DOI:10.1111/j.1750-2659.2011.00302.x
www.influenzajournal.com

Original Article

Influenza viruses in Thailand: 7 years of sentinel surveillance data, 2004–2010

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Accepted 12 August 2011. Published Online 10 November 2011.

Background The re-emergence of avian influenza A (H5N1) in 2004 and the pandemic of influenza A (H1N1) in 2009 highlight the need for routine surveillance systems to monitor influenza viruses, particularly in Southeast Asia where H5N1 is endemic in poultry. In 2004, the Thai National Institute of Health, in collaboration with the US Centers for Disease Control and Prevention, established influenza sentinel surveillance throughout Thailand.

Objectives To review routine epidemiologic and virologic surveillance for influenza viruses for public health action.

Methods Throat swabs from persons with influenza-like illness and severe acute respiratory illness were collected at 11 sentinel sites during 2004–2010. Influenza viruses were identified using the standard protocol for polymerase chain reaction. Viruses were cultured and identified by immunofluorescence assay; strains were identified by hemagglutination inhibition assay. Data were analyzed to describe frequency, seasonality, and distribution of circulating strains.

Results Of the 19 457 throat swabs, 3967 (20%) were positive for influenza viruses: 2663 (67%) were influenza A and able to be subtyped [21% H1N1, 25% H3N2, 21% pandemic (pdm) H1N1] and 1304 (33%) were influenza B. During 2009–2010, the surveillance system detected three waves of pdm H1N1. Influenza annually presents two peaks, a major peak during the rainy season (June–August) and a minor peak in winter (October–February).

Conclusions These data suggest that March–April may be the most appropriate months for seasonal influenza vaccination in Thailand. This system provides a robust profile of the epidemiology of influenza viruses in Thailand and has proven useful for public health planning.

Keywords Influenza, inpatients, outpatients, surveillance, Thailand.

Please cite this paper as: Chittaganpitch *et al.* (2012) Influenza viruses in Thailand: 7 years of sentinel surveillance data, 2004–2010. Influenza and Other Respiratory Viruses 6(4), 276–283.

Introduction

Since 2004, a widespread epidemic of highly pathogenic avian influenza caused by influenza A (H5N1) viruses in animal populations, particularly chickens, has swept through Southeast Asia. The disease poses a considerable public health risk. Not only can viruses infect humans directly, causing severe disease with high mortality,[1] but there is also potential for these viruses to acquire the ability to transmit from human to human either by reassortment with other influenza viruses or by mutation and give rise to new pandemic strains.[2] Avian influenza viruses were first detected in Thailand in January 2004, and through 2006, there were 25 persons infected with laboratory-confirmed influenza A (H5N1) viruses, including 17 deaths, reported to the World Health Organization (WHO).[3] No cases have been identified since 2006.

In response to the spread of avian influenza A (H5N1) viruses, and in recognition that pandemic influenza preparedness is a core communicable disease control function, the Thai National Institute of Health (Thai NIH) at the Ministry of Public Health (MOPH), in collaboration with the US Centers for Disease Control and Prevention (CDC), established a series of influenza surveillance networks. In 2004, Thai NIH set up surveillance sites across the country. The surveillance system was established to monitor the frequency of influenza, identify new strains and describe seasonality.

In this manuscript, we present data on frequency, seasonality, and strain distribution of circulating influenza viruses from 2004 to 2010, at the 11 sentinel sites across the country. These virological and epidemiological data can support the public health service to better understand influenza viruses circulating in Thailand and to better plan effective prevention and control strategies in Thailand.

Methods

Sentinel sites and case sampling

Surveillance was conducted in 11 sites in Thailand (Figure 1). This project was conducted as routine public health surveillance. Sites were chosen from all regions of the country with a focus on border areas. All 11 sites conducted surveillance for influenza-like illness (ILI) in outpatient clinics. Between 2004 and 2009, each site was instructed to enroll a convenience sample of up to five patients per week with ILI for a total of 20 patients per month; patients could be of any age. In September 2009, the case sampling protocol changed to increase the sample size to 10 patients per week including five from children <15 years of age and five from persons aged ≥15 years old for a total of 40 patients per month per site. In January 2010, we also expanded the target group to include all hospitalized patients with severe acute respiratory infection (SARI) in three site hospital inpatient wards (Mae Sot Hospital, Phra Pok Klao Hospital, and Bamrasnaradura Infectious Diseases Institute). Patients enrolled with ILI or SARI provided a throat swab and basic clinical and demographic data were collected on a standard form.

