SEASONAL INFLUENZA B VIRUS STRAINS CIRCULATING IN MALAYSIA, 2005 - 2015

Zarina Mohd Zawawi, Tengku Rogayah Tengku Abdul Rashid, Nur Izmawati Ab Razak, Nur Azrenawaty Mohd Nor, Mohd Apandi Yusof and Ravindran Thayan

Virology Unit, Infectious Disease Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia

Abstract. Seasonal influenza B viruses cause outbreaks in both tropical and temperate regions of the world. In Malaysia, the National Influenza Center, Institute for Medical Research, Kuala Lumpur monitors circulating Influenza virus B strains. All respiratory specimens received from patients presenting with influenza-like illness (ILI) from 2005 to 2015 were included in the study. Specimens were inoculated into Madin-Darby Canine Kidney cells and isolates identified by indirect immunofluorescence antibody technique and hemagglutination inhibition typing assay. The predominant influenza type B virus strains were B/Hong Kong/330/2001-like in 2005, B/Malaysia/2506/2004-like (new strain isolated in Malaysia) in 2006, B/Florida/4/2006-like in 2007 and 2008 and B/Brisbane/60/2008-like in 2009 - 2012, B/Massachusetts/2/2012-like in 2013 - 2014, and B/Phuket/3073/2013-like virus in 2015. During the study period, both B/Victoria and B/Yamagata lineage viruses were circulating in Malaysia. Predictions on upcoming predominant B lineage for influenza vaccine program failed in 2005, 2007 and 2009 as the predominant circulating influenza type B strains did not match the vaccine components. The influenza virus B/Victoria lineage was greater in occurrence compared to B/Yamagata lineage during the 11-year study period. These local surveillance data of the prevalent circulating influenza type B virus strains are important not only for control strategies but also for selection of appropriate virus strains to be used in the annual influenza vaccine formulation.

Keywords: influenza virus B/Victoria lineage, influenza virus B/Yamagata lineage, predominant virus strain, Malaysia

INTRODUCTION

Influenza virus has emerged as one of the major causes of human morbidity and mortality in both temperate and tropical regions of the world (Nicholson *et al*, 2003; Ong *et al*, 2010; Horm *et al*, 2014; Tan *et al*, 2015) with significant number of deaths reported each year (WHO, 2014). Epidemics of seasonal influenza occur annually and affect every person regardless of their age (WHO, 2014). In tropical Malaysia, seasonal influenza cases have been reported to occur all year round with a predominant peak during the dry and wet seasons (Shahidah *et al*, 2003; WHO European Region, 2015).

Although influenza type A viruses

Correspondence: Zarina Mohd Zawawi, Virology Unit, Infectious Diseases Research Centre, Institute for Medical Research, Jalan Pahang, Kuala Lumpur, 50588 Malaysia.
Tel: +603 2616 2671; Fax: +603 2693 8094
E-mail: zarina@imr.gov.my

are considered as having the greatest potential public health impact, influenza B virus infections also contribute to a significant cause of morbidity and mortality worldwide (Seleka *et al*, 2017). Symptoms are usually mild and self-limiting but the infection can also develop into a life-threatening severe acute respiratory illness among elderly and infant patients presenting with chronic medical conditions (Petrovic *et al*, 2011).

Outbreaks of influenza B have been reported worldwide, occurring in locations with close living conditions such as in primary school (Flood *et al*, 2012), nomadic community (Khan *et al*, 2013) and welfare home (Win *et al*, 2010). These reports highlight the need of influenza B surveillance to enhance infection control practices and alleviate the burden of future outbreaks. In Malaysia, recent reports only describe epidemiological and evolutionary dynamics of influenza virus B lineages, categorized as Victoria and Yamagata based on genetic and antigenic properties (Oong *et al*, 2015), circulating in Malaysia from 1995 to 2008 amongst children (Sam *et al*, 2015) and adults from 2012 to 2014 (Oong *et al*, 2015). The only report on circulating influenza virus strains was conducted in 2010 (Zainah *et al*, 2010).

