



Influenza Surveillance at Siriraj Hospital : 1979-1983

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On the basis of ribonucleoprotein and M protein, influenza viruses could be divided into three types namely type A, B and C. Type A is the most common cause of influenza in man and most often undergoes antigenic changes in hemagglutinin (H) and neuraminidase (N). Antigenic variations emerge new subtypes or new variants which consequently lead to pandemic, epidemic or sporadic outbreak. Type B is less common in causing human disease. The antigenic variation is also present but is not quite discriminate enough to establish new H and N. Type C plays a small role and its nature of neuraminidase is less defined⁽¹⁾.

Influenza surveillance for the antigenic changes is a world joint programme contributed by the WHO designated National Influenza Centre of each member country in order to give information and to prevent an influenza outbreak. The extent of the antigenic variations as antigenic drift or antigenic shift will predict the severity of the outbreak situation, and the appropriate strains included in the new vaccine.

Influenza surveillance has been conducted at Siriraj Hospital for over 10 years with collaboration from the Thai National Influenza Center at the Virus Research Institute, Department of Medical Sciences, Ministry of Public Health. The strains isolated in the previous studies are shown in Table 1 and the last report concerned epidemic of the strain closely related to A/USSR/90/77 (H₁N₁) in 1978⁽²⁾. There-

fore, the present study is to report the antigenic variation of influenza isolates at Siriraj Hospital, Bangkok, between 1979 and 1983.

Material and Method

Specimens

Throat swabs were collected from patients aged under 13 years who attended the Pediatric Unit of the Out-Patient Department at Siriraj Hospital during the 5 year-surveillance. All of the patients had symptoms of acute respiratory tract infection. The swabs were transported in nutrient broth supplemented with penicillin 200 units/ml, streptomycin 200 µg/ml and fungizone 2.5 µg/ml. The specimens were clarified by being centrifuged at 2 000 rpm for 20 minutes at 4°C, and the supernatants were kept frozen at -70°C for virus isolation by egg inoculation.

Egg inoculation

Embryonated hen eggs aged between 8-11 days were inoculated by amniotic route with 0.1-0.2 ml of the throat swab specimens in the first isolation passage, and by either allantoic route alone or by both amniotic and allantoic routes in the same eggs for virus propagation. The inoculated eggs were incubated for 3-4 days at 33-35°C in a moist chamber, then chilled and harvested.

Virus identification

Amniotic and allantoic fluids harvested were tested for the presence of influenza virus by

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Table 1. Influenza viruses isolated at Siriraj Hospital from 1968-1978

Virus strains closely related to	Prevalent period
A/Hong Kong/1/68 (H ₃ N ₂)	1968-1972
A/England/42/72 (H ₃ N ₂)	1972-1974
A/Port Chalmers/1/73 (H ₃ N ₂)	1973-1975
A/Victoria/3/75 (H ₃ N ₂)	1975-1977
A/USSR/90/77 (H ₁ N ₁)	1978-
A/Texas/1/77 (H ₃ N ₂)	1978-

hemagglutination activity with 0.5% chick red blood cells. The isolates were sent for final identification at the National Virus Research Institute, at the World Influenza Center in London, and at the International Influenza Center for the Americas, Atlanta, Georgia, U.S.A.

Result

Age and sex distribution of the patients studied

The study comprised 2 036 pediatric patients with acute respiratory diseases (ARD). They were 1 125 males and 911 females which made the male to female sex ratio of 1 : 0.8. Age and sex distribution of the patients are shown in Table 2. Most of the patients (43%) fell into the age-range between 1-3 years.

Influenza is uncommon in young children. Increment in the number of patients linearly correlated with increasing age (Table 3). Approximately 10 per cent of the acute respiratory infected cases in the age range 10-13 years were caused by influenza virus. The average isolation number was 3 per cent in our pediatric patients studied. No correlation between the virus isolation and the sex incidence was shown.

Correlation between clinical diagnosis and virus isolation

All of the subjects in this study had Acute Respiratory Disease (ARD), and more than 90 per cent of the cases were diagnosed as upper respiratory tract infections. Only 3 out of 58 influenza isolates came from cases with lower tract infection (acute bronchitis-Table 4). Based on an individual case, the symptom of influenza is usually atypical, as only 6 cases were clinically diagnosed so.

Nucleoprotein typing of the influenza isolates

Among 58 strains isolated, 54 (93.10%) were nucleoprotein of type A, and 4 (6.9%) were of type B (Table 5). Three of four type B isolates were obtained in July 1981 suggesting the endemicity during that period.

