Original Article

Epidemiology of seasonal influenza in Bangkok between 2009 and 2012

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Abstract

Introduction: This study investigated influenza activity in Bangkok, Thailand between June 2009 and July 2012.

Methodology: Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed to detect influenza viruses among patients with influenza-like illnesses.

Results: Of the 6417 patients tested, influenza virus infection was detected in 42% (n = 2697) of the specimens. Influenza A pH1N1 viruses comprised the predominant strain between 2009 and 2010, and seasonal influenza (H3) had a high prevalence in 2011. Laboratory data showed a prevalence and seasonal pattern of influenza viruses. In 2009, influenza activity peaked in July, the rainy season. In 2010, influenza activity happened in two phases, with the initial one at the beginning of the year and another peak between June and August 2010, which again corresponded to the rainy period. Influenza activity was low for several consecutive weeks at the beginning of 2011, and high H3N2 activity was recorded during the rainy season between July and September 2011. However, from the beginning of 2012 through July 2012, pH1N1, influenza H3N2, and influenza B viruses continuously circulated at a very low level.

Conclusion: The seasonal pattern of influenza activity in Thailand tended to peak during rainy season between July and September.

Key words: influenza; surveillance; prevalence; real-time PCR; Thailand

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Introduction

Influenza viruses are major causes of respiratory tract disease in humans, presenting a significant burden to global public health. In addition to seasonal influenza, occasional outbreaks of pandemic influenza have occurred on a global scale. These outbreaks have invariably been caused by a virus to which the majority of people have not yet had the opportunity to develop sufficient immunity. During early April 2009, while the pH1N1 virus was spreading across the world, there were increasing numbers of pH1N1 infection cases during many influenza seasons in various regions of the world. By August 2010, the World Health Organization (WHO) declared the end of the pandemic phase [1], as there was no immediate concern about the situation. Since then, the pH1N1 virus has been considered with the same level of concern as seasonal influenza and still continues to circulate among human populations.

In Thailand, the first confirmed case of the pH1N1 virus occurred in May 2009 and was reported by the Bureau of Emerging Infectious Diseases, Department of Disease Control, Ministry of Public Health. Thailand is a tropical country in the northern hemisphere with a climate that regularly alternates between wet and dry seasons. The rainy season normally lasts from May to October. The dry season usually lasts from November to April, and the cooler season lasts from November to February. Seasonal influenza prevalence in tropical climates is different from that in temperate climates. However, there are diverse occurrences of influenza among countries in the same climate zone, with the majority of cases occurring during the respective rainy seasons [2-4]. In temperate areas, on the other hand, influenza activity usually peaks during the winter months [5].

Globally circulating strains of influenza viruses vary and can include Influenza A, H3N2 viruses, or Influenza B viruses that are usually identified every year, with the dominant strains of influenza changing from one year to the next influenza viruses are continuously evolving by a process called antigenic drift and antigenic shift; they are mainly subject to antigenic drift, which is caused by an accumulation of changes in the viral antigenic determinants. This is a likely result of host selection pressure, as the two viral genes most prone to mutation are hemagglutinin (HA) and neuraminidase (NA), which code for the external

glycoproteins located on the surface of influenza viruses. These gradual changes in its antigenic sites enable the virus to escape the host's immune response. Hence, predominant strains or subtypes of influenza can circulate for some period of time and will decrease their infection rate as the population acquires protective antibodies sufficiently specific to prevent infection by those subtypes or strains, until the virus begins to undergo antigenic change, a process called herd immunity. Thus, as antibody levels may decline and the viruses evolve, the most efficient protection from influenza is an annual vaccination.

The purpose of this study was to observe seasonal influenza activity along with influenza occurrence and weather conditions based on data collected between June 2009 and July 2012. This epidemiological data will assist in understanding which factors contribute to virus emergence in tropical countries, and thus in developing strategies for outbreak prevention.

Methodology

Geographic location

Bangkok, the capital of Thailand, is located in the central part of the country; with a population of 8 million, it is Thailand's most densely populated city. Overall, the climate is tropical monsoon from May to October and dry between November and April, with the lowest and highest temperatures ranging between 18 and 39 degrees Celsius; the average temperature throughout the year is around 29 degrees Celsius with an average relative humidity of 74%.

