Influenza B Lineages Circulating among Children Attending Yangon Children's Hospital

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Influenza B viruses have also caused a considerable number of paediatric deaths although they are generally less prevalent than influenza A viruses. This cross-sectional study aimed to determine trends in prevalence of influenza B lineages among children attending Out Patient Department of Yangon Children's Hospital. Nasopharyngeal swabs were collected from 316 children with influenza-like illness (ILI) during January 2016 to October 2018. Influenza A and influenza B viruses were detected by conventional reverse transcription-polymerase chain reaction (RT-PCR) targeting matrix gene. Lineages of influenza B virus were identified by conventional RT-PCR targeting haemagglutinin gene. Influenza B virus accounted for 6.5% (10/153), 1.5% (1/68) and 2.1% (2/95) of all ILI cases and 45.5% (10/22), 8.3% (1/12) and 25% (2/8) of influenza virus positive ILI cases in 2016, 2017 and 2018 (up to October), respectively. Age and sex preponderance were not seen among influenza B virus-infected children. Fever, cough and rhinorrhoea were found as main but non-specific symptoms. Majority of influenza B virus-infected children were seen in June, July and August suggesting timing for influenza vaccination. All ten cases of influenza B virus detected in 2016 and the only case in 2017 were of B/Victoria lineage whereas both cases in 2018 were of B/Yamagata lineage highlighting the changing epidemiology of influenza B/lineages in the recent years. This study generated information useful for assessment of influenza B outbreaks, timing for influenza vaccination and selection of influenza vaccine for use in subsequent years in Myanmar.

Keywords: Lineage, Outbreak, Vaccination, Hemisphere, Influenza B

INTRODUCTION

Influenza B viruses have also been associated with a considerable number of paediatric deaths although they are generally less prevalent than influenza A viruses.[1] They are also responsible for about 10% of influenza-associated encephalopathy in young children.[2] After being identified in 1940, they have been detected among laboratory-confirmed influenza cases.[3] A surveillance study in Myanmar found lower detection rate of influenza B viruses compared to influenza A viruses during 2008-2011.[4] Influenza B viruses belong to the genus influenza virus B of the family Orthomyxoviridae. They fall into two distinct lineages namely Victoria-lineage and Yamagata-lineage.[5, 6] During 1991 and 2000, B/Victoria lineage viruses were detected only in eastern Asia.[7, 8] In 2001-2002, Victoria-lineage viruses re-emerged outside of eastern Asia and co-circulated with Yamagata-lineage viruses worldwide.[9, 10] Both B/Victoria and B/Yamagata lineages predominate alternatively with various intervals.[11, 12] A study in Vietnam found predominance of B/Yamagata-lineage in 2007, 2008 and 2012

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and B/Victoria lineage in 2009-2014 except 2012.[13] A study in Myanmar found predominance of B/Victoria lineage in 2005 and 2007 accounting for 75% (17/20) and 100% (112/112) of influenza B virus-infected cases, respectively.[14] Every year, trivalent and quadrivalent influenza vaccines of Southern Hemisphere as well as Northern Hemisphere are produced. Both type of vaccine can protect against two subtypes of influenza A virus (H3N2 and H1N1). However, quadrivalent vaccines for any hemisphere are formulated to protect two lineages of influenza B virus whereas trivalent vaccines against only one lineage of influenza B virus (Victoria lineage or Yamagata lineage).

Therefore, trivalent vaccine of any hemisphere can not protect against the lineage of B viruses which is not included in the vaccine.[15] Therefore, determining predominant lineage of influenza B virus helps in assessment of trivalent influenza vaccine's coverage for influenza B viruses circulating in that season or year.

Justification

Yangon Children's Hospital is one of the tertiary children hospitals in Myanmar. In the hospital, lineage identification of influenza B virus is not routinely done and hence, there is very few data of influenza B lineages circulating among the children attending the hospital. Although some data of influenza A viruses have been published in Myanmar, there has been a few publications of influenza B lineages and hence, little has been known about these viruses in Myanmar especially those circulating among children. Therefore, influenza B viruses should also be studied among children alongside influenza A viruses for assessment of influenza outbreaks and selection of influenza vaccine for use in subsequent years.