Influenza strains isolated in 1979-1983 (Table 6,7)

In 1979, the isolation rate was low (0.58%) only one strain from 171 throat swabs was isolated in August. This was the original strain of Influenza A/Bangkok/1/79 (H₃N₂) which spread throughout the world later. This strain was then included in the vaccine used between 1980-1983.

In 1980, 10 isolates (3.44%) were obtained from 291 throat swab specimens. All of the isolates obtained belonged to the H₃N₂ determinants. Five strains were intermediate between A/Bangkok/1/79 (H₃N₂) and A/Texas/1/77 (H₃N₂); and 5 strains were related to A/Bangkok/1/79 (H₃N₂). The peak of isolation was in August and were positive in 16 per cent of the specimens tested.

Seventeen of 412 specimens (4.13%) were positive isolation during year-round study in 1981. It was noticeable, that 12 strains isolated in June fell into 2 subtypes, i.e., 6 strains were antigenically intermediate between A/Bangkok/1/79 (H₃N₂) and A/Texas/1/77 (H₃N₂); and another 6 strains are the recirculation of A/USSR/90/77 (H₁N₁). The strains of A/England/333/80 (H₁N₁) and B/Singapore/222/79 first appeared in our study in July, and we could not isolate any other strain after that time.

A year-round isolation in 1982 showed that, 13 strains out of 462 specimens were identified. The isolates were 8 strains of A/Bangkok/1/79 (H₃N₂), 3 strains of the intermediate between A/Bangkok/1/79 (H₃N₂) and A/Texas/1/77 (H₃N₂), and one of each of A/England/333/80 (H₁N₁) and B/Singapore/222/79. The isolation rate was highest in September.

While most of the isolates obtained in 1982 belonged to the H₃N₂ antigenic determinants, all of the isolates in 1983 abruptly turned out to be of H₁N₁ determinants. The antigenicity was intermediate between A/Hongkong/2/82 (H₁N₁) and A/Chile/1/83 (H₁N₁). Peak of isolation was in August

Table 2. Age and sex distribution of patients with acute respiratory diseases

Age-range of ARI patients (Yr)								
Year	Sex	<1	1-3	4-6	7-9	10-13	Total	
1979	M	25	38	14	8	3	88	171
	F	19	33	22	7	2	83	
1980	M	52	66	33	12	10	173	291
	F	34	39	31	11	3	118	
1981	M	75	102	36	17	8	238	412
	F	44	78	31	19	2	174	
1982	M	61	115	49	13	9	247	462
	F	43	91	51	24	6	215	
1983	M	97	167	78	28	9	379	700
	F	68	151	77	25	0	321	
Total		518	880	422	164	52	2 036	
		(25.44%)	(43.22%)	(20.73%)	(8.06%)	(2.55%)		

Ratio Male : Female = 1125 : 911
 = 1 : 0.8

Table 3. Age and sex distribution of influenza positive cases during 1979-1983

Age-range	Male patients*		Female patients*		Total	
	No. tested	No. positive	No. tested	No. positive	No. tested	No. positive (%)
< 1	310	2	208	2	518	4 (0.77)
1-3	488	15	392	7	880	22 (2.50)
4-6	210	9	212	6	422	15 (3.54)
7-9	78	7	86	5	164	12 (7.32)
10-13	39	3	13	2	52	5 (9.62)
Total	1125	36 (3.19)	911	22 (2.41)	2036	58 (2.85)

*There was no statistical difference in sex susceptibility to influenza infection (X^2 -test : $P > 0.25$)

Table 4. Clinical diagnosis in patients with positive influenza virus isolation

Clinical diagnosis	No. of influenza virus isolates/year				
	1979	1980	1981	1982	1983
Influenza	0	2	1	2	1
URI	1	3	8	6	4
Common cold	0	1	2	0	1
Tonsilitis	0	0	1	0	0
Pharyngitis	0	4	2	5	5
Pharyngitis with conjunctivitis	0	0	0	0	1
Pharyngotonsilitis	0	0	2	0	1
Rhinopharyngitis	0	0	1	0	1
Acute bronchitis	0	0	0	0	3
	1	10	17	13	17

Table 5. Types of influenza virus isolated during 1979-1983

Year	No. isolated	No. type A	No. type B
1979	1	1	0
1980	10	10	0
1981	17	14	3
1982	13	12	1
1983	17	17	0
Total	58	54 (93.10%)	4 (6.90%)

with 10 per cent of the throat swab specimens being positive.

Discussion

From the 5 year-surveillance study, it was found that most of the cases of ARD (43%) fell into the age-range of 1-3 years. However, influenza infection rate increased with increasing age, and reached the peak in this study at the age-range of 10-13 years, in which the virus was isolated from 10 per cent of them. The average infection rate for all age-groups was 2.85 per cent (Table 3) and no difference in sex incidence was found.