Hence, this report documented in Malaysia seasonal influenza B infection over an 11-year period including previous data (Zainah *et al*, 2010) to provide a broad view on the circulation trend of influenza B cases.

MATERIALS AND METHODS

Samples collection

All influenza-like illness (ILI) specimens received from January 2005 to December 2015 were included in this study. According to WHO criteria, ILI is defined as fever (body temperature ≥38°C) with cough and/or sore throat, and absence of other diagnoses (Julia *et al*, 2017). Specimens (nasopharyngeal swab/aspirate, throat swab/nasal swab, throat gargle, sputum, and bronchoalveolar/tracheal lavage) were collected by government hospitals and outpatient clinics throughout Malaysia and sent to the National Influenza Center (NIC), Institute for Medical Research (IMR), Kuala Lumpur.

This study used retrospective specimens and data were collected as part of a routine influenza public health surveillance. The analysis used only de-identified and aggregated laboratory data. Therefore, the study does not require approval from human subject's ethics review committee.

Laboratory investigations

Samples were identified as positive for influenza virus infection by cell culture technique and indirect immunofluorescence antibody assay. Samples were cultured in Madin-Darby Canine Kidney (MDCK) cells and considered positive for influenza when cytophatic effect (CPE) was present (Zainah *et al*, 2010), and in the indirect immunofluorescence assay (IFA technique) positivity was based on presence of fluorescence (IFA respiratory panel 1 Kit; Millipore, Livingstone, UK) in nucleus or cytoplasm of the cells. Positive samples by cell culture were harvested when cells showed CPE. Only influenza virus isolates (n=1,360) subsequently were submitted to the WHO Collaboration Centre for Reference and Research on Influenza, Melbourne, Australia for hemagglutination inhibition (HAI) typing assay (Zainah *et al*, 2010). Data received on circulating influenza B strains were analyzed locally for evaluating annual incidence and identifying predominant types circulating in Malaysia from 2005 to 2015.

RESULTS

Over the whole study period of 11 years, the NIC, IMR received 19,001 respiratory specimens from patients initially diagnosed with ILI, from which only 1,360 (7.16%) were confirmed positive for influenza virus, with 472/1,360 (34.7%) identified as influenza B and 888 (65.3%) influenza A (Table 1). Influenza type A virus was isolated more often than influenza B virus in 2005 - 2009, 2011, 2014 and 2015, while influenza type B virus was more common in 2010 and 2012-2013 (Fig 1). The highest number (82, 17%) of influenza B cases was reported in 2005 and the lowest (4, 1%) in 2011 (Table 1)

In 2005, three peaks of influenza B were observed (in April, July and November), while only a spike in the number of influenza B cases was observed in 2006 (April). In 2007-2009, no peak in influenza B was noted, but in the following five years, one peak of influenza B incidence was observed in 2010 (September), 2012 (April), 2013 (May), and 2014 (July); In 2011, low incidence of influenza B was present throughout the year. However, in 2015 three influenza B peaks (March, April and October) were noted (Fig 2).

Of the 472 influenza B cases, 330 (70%) were classified into the B/Victoria (208, 63%) and B/Yamagata (122, 37%) lineages (Fig 3). Influenza virus B/Vic-

Table 1
Influenza-like illness (ILI) specimens and percent influenza virus types A and B at the National Influenza Center, Institute for Medical Research (IMR), Kuala Lumpur, Malaysia, 2005 to 2015.

