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Original Article

Influenza Virus Isolation in Bangkok Between 1988-1995
การแยกเชื้อไวรัสไข้หวัดใหญ่ในกรุงเทพมหานคร พ.ศ. 2531-2538

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ABSTRACT

A continuous surveillance of influenza was carried out in Bangkok between 1988-1995. Two thousand seven hundred and thirty-three throat swab specimens were collected from patients with acute respiratory infection (ARI) attending at Public Health Center Number 17 and Ramathibodi Hospital. All specimens were processed in chick embryos and MDCK cell culture and identified using the haemagglutination test. Of the 588 influenza isolates, 270 were of type A, and 318 were of type B. All of the type A isolates were further antigenically characterized : 62 A(H1N1), 193 A(H3N2) and 15 isolates unknown. During the eight years of surveillance, at least 20 influenza virus strains circulated in the Bangkok area. Influenza mainly occurred in those under 15 years old, although all age groups, including adults over 60 years of age, were affected.

บทคัดย่อ

การเฝ้าระวังเพื่อเก็บรวบรวมเชื้อไวรัสไข้หวัดใหญ่ที่แยกได้ในกรุงเทพมหานครเริ่มตั้งแต่ พ.ศ.2531–2538 โดยเก็บตัวอย่างน้ำป้ายคอ จำนวน 2,733 ตัวอย่าง จากผู้ป่วยโรคติดเชื้อเฉียบพลันของระบบหายใจที่เข้ารับการรักษาที่ศูนย์บริการสาธารณสุข 17 ของกรุงเทพมหานครและโรงพยาบาลรามาธิบดี นำตัวอย่างทั้งหมดมาแยกเชื้อในไข่ไก่ฟักและเซลล์เพาะเลี้ยง ส่วนการพิสูจน์เชื้อดูการเกาะกลุ่มของเม็ดเลือดแดงไก่ ผลปรากฏว่า 588 เชื้อสาย (isolate) ที่แยกได้นั้น เป็นทัยป์ A 270 เชื้อสายและทัยป์ B 318 เชื้อสาย ไวรัสทัยป์ A ยังแยกได้เป็น ๒ สัปทัยป์คือ A (H_1N_1) 62 เชื้อสาย A (H_3N_2) 193 เชื้อสาย ส่วนอีก 15 เชื้อสายไม่ทราบสัปทัยป์ ในช่วงเวลา 8 ปี ของการศึกษานี้พบว่า มีไวรัสไข้หวัดใหญ่อย่างน้อย 20 สายพันธุ์ที่แพร่ระบาดอยู่ในเขตกรุงเทพมหานคร แม้ว่าจะพบผู้ป่วยไข้หวัดใหญ่ทุกช่วงอายุ แต่ในเด็กอายุต่ำกว่า 15 ปี พบการติดเชื้อมากที่สุด

INTRODUCTION

Influenza viruses cause recurrent epidemics and pandemics owing to frequent antigenic variation of the viral surface antigens. Antigenic variation is a frequent event with influenza A virus. It occurs less frequently with influenza B virus and has not been seen with influenza C virus. This capacity for mutation, which is difficult to control, helps explain why influenza continues to be a major epidemic disease in humans that cannot be eradicated. Influenza pandemics occurs less frequently than epidemics, however four pandemics have been recorded in this century[1,2]. In Thailand, several outbreaks of influenza have been described. During the most severe pandemic in 1918 to 1919 which caused at least 20 million deaths world-wide, Panpatana reported 2,317,663 cases and 80,263 deaths in Thailand (population then 8,478,566)[3]. In order to control this disease, the World Health Organization formed an international network to collect influenza isolates and epidemiological information. The Thai WHO National Influenza Centre was established at the Virus Research Institute, Department of Medical Sciences in 1972. It is the only center in Thailand to regularly collect and isolate influenza viruses. In 1979, Influenza A/Bangkok/1/79(H3N2), included in the vaccine between 1980-1983, was first isolated at Siriraj Hospital[4]. In Bangkok between 1988 to 1995, 588 influenza virus isolations were made whilst 20 different influenza virus strains identified.

