the school closure may not represent an efficient policy to limit the expansion. As for age distribution of either virus, the novel pandemic H1N1 appears to exclusively affect the younger age groups whereas the contemporary H1N1 virus is equally distributed among all age groups. Moreover, the starting point of the epidemic of novel H1N1 virus was in the schools in the metropolitan area. Therefore, the school closure can more effectively limit the spread of the H1N1 pandemic in Thailand than the same policy in Hong Kong where it has been applied for mitigating seasonal H1N1 expansion.

From the start of the H1N1 2009 outbreak in Thailand from end of June to early July, the target population with high incidence comprised students from 7 to 20 years of age (Fig. 4). Within a couple of weeks, this target will expand to include younger and older people. Therefore, within the next few months, office personnel, prisoners, and factory workers will be affected. Preventive measures should be put in place in the near future.

In conclusion, it will not be possible to stop a pandemic H1N1 outbreak within a few months. The infection will persist in the form of various outbreaks for the next few years and mature to seasonal influenza. Preventive measures are very important to slow down the outbreak and thus enable health care centers to cope with the large number of respiratory tract disease. Everyone should play a part in delaying the spread of this pandemic.

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