Year	Total ILI cases	Influenza virus positive (%)	Influenza virus type A (%)	Influenza virus type B (%)
2005	923	160 (17.3)	78 (49)	82 (51)
2006	459	145 (32)	83 (57)	62 (43)
2007	1,847	236 (12.8)	165 (70)	71 (30)
2008	2,210	225 (10.2)	185 (82)	40 (18)
2009	1,678	227 (13.5)	177 (78)	50 (22)
2010	874	72 (8.2)	30 (42)	42 (58)
2011	1,216	23 (1.9)	19 (83)	4 (17)
2012	1,401	33 (2.4)	7 (21)	26 (79)
2013	2,087	50 (2.4)	20 (40)	30 (60)
2014	2,625	67 (2.6)	43 (64)	24 (36)
2015	3,681	122 (3.3)	81 (66)	41 (34)
Total	19,001	1,360 (7.16)	888 (65.3)	472 (34.7)

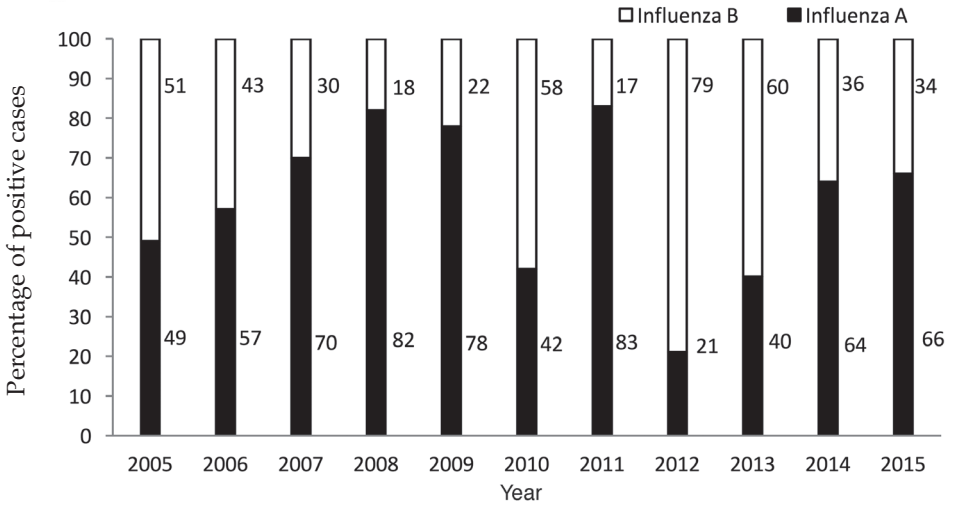

Fig 1 - Circulating influenza type A and B viruses in Malaysia between 2005 and 2015. Data were from influenza-like illness specimens deposited at the National Influenza Center, Institute for Medical Research, Kuala Lumpur, Malaysia and typed using a hemagglutination inhibition assay.

