



## Prevalence of Influenza Virus Type and Subtype at Siriraj Hospital, Bangkok, Thailand During 2013 - 2017

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**Background:** Influenza A (pandemic and seasonal H1/H3) and influenza B viruses were the predominant circulating seasonal influenza strains. Following its massive outbreak in 2009 globally, including Thailand, influenza A (H1N1) pdm09 viruses have replaced the previous seasonal H1 strain and become one of the circulating strains ever since. Both influenza A and B viruses are highly contagious and potentially cause respiratory illness ranging from mild to severe.

**Objective:** To determine the prevalence of types and subtypes of circulating influenza virus strains in Bangkok, Thailand during 2013 - 2017.

**Methods:** The 4385 nasopharyngeal wash specimens were collected from patients presented with influenza-like illness from January 2013 to December 2017 at Siriraj Hospital, Bangkok, Thailand. Influenza virus types and subtypes were determined using real-time RT-PCR technique. Clinical characteristics of patients infected with influenza A viruses and influenza B virus were compared and analyzed.

**Results:** Of 4385 nasopharyngeal wash specimens, the prevalence of influenza virus infection during 2013 - 2017 was 18.22% (n = 799). Of 799 influenza-positive samples, 608 (76.09%) and 191 (23.90%) samples were positive for influenza A and influenza B viruses, respectively. Most patients were presented with fever, cough, and runny nose; however, patients infected with influenza A virus generally had higher severity than those with influenza B virus infection ( $P < .05$ ).

**Conclusions:** The findings provided the characteristics of influenza virus types and subtypes at Siriraj Hospital, Bangkok, Thailand during 2013 - 2017. Sporadic cases of influenza occurred all year round, but the incidence peaked in March 2014 and August 2017. The outcomes of this study are potentially useful for prevention, treatment, and disease monitoring.

**Keywords:** Seasonal influenza, Influenza A (H1N1) 2009 virus, Influenza B virus

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## Introduction

Influenza viruses are the major causes of human respiratory tract disease. This RNA virus belongs to family *Orthomyxoviridae*.<sup>1</sup> Members of this family can be classified into 7 genera, 4 of which can cause influenza in vertebrates, including influenza A, B, C, and D. Influenza A and B viruses are continuously circulated in the human population. Influenza A viruses can be further classified into different subtypes based on the 2 surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). Eighteen different hemagglutinin and 11 neuraminidase subtypes were documented. Epidemiological studies demonstrated the common subtypes include H1N1, H3N2, H5N1, H7N7, and H9N2.<sup>2</sup> Unlike influenza A viruses, influenza B viruses are not further divided into subtypes but can be classified on the antigenic differences into 2 lineages: Victoria and Yamagata lineages.<sup>2</sup>

Clinical symptoms of influenza are fever, myalgia, sore throat, and nonproductive cough. Most patients will recover within 1 - 2 weeks, but some are severe, mostly in children and older adults aged more than 65 years.<sup>3</sup> The clinical symptoms of individuals infected with influenza type A are more severe than those infected with influenza type B.<sup>4</sup>

Influenza viruses cause sporadic outbreaks in many countries via human to human transmission. The most lethal outbreak causing by influenza A H1N1 (Spanish flu) was reported between 1918 and 1920, resulting in approximately 50 million deaths worldwide.<sup>5</sup> Moreover, the recent pandemic occurred in 2009 by influenza A (H1N1) virus was due to genetic exchange between the avian-, swine-, and human-influenza viruses.<sup>6</sup>

In Thailand, the pandemic H1N1 (pH1N1) virus was reported by the Bureau of Emerging Infectious Diseases, Department of Disease Control, Ministry of Public Health in 2009. Presently, influenza A (H1N1) pdm09, H3N2, and influenza B viruses were annually circulated in Thailand. The outbreak of influenza in Thailand usually occurs in the rainy season. Monitoring of genetic change

of influenza viruses was recommended to determine the circulating strains and select the vaccine's specific strains in each region.

This study aimed to elucidate the circulating seasonal influenza virus type between January 2013 to December 2017 at Siriraj Hospital, Bangkok, Thailand.

## Methods

### Clinical Specimens

A total of 4385 nasopharyngeal wash specimens were collected from patients with influenza like-illness from January 2013 to December 2017. These samples were sent to Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand for influenza virus detection.

