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Evolution of Influenza B Virus in Kuala Lumpur, Malaysia, between 1995 and 2008

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Influenza B virus causes significant disease but remains understudied in tropical regions. We sequenced 72 influenza B viruses collected in Kuala Lumpur, Malaysia, from 1995 to 2008. The predominant circulating lineage (Victoria or Yamagata) changed every 1 to 3 years, and these shifts were associated with increased incidence of influenza B. We also found poor lineage matches with recommended influenza virus vaccine strains. While most influenza B virus lineages in Malaysia were short-lived, one circulated for 3 to 4 years.

Influenza B virus causes seasonal epidemics and a significant disease burden in humans, with reported rates of up to 82% of total influenza cases around the world and 22 to 44% of influenza-related child deaths in the United States (1). Since the mid-1970s, influenza B virus has formed two antigenically distinct lineages, "B/Victoria/2/87-like" and "B/Yamagata/16/88-like," referred to as the Victoria and Yamagata lineages, respectively (2). Although the two lineages cocirculate in humans, Vijaykrishna et al. (3) recently demonstrated significant evolutionary and epidemiological differences between the two lineages. The Victoria lineage is subject to stronger seasonal bottlenecks, higher transmission rates, greater antigenic variation, and stronger positive selection and infects mostly younger age groups. In contrast, the Yamagata lineage experiences less severe bottlenecks and infects older people (3). In tropical Kuala Lumpur in Malaysia, there is year-round influenza activity, with biannual epidemics in May to July and November to January (4). A retrospective epidemiological study of hospitalized children in Kuala Lumpur from 1982 to 2008 found that 297 of 2,708 (11%) patients with confirmed respiratory virus infection were positive for influenza virus, making it the third most common respiratory virus affecting children aged <5 years, with higher rates of infection in children aged 1 to 5 years than in children under 12 months old (5). While epidemiological data in Malaysia have been well documented (4–7), no genetic study has been conducted on influenza B virus from Malaysia.

We obtained a total of 338 laboratory-confirmed influenza cases from children and adults (1 month to 49 years old) admitted to the University Malaya Medical Centre in Kuala Lumpur during 1995 to 2008. All patients resided in Kuala Lumpur or the surrounding conurbation. Of these, 88 cases (26.0%) were diagnosed with influenza B viruses, whereas 250 cases (74.0%) were infected with influenza A viruses. Isolates of influenza B virus were cultured in Madin-Darby canine kidney (MDCK) cells and passaged up to three times prior to extraction of viral RNA as described previously (8). Complete genomes of 72 influenza B viruses and partial genomes of 5 influenza B viruses were sequenced (8–10) and deposited in GenBank (see Table S1 in the supplemental material). These sequences represent all full-length hemagglutinin (HA) or complete genome sequences from Malaysia (from 1995 to 2008) available in public databases at the time of writing. Phylogenetic analyses were performed for all eight gene segments (PB2, PB1, PA, HA, NP, NA, MP, and NS) using a combination of Malaysian and global sequences (see Table S1). Temporal phylogenies were reconstructed using the coalescent-based Gaussian Markov random field (GMRF) method with the time-aware smoothing parameter (11) in BEAST v.1.8.1 (12). For all analyses, the uncorrelated log-normal relaxed molecular clock and the SRD06 codon position model (13) with the HKY85+Γ substitution model were used. Three independent analyses of 100 million generations, with sampling every 10,000 generations, were performed.

Our study indicates that 75.4% of positive influenza B cases were in young children aged <5 years, with a median age of 2.0 years (see Table S2 in the supplemental material). The incidence of influenza B virus infection in our study group was generally lower

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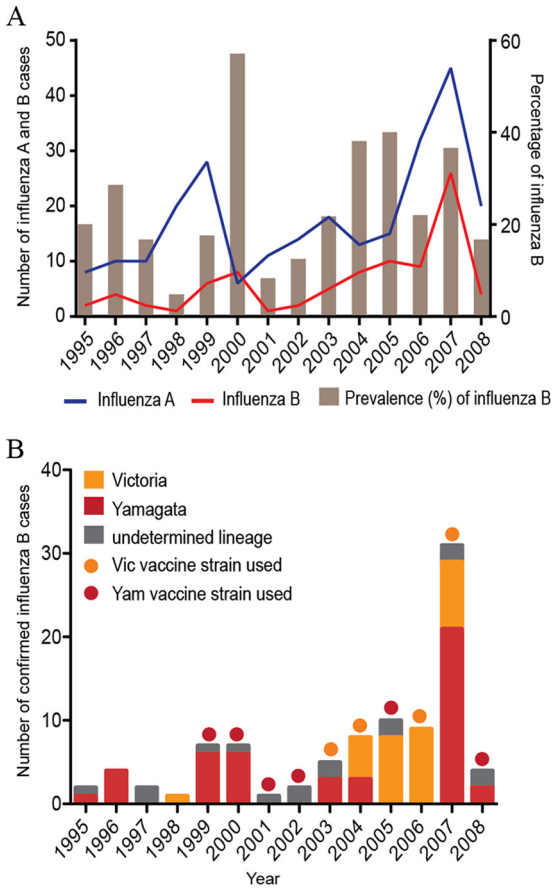