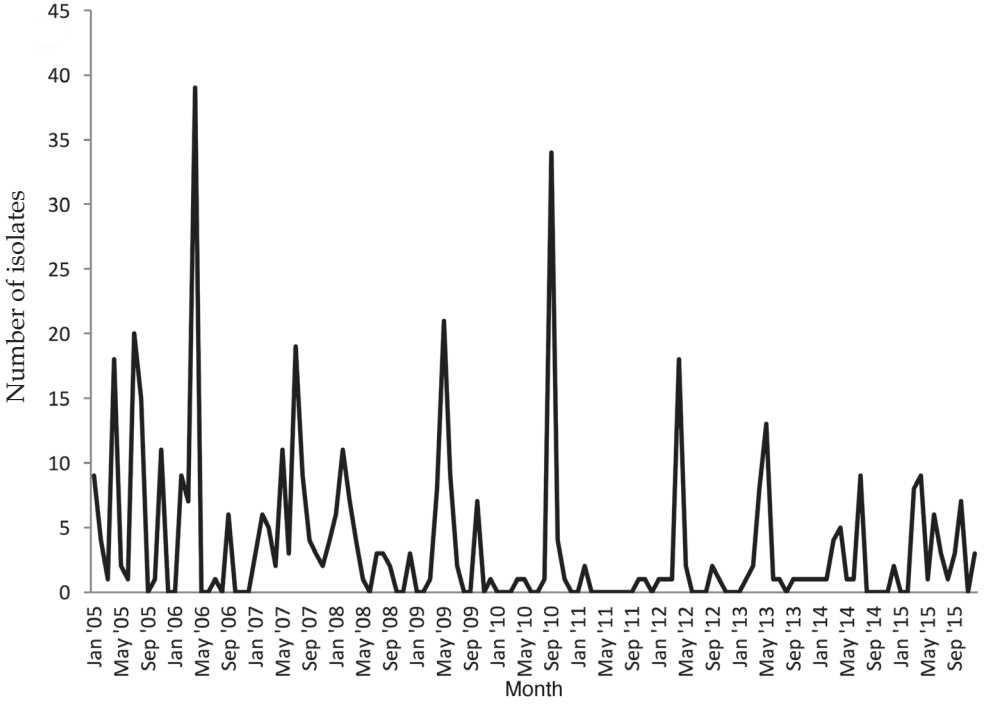

Fig 2 - Monthly distribution of influenza B isolates in Malaysia from 2005 to 2015. Samples are those described in legend to Fig 1.

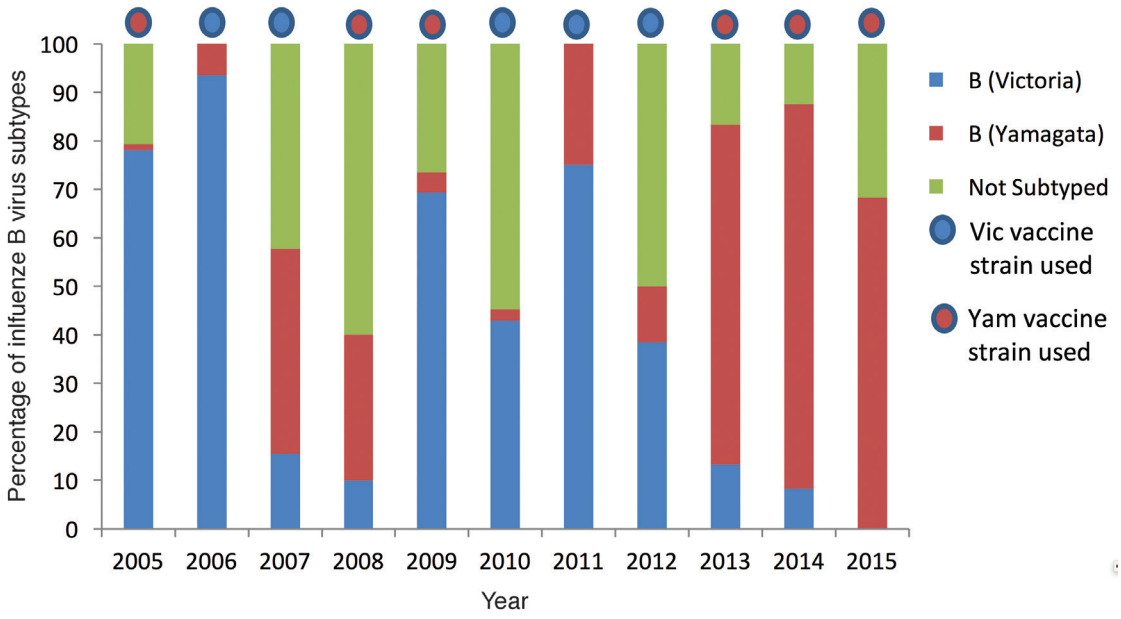

Fig 3 - Percentages of influenza type B viruses subtypes in Malaysia from 2005 to 2015. Samples are those described in legend to Fig 1. Circle on top of column indicates influenza B vaccine formulation. Vic, Victoria; Yam, Yamagata.

toria lineage was predominant in 2005 - 2006 and 2009 -2012, while influenza virus B/Yamagata lineage predominant in the other years of the study period. The major influenza type B virus strains were B/Hong Kong/330/2001-like in 2005, B/Malaysia/2506/2004-like in 2006, B/Florida/4/2006-like in 2007 and 2008, B/Brisbane/60/2008-like in 2009 - 2012, B/Massachusetts/2/2012-like in 2013 and 2014, and B/Phuket/3073/2013-like virus in 2015 (Table 2). The influenza vaccine formulation employed in the country corresponded to the predominant influenza virus B strain only in the years 2006, 2008, 2010, 2011, and 2013 - 2015.

