ISOLATION AND IDENTIFICATION OF INFLUENZA VIRUS STRAINS CIRCULATING IN THAILAND IN 2001

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Abstract. Local influenza surveillance plays an important role in preparing for, and responding to, epidemics and pandemics. Between January and December 2001, the National Institute of Health of Thailand collected a total of 711 throat swab specimens from outpatients affected with acute respiratory symptoms from several centers throughout Thailand, of which 374 were virus-positive. Of these, 338 (90.4%) were positive for influenza virus by immunofluorescence testing. By hemagglutination-inhibition (HI) testing, 155 of the type A viruses were found to be subtype H1N1 strains closely related to A/New Caledonia/20/99, and 70 were subtype H3N2 A/Moscow/10/99-like viruses. For type B, the isolates were antigenically B/Sichuan/379/9-like by HI, although a number of the strains could be shown to be more closely related to earlier influenza B strains by genetic analysis. The strains circulating in Thailand were antigenically similar to strains isolated worldwide during the same period and to strains recommended by the WHO for inclusion in the vaccines for use in 2001-2002.

INTRODUCTION

Due to the frequent antigenic variation of viral surface proteins, the influenza viruses can cause recurrent epidemics and pandemics, resulting in serious public health burdens. Antigenic variation occurs frequently with influenza type A virus and to a lesser extent with influenza type B. This capacity for constant mutation explains why influenza continues to be a major epidemic disease in humans, despite efforts for prevention and control by vaccination. It is for this reason that continuous surveillance efforts are crucial. In order to minimize the impact of this disease, the World Health Organization (WHO) formed an international surveillance network to collect influenza isolates and epidemiological information. At present, over 80 countries (110 centers) worldwide participate in the network. In 1972, a WHO National Influenza Center was established in Thailand at the Virus Research Institute (currently part of the National Institute of Health of Thailand), Ministry of Public Health. In the past, most of the isolates were collected from Bangkok. Specimens have been regularly received from sentinel sites in the four geographical regions of Thailand since 2001.

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MATERIALS AND METHODS

Throat swab specimens were collected by study personnel twice weekly from out-patients with symptoms of acute respiratory infection (ARI) attending Health Center 17 in Bangkok. In addition, clinical specimens were also sent directly from Samitivej Hospital (Bangkok, Thailand), Mae Sot Hospital (Tak Province, northern Thailand), Nong Khai Hospital (Nong Khai Province, northeastern Thailand), Hat Yai Hospital (Songkhla Province, southern Thailand) and Praphok Klao Hospital (Chantaburi Province, central Thailand). Swabs were placed in 3% nutrient broth medium and transported chilled to the Thai National Influenza Center, National Institute of Health, Ministry of Public Health. Upon receipt,

specimens were centrifuged at 3,000 rpm at 4ºC for 30 minutes. The supernatant was then inoculated into both fertilized hens eggs and Madin Darby Canine Kidney (MDCK) cells, as previously reported elsewhere (Kendal, 1982; Thawatsupha *et al*, 2000).

Respiratory virus typing

A commercially available indirect fluorescent antibody screening kit (Respiratory Panel 1 Viral Screening and Identification IFA kit, Chemicon International Inc, USA) was used to screen for influenza virus, parainfluenza virus, respiratory syncytial virus and adenovirus. Those isolates from eggs and MDCK cells positive for influenza virus were typed and subtyped by the hemagglutination inhibition (HI) test against three reference antisera for influenza A (H3N2), A (H1N1) and B, which were selected by the WHO Collaborating Center for Influenza Research and Reference in Melbourne, Australia. In 2001, the reference antisera consisted of A/New Caledonia/20/99-like (H1N1), A/Panama/2007/99-like (H3N2), and B/Johannesburg/5/99-like strains. Isolates were sent for further antigenic analysis to the same WHO Collaborating Center in Melbourne.