FIG 1 Influenza virus activity in Kuala Lumpur, Malaysia, between 1995 and 2008. (A) The numbers of laboratory-confirmed human cases of influenza A and B viruses are shown for each year. The vertical bars represent the yearly proportion (percentage) of influenza B virus cases against the total number of positive influenza virus cases recorded. (B) Bars represent the prevalence (number of cases) of Victoria and Yamagata lineages. Cases of undetermined lineage refer to samples that tested positive for influenza B virus by immunofluorescence but were not successfully cultured for sequencing.

than that of influenza A virus infection; however, a higher incidence of influenza B virus (>30% of influenza cases) was observed in 2000, 2004, 2005, and 2007 (Fig. 1A), coinciding with shifts in the predominant lineage. In most years, only a single influenza B virus lineage was detected, with Yamagata viruses detected exclusively in 6 of the 14 study years, while only Victoria strains were detected in just 3 years (Fig. 1B). Cocirculation of both lineages was detected only in 2004 and 2007 in our study, in contrast to a recent study that showed cocirculation in Australia and New Zealand in most years (3). Although our study obtained a small number of sequences in most years, with no influenza virus detected in three of the years, the predominant influenza B virus lineages circulating in Malaysia as a whole from 2005 to 2008 (6), identified by hemagglutination inhibition, were similar to our data from Kuala Lumpur.

The HA phylogeny indicated that there had been multiple independent introductions of the two influenza B virus lineages into Malaysia that occurred sporadically throughout the study period (Fig. 2A). The markedly greater influenza B virus activity observed in 2007 (Fig. 1) was largely due to multiple introductions of Yamagata viruses (denoted by red branches in Fig. 2A), including a monophyletic clade that may indicate that a point source outbreak occurred in Kuala Lumpur (Fig. 2B). Genetic analysis also showed that the Victoria lineage viruses, which we found were more prevalent from 2004 to 2007 than in previous years (Fig. 1B), were B/Malaysia/2506/2004-like viruses (Fig. 2A). This strain was the WHO-recommended influenza B virus vaccine component in the Northern and Southern Hemispheres from 2006 to 2007 (14). These viruses appear to have circulated in Malaysia continuously from 2004 to 2007, and the Malaysian strains fall in a position basal to all other B/Malaysia/2506/2004-like viruses (Fig. 2C). Mapping of amino acid substitutions at the node leading to the B/Malaysia/2506/2004-like viruses showed two HA mutations, K95R and K144N (Fig. 2C). Residue 144 is a known antigenic site (15), and the K144N mutation may have led to antigenic drift of these viruses. Interestingly, these basal B/Malaysia/2506/2004-like viruses were isolated from pediatric patients aged 2 to 10 months old (see Table S2 in the supplemental material), although this may be a sampling artifact due to the undersampling of adult and elderly patients in our study. Given that B/Malaysia/2506/2004-like viruses made up 23/25 (92%) influenza B viruses collected between 2004 and 2006 from our study (see Fig. S4 in the supplemental material) and over half of the 165 influenza B viruses collected in 2005 and 2006 from around Malaysia (6), we speculate that these B/Malaysia/2506/2004-like viruses may have caused an epidemic in Malaysia before spreading to other countries.

Cocirculation of the two lineages in humans may lead to reassortment between Yamagata and Victoria viruses (3, 16). While the NP and MP phylogenies were consistent with the HA tree, phylogenies of the other internal genes showed that 6 of the Malaysian strains were reassortants (see arrows in Fig. S1 to S8 in the supplemental material). Despite frequent interlineage reassortments, the distinction between the PB1–PB2–HA gene complexes of the Yamagata and Victoria lineages was maintained (16).

The World Health Organization recommends trivalent influenza virus vaccine composition on a semiannual basis. Influenza virus vaccination control in Malaysia follows the Southern Hemisphere recommendations (6), but we found mismatches in 3 of 8 years (37.5% mismatch rate) for which sequence data were available in Kuala Lumpur (Fig. 1B), compared with global vaccine mismatch rates of 38 to 54% for influenza B virus (17, 18). However, influenza virus vaccination rates are low in Southeast Asian countries, including Malaysia (19). If increased vaccine use were to be considered in Malaysia, influenza B vaccine mismatch rates could be improved either by considering rapid adoption of the Northern and Southern Hemisphere recommended formulations or by using the quadrivalent vaccine that includes influenza B virus strains from both lineages.

Malaysia's influenza surveillance system is based on two major components, disease-based and laboratory-based surveillance. Disease-based surveillance collects influenza-like illness data from sentinel sites, i.e., health clinics and general practices, but these data are not publicly available. Our data from Kuala Lumpur consist of laboratory-confirmed influenza, and we show that influenza B virus accounts for 26% of all influenza cases, suggesting a considerable national burden of disease, which is consistent with previously reported data indicating that influenza B virus accounted for 9 to 48% of confirmed influenza cases in Malaysia from 2006 to 2014 (4, 6, 20). Our study also showed an overwhelming burden of influenza B virus cases in young children, consistent with previous studies globally (3, 21–24).