We defined ILI as fever (history or documented temperature >38°C) and cough or sore throat in a person of any age presenting to a sentinel outpatient clinic. We defined SARI as fever >38°C and cough or sore throat and shortness of breath or difficulty breathing requiring hospitalization in a person presenting to a sentinel hospital.[4]

Laboratory methods

Throat swab specimens were collected from patients meeting the case definition and put into 2·0 ml of viral transport media. The vials were kept on ice for up to four hours, then moved to a liquid nitrogen tank at the hospital laboratory and subsequently transported weekly to Thai NIH in Bangkok. For the first 4 years, all specimens were placed on Madin-Darby canine kidney (MDCK) cells to obtain viral isolates and any virus was identified by immunofluorescence assay. Strain analysis was carried out by hemagglutination inhibition (HAI) per WHO guidelines.[5] The HAI Influenza Diagnostics Kit was provided by WHO Collaborating Center for Reference and Research on Influenza, Melbourne, Australia, and US CDC.

Starting in November 2008, specimens were first tested by real time-reverse transcription polymerase chain reaction (rRT-PCR). The specimens were tested for influenza A and B viruses using the standard WHO[6] and US CDC protocol for rRT-PCR.[7] Influenza A viruses were then subtyped with specific primers from US CDC. All specimens from sentinel sites positive by rRT-PCR were selected for virus isolation in MDCK cells. Influenza A viruses that were not able to be typed by RT-PCR were further tested by viral isolation. If the virus did not grow, it was sent to a WHO Collaborating Center for additional testing. A sample of isolates collected throughout the year (around 200 isolates per year) was sent to the WHO Collaborating Center for Reference and Research on Influenza at Melbourne, Australia, and US CDC for strain confirmation.

No.	Month/Year*	Region	Site Name	Province
1	Sep 2004	North	Mae Sot Hospital	Tak
2	Sep 2004	Central	Health Care Center 17	Bangkok
3	Sep 2004	East	Phra Pok Klao Hospital	Chantaburi
4	Jun 2005	South	Hat Yai Hospital	Songklha
5	Jul 2005	Northeast	Nong Khai Hospital	Nong Khai
6	Nov 2005	North	Mae Chan Hospital	Chiang Rai
7	Nov 2005	North	Chiang Saen Hospital	Chiang Rai
8	Sep 2006	East	Koh Chang Hospital	Trat
9	Sep 2006	South	Bangkok Hospital Samui	Surat Thani
10	Oct 2006	South	Koh Samui Hospital	Surat Thani
11	Jan 2010	Central	Bamrasnaradura Hospital	Nonthaburi

*Started specimen collection

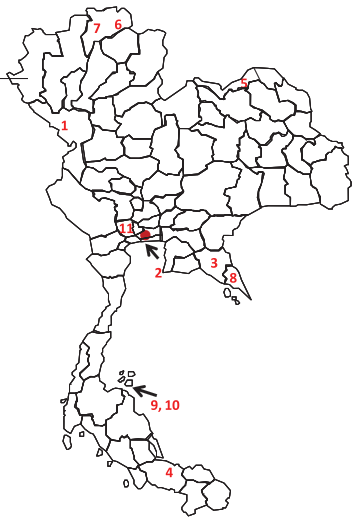

Figure 1. Location of the 11 sentinel influenza surveillance sites, Thailand, 2004–2010.

