MAJOR ARTICLE

Influenza B Lineage Circulation and Hospitalization Rates in a Subtropical City, Hong Kong, 2000–2010

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Background. A need for quadrivalent vaccines to cover both lineages of influenza B has been raised. Information on the circulation status of influenza B lineages and the associated hospitalization rates is important to assist evidence-based decision making. This retrospective study revealed the situation in a subtropical city over a 10-year period.

Methods. Sequences of 268 influenza B isolates were analyzed to identify the circulating pool of virus lineages for each year. Hospital records and population census data were used to estimate annual age-specific hospitalization rates.

Results. Cocirculation with 2 influenza B lineages was found in 9 of the 10 years. Only in 6 of the 10 years had the vaccine strain successfully matched with the lineage that was found in >50% of the circulating pool. Six years were predominated by one lineage (occupying >80% of the circulating pool), and these years had higher (average, 1.4-fold) hospitalization rates. Matching between vaccine and circulating lineage was achieved only in 2 of the 6 "predominated years." The Yamagata lineage accounted for most (5/6) of the predominated years. Overall, 24% of influenza admissions were due to influenza B, and influenza B contributed to a higher proportion (41.9%) among children and young teenagers (5–14 years old).

Conclusions. Cocirculation with 2 influenza B lineages is common in the subtropical region. To predict the next predominant lineage proves to be difficult. Influenza B accounts for a substantial fraction of influenza-associated hospitalizations, especially among children and young teenagers. Quadrivalent vaccines may improve the effectiveness of influenza vaccination programs.

Keywords. influenza; hospitalisation; lineage; Yamagata; Victoria.

Four antigenically distinct groups of influenza viruses including 2 subtypes of influenza A (A/H1N1 and A/H3N2) and 2 lineages of influenza B (B/Victoria and B/Yamagata) are currently cocirculating in humans. Since 2009, the previously circulating seasonal H1N1 has been replaced by the swine-origin A(H1N1) pdm09 virus. Prior to the reemergence of A/H1N1 in 1976, a bivalent vaccine was recommended to cover the circulating A/H3N2 and B viruses. Since 1977, it was shifted to a trivalent vaccine with the addition of an A/H1N1 component because of the lack of cross-protection between the 2 subtypes of influenza A viruses. In contrast to influenza A, influenza B viruses mutate more slowly and have no animal reservoir. Until the mid-1980s, only 1 lineage of influenza B was circulating globally [1]. The Victoria lineage, which emerged in China in the 1970s, became widely circulated worldwide by the 1980s [2]. As with influenza A, 2 antigenically distinct lineages of influenza B viruses have been cocirculating in the past 20 years. However, influenza B is being dealt with differently, for which

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only 1 lineage is selected as a component of trivalent seasonal influenza vaccines [3]. Surveillance from the United States and Europe over the last 10 years showed that the chance of a correct matching between the vaccine and the predominant circulating lineage of influenza B had only been 50% [4]. Given the limited cross-lineage protection offered by current influenza vaccines [5], there is a call to shift to quadrivalent vaccines incorporating a prevalent strain from each of the 2 influenza B lineages [4, 6, 7].

To improve our understanding on the disease impact of influenza B lineages in the subtropical region, we identified the virus lineages circulating in Hong Kong as well as the associated age-specific hospital admission rates over a 10-year period.

MATERIALS AND METHODS

Case Identification and Study Population

Patients with laboratory-confirmed influenza A or B infection admitted to the Prince of Wales Hospital from 1 January 2000 to 31 December 2010 were identified from computerized hospital records. In most situations, the presence of influenza virus in nasopharyngeal aspirate samples was detected by immunofluorescence-based antigen test and virus isolation in parallel. Molecular detection using real-time polymerase chain reaction was used upon special requests for urgent and severe cases. The Prince of Wales Hospital is a 1400-bed general acute district teaching hospital serving a population of about 0.6 million (9% of the entire population in Hong Kong), of which 3% are children aged <5 years, and 10% are elderly persons aged >65 years [8]. The age-specific resident population data obtained from the 2006 population bi-census were used to estimate the annual hospital admission rate due to influenza infection [8].