Specimen collection

In total, 6417 nasopharyngeal or throat swabs were collected from patients with ILI who had attended hospitals located in Bangkok, Thailand between June 2009 and July 2012. All specimens were obtained during the patients' routine examinations for respiratory tract viruses for management and treatment. The inclusion criteria for influenza-like illnesses were based on symptoms such as fever, sore throat, cough, nasal congestion, and myalgia. During transport to the Center of Excellence in Clinical Virology, all samples were stored in a transport medium (penicillin G (2 x 10^6 U/L), streptomycin 200 mg/L) at 4^{o}C, and then transferred to -20^{o}C within 24 hours. The samples were tested for influenza A (pH1N1, seasonal H1N1, seasonal H3N2), and influenza B viruses by real-time reverse transcription polymerase chain reaction (RT-PCR).

This study examined patients' samples once routine service had been finalized. Patient identification, including personal information and hospitalization or admission number, was deleted from the data records to protect patient confidentiality. The research protocol was approved by the Ethics Committee of the Faculty of Medicine Chulalongkorn University (IRB No. 350/54). Permission to use those data was granted by the director of Chulalongkorn Hospital, and all information was used for research purposes only. The Institutional Review Board waived the need for consent because the data used was kept anonymous.

Meteorological Data

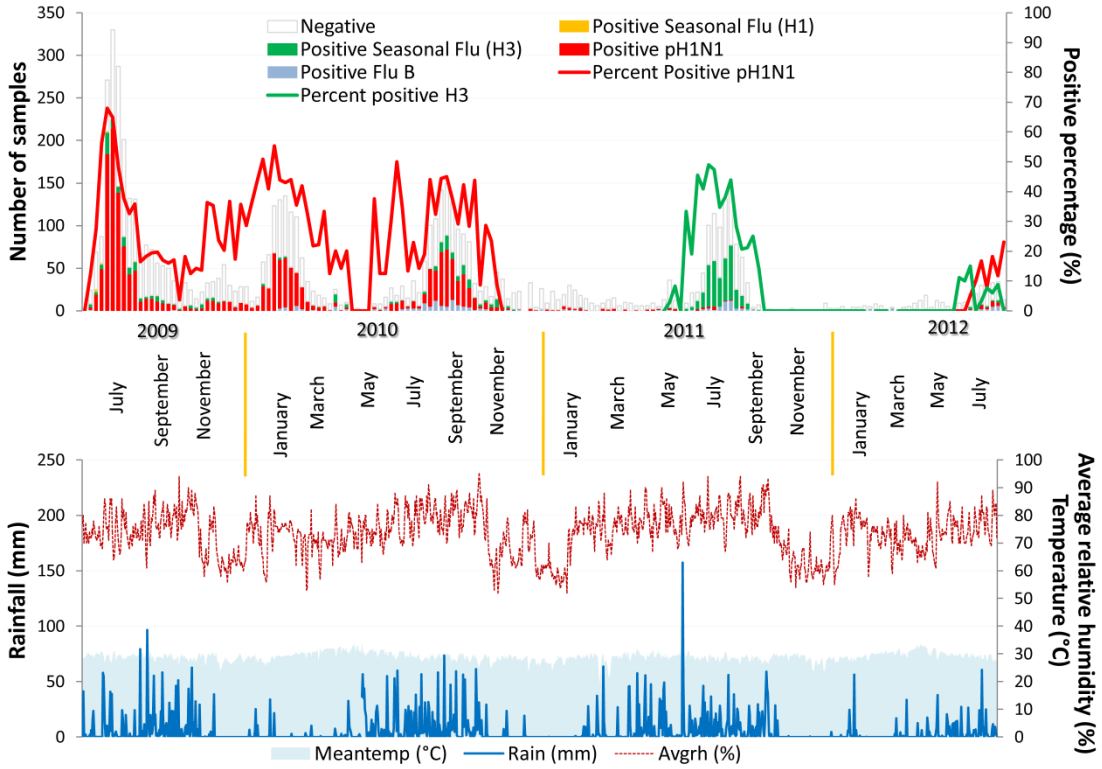
Data on temperature and average rainfall were provided by the Thai Meteorological Department. These data were collected daily in the Bangkok metropolitan area for the entire duration of this study. Average temperature, average relative humidity, and rainfall were measured in degrees Celsius and millimeters, respectively.

Laboratory methods

Detection of influenza viruses by real-time RT-PCR assay

RNA was extracted using the Viral Nucleic Acid Extraction Kit (RBC Bioscience Co, New Taipei City, Taiwan) according to the manufacturer's instructions. To create amplification of the target genes, primers were designed by retrieve nucleotides of various influenza strains from the GenBank database. After the alignment of the nucleotide, the most conserve region was selected as the primer to amplify cDNA during the assay. Degenerate primers were designed for nucleotide data sets with a low conservative region to ensure sequence coverage. The first reaction included primers and probes designed to specifically detect influenza A or B viruses; the second reaction was designed to detect pH1N1 and seasonal H1, H3, H5 subtypes. TaqMan probes, primers, and thermal profiles for the reaction were as described previously [6-8]. RT-PCR was performed using the SuperScript III Platinum One-Step RT-PCR system (Invitrogen, Foster City, USA) in a Rotor-Gene 3000 (Corbett Research, Mortlake, Australia). With this method, only the type and subtype of influenza viruses could be identified. Samples were defined as positive cases upon confirmation of any influenza virus by real-time RT-PCR.