MATERIALS AND METHODS

Study design

It was a cross-sectional study conducted at Out-Patient Department (OPD) of Yangon Children's Hospital from January 2016 to October 2018. Children who attended the OPD due to influenza-like illness without nasal polyp or respiratory distress were included in this study.

Sampling procedure

All children who met the criteria mentioned above were recruited at OPD on every Monday, Wednesday and Friday within the office hours during the study period.

Data collection

Demographic and clinical data were collected from children's medical records as well as by interviewing the guardians. Laboratory data were obtained by specimen processing.

Specimen collection

Nasopharyngeal swab specimens were collected from the children, placed into the tubes containing Viral Transport Medium (VTM) and transported to the laboratory of Virology Research Division, Department of Medical Research within 8 hours after the specimen collection.

Extraction of viral RNA

Viral RNAs in the specimens were extracted by QIAamp® Viral RNA kits (Qiagen, Germany).

Typing of influenza virus

Influenza viruses were typed by multiplex reverse transcription-polymerase chain reaction (RT-PCR) targeting matrix gene of influenza A and influenza B virus. One step RT-PCR kit (Qiagen, Germany) was used for PCR reagents. Each PCR mixture contained 10 µl of deionized water, 5 µl of 5X buffer, 1 µl of dNPT, 0.5 µl of forward primer of influenza A matrix gene (ATGAGYCTTYTAACCGAGGTCGAAAC G), 0.5 µl of reverse primer of influenza A matrix gene (TGGACAAANCGTCTACGC TGCAG), 0.5 µl of forward primer of influenza B matrix gene (ATG TCG CTG TTT GGA GAC ACA AT), 0.5 µl of reverse primer of influenza B matrix gene (TCA GCT AGA ATC AGR CCY TTC TT) and 1 µl of enzyme mixture and 5 µl of RNA

template. Both positive and negative RNA templates were tested for quality control. Gel electrophoresis of PCR products were done at 100 volts for 45 minutes. The gel image of PCR products was visualized by molecular imager (BIORED, USA). The PCR product with 244 bp and 381 bp represented matrix gene of influenza A and influenza B virus, respectively.[16, 17]

Lineage identification of influenza B virus

RNA templates containing matrix gene of influenza B virus were subjected to lineage identification. Victoria and Yamagata lineage of influenza B virus were identified by multiplex RT-PCR targeting their haemagglutinin gene.

One step RT-PCR kit (Qiagen, Germany) was also used for PCR reagents. Each PCR mixture contained 10 µl of deionized water, 5 µl of 5X buffer, 1 µl of dNPT, 0.5 µl of forward primer of influenza B/Victoria HA gene (ACA TAC CCT CGG CAA GAG TTT C), 0.5 µl of reverse primer of influenza B/Victoria HA gene (TGC TGT TTT GTT GTT GTC GTT TT), 0.5 µl of forward primer of influenza B/Yamagata HA gene (ACA CCT TCT GCG AAA GCT TCA), 0.5 µl of reverse primer of influenza B/ Yamagata HA gene (CAT AGA GGT TT TCA TTT GGG TTT) and 1 µl of enzyme mixture and 5 µl of RNA template.

Both positive and negative RNA templates were tested for quality control. Gel electrophoresis of PCR products were done at 100 volts for 45 minutes. The gel image of PCR products was visualized by molecular imager (BIORED, USA). The PCR product with 284 bp and 388 bp represented HA gene of B/Victoria and B/Yamagata lineage virus, respectively.[16]

Data analysis

Data analysis was done by SPSS (version 11). Chi-square test was applied to determine the association between categorical variables. The association was considered to be statistically significant with 95% confident interval, if p-value is less than 0.05.

Ethical consideration

Ethical approval to conduct this study was obtained from Ethics Review Committee, Department of Medical Research.

RESULTS

From January 2016 to October 2018, a total of 316 children with ILI (153 children in 2016, 68 children in 2017 and 95 children in 2018) who attended Yangon Children's Hospital were studied. Influenza B virus was detected in 6.5% (10/153), 1.5% (1/68) and 2.1% (2/95) of all ILI cases, whereas influenza A virus in 7.9% (12/153), 16.2% (11/68) and 6.3% (6/95) in 2016, 2017 and 2018 (up to October), respectively. Therefore, overall prevalence of influenza virus among ILI cases was 14.4% (22/153), 17.7% (12/68) and 8.4% (8/95), respectively in 2016, 2017 and 2018 (Fig. 1).