Data collection and analysis

A standard 2-page surveillance form was completed on patients providing specimens. Between 2004 and 2006, paper forms were mailed to Bangkok and data were entered into a centrally maintained Access database. Starting in 2006, an Internet-based system was implemented, and sites entered the data from the paper forms online. Paper forms sent to Bangkok are now used to check data entry from the sites. Data were analyzed using Excel (Microsoft Office).

Results

We conducted surveillance for human influenza between September 2004 and December 2010 in 11 sites in Thailand. Of the 11 sites, 10 were in hospitals (one tertiary, four general, four community, and one private) and one was in a health center. Specimens were collected from 19 116 ILI cases (9638 in children <15 years and 9478 in persons ≥15 years) and 336 SARI cases (119 in children <5 years, 177 in persons 6–64 years and 40 in elderly ≥65 years). Of the 19 457 throat swabs, 3967 (20%) were positive for influenza viruses. Of the 3967 influenza positive specimens, 98% were from patients with ILI.

Among the 3896 influenza viruses from patients with ILI, 2612 (67%) were influenza A viruses and able to be subtyped [21% influenza A (H1N1), 25% influenza A (H3N2), 21% pandemic (pdm) H1N1] and 1284 (33%) were influenza B viruses (Table 1). No influenza A (H5N1) virus was identified. Less than 1% of influenza A viruses were not able to subtyped (data not shown). The proportion of samples that tested positive for influenza viruses ranged from a low of 15% in 2006 to a high of 25% in 2010 (Table 1). After implementing rRT-PCR, the percent of samples that was influenza positive increased from 19% (1564/8139) in 2004–2007 to 23% (2332/9982) in 2008–2010 ($P < 0{\cdot}001$). The most frequently identified influenza virus in circulation by year was influenza A (H3N2) in 2005, influenza A (H1N1) in 2006, influenza A (H3N2) and influenza B in 2007, all three viruses in 2008 and pdm H1N1 in 2009 and 2010. The proportion positive in children <5 years ranged from a low of 10% in 2006 and 2009, where >60% of viruses identified were influenza A (H1N1) and pdm H1N1 virus, respectively, to a high of 18% in 2008, where influenza A (H1N1), influenza A (H3N2), and influenza B viruses circulated in roughly equal proportions. In addition to the general predominance of influenza A strains, the system also detected the emergence of pdm H1N1 in June 2009 and its persistence into 2010, and the emergence of a new variant of influenza, A/Brisbane/10/2007 (H3N2), which caused an outbreak in January 2007.

Among hospitalized patients in 2010, 21% had an influenza virus identified. The age group 5–64 years had a 28% influenza positive rate while young children <5 years and elderly ≥65 years had a similar influenza positive rate of 13%. Among children <5 years, influenza B virus was most commonly identified (44%), among persons aged 5–64 it was pdm H1N1 virus (64%) and among persons ≥65 years it was influenza A (H3N2) viruses (60%) (Table 2).

Influenza viruses occurred throughout the year but the major peaks of influenza viruses in most regions were found in the rainy season from June through August (average 3-month range, 18–33% positive) with influenza A viruses dominating and a minor peak in the winter from October through February (average 5-month range, 11–17% positive) with influenza B viruses dominating circulation (Figure 2). In general, the peaks became more diffuse as the sites progressed south (Figure 3). In the 7 years of surveillance, the southern sites had only 2 months where no influenza virus was identified. In 2009, pdm H1N1 virus was first detected in the surveillance sites in June in Mae Sot, Phra Pok Klao, Koh Chang, Bangkok Samui, Koh Samui, and the Health Center in Bangkok. The number rapidly increased, and the greatest percent positive, 38%, was seen in August 2009. The second wave of pdm H1N1 virus began in November 2009 and peaked in mid-February 2010, and the third wave began in July 2010 and peaked in September 2010. During 2009–2010, pdm H1N1 virus was the predominant strain in both years and seasonal influenza A (H1N1) disappeared after September 2009. Among patients with ILI, influenza A (H3N2) and influenza B viruses were detected before September 2009 and throughout 2010, and the proportion positive for influenza A (H3N2) and influenza B viruses was similar in 2009 (13%), but in 2010, influenza B increased to 33% and influenza A (H3N2) was 13%.