Influenza B Lineage Identification

The stored virus isolates were randomly selected for sequencing of a fragment of the hemagglutinin (HA) 1 gene. A previously published protocol was adopted using primers 5′-ATA ACA TCG TCA AAC TCA CC-3′ and 5′-GCA CCA TGT AAT CAA CAA CA-3′ to amplify a 739-bp fragment corresponding to amino acids 22–267 of the HA1 protein [9]. A maximum-likelihood tree was constructed using MEGA 5.0, and rooted to B/Lee/40 sequence (GenBank accession No. K00423) [10]. The data were bootstrap-resampled 1000 times. To verify whether a phylogenetic tree based on this HA1 fragment was robust enough to identify the lineage of influenza B, 21 sequences of known lineage published from 2000 to 2010 downloaded from GenBank were included in the tree construction.

Data Analysis

For the purpose of presenting the results in this study, a "predominated year" was defined as one with either the Victoria or Yamagata lineage found in >80% of the specimens, whereas a "mixed year" was defined as one when either lineage existed in 20%–80% of the specimens.

Hong Kong adopts the northern hemisphere version of influenza vaccine recommended by the World Health Organization (WHO). The vaccine is administered mainly during October–December to prepare for the seasonal peaks that usually occur in the following months of February–March and June–July [11]. Therefore, when assessing the matching between vaccine and circulating virus, the 1999–2000 northern hemisphere vaccine components were correlated with the virus lineages circulating in 2000 in Hong Kong, and similarly for the other years.

When expressing the hospital admission rates, medians and interquartile ranges (IQRs) were reported because the Shapiro-Wilk test showed that the outcome measures were not normally distributed. Similarly, the Mann-Whitney *U* test was used to compare hospitalization rates between groups.

Differences between the proportion of influenza A and B within a group were assessed by binomial test, and those between groups were assessed by the χ^2 test or the Fisher exact test as appropriate. Statistical tests were performed using the Statistical Package for Social Sciences (version 18.0.0, SPSS IBM). Two-tailed *P* values <.05 were regarded as significant.

RESULTS

Circulation Pattern of Influenza B Lineages

Altogether, 268 influenza B isolates collected during 2000–2010, with an average of 27 (range, 19–32) samples per year, were examined. Since 2009 was predominated by the A (H1N1)pdm09 virus, samples in 2009 were not included in this study. The phylogenetic tree constructed from these study samples and the published reference sequences is shown in Supplementary Figure 1. As expected, the Victoria and Yamagata reference sequences clustered respectively into 2 main branches, confirming the robustness of this HA1 fragment for lineage identification.

Both lineages were detected in 9 of the 10 years examined (Figure 1). Year 2000 was the only year with 1 lineage found. Six years (2000, 2001, 2004, 2006, 2007, and 2008) were regarded as "predominated years," as one of the lineages occupied >80% of the circulating pool. Similarly, 4 years (2002, 2003, 2005, and 2010) were regarded as "mixed years," as one of the lineages was found in 20%–80% of the circulating pool. Five of the 6 predominated years were due to Yamagata lineage. The proportion of a lineage over time displayed a zigzag pattern. The Yamagata lineage was replaced gradually by the Victoria lineage over a period of 3–4 years, whereas the Victoria lineage was replaced more quickly over a single season (2003–2004 and 2006–2007; Figure 1).