Figure 1: Weekly percentage of laboratory confirmed influenza cases by type from influenza-like illness patient visits to hospital in Bangkok area, and daily average temperature, average rainfall in Bangkok from June 2009 to July 2012.



Data analysis

The epidemiological surveillance study was conducted based on recorded information from routine service. All data were depicted as a weekly percentage of laboratory-confirmed influenza cases by influenza type and distribution of influenza positive cases by age group. Statistical Pearson correlation analysis was performed by using SPSS software for Windows version 17.

Results

Of the 6417 samples collected between June 2009 and July 2012, 3102 were from males and 3315 were from females; the age of the patients ranged from one day to 98 years, with a mean age of 23. Due to insufficient quantity of cellular material, the housekeeping gene could not be amplified in 136 specimens. Six thousand and fifty specimens, comprised of 3035 males and 3246 females, were included in the analysis. Of those, 2969 (42%) were found positive for influenza viruses. Samples positive for influenza A virus were subtyped into seasonal H1N1, seasonal H3N2, pandemic (H1N1) 2009 viruses, and influenza B virus. None of the specimens were positive for H5N1.

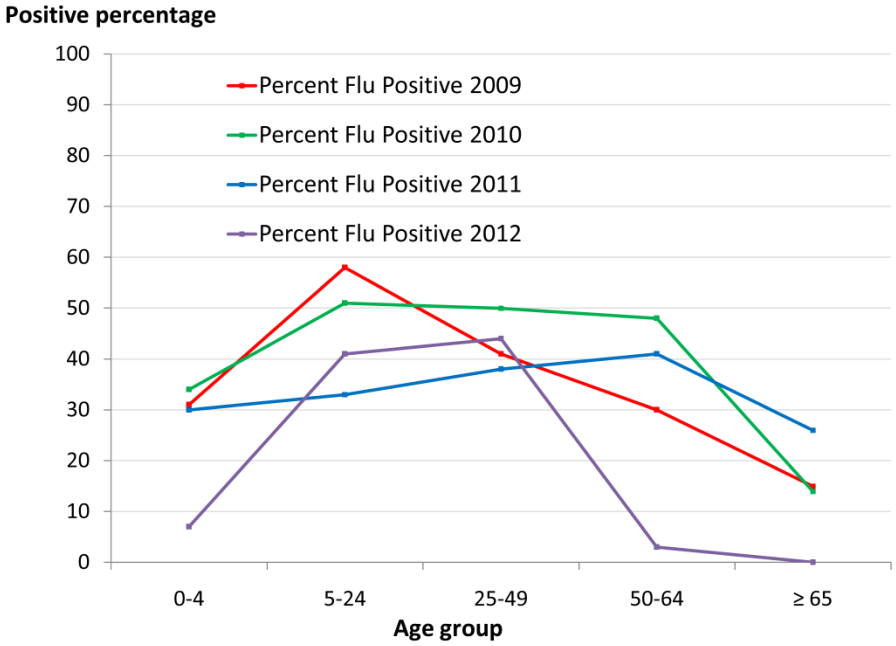
Prevalence distribution

The pH1N1 virus was the most common influenza strain found in positive samples. During the study period, 40% of ILI cases had an influenza A virus infection. Seasonal H1N1 subtype was identified in 0.2%, pH1N1 in 30%, and H3N2 in 10%. Three per cent of tested samples were positive for the influenza B virus.

Figure 1 shows the total number of patients with acute respiratory tract symptoms per week, as well as percentages of pH1N1 and influenza A (H3). In June 2009 (week 24), influenza activity – most notably pH1N1 – started to increase until August 2009 (week 35). Of the 1621 specimens tested during this wave of influenza activity, 813 (54%) were positive for pH1N1.

Moreover, the small proportions of co-circulating strains were influenza A (H3) and, to a lesser extent, influenza A (H1). After August 2009 (week 35), influenza activity declined for several months with a

Figure 2: Percent positive of influenza by age group from June 2009 to July 2012



small number of positive cases of pH1N1 and influenza A (H1) remaining.