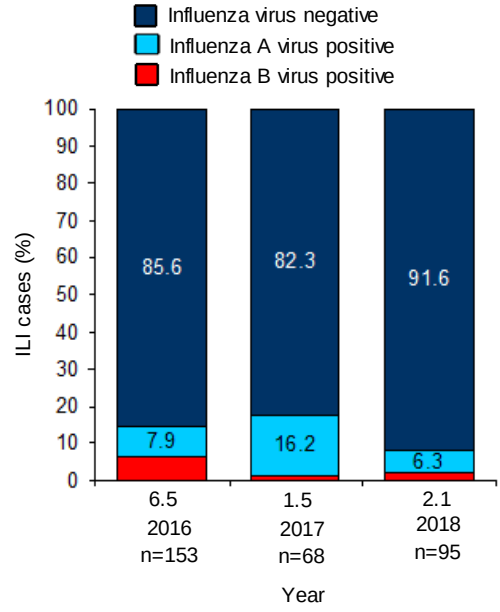

Fig. 1. Distribution of ILI cases attending Yangon Children's Hospital by positivity of influenza B virus (2016-2018)

The proportion of influenza B virus among influenza virus positive cases was found to be 45.5% (10/22), 8.3% (1/12) and 25% (2/8) in 2016, 2017 and 2018, respectively (Fig. 2).

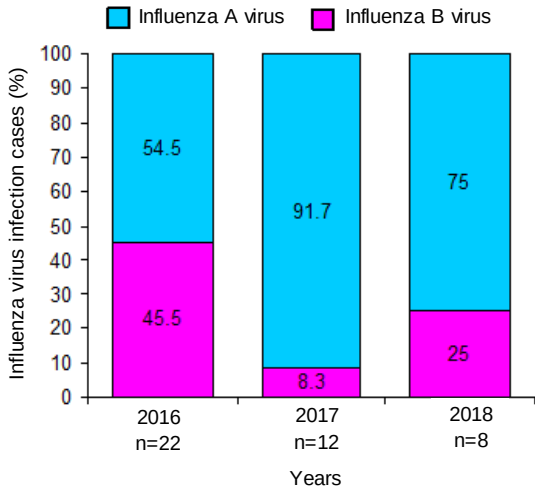

Fig. 2. Proportion of inflenza B infected cases among laboratory-confirmed influenza cases attending Yangon Children's Hospital (2016-2018)

Table 1. Demographic characteristics of influenza B virus infected ILI cases attending Yangon Children's Hospital

	ILI cases				p value
	Influenza B virus positive n(%)	Influenza A virus positive n(%)	Influenza virus negative n(%)	Total n(%)	
Age group (years)					
<5	7(3.2)	17(7.8)	195(89.0)	219(100)	0.22
5-12	6(6.2)	12(12.4	79(81.4)	97(100)	
Sex					
Male	9(5.1)	18(10.2)	149(84.7)	176(100)	0.32
Female	4(2.9)	11(7.9)	125(89.2)	140(100)	

Seven cases (3.2%) of influenza B virus was detected in 219 children under 5 years of age and 6 cases (6.2%) in 97 children aged between 5 to 12 years. There was no significant association between age and prevalence of influenza B virus (p=0.22). Regarding sex prevalence, influenza B virus was detected in 5.1% (9/176) of males and 2.9% (4/140) of females. Association between sex and pre-valence of influenza B virus was also not statistically signi-ficant (p=0.32). Therefore, age and sex preponderance of influenza B virus- infected children were not seen in this study (Table 1).