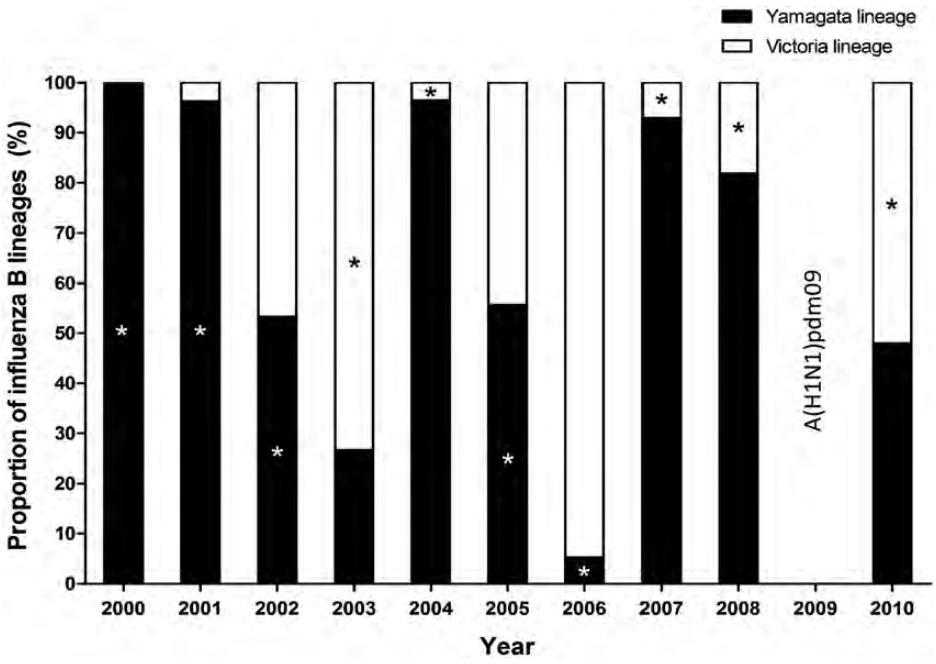

Figure 1. Distribution of influenza B lineages in Hong Kong, 2000–2010. Black boxes represent Yamagata lineage; white boxes represent Victoria lineage. Asterisks indicate the World Health Organization–recommended influenza B vaccine strain of that year. Data from 2009 were not included as it was predominated by the A(H1N1)pdm09 virus.

In 6 of the 10 studied years, the WHO-recommended vaccine component matched with the lineage that accounted for >50% of the contemporary circulating pool of influenza B viruses (Figure 1), whereas the WHO-recommended vaccine strain matched with the predominant lineage in only 2 of the 6 predominated years.

Age-Specific Annual Hospital Admission Rates of Influenza

Influenza A

The mean annual hospital admission rate of influenza A for all age groups recorded over the 10 years (2000–2010, excluding 2009) was 65.1 per 100 000 per year. The age-specific annual hospital admission rates displayed a U-shape curve with higher hospitalization rates for the extremes of age (Figure 2). Young children aged <5 years had the highest hospitalization rate (median, 921 [IQR, 833–1196] per 100 000 per year), followed by elderly persons aged ≥80 years (median, 296 [IQR, 96–563] per 100 000 per year), and then closely by children aged 5–9 years (median, 188 [IQR, 160–228] per 100 000 per year). The median hospitalization rate for the remaining age groups ranged from 13 to 76 per 100 000 per year.

Influenza B

The mean annual hospital admission rate of influenza B for all age groups recorded over the 10 years was 20.6 per 100 000 per year. The highest annual hospitalization rate of influenza B also occurred in young children aged <5 years (median, 238 [IQR, 170–352] per 100 000 per year; Figure 2). However, in contrast to influenza A, older children aged 5–9 years ranked second for influenza B (median, 152 [IQR, 133–171] per 100 000 per year), and was 3 times higher than that of elderly persons aged ≥80 years (median, 44 [IQR, 7–74] per 100 000 per year). The median hospitalization rates for remaining age groups ranged from 3 to 25 per 100 000 per year.

Comparison Between Influenza A and B

The median annual hospitalization rate of influenza A was consistently higher than influenza B across all age groups (Figure 2). The biggest difference was observed among elderly persons aged ≥65 years, for whom the median annual hospitalization rate of influenza A was 6.7-fold higher than that of influenza B, whereas children aged 5–9 years showed the smallest difference (1.2-fold), followed by young adolescents aged 10–14 years (1.7-fold). The difference in annual admission rate between influenza A and B was statistically significant across all age groups (P values ranged from < .001 to .035 by Mann-Whitney U test), except for children aged 5–9 years (P = .075 by Mann-Whitney U test).

Influenza B Lineage and Hospital Admission Rates

All Ages Combined

During the 6 predominated years (2000, 2001, 2004, 2006, 2007, and 2008), an average of 140.6 (range, 61–197) influenza B admissions per year were recorded, which was approximately

