Circulation and antigenic drift in human influenza B viruses in SE Asia and Oceania since 2000

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Abstract

During annual influenza epidemics, influenza B viruses frequently co-circulate with influenza A viruses and in some years, such as 2005, large outbreaks have occurred while in other years, the virus virtually disappears. Since 1987 there have been two lineages of influenza B viruses co-circulating in various countries and causing disease in humans. The proportions of these two lineages vary from year to year and country to country. For example, in 2005, the B/Victoria/2/87 lineage was predominant in New Zealand while in Australia the B/Yamagata/16/88 lineage was more common. Antigenic and genetic analysis has revealed gradual movement in the both lineages. Careful monitoring of the two virus lineages is important, as they are antigenically distinct. This is an important consideration for influenza vaccine formulation decisions, as only one influenza B component is traditionally included in the annual trivalent influenza vaccine. *Commun Dis Intell* 2006;30:350–357.

Keywords: Influenza B, serology, vaccines, vaccination, phylogenetic, evolution

Introduction

Influenza type B makes up an important component of the overall disease burden of influenza in humans. The proportion of influenza type A and influenza type B viruses circulating varies each year and in each country, as does the lineage of influenza B strains. In Australia in 2005, 26.6 per cent of the laboratory confirmed influenza cases that were typed were influenza type B,[1] while in New Zealand 87.2 per cent of influenza viruses typed were type B[2] and influenza B infection was associated with three deaths in adolescents.[3] During the early 1980s, a new lineage of B influenza emerged in humans that was antigenically and genetically distinct from the existing lineage of influenza B. Since then this lineage and the existing influenza B lineage have co-circulated and caused seasonal outbreaks in Australia and the Asia-Pacific region. The two lineages are represented by the reference strains B/Victoria/2/87 and B/Yamagata/16/88. These two lineages are antigenically quite distinct as antisera raised in ferrets to one lineage have no cross-reactive neutralising antibody against the second lineage.[4] They also form distinctly divergent genetic groups based on their haemagglutinin genes where there are some 27 amino acid differences.[5]

During the 1980s B/Victoria-like viruses were the predominant B lineage throughout the region, while from 1991 to 2000 the B/Yamagata lineage predominated in many countries with the B/Victoria lineage confined mainly to East Asia.[6] From 2000 onward, the B/Victoria lineage was again seen in increasing proportions outside Asia and was the predominant B lineage in the region in 2002 and in many countries in 2005. Each year the Australian influenza vaccine formulation is updated to incorporate new variants based on strains currently circulating or anticipated to circulate in the region. Currently only one type B strain, representing one of the two lineages, can be incorporated into the vaccine. As the two lineages have no cross reactivity, in years where both strains are circulating, the decision as to which lineage is selected can be difficult to determine. In this article we describe the distribution of the B/Victoria and B/Yamagata lineages in Australia and the Asia-Pacific region from 2000 to 2005 and compare the antigenic and genetic drift of these two lineages over this period.

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Methods

Viruses and antigenic analysis

Influenza B viruses were received from World Health Organization (WHO) national influenza centres; WHO influenza collaborating centres; Environmental Science and Research, Wellington, New Zealand; and other regional laboratories and hospitals from Australia, New Zealand, and the Asia-Pacific region. Viruses were received as isolates passaged in cell culture or as original clinical samples in which influenza B antigen had been detected by immunofluorescence or were positive for influenza B by (RT-PCR). Once received at the centre, the isolates were cultured in MDCK cells and monitored for growth by cytopathic effects and the presence of haemagglutination activity using turkey red blood cells (RBCs) as previously described.[7] Positive samples were typed using the haemagglutination inhibition assay (HAI) against a panel of known standard reference viruses and their homologous ferret antiserum.[7] Ferret antisera were pre-treated with Receptor destroying enzyme (RDE) (Denka Seikan, Japan), to remove non-specific inhibitors prior to use.