During most years, the majority of circulating influenza A strains were well matched to the influenza strains in both the Southern and Northern Hemisphere vaccine compositions (Table 3). In 2007, the new A (H3N2) variant, A/Brisbane/10/2007, was first introduced and caused infections among persons presenting in most sentinel sites in January 2007. During 2004–2008, influenza B viruses of both Victoria and Yamagata lineages co-circulated and each year there was a change in lineage predominance suggesting that nearly half of the circulating influenza B viruses were mismatched with the influenza B component of the annual vaccine. In 2009 and 2010, only influenza B viruses of the Victoria lineage circulated so there was a complete match with the annual vaccine in these years.

Discussion

We found that from 2004 to 2010, influenza viruses caused a substantial proportion of disease among ILI (20%) and SARI (21%) patients in Thailand. In the hospitalized patients from 2010, the age group 5–64 years had a 28%

Table 1. Number and percent positive for influenza viruses by type/subtype and age groups among outpatients with ILI, September 2004–December 2010

		% influenza positive in ILI		Distribution of influenza virus type/subtype			
Year	Age group (in years)	No. tested for ILI	Number positive for Influenza viruses (%)	A (H1N1) *n* (%)	A (H3N2) *n* (%)	A (pdm H1N1) *n* (%)	B *n* (%)
2004	<5	0	0	0	0	–	0
	5–64	10	4 (40)	2 (50)	0	–	2 (50)
	≥65	1	0	0	0	–	0
	Total	11	4 (36)	2 (50)	0	–	2 (50)
2005	<5	301	39 (13)	2 (5)	20 (51)	–	17 (44)
	5–64	981	218 (22)	6 (3)	138 (63)	–	74 (34)
	≥65	48	4 (8)	0	3 (75)	–	1 (25)
	Total	1330	261 (20)	8 (3)	161 (62)	–	92 (35)
2006	<5	746	71 (10)	52 (73)	3 (4)	–	16 (23)
	5–64	2675	432 (16)	263 (61)	44 (10)	–	125 (29)
	≥65	66	3 (5)	3 (100)	0	–	0
	Total	3487	506 (15)	318 (63)	47 (9)	–	141 (28)
2007	<5	1076	171 (16)	42 (25)	80 (47)	–	49 (29)
	5–64	2119	612 (20)	88 (14)	257 (42)	–	267 (44)
	≥65	116	10 (9)	0	8 (80)	–	2 (20)
	Total	3311	793 (24)	130 (16)	345 (44)	–	318 (40)
2008	<5	995	184 (18)	59 (32)	53 (29)	–	72 (39)
	5–64	2636	710 (27)	210 (30)	189 (27)	–	311 (44)
	≥65	104	12 (12)	2 (17)	5 (42)	–	5 (42)
	Total	3735	906 (24)	271 (30)	247 (27)	–	388 (43)
2009	<5	639	67 (10)	17 (25)	12 (18)	34 (51)	4 (6)
	5–64	2352	554 (24)	69 (12)	65 (12)	344 (62)	76 (14)
	≥65	87	9 (10)	1 (11)	4 (44)	4 (44)	0
	Total	3078	630 (20)	87 (14)	81 (13)	382 (61)	80 (13)
2010	<5	885	119 (13)	0	21 (18)	50 (42)	48 (40)
	5–64	2157	654 (30)	0	74 (11)	370 (57)	210 (32)
	≥65	127	23 (18)	0	11 (48)	7 (30)	5 (22)
	Total	3169	796 (25)	0	106 (13)	427 (54)	263 (33)
Total		19 121	3896 (20)	816 (21)	987 (25)	809 (21)	1284 (33)

ILI, influenza-like illness; pdm, pandemic.