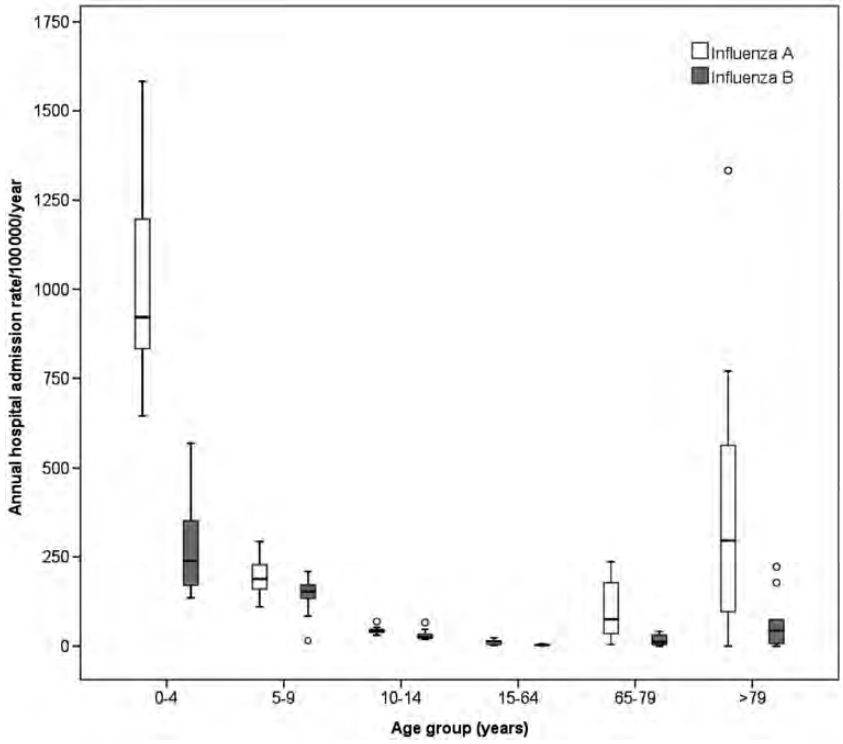

Figure 2. Age-specific annual hospital admission rates of influenza A and B, 2000–2010. Empty boxes represent influenza A. Shaded boxes represent influenza B. Boxes indicate interquartile ranges of annual hospital admission rate; the lines extending from each box represent the extremes of values, and the line across each box indicates the median. Outliers are indicated by circles. Data for 2009, which was predominated by the A(H1N1)pdm09 virus, are not included.

1.4-fold higher than that of mixed years (2002, 2003, 2005, and 2010; average, 102.3 [range, 82–131] admissions per year).

Age Stratified

A similar age-related pattern of hospitalization rates was observed for years predominated by one lineage as compared to those with mixed lineages (Figure 3). The hospitalization rate for young children aged <5 years appeared to be higher for predominated years (2000, 2001, 2004, 2006, 2007, and 2008) compared with mixed years (2002, 2003, 2005, and 2010). However, the differences did not reach statistical significance (median, 326 [IQR, 199–364] vs 190 [IQR, 153–238] per 100 000 per year; $P = .171$ by Mann-Whitney U test).

The hospitalization rates for years predominated by the Yamagata lineage appeared to be higher for children <5 years and for elderly persons aged >79 years compared to other years. However, the differences did not reach statistical significance.

Influenza B Lineage and Ratio of Influenza A to Influenza B Admissions

All Ages Combined

Overall, influenza B was attributed to 24% (1253/5210) of influenza-associated admissions recorded over the 10 years (Table 1). Throughout the 10 years, influenza A accounted for a significantly larger proportion of admissions, with ratios of influenza A to influenza B ranging from 1.9 to 8.5 ($P < .001$ for all years by binomial test). During the 6 years that were predominated by one lineage, the ratios of influenza A to influenza B ranged from 1.9 to 8.5 (mean, 3.3), whereas those for the 4 mixed years were 1.9–7.6 (mean, 4.4). The overall proportion of influenza B admission for predominated years was significantly higher than that of mixed years (27.2% vs 19.5%, $P < .001$ by χ^2 test).

Age-Stratified

The age-specific proportions of influenza admission due to influenza B are shown in Figure 4. When all years were combined, the proportion due to influenza B was significantly higher among children aged 5–9 years (42.2%, 376/890) and young teenagers aged 10–14 years (40.9%, 110/269), compared to those of other age groups (13.5%–21.4%; $P < .001$ for 2-by-2 comparisons with all age groups by χ^2 test). This age-related pattern was reproduced and the association remained significant for both predominated and mixed years.