### Viral RNA Extraction

NucliSens nucleic acid extraction kit (Biomerieux, Marcy l'Etoile, France) was used to extract influenza virus RNAs from 200  $\mu$ L of nasopharyngeal wash. Extraction was performed according to the manufacturer's instructions. Viral RNA was eluted with 80  $\mu$ L of elution buffer.

### Detection and Subtyping of Influenza A Virus

Influenza viruses were identified and subtyped using Allplex™ Respiratory Panel 1 (Seegene Inc, Seoul, Korea), a real-time reverse transcriptase - polymerase chain reaction (RT-PCR) assay. The amplification and detection of viral genes were automatically performed using Seegene Viewer software (Seegene Inc, Seoul, Korea), according to the manufacturer's instructions.

### Statistical Analysis

Clinical characteristics of patients infected with influenza A viruses subtype H3, pdm09, and influenza B virus were compared and analyzed using the Pearson's chi-square test. A *P* value less than .05 was considered statistically significant. Analyses were performed using statistic software package, SPSS version 13.0 (SPSS for Windows, Version 13.0. Chicago, SPSS Inc; 2004).



### Ethical Considerations

The study protocol was approved by the institutional review board and ethics committee for research involving human subjects at Faculty of Medicine Siriraj Hospital, Mahidol University (No. Si161/2018 on March 15, 2018).

rainy season (June to October). In 2014, influenza peaked in March (summer), while in 2017, influenza infections increased in August, which matched the rainy season in Thailand, and decreased during summer in March (Figure 1).

## Results

### Prevalence of Influenza Virus

During 2013 - 2017, a total of 4385 nasopharyngeal wash specimens were processed for influenza virus identification. Real-time RT-PCR was used and found 799 (18.22%) laboratory-confirmed cases of influenza virus infection. Of these numbers, 608 (76.09%) cases were infected with influenza A virus (H1N1 pdm09 and H3N2), while 191 (23.90%) cases were infected with influenza B virus (Table 1).

### Characteristics of Patients With Influenza

The median age of 799 influenza virus-infected patients was 62 years (range, 0.10 - 107 years). Among these patients, there were 286 (35.79%) of male patients and 513 (64.21%) of female patients. The median age of female patients was 59 years (range, 0.10 - 107 years), while male patients' median age was 64 years (range, 0.6 - 97 years). According to clinical data of influenza patients each year during 2013 - 2017, the main signs and symptoms were high fever with body temperature greater than 38 °C (50% - 76%), cough (57% - 85%), and runny nose (38% - 74%). Around 3% to 23% of patients had pneumonia, and 2% to 6% developed respiratory failure. Oseltamivir was prescribed in 54% to 82% of patients diagnosed with influenza infection (Table 2).

### Weather Conditions and Prevalence

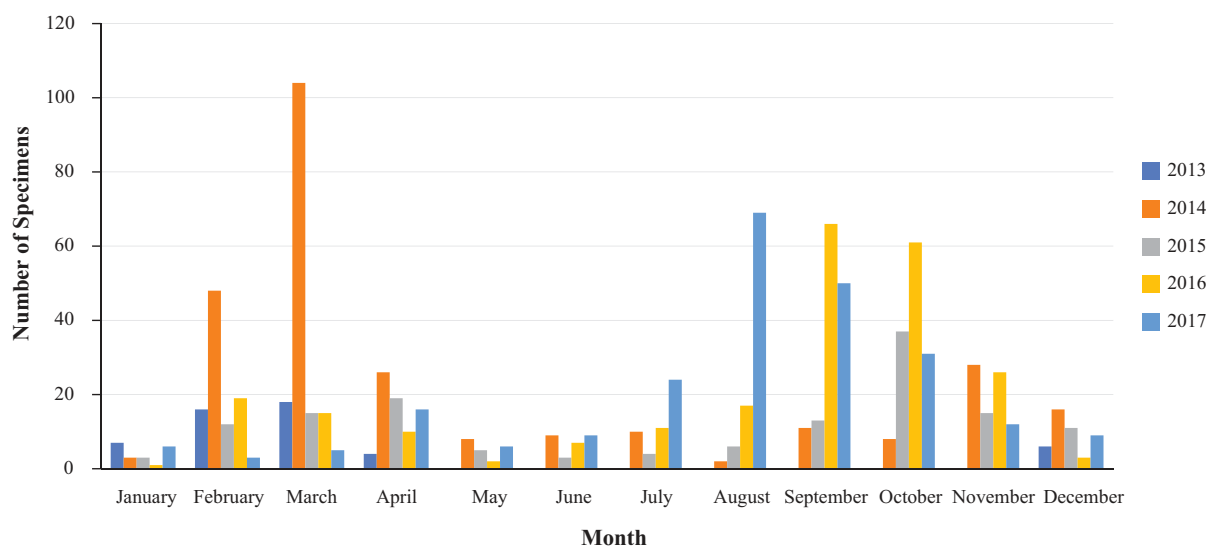
There are 3 seasons in Thailand, including the winter (November to February), summer (March to May), and