Table 2. Number and percent positive for influenza viruses by type/subtype among hospitalized patients with SARI in three sites, September–December 2010

	% influenza positive in SARI		Distribution of influenza virus type/subtype			
			A (H1N1)	A (H3N2)	A (pdm H1N1)	B
Age group (in years)	No. Tested for SARI	Number positive for influenza viruses (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
<5	119	16 (13)	–	5 (31)	4 (25)	7 (44)
5–64	177	50 (28)	–	5 (31)	32 (64)	13 (26)
≥65	40	5 (13)	–	3 (60)	2 (40)	–
Total	336	71 (21)	–	13 (18)	38 (54)	20 (28)

SARI, severe acute respiratory infection; pdm, pandemic.

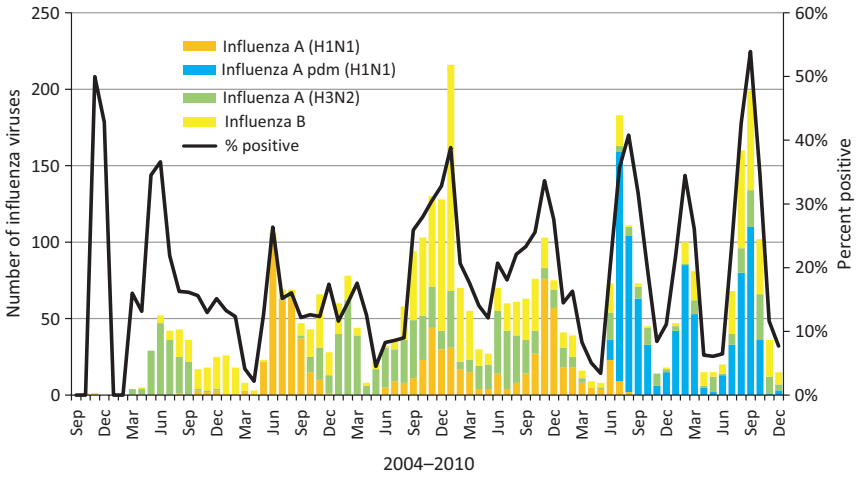

Figure 2. Number of influenza cases and percentage of influenza-like illness and severe acute respiratory infection cases positive for influenza virus by type/subtype and month, Thailand, September 2004–December 2010.

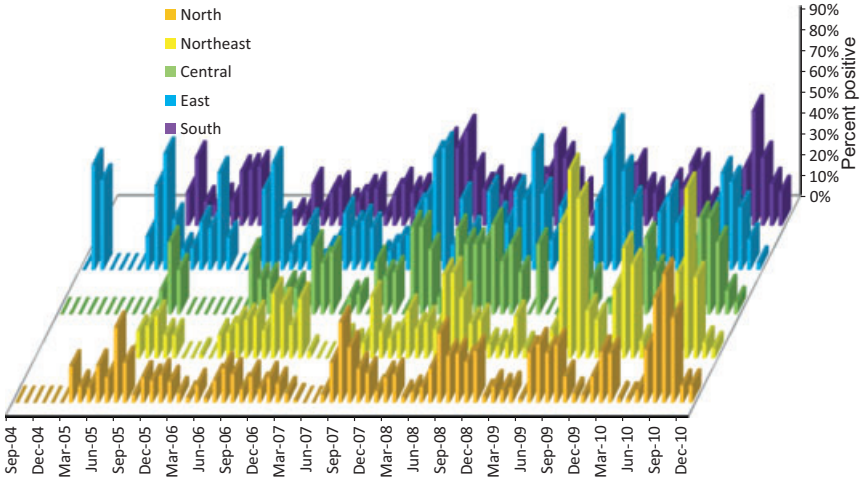

Figure 3. Percent positive of influenza cases by region (North, Northeast, East, South, and Central), September 2004–July 2010.

influenza positive rate while young children <5 years and elderly ≥65 years had a similar influenza positive rate of 13%. Although respiratory syncytial virus (RSV) is known to cause respiratory illness in young children,[8] Thailand has limited data on the role of RSV in children.[9] Our study demonstrates that the burden of disease caused by influenza viruses in young children is substantial and in some years (18% in 2008) approached the average level seen from RSV (19% in 2004–2007).[9]