The proportion of influenza admissions due to influenza B was significantly higher for predominated years than mixed

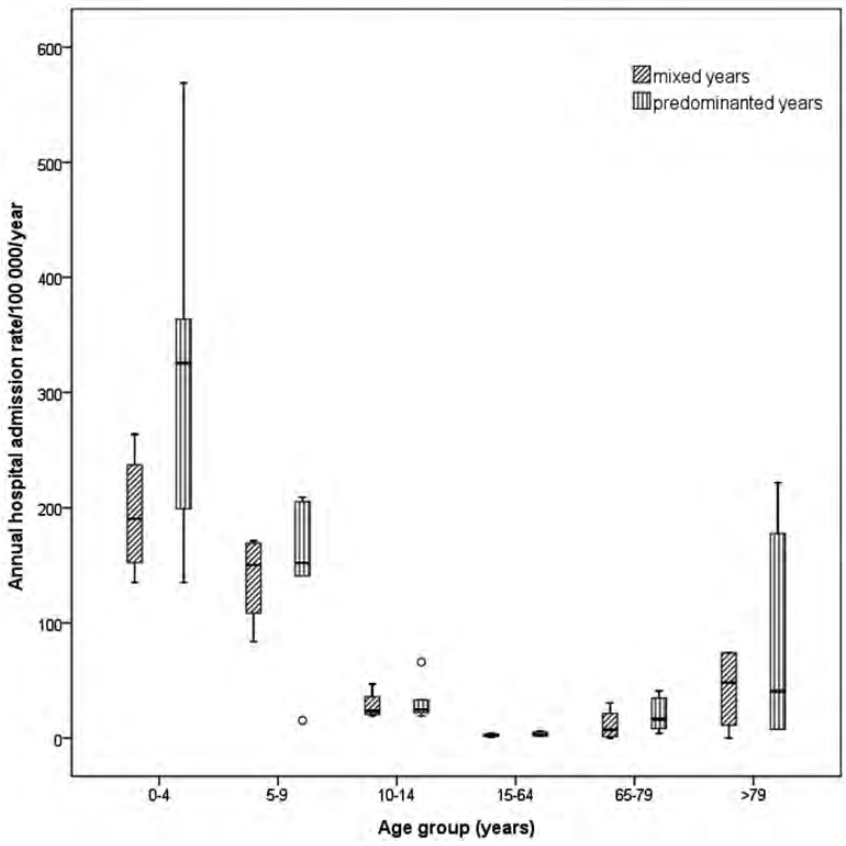

Figure 3. Age-specific annual hospital admission rates according to the circulation status of influenza B lineages. Six years (2000, 2001, 2004, 2006, 2007, and 2008) were regarded as "predominated years," as one of the lineages occupied >80% of the circulating pool. Four years (2002, 2003, 2005, and 2010) were regarded as "mixed years" as one of the lineages occupied 20%–80% of the circulating pool. Boxes indicate interquartile ranges of annual admission rate; the lines extending from each box represent the extremes of values, and the line across each box indicates the median. Outliers are indicated by circles. Data for 2009, which was predominated by the A(H1N1)pdm09 virus, are not included.

Table 1. Influenza-Associated Hospital Admissions and Circulation Status of Influenza B Lineages

		No. of Influenza-Associated Hospital Admissions for All Age Groups Combined		
Year	Influenza B Lineage Circulation Status[a]	Influenza A	Influenza B	Influenza A/Influenza B
2000	Predominated (Yamagata, 100%)	348	161	2.2
2001	Predominated (Yamagata, 96%)	245	125	2.0
2002	Mixed (Yamagata, 53%)	341	85	4.0
2003	Mixed (Victoria, 73%)	216	111	1.9
2004	Predominated (Yamagata, 96%)	520	61	8.5
2005	Mixed (Yamagata, 56%)	511	131	3.9
2006	Predominated (Victoria, 95%)	363	127	2.9
2007	Predominated (Yamagata, 90%)	457	197	2.3
2008	Predominated (Yamagata, 86%)	330	173	1.9
2009	Influenza A(H1N1)pdm predominated	1506	17	88.6
2010	Mixed (Victoria, 52%)	626	82	7.6

[a] Predominated circulation was defined as either one of the lineages being found in >80% of the circulating pool. Mixed circulation was defined as having one of the lineages found in 20%–80% of the circulating pool.