Human serology

CSL Limited provided sera from vaccinated adults (aged 18–60 years) and the elderly (61–85 years) undergoing vaccination field trials in 2004 and 2005. The vaccine used in these trials (Fluvax™ ,CSLLimited, Australia) contained influenza strains representing the currently circulating strain as recommended by the Australian Influenza Vaccine Committee (AIVC) and the Therapeutic Goods Administration (TGA). Influenza A(H3N2), A(H1N1) and type B, at a concentration of 15 ug/ml haemagglutinin were included in the vaccine. The vaccines given in 2004 and 2005 differed in their type B component, with the 2004 vaccine containing B/Brisbane/32/2002 (B/Victoria lineage), and the 2005 vaccine containing B/Jiangsu/10/2003 (B/Yamagata lineage). Blood samples were taken prior to vaccination and four weeks later. Pre- and post-vaccination sera were RDE treated and antibody levels tested by haemagglutination inhibition assay using turkey RBCs as the indicator cells against the vaccine strains and selected strains from the current 2004 and 2005 influenza seasons. For the 2004 samples, sera were assayed against egg grown B/Brisbane/32/2002 while for the 2005 samples, sera were assayed against egg grown B/Jiangsu/10/2003, the strains contained in the respective vaccines. The panels of sera were pre-selected from the subjects who showed a significant rise in post-vaccination titre compared to the pre-vaccination titre. Geometric titres and the number of subjects with HAI titres ≥ 40 were determined for each group. Prior to use in HAIs, B viruses were 'split' using an ether treatment method as previously described.[8] Briefly, viruses were mixed with an equal volume of Diethyl Ether (Merck) and vigorously stirred without frothing for four hours by magnetic stirrer. After mixing, the two layers were allowed to separate and the lower layer containing the split virus was removed. Residual ether was removed from the virus layer by slowly bubbling through gaseous nitrogen.

Sequencing RNA extraction, RT-PCR and sequencing were performed as previously published.[9]

Sequences were assembled using the Lasergene Seqman package IV (DNAStar V5.3) and phylogenetic relationships determined with PHYLIP V 3.5.7,[10] using the neighbour-joining method on Australian National Genomic Information Service and dendrograms were drawn using Treeview.[11]

Results

Haemagglutination inhibition assays

Table 1 shows the HAI assay of B viruses from the region representative of B/Victoria and B/Yamagata lineages from 2004 to 2005. Ferret sera were raised against reference strains representing the B/Victoria lineage (B/Brisbane/32/2002) and B/Yamagata (B/Shanghai/361/2002) and tested by HAI against isolates received at the WHO Collaborating Centre. The B/Victoria and B/Yamagata lineages were serologically distinct, for example the ferret sera raised to B/Brisbane/32/2002, a B/Victoria lineage virus, gave good HAI titres to B/Victoria-like strains but none against viruses from the B/Yamagata lineage. The converse was also true for ferret sera raised to B/Shanghai/361/2002, a B/Yamagata lineage virus, which reacted with B/Yamagata-like viruses but showed no cross reactivity to strains of the B/Victoria lineage. Two viruses associated with the deaths in children/adolescents in New Zealand in 2005, B/Wellington/21/2005 and B/Waikato/28/2003 are also shown in Table 1 and both were of the B/Victoria lineage and reacted similarly to other B/Victoria-like viruses tested.