RESULTS

A total of 711 throat swabs were received from the 6 centers throughout Thailand. Three hundred and seventy-four (52.60%) were virus-positive. Of these, 338 (90.37%) were positive for influenza virus, with the remaining isolates being identified as adenovirus (5.08%), parainfluenza virus (2.41%), respiratory syncytial virus (1.60%) and herpes simplex virus (0.53%) (Table 1). Further subtyping of the influenza isolates showed that 155 (45.86%) were subtype A(H1N1), 70 (20.71%) were subtype A(H3N2), 11 (3.25) were untyped A and 102 (30.18%) were type B (Table 2).

Influenza viruses were isolated throughout the year, with a major peak in incidence during the rainy season, from June to October. Antigenic analysis indicated that 3 different strains predominated in 2001. A/New Caledonia/20/99-like (H1N1), A/Moscow/10/99-like (H3N2), B/Sichuan/379/99-like and B/Shenzen/654/99-like strains represented 45.86% (155/338), 20.71% (70/338), 21.30% (72/338) and 6.80% (23/338) of all influenza isolates, respectively (Fig 1). A/New Caledonia/20/99(H1N1)-like strains, which comprised the majority of type A isolates, were isolated mainly between May and December, while A/Moscow/10/99(H3N2)-like viruses were seen predominantly during the first half of 2001. B/Sichuan/379/99-like influenza was seen at low levels throughout the year (Fig 2). Although, B/Shenzen/654/99, a type B virus genetically more closely related to an earlier reference strain, B/Harbin/7/94, was also identified later in the year. However, this virus was not readily distinguishable from B/Sichuan/379/99 by standard HI tests.

Table 1
Laboratory diagnosis of acute respiratory infection.

Virus	Number	Percent
Adenovirus	19	5.08
Parainfluenza virus	9	2.41
Respiratory syncytial virus	6	1.60
Herpes simplex virus	2	0.53
Total influenza	338	90.37
Total isolates	374	100.00

Table 2
Breakdown of influenza isolates by subtype.

Subtype	Number	Percent
Influenza type A/H1N1	155	45.86
Influenza type A/H3N2	70	20.71
Influenza A untyped	11	3.25
Influenza type B	102	30.18
Total influenza	338	100.00

DISCUSSION

Influenza can be a major public health problem. In addition to causing acute respiratory infections, the virus can exacerbate pre-

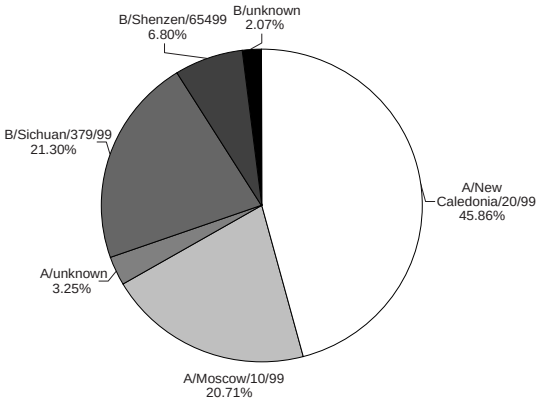

Fig 1–Breakdown of influenza isolates by strain.

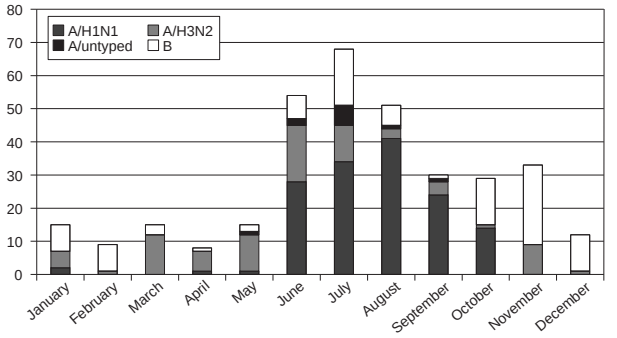

Fig 2–Breakdown of influenza strains by month.