**Table 1. Comparison of Clinical Differences Among Influenza A Virus and Influenza B Virus Infected Patients From 2013 to 2017**

Characteristic	No. (%)		P Value*
	Influenza A Virus (n = 608)	Influenza B Virus (n = 191)	
Clinical features			
Body temperature (>38 °C)	302 (49.67)	83 (43.46)	.13
Oseltamivir treatment	392 (64.47)	86 (45.03)	< .05
Symptoms			
Cough	469 (77.14)	94 (49.21)	< .05
Runny nose	306 (50.33)	39 (20.42)	< .05
Lower respiratory infection			
Pneumonia	116 (19.09)	10 (5.24)	< .05
Respiratory failure	30 (4.93)	0 (0)	< .05
Bacterial infection	5 (0.82)	0 (0)	-
Underlying disease	396 (65.13)	74 (38.74)	< .05

\* P < .05 was considered statistically significant.

**Figure 1. Monthly Distribution of Influenza Viruses in Thailand During January 2013 to December 2017**



**Table 2. Demographic Data and the Characteristics of Influenza Virus Infected Patients From 2013 to 2017**

Characteristic	No./Total (%)					P Value*
	Year					
	2013	2014	2015	2016	2017	
Demographic feature						
Age, mean (median), y	35 (39)	14 (48)	49 (65)	50 (64)	44 (69)	< .05
Gender						
Male	45/102 (44.12)	75/188 (39.89)	45/135 (33.33)	54/175 (30.86)	67/199 (33.67)	< .05
Female	57/102 (55.88)	113/188 (60.11)	90/135 (66.67)	121/175 (69.14)	132/199 (66.33)	< .05
Clinical features						
Body temperature (> 38 °C)	78/102 (76.47)	110/188 (66.23)	74/135 (54.81)	89/175 (50.86)	113/199 (56.78)	< .05
Oseltamivir treatment	84/102 (82.35)	103/188 (54.79)	82/135 (60.74)	127/175 (72.57)	149/199 (74.87)	< .05
Symptoms						
Cough	87/102 (85.29)	109/188 (57.98)	116/135 (85.93)	148/175 (84.57)	168/199 (84.42)	< .05
Runny nose	76/102 (74.51)	72/188 (38.30)	71/135 (52.59)	90/175 (51.43)	142/199 (71.36)	< .05
Lower respiratory infection						
Pneumonia	16/102 (15.67)	6/188 (3.19)	27/135 (20.00)	31/175 (17.71)	47/199 (23.62)	< .05
Respiratory failure	6/102 (5.88)	5/188 (2.66)	3/135 (2.22)	4/175 (2.29)	12/199 (6.03)	.21
Bacteria infection	1/102 (0.98)	2/188 (1.06)	2/135 (1.48)	0/175 (0)	0/199 (0)	-
Underlying disease	71/102 (69.61)	79/188 (42.02)	80/135 (59.26)	140/175 (80.00)	169/199 (84.92)	< .05

\*  $P < .05$  was considered statistically significant.

This study compared the clinical characteristics of patients infected with influenza A and influenza B viruses. Most patients had a fever, cough, and runny nose. Fever was defined as having an axillary temperature of 38°C or higher. Rate of having fever did not differ significantly between

patients infected with influenza A virus versus influenza B virus ( $P = .13$ ). However, cough and runny nose were significantly found in patients with influenza A infection than in patients with influenza B infection ( $P < .05$ ). Of note, patients with influenza A infection tended to



develop pneumonia and respiratory failure more than patients with influenza B infection ( $P < .05$ ) (Table 1). Five influenza A-infected patients also had secondary bacterial infections, including *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Acinetobacter baumannii*. In the present study, secondary bacterial infection was not observed in patients infected with influenza B virus. Around 65% ( $n = 396$ ) of patients with influenza A infection and 38% ( $n = 74$ ) of patients with influenza B infection had underlying diseases ( $P < .05$ ). The underlying diseases included diabetes type 2, hypertension, allergy, asthma, sinusitis, chronic kidney disease, and papillary thyroid cancer.