Most of the influenza infected cases were clinically diagnosed as URI except 3 cases of acute bronchitis (Table 4). Since our study was limited to the Out-Patient Department, thus, symptoms in these groups of patients were not so serious.

In our study, the influenza isolation rate increases to 10-16 per cent at the peak of infection during the rainy season, *viz* June to September. The isolation rate declines in the cool season and usually diminishes in summer. Information provided by the WHO is that seasonal incidence of influenza will strike in the rainy season or winter in the southern hemisphere and in the winter months in the northern hemisphere, but the incidence in equatorial areas was rarely mentioned⁽³⁾.

Our study agrees with the observation in other geographical areas that most of the isolates predominating in each year are of ribonucleoprotein type A⁽³⁾. Among 58 strains isolated during a 5 year-period, 54 (93.10%) were of type A, 4 (6.90%) were of type B, but type C has never been detected (Table 2).

Table 6. Influenza surveillance for antigenic variation during 1979-1983

No. Isolates/No. specimen tested by month					
Month	1979	1980	1981	1982	1983
Jan	ND	ND	0/33	0/15	0/92
Feb	ND	ND	0/26	0	0/65
Mar	ND	ND	0/29	0/24	0/63
Apr	ND	ND	0/12	0	0/40
May	ND	0/7	0/30	0	0/39
Jun	0/9	0/11	12/72 (16.67%)	0/31	2/50 (4.0%)
Jul	0/14	3/57 (5.26%)	5/35 (14.29%)	0/72	5/90 (5.56%)
Aug	1/71 (1.41%)	4/25 (16.0%)	0/31	1/52 (1.92%)	5/47 (10.64%)
Sept	0/24	0/52	0/20	5/52 (9.62%)	1/66 (1.52%)
Oct	0/53	2/67 (2.99%)	0/34	2/57 (3.51%)	1/45 (2.22%)
Nov	ND	0/20	0/41	4/81 (4.94%)	0/54
Dec	ND	1/52 (1.92%)	0/49	1/78 (1.28%)	3/49 (6.12%)
Average isolation					
No./year	1/171 (0.58%)	10/291 (3.44%)	17/412 (4.13%)	13/462 (2.81%)	17/700 (2.43%)

ND = not done

Table 7. Antigenicity of influenza isolates during 1979-1983

Antigenicity related to	No. of isolates in year				
	1979	1980	1981	1982	1983
A/Bangkok/1/79 (H ₃ N ₂)	1	5	0	8	0
Intermediate between A/Bangkok/1/79 (H ₃ N ₂) and A/Texas/1/77 (H ₃ N ₂)	0	5	6	3	0
A/USSR/90/77 (H ₁ N ₁)	0	0	6	0	0
A/England/333/80 (H ₁ N ₁)	0	0	2	1	0
Intermediate between A/Hongkong/2/82 (H ₁ N ₁) and A/Chile/1/83 (H ₁ N ₁)	0	0	0	0	17
B/Singapore/222/79	0	0	3	1	0
	1	10	17	13	17
Total = 58 isolates					

Influenza virus infection may be asymptomatic or produce various disease manifestations ranging from mild URI to pneumonia and death. The epidemics are frequently associated with serious illness especially when it occurs primarily in high risk groups e.g., malnourished children and old people. Annual vaccination against influenza with inactivated or split vaccine composed of current strains has been recommended since 1963 for high risk individuals. Target groups for vaccination may include medical personnel who come in contact with those high risk persons(4).

As recommended by Centers for Disease Control of the USA., influenza vaccine used during 1979-1980 was composed of A/Brazil/11/78 (H₁N₁), A/Texas/1/77 (H₃N₂) and B/Hongkong/5/72(5). In the vaccines used during 1980-1983, A/Texas/1/77 (H₃N₂) was replaced by a strain isolated from our laboratory A/Bangkok/1/79 (H₃N₂), and B/Hongkong/5/72 was also replaced by B/Singapore/222/79, but A/Brazil/11/78 (H₁N₁) was still(6,7,8). During 1983-1984, the valent A/Bangkok/1/79 (H₃N₂) was replaced by its variant A/Philippines/2/82 (H₃N₂); and the new vaccine for 1984-1985 as recommended by the WHO will consist of A/Chile/1/83 (H₁N₁), A/Philippines/2/82 (H₃N₂) and B/USSR/100/83(9,10).

Surveillance for new variants is important in searching for the appropriate strains included in the vaccine for each coming year. In Japan where vaccines have been produced by themselves, the strains included in the new vaccine could be predictable from the preceding isolates in Thailand(11). Nevertheless, with consideration from the above information, the strains being prevalent in Thailand do not always match the valents of the imported vaccine available at that time, e.g., most of those isolated in Thailand in 1983 were antigenically intermediate between A/Hongkong/2/82 (H₁N₁) and A/Chile/1/83 (H₁N₁) which was not introduced into the vaccine until 1984.