The low activity of influenza viruses continued until early 2010, when influenza activity started to increase with the same predominant strain, pH1N1, as during the previous year. In the course of this wave, which lasted until early March (week 10), 373 of 849 (44%) specimens were positive for pH1N1. Low levels of influenza B viruses as co-circulating strains were also detected in this wave, which lasted approximately three months. From the end of March 2010 (week 12) to the end of May (week 21), influenza activity remained low, with minor pH1N1 activity. By August 2010 (week 31), high levels of pH1N1 were detected. During this wave, influenza B viruses and seasonal influenza (H3) co-circulated until October 2010 (week 40), when the overall activity started to decrease. Out of the 983 specimens tested during this time, 371 (38%) cases were positive for pH1N1, 67 (7%) for influenza B viruses, and 75 (8%) for seasonal influenza (H3). From the end of October 2010 (week 42) to July 2011 (week 26), pH1N1 prevalence remained very low. From July to mid-September 2011 (weeks 26-37), 328 of 859 (38%) collected specimens harbored influenza A virus subtype H3N2, with 43 (5%) samples positive for influenza B, and 14 (2%) for pH1N1. From mid-September 2011 through June 2012, the frequency of influenza virus infections continued to decrease, with little influenza activity. Of a total of 171 specimens collected at the beginning of 2012, 35 (20%) specimens were positive for influenza B, 26 (15%) were positive for pH1N1, and 15 (9%) for influenza A (H3N2). From late June to the end of July, the numbers of pH1N1-positive cases increased. There were a total of 51 influenza-positive cases from the 159 specimens tested. The predominant influenza viruses during this month were the pH1N1 and influenza B viruses, which account for 23 (14%) and 20 (13%) positive cases, respectively; 8 (5%) specimens were positive for the influenza A virus subtype H3N2.

Age stratification

Between June 2009 and July 2012, influenza virus activity was divided into four waves: June to August 2009, January 2010 to March 2010, August to October 2010, and July to September 2011. To determine the case distribution per age group during these periods, the percentages of influenza-positive cases related to age group were compared to the pandemic (H1N1) 2009 virus targeted school-age children and adolescents (between five and 24 years of age) in 2009 during the first wave of pH1N1 (mean age = 19 years). In 2010, the prevalence of the pandemic (H1N1) 2009 virus was still predominant in the school-age group,

but the older age group was increasingly affected when compared with the first period. But in 2011, the proportion of positive cases among those of working age (25-49 years) and the elderly groups (over 50 years) increased, as the predominant strain had shifted from pandemic (H1N1) 2009 to seasonal influenza H3. In 2012, most positive cases were among children and adolescents (5-24 years) and the working age group (25-49 years).

Weather conditions and prevalence

The weather in Bangkok and its surrounding areas was reviewed for the study period between June 2009 and July 2012 and was compared with influenza activity in this area as shown in Figure 1. The overall percentages of influenza-positive cases among acute respiratory tract infection patients are depicted in Figure 2 as percent positive in each age group from each year.

In the first wave of infection, between June and August 2009, the average temperature in Bangkok, the capital of Thailand was 29.2°C (range 26.7 to 31.4°C), and rainfall averaged at 8.1 millimeters per day (range 0 to 96.6 millimeters per day). This period constituted the rainy season. During this period, the pandemic (H1N1) 2009 virus was the most prevalent. The seasonal H3N2 virus was also detected throughout this season.

Between January 2010 and March 2010, the pandemic (H1N1) 2009 virus was still predominant. The average temperature in this period was 29.1°C (range 25.7 to 31.8°C). The average rainfall in this period was lower than during the previous period, 1.30 millimeters per day (range 0 to 34 millimeters per day). In the second period of infection, the prevalence of seasonal H3N2 and B virus decreased in comparison with the rainy season, with seasonal H1N1 absent; however, there was a high frequency of pandemic (H1N1) 2009.

During the following wave, from August to October 2010, the average temperature and rainfall was 29.4°C (range 24.9 to 30.4°C) and 10.97 millimeters per day (range 0.0 to 73.5 millimeters per day), respectively. The average rainfall during this season was higher than in the previous year. During this period, the pandemic (H1N1) 2009 virus was still predominant. During the wave in 2011 from July to September, the average temperature was 28.4°C (range 26.1 to 31.1°C), with average rainfall of 9.11 millimeters per day (range 0 to 56.1 millimeters per day). During this period, seasonal influenza H3 was predominant.

Between January and July 2012, the average temperature and rainfall was 29.62°C (range 21.8 to 40°C) and 1.5 millimeters per day (range 0 to 56.4 millimeters per day), respectively. The correlation analysis between average temperature, average rainfall, and average relative humidity did not show any significant correlation with the number of influenza-positive cases from 2009 through 2012 ($r = -0.091$, $r = 0.189$ and $r = 0.238$; $p > 0.05$, respectively).