Circulation of influenza type B lineages in the Asia-Pacific region

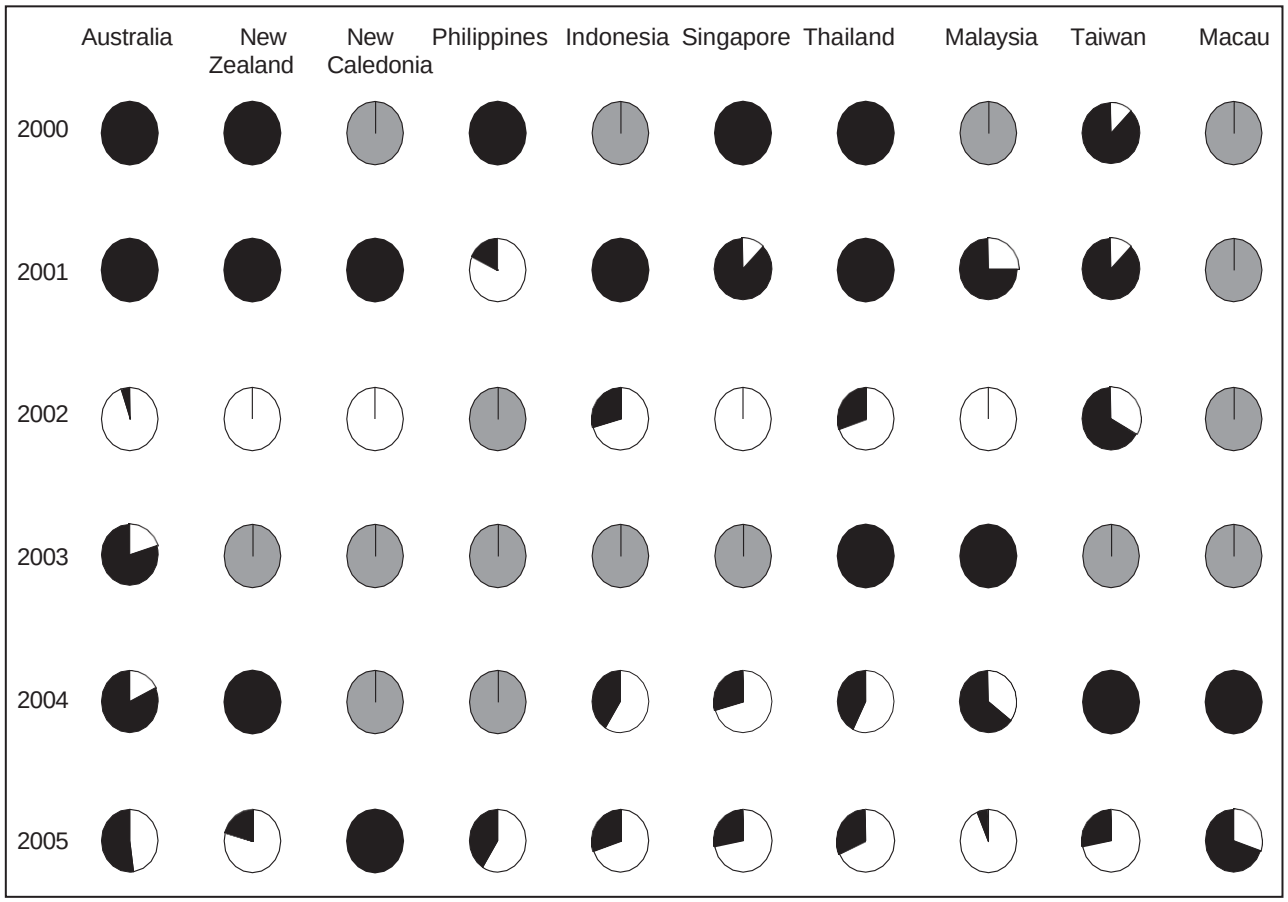
The distribution of the two type B lineages in the Asia-Pacific region were based on samples received at the centre from 2000 to 2005 and typed by HAI analysis as shown in Figure 1. Countries where less than five B viruses were detected in that year were not included.

In 2000, the B/Yamagata lineage viruses predominated throughout the Asia-Pacific region with five of the six countries studied having B/Yamagata-like viruses exclusively. In 2001 although the B/Yamagata lineage once again predominated, the prevalence

Table 1. Haemagglutination inhibition assay of B/Victoria and B/Yamagata-like viruses

	Ferret antiserum to		Lineage
	B/Brisbane/32 (B/Victoria lineage)	B/Shanghai/361 (B/Yamagata lineage)	
Reference antigens			
B/Brisbane/32/2002	320	<20	B/Victoria
B/Malaysia/2506/2004	320	<20	B/Victoria
B/Shanghai/361/2002	<20	640	B/Yamagata
B/Jiangsu/10/2003	<20	1,280	B/Yamagata
Test antigens			
B/Singapore/18/2004	160	<20	B/Victoria
B/Waikato/222/2005	80	<20	B/Victoria
B/Perth/112/2005	160	<20	B/Victoria
B/Malaysia/737/2005	160	<20	B/Victoria
B/Wellington/21/2005	160	<20	B/Victoria
B/Waikato/28/2005	160	<20	B/Victoria
B/Macau/131/2004	<20	640	B/Yamagata
B/Taiwan/142/2005	<20	640	B/Yamagata
B/Christchurch/103/2005	<20	640	B/Yamagata
B/Thailand/299/2005	<20	320	B/Yamagata
B/Victoria/501/2005	<20	640	B/Yamagata
B/Philippines/561/2005	<20	320	B/Yamagata

Figure 1. Circulation of influenza type B viruses in the Asia-Pacific region, 2000 to 2005



Black = the proportion of viruses typed as B/Yamagata-lineage.