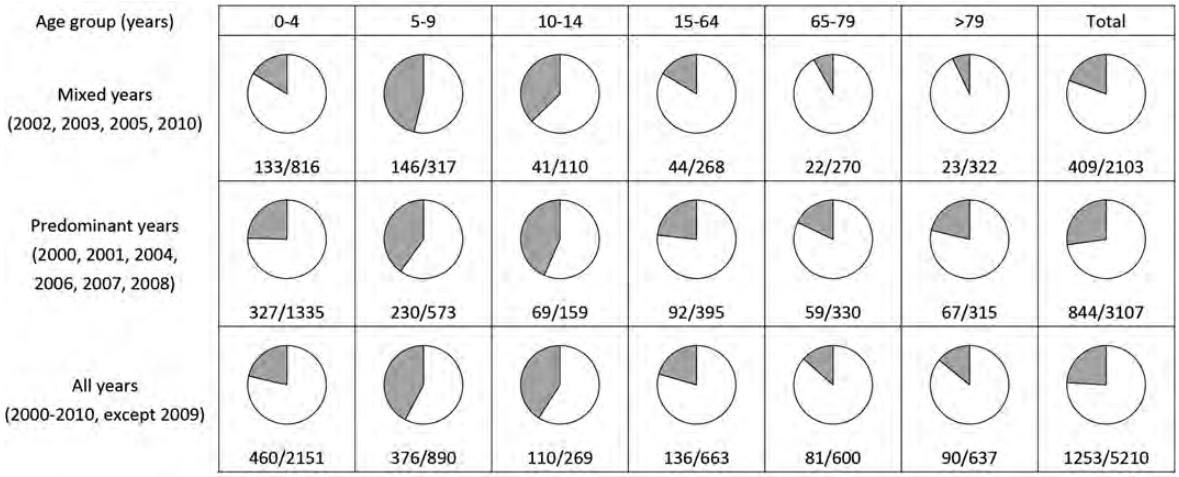

Figure 4. Age-specific proportions of influenza-associated admissions due to influenza B. Mixed years defined as those years with either the Victoria or Yamagata lineage occupying 20%–80% of the circulating pool. Predominated years defined as those years with either lineage occupying >80% of the circulating pool. Data for 2009, which was predominated by the A(H1N1)pdm09 virus, are not included. Shaded areas of pies indicate proportions of influenza B admission. Figures below pies indicate No. of admissions due to influenza B/No. of admissions due to influenza A and B.

years (1.4–3.0-fold, *P* values ranged from .031 to < .001) for most of the age groups, with the exception of children aged 5–9 years and young adolescents aged 10–14 years (Figure 4).

DISCUSSION

The seasonality of influenza activity is more variable in the tropical and subtropical regions compared with the temperate zones [11–13]. Successive epidemics occurring at different times and locations within the region allow continuous circulation and evolution of influenza viruses. Whereas the East and Southeast Asian region is recognized as an epicenter for the emergence of new variants of influenza A viruses [14–17], little is known about the behavior of influenza B viruses in this region [18]. Hong Kong is a densely populated city situated in the East and Southeast Asian circulation network. Our data demonstrated that cocirculation of both lineages of influenza B viruses was common, as it has been demonstrated in the temperate zones such as the United States and Europe [4]. To achieve a good match between the vaccine component and the contemporary circulating influenza B virus was no better than chance alone. During the 10 years of our study, only in 6 years had the vaccine strain matched with the lineage found in >50% of the contemporary circulating pool. Of note, only in 2 of the 6 predominated years did the vaccine strain match with the predominant lineage. Such a poor chance of success in matching seems to be a global problem [19, 20].

Influenza has long been recognized as an important disease with a high hospital admission rate and mortality among the elderly, whereas the impact on children and adults has been less well documented. In this study, we observed an age-related disparity in disease burden between influenza A and B. Although both influenza A and B displayed a U-shape curve of age-specific hospitalization rate, some notable differences were observed. First, a sharp increase in hospitalization rate among elderly persons aged >65 years was observed for influenza A, but only a modest increase was observed for influenza B. Second, the hospitalization rate of influenza A dropped sharply with increase in age from <5 to 5–9 years, but only a modest drop was observed for influenza B. Third, the admission rate of influenza B relative to influenza A was highest among older children and young teenagers aged 5–14 years. A similar predilection of influenza B for children and young adults has also been reported from other studies [21, 22]. These observations are in line with a previous report that children accumulated natural immunity to influenza B more slowly than to influenza A [23].