DISCUSSION

The aim of influenza surveillance in Malaysia is to alert the Ministry of Health of an upcoming influenza pandemic. During the influenza A/H1N1pdm09 pandemic in 2009, influenza surveillance systems monitored the severity and trend of pandemic disease (Fisher *et al*, 2011). Management of surveillance data during that time was important so that a rapid risk assessment was carried out to alert public health action in identifying unusual cluster such as high death rate as well as initiating appropriate responses.

This study reveals influenza B cases in Malaysia were seen throughout the year with no consistent month(s) of peak incidence during 2005 to 2015. There were usually 1-3 influenza B virus strains co-circulating simultaneously each year. Tropical countries such as Malaysia do not have a well-defined influenza seasons compared to countries in temperate regions (Viboud *et al*, 2006). Both influenza A and B viruses were circulating during all the 11-year study period, with higher percent influenza A than influenza B viruses

Table 2
Predominant influenza type B virus strains circulating in Malaysia from 2005 to 2015 and influenza vaccine formulation employed.

Year	Circulating Influenza type B virus strain in Malaysia	Percent	WHO vaccine formulation
2005*	B/Hong Kong/330/2001-like virus[a]	79.0	B/Shanghai/361/2002-like virus[b]
	B/Malaysia/2506/2004-like virus[a]	19.8	
	B/Shanghai/361/2002-like virus[b]	1.2	
2006*	B/Malaysia/2506/2004-like virus[a]	94.2	B/Malaysia/2506/2004-like virus[a]
	B/Shanghai/361/2002-like virus[b]	5.8	
2007*	B/Florida/4/2006-like virus[b]	44.1	B/Malaysia/2506/2004-like virus[a]
	B/Shanghai/361/2002-like virus[b]	28.8	
	B/Malaysia/2506/2004-like virus[a]	27.1	
2008*	B/Florida/4/2006-like virus[b]	100.0	B/Florida/4/2006-like virus[b]
2009*	B/Brisbane/60/2008-like virus[a]	67.6	B/Florida/4/2006-like virus[b]
	B/Malaysia/2506/2004-like virus[a]	29.7	
	B/Florida/4/2006-like virus[b]	2.7	
2010	B/Brisbane/60/2008-like virus[a]	94.7	B/Brisbane/60/2008-like virus[a]
	B/Florida/4/2006-like virus[b]	5.3	
2011	B/Brisbane/60/2008-like virus[a]	75.0	B/Brisbane/60/2008-like virus[a]
	B/Florida/4/2006-like virus[b]	25.0	
2012	B/Brisbane/60/2008-like virus[a]	76.9	B/Wisconsin/1/2010-like virus[b]
	B/Wisconsin/1/2010-like virus[b]	23.1	
2013	B/Massachusetts/2/2012-like virus[b]	76.5	B/Massachusetts/2/2012-like virus[b]
	B/Brisbane/60/2008-like virus[a]	23.5	
2014	B/Massachusetts/2/2012-like virus[b]	90.5	B/Massachusetts/2/2012-like virus[b]
	B/Brisbane/60/2008-like virus[a]	9.5	
2015	B/Phuket/3073/2013-like virus[b]	100.0	B/Phuket/3073/2013-like virus[b]

*From Zainah *et al* (2010). [a]B/Victoria lineage. [b]B/Yamagata lineage.

except in year 2005, 2010, 2012, and 2013.

In Singapore, influenza viruses circulate throughout the year (Ang *et al*, 2016) but with bimodal pattern of influenza incidences at the start and middle of the year (Lim *et al*, 2014), similar to an earlier report in Malaysia by Shahidah *et a*l (2003). This discrepancy with the current study might be due to variability of climate and other environmental factors such as temperature, humidity and El Nino effect, which could impact influenza prevalence (Shoji *et al*, 2011; Yang *et al*, 2011).