White = the proportion of viruses typed as B/Victoria-lineage.

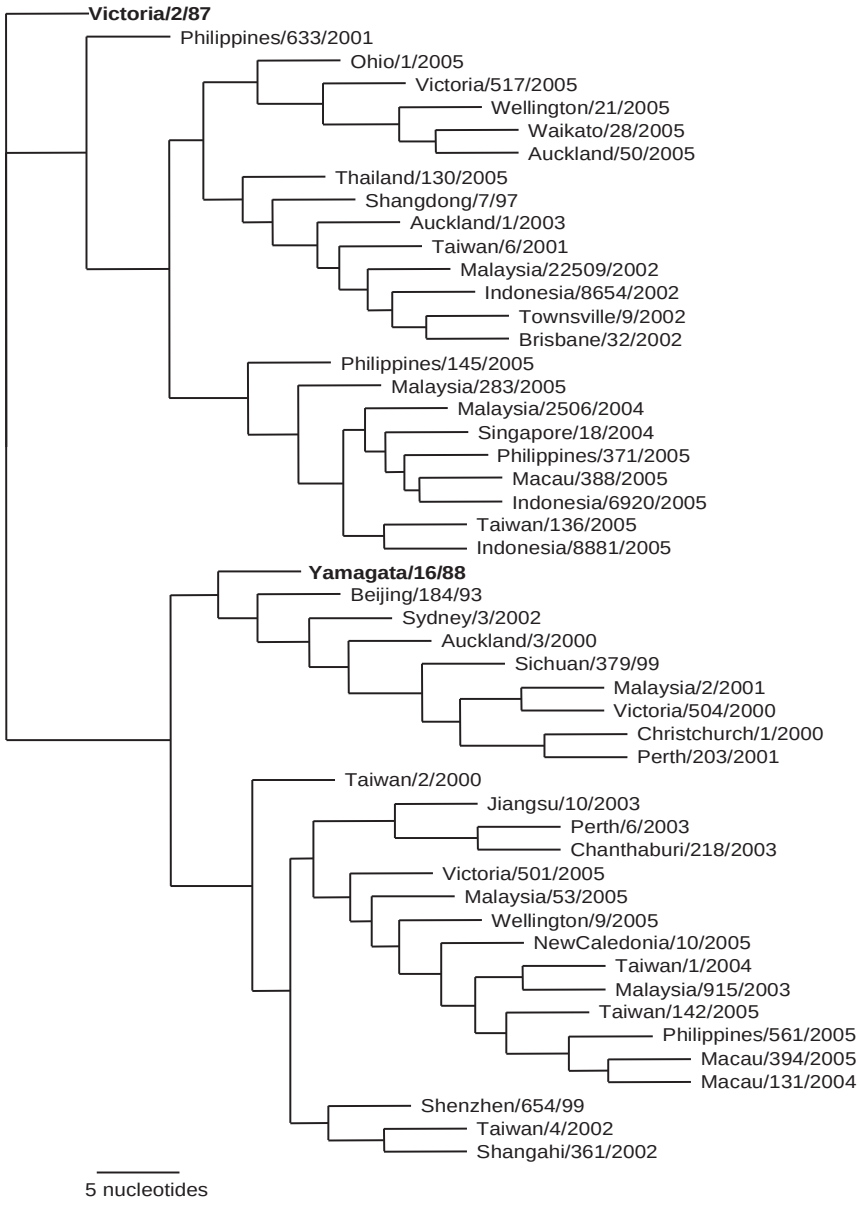
Grey circles indicate insufficient samples (<5) to determine proportions.

of the B/Victoria lineage in the region was beginning to increase. Four of nine countries had some B/Victoria- like virus activity and in the Philippines 82 per cent of isolates tested were from this lineage. In 2002 the B/Victoria lineage predominated almost exclusively in the region, yet Taiwan still had a greater proportion of B/Yamagata-like isolates (67%). The year 2003 was predominantly an A(H3) year and influenza B was virtually absent from the region with the exception of low levels of activity in Australia, Taiwan and Malaysia which mainly were of B/Yamagata lineage. In 2004 country to country variation was at its greatest with New Zealand, Taiwan and Macau almost exclusively B/Yamagata, while Indonesia, Singapore and Thailand had predominantly B/Victoria-like viruses, with mixed lineages in Australia and Malaysia. In 2005, type B activity was widespread throughout the region. Influenza type B activity in New Zealand was at epidemic levels and was dominated by the B/Victoria lineage (79%), while Australia had a predominantly A(H3) season,[1,2] and the B viruses were almost evenly divided between the two lineages. The B/Victoria lineages predominated in the Asia-Pacific region in 2005, with the exception of New Caledonia, which had viruses exclusively from the B/Yamagata lineage.