## Discussion

Influenza A (pandemic and seasonal H1/H3) and influenza B viruses predominantly circulate in the human population globally. In tropical countries, the seasonal distribution of influenza viruses may be varied and may not display the defined seasons, as seen in temperate countries. In Thailand, the influenza season usually starts in July and ends in April, which coincides with the rainy season and winter season. Rainfall is associated with a peak in influenza activity in neighboring Southeast Asian countries. This study indicated that most patients presented with acute respiratory infection who visited the hospital during the influenza season were diagnosed with influenza infection.

The present study demonstrated that of all patients with influenza virus infection, 76.09% were infected with influenza A (H1N1, H3N2) virus, while 23.90% were infected with influenza B virus. The result showed similar detection rates with other studies in China, Korea, and Thailand.<sup>11, 12, 13</sup> In this study, the most frequently detected influenza virus was influenza A (H3N2), even though the seasonal influenza vaccination rate was 90%. Here, this study showed that the median age of influenza virus-infected patients was 62 years, which is closed to the age group at risk for influenza infection ( $> 65$  years), as defined by World Health Organization (WHO).

An axillary fever ( $\geq 38$  °C) was a significant characteristic found in patients with influenza infection compared to other respiratory viruses<sup>7</sup>.

This finding was also observed in the pandemic H1N1 outbreak in Thailand during 2009 - 2011, which was consistent with the previous study.<sup>8</sup> The present study showed that influenza infection generally occurred at low levels throughout the year with some degree of seasonal variation between different years, (eg, July to November or December to March). Some nearby countries within the Southeast Asian region demonstrate annual seasons of influenza viruses similar to Thailand's. Both Malaysia (November to March and July to September) and Singapore (May - June and December - January) have bimodal peaks, while Cambodia (October - December) and Indonesia (November - March) have a single peak. Many factors, including the weather, can be associated activity influenza virus,<sup>5</sup> as observed and in the previous study on cases with influenza B infection in Bangkok during 2011 - 2014.<sup>9</sup>

The present study showed the prevalence of influenza A and B viruses at Siriraj Hospital, Bangkok during 2013 - 2017, and also noticed that when cases with influenza A (H3) virus infection increased, there was a decrease in cases with influenza A (H1N1) pdm09 virus and influenza B virus infection. Patients infected with influenza A virus had a higher severity (higher rate of developing pneumonia and respiratory failure) than those infected with influenza B virus, suggesting the role of virological factors in clinical outcomes. Moreover, 65% of influenza A-infected patients were reported to have underlying diseases, while only 38% of influenza B-infected patients had these predisposing factors. This host factor would also affect the severity of the patients observed in this study. Accordingly, weaker immunity and a higher incidence of comorbidities would promote influenza-related clinical outcomes.

During the year 2013 - 2017, the influenza vaccine used in Thailand was composed of influenza A H1N1 (A/California/7/2009(H1N1)pdm09(2032016) and A/Michigan/45/2015(H1N1)pdm09(2017), Influenza A H3N2(A/Victoria/361/2011(H3N2)(2013/2014), A/Switzerland/9715293/2013



(H3N2)(2015-2016), A/HongKong/4801/2014(H3N2)(2017) and Influenza B(B/Wisconsin/1/2010likevirus(20132014), B/Phuket/3073/2013(2015/2016), B/Brisbane/60/2008(2017). This reveal that even though the vaccine coverage was sufficient, vaccine's efficacy might decline from the beginning of the year and the end of the year due to genetic drift of influenza virus.

Oseltamivir was used for treatment and prophylaxis of influenza diseases since 2011. This antiviral drug is effective and safe for the prevention of influenza-associated illness.<sup>10</sup> The present study demonstrated that 68.21% of oseltamivir was used to treat influenza infection in both hospitalized patients and outpatients, which should reduce the mortality and morbidity of infected patients.