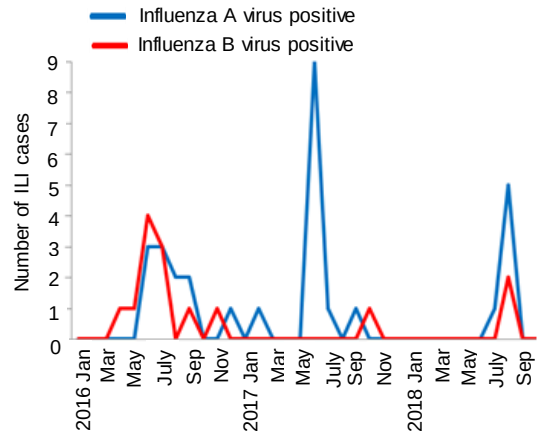

Fig. 3. Monthly distribution of influenza B virus infected cases attending Yangon Children's Hospital (2016-2018)

From January 2016 to October 2018, influenza B viruses were mainly detected in rainy season especially in June, July and August. Majority of influenza A viruses were also detected in these months. Therefore, influenza B viruses showed the seasonal pattern similar to influenza A viruses (Fig. 3).

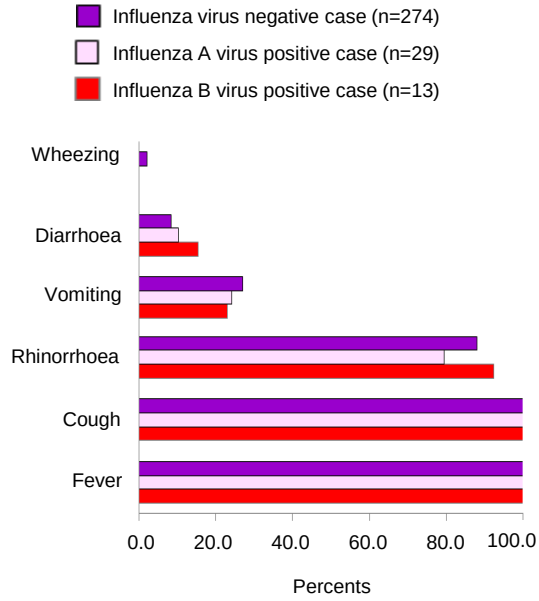

Fig. 4. Clinical presentations of influenza B virus infected children attending Yangon Children's Hospital (2016-2018)

Majority of influenza B virus infected children presented with fever (100%), cough (100%) and rhinorrhoea (79.3%) and a few cases with vomiting (24.1%) and diarrhoea (10.3%). Such frequency distribution of clinical presentations was also seen in influenza A virus-infected children and influenza virus negative children. Therefore, fever, cough and rhinorrhoea were found as main but non-specific symptoms of influenza B virus-infected children (Fig. 4).

Table 2. Lineage distribution of influenza B viruses-infected children attending Yangon Children's Hospital (2016-2018)

	Influenza B virus infected ILI cases		
	B/Victoria-lineage	B/Yamagata-lineage	Total
2016	10	0	10
2017	1	0	1
2018 (up to October)	0	2	2

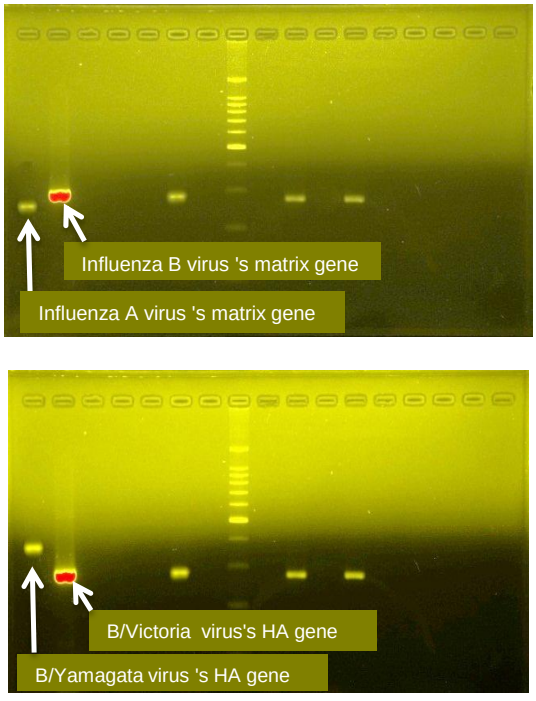

Plate. 1. Gel images showing PCR products of matrix gene of influenza A and influenza B virus and HA gene of B/Victoria and B/Yamagata virus

There were ten cases of influenza B virus detected in 2016 which are of B/Victoria-lineage. In 2017, only one case of influenza B virus was detected and it was also of B/Victoria-lineage. However, in 2018, two cases of influenza B virus were detected and they were of B/Yamagata-lineage. Therefore, B/Victoria-lineage viruses predominated in 2016 and 2017 but B/Yamagata-lineage viruses predominatedin 2018 (Table 2).