MATERIAL AND METHODS

Specimens

Throat swab specimens were collected twice weekly from patients with symptoms of acute respiratory infection (ARI) attending Public Health Centre Number 17 in Bangkok by Virus Research Institute personnel, or, sent directly from Ramathibodi Hospital. Patients were of low to middle socioeconomic status. Swabs were placed in 3% beef broth medium and immediately transported chilled to the Virus Research Institute. Upon arrival specimens were spun at 3,000 RPM at 4 °C for 30 minutes. Supernatant was then inoculated into both fertilized hens eggs and Madin Darby Canine Kidney (MDCK) cells.

Egg inoculation: Two isolation passages in fertilized hens eggs 9 to 11 days old incubated at 33 °C for 3 days were carried out. In the first, 0.2 ml of supernatant was inoculated via the amniotic route. In the second, 0.1 ml of amniotic or allantoic fluid demonstrating positive haemagglutination (HA) was inoculated into the allantoic sac of a new egg. HA titre was then repeated[2].

Cell culture isolation: 0.1 ml of supernatant followed by 1-2 g/ml trypsin were inoculated into MDCK cells. The cell culture was observed daily for a cytopathic effect (CPE) characterised by a rounding of cells starting in small clusters and spreading to affect the entire cell sheet. Infected cells were harvested and checked for HA titre.

Virus typing:

Influenza virus isolated from eggs and MDCK cells were typed and subtyped by haemagglutination inhibition (HI) test against three to four reference antisera for influenza A (H_3N_2), A (H_1N_1) and B annually selected by WHO. For example, in 1995 the reference antisera were A/Taiwan/1/86 (H_1N_1), A/Johannesberg/133/94 (H_3N_2), B/Beijing/184/93 and B/Guangdong/8/93. Two pools of mouse ascitic fluids specific for influenza A and B, and an anti-mouse IgG FITC conjugate, for indirect fluorescent antibody (IFA) assay were also used. Isolates significantly different from the reference antisera were sent for further antigenic analysis at the WHO Influenza Collaboration Centres in Atlanta or London.

RESULTS

Age and sex distribution

Between 1988-1995, 2,733 throat swabs were collected, of which 2,555 (93%) came from Public Health Center Number 17 and 178 (7%) from Ramathibodi Hospital. The majority of patients presenting with ARI were children: 1,275

(46.7%) under 4 years of age and 867 (31.7%) between 5-14 years old. The male to female ratio was 1:1.10.

Influenza viral isolation was possible in 588 (21.5%) out of the 2,733 throat swabs. Influenza occurred in all age groups. Of the isolates 16.9% (215/1,275) were from patients 0-4 years, 26.8% (232/867) from patients 5-14 years, 26.8% (64/239) from patients 15-24 years, 21.9% (70/319) from patients 25-59 years, and 21.2% (7/33) from patients greater than 60 years old. The male to female ratio was not significantly different at 1:1.06.

Strains

Influenza B viruses predominated and accounted for 318 (54%) of all the influenza isolates. Influenza A virus accounted for 270 (46%) of the 588 isolates. Of these isolates subtyped, 62 (10%) were influenza A (H_1N_1) 193 (33%) were influenza A (H_3N_2), and 15 (3%) unknown. The antigenicity of influenza isolates found and their prevalent period is shown in Table 1.

Seasonality

Influenza A and B were isolated throughout the year. The combined peak incidence, accounting for 67% of total isolates, occurred during the rainy season between June-October. September accounted for the highest incidence of 20%. Influenza A isolation rate gradually increased from June to August, sharply increased to a peak in September, and declined steeply from October onwards to a base line level. Influenza B had a bimodal increase with a small peak in March, declining in April and May, and increasing to a plateau between June to September, as shown in Figure 1.