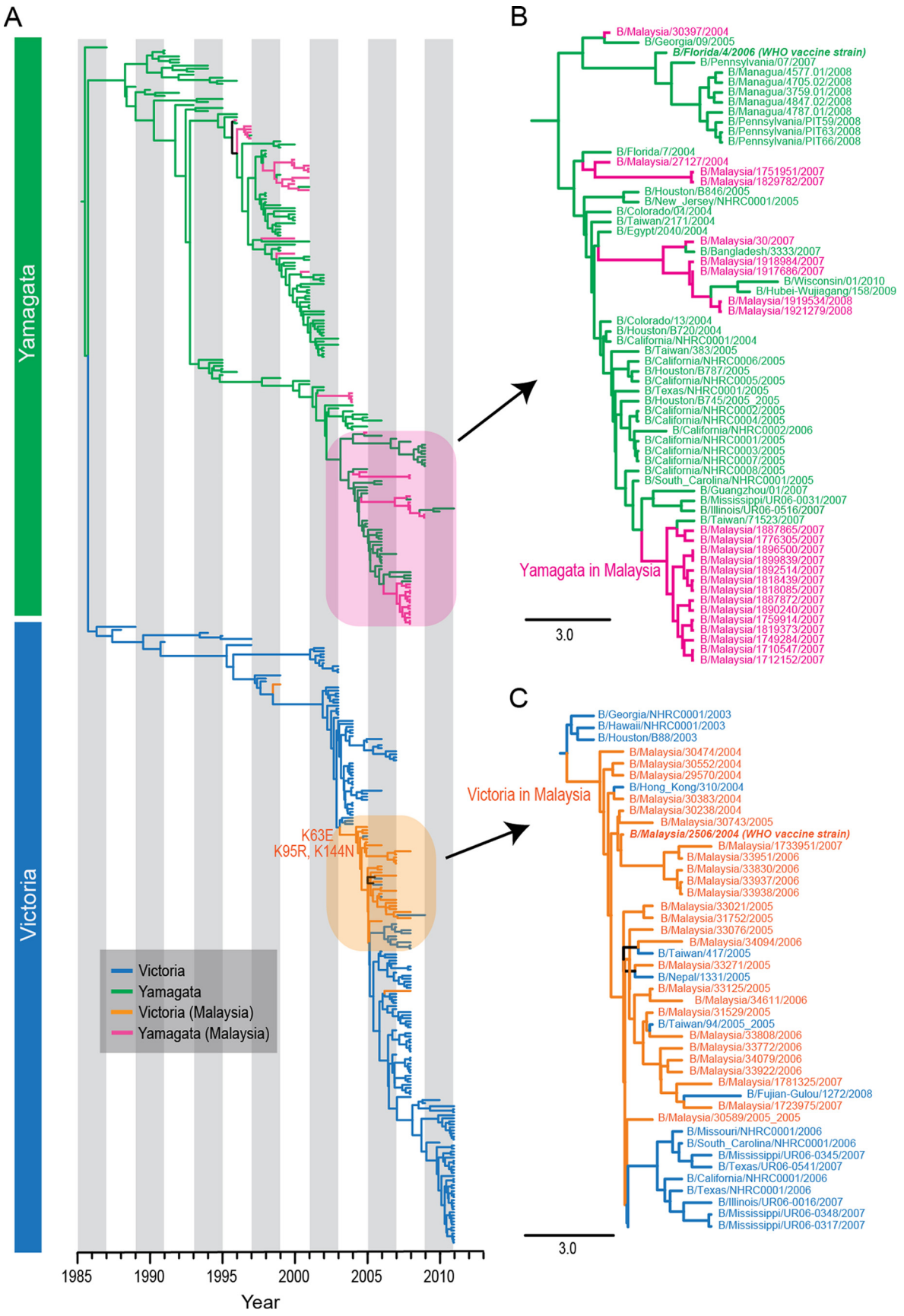

FIG 2 (A) Phylogeny of the HA gene of influenza B viruses, stratified by date. Victoria and Yamagata lineages are represented by blue and green branches, respectively, whereas Malaysian isolates of the Victoria and Yamagata lineages are represented by orange and pink branches, respectively. (B) Magnification of Yamagata lineages shown in the pink box in panel A. (C) Magnification of Victoria lineages shown in the orange box in panel A. The virus strain B/Malaysia/2506/2004 was previously selected as a WHO vaccine strain. The horizontal scale bar represents the number of substitutions per site.

Active coordination of surveillance efforts in sentinel clinics and hospitals, including the standardization of patient and epidemiological data collected, is needed to effectively monitor influenza disease dynamics in Malaysia. This study highlights the necessity of establishing systematic and ongoing disease surveillance programs in Malaysia in order to provide accurate information on circulating influenza virus strains that may be used to improve vaccination strategies in the country.

Nucleotide sequence accession numbers.

The sequences generated in this study were deposited in GenBank under accession numbers CY118283 to CY118417, CY119546 to CY120017, and CY120029 to CY120046.

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