Additionally, we detected influenza viruses in all months throughout the year, especially in the southern region of Thailand. While we cannot explain the differences in seasonality between Northern and Southern Thailand, it may be that climate or tourist differences play a role by contributing to viral persistence or viral re-introduction, respectively. The one private hospital that treated both Thai and foreign expatriates had less pronounced periods of viral activity (data not shown). Despite the annual presence of viruses, influenza viruses in Thailand annually presents two peaks, a major peak during the rainy season (June–August) and a minor peak in winter (October–February). Neighboring countries, such as Burma, Cambodia, Laos, and Vietnam, appear to have a similar peak of influenza viruses in the rainy season but the secondary peak was not as consistent.[10–13] Our study suggests that March and April may be the most appropriate months for seasonal influenza vaccination in Thailand.

Our surveillance system is important to monitor for novel subtypes or new strains of an existing subtype that could affect the normal seasonality. An example is our detection of the new variant, A/Brisbane/10/2007 (H3N2), in January 2007, which produced a low HI titer with the

Table 3. Influenza strains isolated in Thailand and Northern and Southern Hemisphere vaccine strains, 2004–2010

Year	No. isolates	Thai strains						Vaccine strains for Northern Hemisphere	Vaccine strains for Southern Hemisphere
		A (H1N1)		A (H3N2)		B			
		Strain	%	Strain	%	Strain	%		
2004	344	A/New Caledonia/20/99	34	A/Fujian/411/2002	24	B/Shanghai/361/2002	5·2	A/New Caledonia/20/99(H1N1)	A/New Caledonia/20/99(H1N1)
				A/Wellington/1/2004	20	B/Hong Kong/330/2001	11	A/Fujian/411/2002(H3N2)	A/Fujian/411/2002(H3N2)
				A/California/7/2004	6·4			B/Shanghai/361/2002	B/Hong Kong/330/2001
2005	263	A/New Caledonia/20/99	3·4	A/California/7/2004	39	B/Shanghai/361/2002	19	A/New Caledonia/20/99(H1N1)	A/New Caledonia/20/99(H1N1)
				A/Wellington/1/2004	22	B/Hong Kong/330/2001	8·0	A/California/7/2004(H3N2)	A/Wellington/1/2004(H3N2)
				A/Wisconsin/67/2005	3·0	B/Malaysia/2506/2004	4·6	B/Shanghai/361/2002	B/Shanghai/361/2002
2006	353	A/New Caledonia/20/99	52	A/Wisconsin/67/2005	16	B/Shanghai/361/2002	13	A/New Caledonia/20/99(H1N1)	A/New Caledonia/20/99(H1N1)
		A/Hawaii/15/2001	1·7			B/Malaysia/2506/2004	9·6	A/Wisconsin/67/2005(H3N2)	A/California/7/2004 (H3N2)
		A/Solomon Islands/03/2006	1·7			B/Ohio/1/2005	3·7	B/Malaysia/2506/2004	B/Malaysia/2506/2004
						B/Hong Kong/330/2001	2·6		
2007	218	A/Solomon Islands/03/2006	11	A/Wisconsin/67/2005	28	B/Ohio/1/2005	13	A/Solomon Islands/3/2006(H1N1)	A/New Caledonia/20/99(H1N1)
		A/Brisbane/59/2007	3·2	A/Brisbane/10/2007	24	B/Florida/7/2004	13	A/Wisconsin/67/2005(H3N2)	A/Wisconsin/67/2005(H3N2)
						B/Malaysia/2506/2004	7·8	B/Malaysia/2506/2004	B/Malaysia/2506/2004
2008	168	A/Brisbane/59/2007	17	A/Brisbane/10/2007	41	B/Florida/7/2004	24	A/Brisbane/59/2007(H1N1)	A/Solomon Island/3/2006(H1N1)
		A/Solomon Islands/03/2006	1·2			B/Malaysia/2506/2004	16	A/Brisbane/10/2007 (H3N2)	A/Brisbane/10/2007 (H3N2)
								B/Florida/04/2006	B/Florida/04/2006
2009	108	A/California/7/2009	60	A/Brisbane/10/2007	10	B/Brisbane/60/2008	9·3	A/Brisbane/59/2007(H1N1)	A/Brisbane/59/2007(H1N1)
		A/Brisbane/59/2007	13	A/Perth/16/2009	4·6	B/Malaysia/2506/2004	2·8	A/Brisbane/10/2007 (H3N2)	A/Brisbane/10/2007 (H3N2)
								B/Brisbane/60/2008	B/Florida/04/2006
2010	131	A/California/7/2009	52	A/Perth/16/2009	13	B/Brisbane/60/2008	31	A/California/07/2009 (H1N1)	A/California/07/2009 (H1N1)
						B/Malaysia/2506/2004	3·8	A/Perth/16/2009 (H3N2)	A/Perth/16/2009 (H3N2)
								B/Brisbane/60/2008	B/Brisbane/60/2008