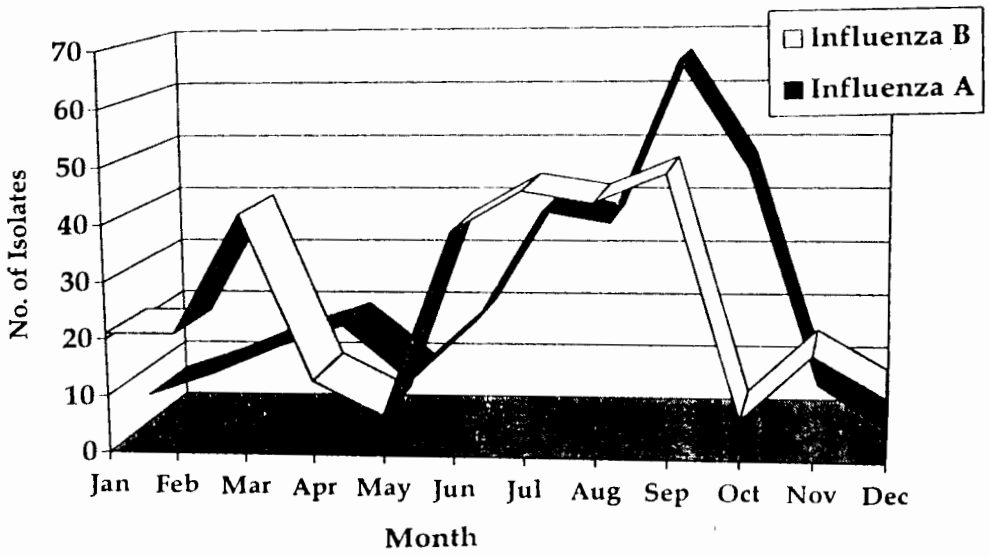
Table 1 Antigenicity of influenza virus isolates in Bangkok during 1988-1995

Virus strains closely related to:	Prevalent period
A/Taiwan/1/86 (H_1N_1)	1988-1989,1992,1995
A/Shanghai/11/87 (H_3N_2)	1988
A/England/427/88 (H_3N_2)	1990-1992
A/Hokkaido/20/89 (H_1N_2)	1989
A/Beijing/353/89 (H_3N_2)	1990-1993
A/Hong Kong/25/90 (H_3N_2)	1991
A/Shanghai/06/90(H_3N_2)	1991
A/Hong Kong/34/90 (H_3N_2)	1992
A/Brazil/02/91 (H_3N_2)	1992
A/Washington/15/91 (H_3N_2)	1992
A/Beijing/46/92 (H_3N_2)	1992
A/Beijing/32/92 (H_3N_2)	1992-1994
A/Shangdong/9/93 (H_3N_2)	1994
A/Johannesburg/33/94 (H_3N_2)	1995
A/Hong Kong/01/94 (H_3N_2)	1995
B/Yamagata/16/88	1988-1989,1993-1994
B/Hong Kong/22/89	1990
B/Panama/45/90	1991-1994
B/Qingdao/102/91	1991
B/Beijing/184/93	1995

DISCUSSION

Of all the patients with symptoms of ARI swabbed, influenza virus was isolated in 21.5%. Our findings that most of the influenza occurred in children may simply reflect the age bias of the patients attending public health centres. Illness severity and complications were not assessed. Thailand spans a region North of the Equator and exhibits the characteristic seasonal pattern of tropical climates with influenza activity occurring throughout the year. Singapore is 1.5 ° North of the Equator and as occurs in most tropical climates has an influenza season which extends throughout the year. The circulation of influenza viruses throughout the year makes tropical and subtropical regions unique locations for the early detection of variants. Whilst influenza virus could be isolated year round an increase was apparent from June to October during the rainy season. In temperate regions, such as in North America and

Figure 1. Combined monthly distribution of influenza isolates, 1988-1995



Europe, influenza occurs in winter[5].

Our study demonstrates the circulation of both influenza A and B virus Influenza A predominated every year except in 1991, 1993 and 1994. Antigenic characterization demonstrated at least 20 influenza strains in this period. Some strains were simultaneously distributed in other regions of the world. Several strains became the components of the vaccine during 1988 to 1995[10, 11]. A lag of usually one year, but up to two years, was often seen between the original isolation and first isolation in Bangkok. Some strains circulated in Bangkok for only one year whilst others persisted such as B/Panama/45/90 isolated between 1991-1994. Those found over a number of years mainly occurred in consecutive years, however some reappeared after a break such as A/Taiwan/1/86 (H_1N_1) which appeared first in 1988-1989, then again in 1992 and 1995.

Many areas remain to be studied. What strains circulate beyond Bangkok? Does southern Thailand prone to monsoons experience a different influenza season? Northern Thailand's proximity to Southern China, given China's presumed role as the origin of viral antigenic mutations, may provide a gateway to map virus movement. The constant flux of travellers in todays age of jet travel demands that health authorities be on the alert should another global influenza epidemic manifest. Local surveillance linked to world-wide networks remain essential in gathering data for the formulation of effective vaccines to prevent this disease.

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