existing conditions in patients with underlying chronic disease. Historically, epidemics have occurred frequently and pandemics have occurred 3-4 times per century, although at irregular intervals. In the last century, there were three pandemics: the 1918 Spanish influenza, which is believed to have caused up to 40 million deaths worldwide, the 1957 Asian influenza and the 1968-69 Hong Kong influenza. Experts believe that further pandemics are inevitable. For this reason, the World Health Organization has started planning for a future pandemic (WHO, 1999; Gust *et al*, 2001). An essential element of this is the international surveillance network. In addition to its role in providing an alert for impending epidemics, the network also provides data on the most appropriate strains for inclusion in the twice-yearly update of influenza vaccine formulations: in February for the Northern Hemisphere and in October for the Southern Hemisphere. Thailand, located just north of the equator, has a tropical climate that does not exactly correspond to the seasonality of either hemisphere. Therefore, consistent surveillance of locally circulating strains takes on an even greater importance in deciding when vaccination should take place, and which influenza vaccine should be used. In 2001, three types of strains predominated in Thailand: A/New Caledonia/20/99(H1N1)-like, A/Moscow/10/99(H3N2)-like, and B/Sichuan/379/99-like. Viruses of each of these three strains have been included in the WHO vaccine formulation for use in the 2001 and 2002 Southern Hemisphere influenza seasons (WHO, 2000a; 2001) and the 2001-2002 Northern Hemisphere influenza season (WHO, 2000b). Viruses related to these three strains have predominated worldwide (CDC, 2001). Some of the influenza B viruses isolated late in the year were more closely related to earlier influenza B strains similar to B/Harbin/7/94. However, antisera to B/Sichuan/379/99 reacted well with these viruses and they could only be definitively differentiated by genetic sequencing.

Influenza is a reportable disease in Thailand. The number of clinical cases of influenza-like illness reported to the Ministry of Public Health has remained relatively stable over the past few years, with around 40 to 50 thousand cases per year. In 1990, the number of reported cases rose as high as 70,000 (Division of Epidemiology, 2001). These numbers, when compared with western countries, may seem relatively low. However, the reports are based on clinical diagnoses alone and it is believed that influenza is being underreported due to misdiagnosis with the common cold or other respiratory pathogens. The clinical specimens collected by the Thai National Influenza Center are from patients presenting with acute respiratory infection, which can also be caused by a variety of different pathogens. However, in 2001, influenza A and B were isolated in 90.37% of the specimens. Between 1994 and 1999, influenza accounted for over 50% of virus-positive specimens received by the Center (Prasittikhet *et al*, 1998; Thawatsupha *et al*, 2000), indicating that influenza does indeed contribute to a high disease burden.

In tropical countries, such as Thailand, influenza is present all year round. A base level of approximately 3,000 cases of clinical influenza-like illness is reported to the Ministry of Public Health each month (Division of Epidemiology, 2001). Months when the number of cases rises above this level are considered to be peak seasons. Historically, the main peak has usually occurred during the rainy season, from June to October. In addition to this, a smaller peak has often been seen around the cool season, between January to March. During 2001, an increase in the number of influenza-positive isolates was seen at periods roughly coinciding with the rainy season and the cool season.

Local surveillance of virus strains remains an integral part of the WHO global program for influenza. With the boundaries between countries becoming less and less apparent and cross-continental travel becoming more and more frequent, the opportunity for disease spread has become even greater than a decade ago. Influenza constitutes an ongoing epidemic problem and a potential pandemic threat. In addition to serving as an advance warning for upcoming epidemics and potential pandemics, local surveillance provides essential data for the annual updating of the influenza vaccine formulation. At present, vaccination is the only effective method of disease prevention and control. In Thailand, vaccination is generally recommended between March and June, prior to the start of the rainy season. This coincides with the availability of the Southern Hemisphere vaccine formulation, which has historically been well-matched to locally circulating strains. However, in non-temperate regions, each country needs to determine which vaccine formulation is most appropriate for its needs, based on local strain circulation, seasonality and the timing of vaccine availability.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the staff members of Health Center 17, Samitivej Hospital, Mae Sot Hospital, Nong Khai Hospital, Hat Yai Hospital and Praphok Klao Hospital for their collaboration in collecting clinical specimens; the WHO Collaborating Center for Influenza Reference and Research, Melbourne, Australia, for influenza strain identification; Alan Hampson for manuscript review; and Aventis Pasteur (Thailand) Ltd for logistical support in transporting isolates for strain identification.

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