Discussion

This study indicated that most patients who attended a hospital during the influenza season were diagnosed with influenza viruses that caused acute respiratory tract infections. This study also indicated that influenza viruses are important causes of respiratory tract disease, in addition to other known viruses associated with respiratory tract infection such as rhinovirus, adenovirus, respiratory syncytial virus, and parainfluenza virus [9,10]. Also, influenza A virus strains circulated during this study, with pH1N1 and seasonal influenza H3 predominant in 2009 through 2010 and 2011, respectively.

From the end of 2011 through July 2012, the number of patients admitted to a hospital with acute respiratory tract diseases decreased due to lower overall public awareness of pH1N1, because the symptoms of influenza were relatively mild in patients without any underlying disease. The number of positive cases may therefore be underestimated.

In 2009, the prevalence of pH1N1 was much higher than at the peak of the influenza season in 2010. This may be due to a large proportion of the population not yet having developed antibodies against pH1N1, which at that time constituted a new pandemic strain.

In this study, cases of pH1N1 were detected in June 2009. The first confirmed case in Thailand occurred in May 2009 and was reported by the Bureau of Emerging Infectious Diseases, Department of Disease Control, Ministry of Public Health. Upon its emergence, a large number of positive cases were confirmed in this period. The seasonal H1N1 virus decreased in prevalence and, since the emergence of the pandemic (H1N1) 2009 virus, has been detected rarely.

At the emergence of pandemic (H1N1) in 2009, the mean age of infected patients was 19 years, which correlates with other studies that have reported that pH1N1, in contrast to seasonal influenza, specifically targets younger age groups [11,12]. This may be

explained by the fact that older adults have developed some level of protective antibody against pH1N1 due to previous infection with the related H1N1 virus [13].

The influenza positive rate of the 5-24 year age group decreased in the following year because this group had acquired some level of seroprotective antibody after pH1N1 infection, while the infection became more prevalent in older age groups [14]. Thus, in 2010, the mean age of patients was 19 years, which increased to 29 years in 2011. It was observed that once the pandemic (H1N1) 2009 virus had become the seasonal flu, people's attentiveness to good personal hygiene dropped in comparison with the first infection period.

In 2011, the seasonal influenza (H3) was the major circulating strain, affecting the 50-64 year age group, which correlates with numerous studies that have reported that most seasonal influenza viruses tend to attack older people due to their weaker immune systems and causes more severe complications [15].

However, from the beginning of 2012 through June, the overall influenza activity in Bangkok declined, with few patients attending the hospital. From late June to July, influenza activity started to increase. This may be because influenza activity is usually most pronounced during the rainy season, despite the apparent lack of any significant correlation between influenza positive cases and rainfall or any other two environmental factors in this study.

Influenza activity in Bangkok and its surroundings was different from other regions in Thailand. The predominant strain in Nakhon Si Thammarat province (south of Thailand) was seasonal influenza (H3), with lower levels of the pH1N1 and influenza B virus co-circulating during the 2010 season [16], while the pH1N1 virus predominated during the same season in Khon Kaen province (northeast of Thailand) with low-level co-circulation of seasonal influenza (H3) and influenza B viruses [17]. In Bangkok, pH1N1 was a predominant strain throughout the year with co-circulation of seasonal influenza (H3) and influenza B viruses. However, during the 2011 season, seasonal influenza (H3) became the predominant strain.

Despite minor differences in influenza activity patterns at the same time of year, which might have been influenced by weather variations, the peak influenza activity trend was similar [18,19]. In south Thailand, the climate has only two seasons, rainy and dry [20], with more prolonged periods of rain than in other regions. In the northeast, on the other hand, the climate is usually semi-humid and dry with generally little rainfall compared to other regions. In the neighboring countries and other countries in tropical climate zones, influenza activity has been reported to peak in the rainy season [21-24]. Also, in this study, the peak of influenza activity was usually observed during and immediately after the rainy season, which might have had a direct or indirect effect on influenza seasonality. However, other environmental factors such as temperature, humidity, El Niño, and the annual variability of climate have been reported to affect influenza prevalence [25,26].

Continuing surveillance of influenza circulation patterns could constitute part of the epidemiological data required for preventive measures such as vaccination campaigns for high-risk groups among the Thai population. Although epidemiological studies will assist in improving strategies aimed at future prevention of epidemics or pandemics, the best prevention strategies are raising awareness about the importance of personal hygiene.

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