The other important focus of influenza surveillance is to monitor virus strains to be used in the annual influenza vaccine formulation. From 2005 to 2014, both influenza B/Victoria and B/Yamagata lineages were found to be circulating in Malaysia, but only viruses of the B/Yamagata lineage were detected in 2015. In 2004, a new circulating influenza B

strain was discovered and characterized as B/Malaysia/2506/2004-like virus in the B/Victoria lineage virus (Sam *et al*, 2015). This virus appeared to be circulating in Malaysia continuously from 2005 to 2008, with the highest predominance among influenza B strains (94.2%) in 2006; however, the strain was not found after 2009. WHO recommended the B/Malaysia/2506/2004-like strain be included in vaccine formulation for Northern and Southern Hemisphere for 2006 and 2007 (WHO, 2006). We believe this B/Malaysia/2506/2004-like virus may have caused an epidemic in Malaysia before spreading to other countries (Sam *et al*, 2015).

In Malaysia, influenza B vaccine components perfectly matched the circulating strains except in 2005, 2007, 2009, and 2013. From 2010 to 2012 the predominant influenza type B virus strains were identified as B/Victoria lineage while B/Yamagata lineage virus was the predominant strain from 2013 to 2014. It was also observed, even though B/Victoria lineage was predominant in 2011 and 2012, around 25% of influenza B circulating in Malaysia were B/Yamagata lineage viruses, while in 2013, B/Yamagata lineage was predominant with B/Victoria lineage accounting for only 25%.

This study has a major strength in that the ILI clinical samples were collected countrywide by the practitioners of sentinel sites and all influenza isolates were analyzed by the WHO Collaborating Centre in Melbourne, Australia. Therefore, the data provided a comprehensive picture of circulation trends of influenza B viruses in Malaysia during the study period. However, a number of limitations were encountered: (i) only 70% of the influenza B cases were successfully recovered and grouped into subtypes due to loss of the virus viability during transportation of specimens and limitation in sensitivity of HI analysis, and (ii) because not all patients with ILI symptoms sought treatment resulting in an underestimation of the actual incidence of influenza in the country. We also noticed criteria for ILI (Navarro-Mari *et al*, 2005) poorly related to laboratory-confirmed influenza as only 7.2% of ILI cases were confirmed positive for influenza viruses (A and B).

In conclusion, this current study provides information on temporal pattern of influenza B incidences and reaffirmed the need of a local influenza surveillance program to inform on the appropriateness of annual influenza vaccines as well as for outbreak control.

ACKNOWLEDGEMENTS

The study was funded by the Ministry of Health, Malaysia. The authors thank the Director, Institute for Medical Research and the Director General, Ministry of Health, Malaysia for permission to publish the work.

REFERENCES

Ang LW, Tien WS, Lin RT, *et al*. Characterization of influenza activity based on virological surveillance of inlfulenza-like illness in tropical Singapore, 2010-2014. *J Med Virol* 2016; 88: 2069-77.

Fisher D, Hui DS, Gao ZC, *et al*. Pandemic response lessons from influenza H1N1 2009 in Asia. *Respirology* 2011, 16: 876-82.

Flood L, Raupach J, D'Onise K, Russo D. Influenza B outbreak in a primary school in Adelaide, Australia, 2011. *West Pac Surveill Resp J* 2012; 3: 76-82.

Horm SV, Mardy S, Rith S, *et al*. Epidemiological and virological characteristics of influenza viruses circulating in Cambodia from 2009 to 2011. *PLOS One* 2014; 9: e110713.

Julia F, Saba Q, Anthony WM, *et al*. Revision of clinical case definitions: influenza-like illness and severe acute respiratory infection. *Bull World Health Organ* 2017; 96: 122-8.

Khan UH, Mir MA, Ahmad F, *et al*. An outbreak of influenza B in an isolated nomadic community in Jammu & Kashmir, India. *Indian J Med Res* 2013; 138:1012-5.