Victoria lineage: B/Hong Kong/330/2001, B/Malaysia/2506/2004, B/Ohio/1/2005, B/Brisbane/60/2008.
Yamagata lineage: B/Shanghai/361/2002, B/Florida/7/2004.

WHO subtying reagent kit. This strain was identified in Thailand prior to its being included as a vaccine component in either the Southern or Northern Hemisphere influenza vaccines or the subtying reagent kit provided by WHO. This new variant subsequently caused sporadic outbreaks in many countries.[14] This event highlights the importance of these data for international vaccine strain selection.

Our surveillance system has several limitations. Taking a convenience sample at each site may limit the generalizability of our data if there were biases in how these persons were selected. Our data on hospitalized SARI cases are too few to make inferences on more severe clinical forms of the disease, and we recognize the need to improve and strengthen this aspect of surveillance by expanding our surveillance for hospitalized patients. We also need to improve the linkage of virological data and epidemiological data to be more rapid and complete with information for an early warning system. Despite these limitations, we believe that our system provides a robust profile of the epidemiology of influenza in Thailand and has proven useful for public health planning and outbreak control.

During the past 7 years, the virologic surveillance system in Thailand was dramatically expanded and improved. Molecular diagnostics were added in 2008, resulting in an overall increase in the proportion of influenza virus detection. Because of the recent influenza pandemic from a novel swine origin influenza A (H1N1) virus and the continued occurrence of avian influenza outbreaks worldwide, there needs to be a continued emphasis on developing and improving the existing surveillance system in Thailand and support to strengthen the early warning system. Harmonization of virologic and epidemiologic surveillance has just been established using existing passive surveillance data from the Bureau of Epidemiology in the MOPH, which is currently collected weekly using ICD-10 codes, and virologic surveillance data from Thai NIH for the purpose of establishing a sensitive and timely influenza surveillance system capable of detecting and reporting increases in influenza activity that lead to public health action. To maintain its sustainability, the sentinel influenza surveillance system will need to continue to be flexible and meet new needs as they arise.

Acknowledgements

We would like to thank the following for their valuable assistance in this study: the staff of Thai National Influenza Center, National Institute of Health, Department of Medical Science; the staff of Bureau of Epidemiology, Thailand Ministry of Public Health; the staff of Health Center 17, Mae Sot Hospital, Nong Khai Hospital, Pra Pok Klao Hospital, Chiang Saen Hospital, Mae Chan Hospital, Koh Chang Hospital, Koh Samui Hospital, Bangkok Hospital Samui, Hat Yai Hospital, and Bamrasnaradura Infectious Diseases Institute. We are grateful to WHO Collaborating Centers in Melbourne, Australia and US CDC, Atlanta, USA for providing reagents kits and for their technical assistance, and to Ann Moen, Influenza Division, National Center for Immunization and Respiratory Diseases, US CDC and Ms. Pranee Thawatsupha, Dr. Busarawan Sriwantana and Dr. Natteewan Poonwan from National Institute of Health, Department of Medical Sciences for their valuable advice and technical assistance. This work was supported by funding from the Department of Medical Sciences, Thailand Ministry of Public Health and US CDC (cooperative agreement 5U51IP000345).

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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