DISCUSSION

Influenza B virus is usually responsible for 25% of laboratory-confirmed influenza cases.[18] However, the proportion may be between 0-90% in any year.[19] Previous studies in Yangon Children's Hospital in 2013-2015 found no case of influenza B virus among influenza cases.[20, 21, 22] During this study, influenza B virus's proportion was highest in 2016 accounting for 45% and approaching the proportion of influenza A virus. Such proportion of influenza B virus was also seen in a study in Bangladesh during 2008-2009 and a study in Philippine during 2010-2013.[23, 24] In some years, proportion of influenza B virus exceeds to influenza A virus. A surveillance study in Myanmar found such higher proportion of influenza B virus in 2005.[14] These findings proved the changing proportion of influenza B virus among influenza cases with the period of time.

In 2016, not only proportion of influenza B virus in laboratory-confirmed influenza cases but its prevalence among children with ILI was highest in 2016 reflecting its epidemics.[20, 21, 22] Both proportion of influenza B virus in influenza cases and prevalence of influenza B infection among children significantly decreased in 2017 in which influenza outbreak due to influenza A (H1N1) pdm09 occurred. In 2018, both proportion and prevalence of influenza B virus slightly increased highlighting changing pattern of influenza B virus in term of epidemiology.

Except one case in 2017, most cases of influenza B virus were detected in June, July and August during the study period. In general, seasonal pattern of influenza B virus

coincided during rainy season like influenza A virus suggesting that seasonal influenza vaccination should be started before June in Myanmar.

Influenza B virus-infected children frequently presented with fever, cough and rhinorrhoea and infrequently as vomiting and diarrhoea. Clinical presentations of influenza B-infected children were not significantly different with those of influenza A-infected children and other children with ILI proving the importance of laboratory diagnosis in confirmation of influenza B virus infection.

In 2016 and 2017, B/Victoria-lineage was predominant which was covered by the trivalent vaccine of Southern Hemisphere for those years that contained B/Brisbane/ 60/ 2008-like virus (B/Victoria-lineage). B/ Yamagata became the predominant lineage among children in 2018 but fortunately, B/Phuket/3073/2013-like virus B/Yamagata-lineage) was contained in the trivalent vaccine of Southern Hemisphere for that year.

Therefore, trivalent vaccines of Southern Hemisphere were found to cover the B/lineages circulating among children in Yangon in the recent years. On the other hand, the Northern Hemisphere trivalent vaccine in 2015-2016 season contained a B/Phuket/3073/ 2013-like virus whereas that in 2016-2017 and 2017-2018 season contained a B/ Brisbane/ 60/ 2008-like virus. Thus, it could cover only B/lineage circulating in 2016-2017 season.[25, 26, 27, 28, 29, 30]

However, vaccine efficacy for each year or season might depend on the strain match and host factors.[31] Since the predominant lineage among the children in subsequent years can not be predicted, the Southern hemisphere vaccine of quadrivalent type, if available, should be used to cover both lineages.

Limitation of study

- Symptoms such as headache, myalgia and arthralgia could not be assessed in the study due to inability of very young children to complain of these symptoms.
- Lineage shift of influenza B virus could not be definitely determined due to small number of influenza B cases in 2017 and 2018.

Conclusion

This study generated information useful for assessment of influenza B outbreaks, timing for influenza vaccination and selection of influenza vaccine for subsequent years in Myanmar. Continuous large-scale studies should be carried out to better understand the circulating pattern and lineage shift of influenza B lineages in Myanmar.

*Recommendation*s

- Influenza B virus should also be considered as an important human respiratory pathogen and should be continuously monitored for assessment of their epidemics.
- Seasonal influenza vaccination should be started before rainy season especially before June in Myanmar.
- The Southern Hemisphere vaccine especially of quadrivalent type, if available, should be used to cover both lineages of influenza B virus which seem to be prevalent alternatively in Myanmar.

Competing interests

The authors declare that they have no competing interests.

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