Lim C, Ang LW, Ma S, Cui L, James L, Lin RTP. Virological surveillance of influenza-like illness in the community in Singapore, 2010–2013. *Epidemiol News Bull* (Singapore) 2014; 40: 53-8.

Navarro-Marí JM, Pérez-Ruiz M, Cantudo-Muñoz P, Petit-Gancedo C, Jiménez-Valera M, Rosa-Fraile M. Influenza-like illness criteria were poorly related to laboratory-confirmed influenza in a sentinel surveillance study. *J Clin Epidemiol* 2005; 58: 275-9.

Nicholson KG, Wood JM, Zambon M. Influenza. *Lancet* 2003; 362: 1733-45.

Ong JBS, Chen MI, Cheng, *et al*. Real-time epidemic monitoring and forecasting of H1N1-2009 using influenza-like illness from general practice and family doctor clinics in Singapore. *PLOS One* 2010; *5*: e10036.

Oong XY, Ng KT, Lam TT-Y, *et al*. Epidemiological and evolutionary dynamics of influenza B viruses in Malaysia, 2012-2014. *PLOS One* 2015; 10: e0136254.

Petrovic V, Seguljev Z, Cosic G, *et al*. Overview of the winter wave of 2009 pandemic influenza A(H1N1)v in Vojvodina, Serbia. *Croat Med J* 2011; 52: 141-50.

Sam I-C, Su YCF, Chan YF, *et al*. Evolution of influenza B virus in Kuala Lumpur, Malaysia between 1995 and 2008. *J Virol* 2015; 89: 9689-92.

Seleka M, Treurnicht FK, Tempia S, *et al*. Epidemiology of influenza B/Yamagata and B/Victoria lineages in South Africa, 2005-2014. *PLOS One* 2017; 12: e0177655.

Shahidah N, Merican I, Ismail R. Influenza surveillance in Malaysia :1997-2001. *Malaysia J Public Health Med* 2003; 3: 11-5.

Shoji M, Katayama K, Sano K. Absolute humidity as a deterministic factor affecting seasonal influenza epidemics in Japan. *Tohoku J Exp Med* 2011; 224: 251-6.

Tan AL, Virk RK, Tambyah PA, *et al*. Surveillance and clinical characterization of influenza in a university cohort in Singapore. *PLOS One* 2015; 10: e0119485.

Win MK, Chow A, Chen M, Lau YF, Ooi EE, Leo YS. Influenza B outbreak among influenza-vaccinated welfare home residents in Singapore. *Ann Acad Med Singapore* 2010; 39: 448-52.

World Health Organization (WHO). Recommendations for influenza vaccine composition. Southern hemisphere: 2006. Geneva: WHO, 2006. [Cited 2017 Apr 20]. Geneva: http://www.who.int/influenza/vaccines/vaccinerecommendations1/en/index8.html

World Health Organization (WHO). Influenza (Seasonal). Fact sheet No. 211. Geneva: WHO, 2014. [Cited 2017 Mar 25]. Available from: http://www.who.int/mediacentre/factsheets/fs211/en/

World Health Organization Regional Office for Europe. Influenza surveillance in the WHO European Region. Copenhagen: WHO Regional Office for Europe,. 2015. [Cited 2017 Mar 20]. Available from: http://www.euro.who.int/en/health-topics/communicable-diseases/influenza/surveillance-and-lab-network

Viboud C, Alonso WJ, Simonsen L. Influenza in tropical regions. *PLOS Med* 2006; 3: e89.

Yang L, Chen PY, He JF, *et al*. Effect modification of environmental factors on influenza-associated mortality: a time-series study in two Chinese cities. *BMC Infect* 2011; 11: 342.

Zainah S, Abdul Rashid TRT, *et al*. Seasonal influenza virus strains circulating in Malaysia from 2005 to 2009. *Southeast Asian J Trop Med Public Health* 2010; 41: 1368-73.