All of the information in this study came from outpatient-pediatric cases under 13 years of age but may represent in some degree the circulation of influenza virus in the country. Several points are needed for further investigation e.g., incidence in admitted cases, and also in high risk people. The

influenza study in coordination with those other etiologic agents causing disease of respiratory tract in various age-groups will be beneficial to the strategy to control ARD in Thailand. A vaccination programme and management of the patients, could then be evaluated.

Summary

The present communication reported influenza surveillance at Siriraj Hospital between 1979 and 1983 by using egg inoculation technique. Two thousands and thirty-six throat swab specimens were collected from patients aged under 13 years who attended the Pediatric Unit of the Out-patient Department with the diagnosis of acute respiratory infection, and the isolation rate was 2.85 per cent. Influenza was prevalent in older children, especially at age 10 to 13 years whose isolation rate was 10 per cent. The sex difference was not found.

Of the 58 influenza isolates during 5 year-surveillance, 54 were of type A and in a smaller percentage were of type B. In 1979, a strain designated A/Bangkok/1/79 (H₃N₂) was first isolated, and then spread throughout the world. This strain was prevalent in Thailand during 1979-1982, and it was one of the influenza vaccine components recommended between 1980-1983. In addition to A/Bangkok/1/79 (H₃N₂), the strains isolated in the later years were the intermediate between A/Bangkok/1/79 (H₃N₂) and A/Texas/1/77 (H₃N₂), A/USSR/90/77 (H₁N₁), A/England/333/80 (H₁N₁) and B/Singapore/222/79. In 1983, all of the isolates were H₁N₁ in which the antigenicity related to intermediate between A/Hongkong/2/82 (H₁N₁) and A/Chile/1/83 (H₁N₁).

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การเฝ้าระวังไข้หวัดใหญ่ระบาดที่โรงพยาบาลศิริราช ระหว่างปี 2522 - 2526

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การเฝ้าระวังไข้หวัดใหญ่ระบาดด้วยวิธีแยกเชื้อไวรัสในไขไก่ฟัก โดยใช้ตัวอย่างตรวจจากน้ำป้ายคอ การศึกษานี้ได้ทำการเก็บน้ำป้ายคอจำนวน 2 036 ตัวอย่าง จากผู้ป่วยอายุต่ำกว่า 13 ปี ที่เป็นโรคติดเชื้อระบบทางเดินหายใจชนิดเฉียบพลัน และมารับการตรวจที่ห้องตรวจโรคเด็ก แผนกผู้ป่วยนอก ร.พ.ศิริราช ระหว่างปี 2522-2526 การติดเชื้อไวรัสไข้หวัดใหญ่เกิดขึ้นในเด็กโตบ่อยกว่าในเด็กเล็ก ในช่วงอายุ 10-13 ปี พบได้ถึงร้อยละ 10 ในขณะที่อัตราเฉลี่ยของการติดเชื้อในเด็กทุกช่วงอายุมีประมาณร้อยละ 3 เท่านั้น แยกเชื้อได้จากเด็กหญิงและเด็กชายเท่า ๆ กัน ในช่วง 5 ปีของการศึกษาครั้งนี้ แยกเชื้อไวรัสไข้หวัด-

ใหญ่ได้ทั้งหมด 58 เชื้อสาย เป็นหัยป์ เอ 54 เชื้อสาย หัยป์ บี 4 เชื้อสาย ในปี 2522 แยกได้เชื้อ A/Bangkok/1/79 (H₃N₂) เป็นครั้งแรก ต่อมาพบเชื้อนี้ได้ทุกภูมิภาคโลก และอยู่ในวัคซีน ซึ่งใช้กันอยู่ในช่วงปี 2523-2526 นอกจากเชื้อตัวนี้แล้ว เชื้อสายอื่นที่แยกได้ใน ร.พ.ศิริราชในปีต่อ ๆ มามีลักษณะแอนติเจนอยู่กึ่งกลางระหว่าง A/Bangkok/1/79 (H₃N₂) กับ A/Texas/1/77 (H₃N₂), หรือมีแอนติเจนใกล้เคียงกับ A/USSR/90/77 (H₁N₁), A/England/333/80 (H₁N₁) และ B/Singapore/222/79 ในปี 2526 เชื้อไวรัสที่แยกได้ทั้งหมดมีลักษณะแอนติเจนอยู่ระหว่าง A/Hongkong/2/82 (H₁N₁) และ A/Chile/1/83 (H₁N₁)

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