## Conclusions

This study provided the characteristics of influenza virus types and subtypes at Siriraj Hospital, Bangkok, Thailand during 2013 - 2017. Sporadic cases of influenza occurred all year round, but the incidence peaked in March 2014 and August 2017. This study showed that the severity of patients infected with influenza virus A virus was higher than those infected with influenza B virus.

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## การศึกษาความชุกชนิดและชนิดย่อยของเชื้อไวรัสไข้หวัดใหญ่ในโรงพยาบาลศิริราช กรุงเทพฯ ประเทศไทย ระหว่างปี พ.ศ. 2556 – 2560

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**บทนำ:** การระบาดของฤดูกาลของไวรัสไข้หวัดใหญ่ส่วนใหญ่เกิดจากไวรัสไข้หวัดใหญ่ชนิด A (สายพันธุ์ A/H1N1/2009 และสายพันธุ์ seasonal H1 และ H3) และไข้หวัดใหญ่ชนิด B โดยภายหลังการระบาดทั่วโลกครั้งใหญ่ของสายพันธุ์ A/H1N1/2009 ในปี พ.ศ. 2552 สายพันธุ์ดังกล่าวได้เข้ามาแทนที่ seasonal H1 เดิม และถูกพบเรื่อยมาจนถึงปัจจุบัน ไวรัสไข้หวัดใหญ่ชนิด A และ B สามารถติดต่อแพร่กระจายได้ง่ายและก่อโรคติดเชื้อในระบบทางเดินหายใจได้ตั้งแต่มีอาการเล็กน้อยไปจนถึงอาการรุนแรง

**วัตถุประสงค์:** เพื่อศึกษาความชุกของชนิดและชนิดย่อยของเชื้อไวรัสไข้หวัดใหญ่ ในกรุงเทพฯ ประเทศไทย ตั้งแต่ปี พ.ศ. 2556 - 2560

**วิธีการศึกษา:** การรวบรวมข้อมูลการติดเชื้อไวรัสไข้หวัดใหญ่และจำแนกเชื้อระหว่างปี พ.ศ. 2556 - 2560 โดยใช้เทคนิคทางอณูโมเลกุลด้วยวิธี real-time RT-PCR ในการวิเคราะห์จำแนกสายพันธุ์ของเชื้อไวรัสและเปรียบเทียบข้อมูลอาการทางคลินิกของผู้ป่วยที่ติดเชื้อไข้หวัดใหญ่ชนิด A และชนิด B

**ผลการศึกษา:** สิ่งส่งตรวจจำนวนทั้งหมด 4,385 ตัวอย่าง จากผู้ป่วยที่มีอาการคล้ายไข้หวัดใหญ่ตั้งแต่เดือนมกราคม พ.ศ. 2556 ถึงเดือนธันวาคม พ.ศ. 2560 พบว่าความชุกของเชื้อไวรัสไข้หวัดใหญ่อ้อยละ 18.22 (799 ตัวอย่าง) โดยเป็นเชื้อไวรัสไข้หวัดใหญ่ชนิด A จำนวน 608 ตัวอย่าง (ร้อยละ 76.09) และไข้หวัดใหญ่ชนิด B จำนวน 191 ตัวอย่าง (ร้อยละ 23.90) ผู้ป่วยส่วนใหญ่มาด้วยอาการไข้ ไอ น้ำมูก โดยผู้ป่วยที่ติดเชื้อไข้หวัดใหญ่สายพันธุ์ A จะมีอาการรุนแรงกว่าไข้หวัดใหญ่สายพันธุ์ B

**สรุป:** เชื้อไข้หวัดใหญ่ชนิด A และ B เกิดขึ้นเป็นระยะๆ ตลอดทั้งปี แต่มีอุบัติการณ์เกิดขึ้นสูงในเดือนมีนาคม พ.ศ. 2556 และเดือนสิงหาคม พ.ศ. 2560 ซึ่งจะเป็ประโยชน์ต่อการดูแลแนวโน้มของชนิดเชื้อไข้หวัดใหญ่ เพื่อป้องกัน รักษา และเฝ้าระวัง รวมถึงเป็นแนวทางสำหรับการผลิตวัคซีน

**คำสำคัญ:** ไข้หวัดใหญ่ชนิด A สายพันธุ์ (H1N1) 2009 ไข้หวัดใหญ่ตามฤดูกาล ไข้หวัดใหญ่ชนิด B

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