Our results showed that influenza B was attributed to 24% of influenza-related hospital admissions overall, and was as high as 41.9% for older children and young adolescents aged 5–14 years. This observation is in line with reports that influenza-associated hospitalization among young children was substantial [24–26]. Given the higher disease burden of influenza B among children and young adolescents compared with other age groups, quadrivalent vaccines are necessary to improve the effectiveness of childhood vaccination programs.

The current evidence suggests that immunizing children with either live-attenuated or inactivated vaccines confer indirect protection for other age groups against infection with influenza in general [27, 28]. The current data do not provide a

clear answer whether the indirect protective effect varies between the 2 groups of influenza. Theoretically, there is no reason to suspect this will be the case, as both groups of influenza virus share a similar mode of transmission. Therefore, in countries that can achieve a high coverage of childhood influenza vaccination, quadrivalent vaccines are expected to have a substantial benefit in reducing the childhood hospitalization rate, and the associated decrease in the circulating pool of viruses may eventually decrease the attack rate of other age groups. Furthermore, the good safety profile of quadrivalent vaccines as revealed by recent clinical trials is another argument for adopting them in routine immunization programs [29–31].

Our data revealed a zig-zag distribution of influenza B lineages over time. Interestingly, the 2 lineages seemed to replace each other in a different speed. The Yamagata lineage was replaced more gradually over a few years, whereas the Victoria lineage was replaced over a single season. This observation may suggest that the population immunity to Victoria lineage disappears more quickly than that to Yamagata lineage. We observed that when an influenza B lineage predominated (>80%) in the circulation, a higher hospital admission rate for influenza occurred. This could be a result of lack of immunity toward newly emerged variants together with a poor match between vaccine and circulating strains. We hypothesize that the higher admission rate could also be due to a higher virulence of the Yamagata lineage that had accounted for the majority (5/6) of the years with a predominated lineage. Should trivalent vaccines remain, and thus only 1 influenza B lineage can be selected, Yamagata lineage should have a higher priority when the surveillance data predict a similar likelihood for both lineages.

Although quadrivalent vaccines including 2 influenza B lineages can improve matching with the circulating pool of viruses, other factors must be carefully evaluated before it can be adopted for routine use. In this study, we examined the disease impact of influenza B in association with the status of lineage circulation. These results are important considerations when balancing the pros and cons of adopting quadrivalent vaccines. We chose to use hospitalized, laboratory-confirmed cases as this can reflect more closely the burden to the healthcare system. Nevertheless, one should be aware that many influenza cases are managed in outpatient settings.

Although this study might have underestimated the hospitalization rate owing to admission to private hospitals or to public hospitals in other districts, this was likely to be small and should not affect our comparisons in admission rates between influenza A and B, and among the 2 influenza B lineages. One limitation of this study is that the number of samples examined per year was relatively small. Therefore, this study could not accurately predict the proportions of viruses circulating. Nevertheless, this study should be able to identify the predominant lineage circulated during those years.

On the basis of the data collected over a period of 10 years in a subtropical city, we found that influenza B accounted for a substantial proportion of influenza-associated hospitalizations, and it posed a higher weight toward children and young teenagers. Cocirculation of both lineages was common, and the chance of success in predicting the predominant lineage proved to be difficult. Quadrivalent influenza vaccines may improve the effectiveness of influenza vaccination programs.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. P. K. S. C. was responsible for the study design, data analysis, and manuscript preparation; N. L., T. F. L., E. A. S. N., and D. S. C. H. conducted the clinical sample collection and data analysis; M. C. W. C., M. C. S. W., and A. C. M. Y. were responsible for data analysis and manuscript preparation; J. L. K. C. and K. L. K. N. provided the laboratory analysis.

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Potential conflicts of interest. P. K. S. C. has received consultancy fees and research funding from F. Hoffmann-La Roche and support for attending academic conferences from GlaxoSmithKline; N. L. has received grant support from F. Hoffmann-La Roche on principal investigator–initiated clinical influenza research, honoraria for consultancy work from GlaxoSmithKline, and conference support from Sanofi-Aventis Hong Kong Ltd, MSD (Asia) Ltd, and Pfizer Hong Kong; E. A. S. N. has received funding support from Pfizer for an investigator-initiated respiratory disease surveillance study, has participated in vaccine studies funded by Baxter, GlaxoSmithKline, MedImmune, and Wyeth, and has received lecture fees and travel support from GlaxoSmithKline, Merck, Intercell, and Pfizer (Wyeth). All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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