Phylogenetic analysis

The phylogenetic tree (Figure 2) shows the two divergent Influenza type B lineages based on nucleotide differences in the HA1 region of the haemagglutinin gene. The recent vaccine strains for the two lineages were B/Brisbane/32/2002 (B/Victoria) in 2003 and

Figure 2. Phylogenetic tree showing the two distinct lineages of B influenza viruses and representative isolates from the region during 2000 to 2005



Reference viruses shown in bold

2004 and B/Jiangsu/10/2003 (B/Yamagata) in 2005. Both lineages showed continued evolution with time, however the B/Victoria lineage showed little drift in the early years (2000–03) with viruses appearing similar to B/Shangdong/7/97 but more extensive drift has been seen recently (2004–05). Australian B/Victoria-like viruses isolated in 2005 phylogenetically grouped mainly around the B/Malaysia/2506/2004 clade (data not shown) or the B/Ohio/1/2005 clade (Figure 2). The B/Yamagata viruses in contrast, have shown slow but continued drift over the same period.

Cross protective efficacy in vaccinated subjects to the B/Victoria and B/Yamagata lineages

Tables 2a and 2b show the antibody response in vaccinated adult (18–64 years) and elderly (65–80 years) subjects against the vaccine strain received and representative viruses from strains representing the B/Victoria and B/Yamagata lineages. The 2004 vaccine contained a B/Victoria lineage virus (B/Brisbane/32/2002, Table 2a), while the 2005 vaccine contained a B/Yamagata lineage virus (B/Jiangsu/10/2003, Table 2b). Adults vaccinated against one B lineage type had reduced post-vaccination geometric mean titres and had a lower percentage of titres, ≥40 to viruses from the alternative lineage. This indicated that adults or the elderly vaccinated with influenza vaccine against one type B lineage would have reduced protection against infection with the alternative lineage if it were circulating. However there was a moderate rise in antibody geometric mean titre (GMT) levels against viruses representing the alternative lineage with both the 2004 and 2005 vaccines in both adults and the elderly sera examined, albeit some fivefold lower GMTs than viruses from the matched lineage.

Table 2a. Antibody response from vaccinees with the 2004 Australian influenza vaccine containing B/Brisbane/32/2002 (B/Victoria lineage)

Population	n	Antigen	GMT		% HAI titre ≥40	
			Pre	Post	Pre	Post
Adults	24	B/Brisbane/32/2002*	18.9	174.4	25	96
		B/Wulumuqi/26/04*	19.4	190.2	25	96
		B/Victoria/501/2005†	23.1	106.8	46	96
		B/Jiangsu/10/2003†	14.1	50.4	46	79
Elderly	24	B/Brisbane/32/2002*	14.6	195.8	17	96
		B/Wulumuqi/26/04*	14.1	179.5	8	96
		B/Victoria/501/2005†	15.4	51.9	21	67
		B/Jiangsu/10/2003†	10.0	41.2	54	88

* B/Victoria/2/87 lineage.

† B/Yamagata/16/88 lineage.

Table 2b. Antibody response from vaccinees with the 2005 Australian influenza vaccine containing B/Jiangsu/10/2003 (B/Yamagata lineage)

Population	n	Antigen	GMT		% HAI titre ≥40	
			Pre	Post	Pre	Post
Adults	24	B/Jiangsu/10/2003†	18.3	109.9	33	88
		B/Florida/7/2004†	35.6	155.4	50	96
		B/Malaysia/2506/2004*	23.1	36.7	42	58
		B/Brisbane/32/2002*	26.7	54.9	54	79
Elderly	24	B/Jiangsu/10/2003†	15.4	123.3	21	92
		B/Florida/7/2004†	20.0	119.8	33	83
		B/Brisbane/3/2005†	13.3	65.3	29	79
		B/Malaysia/2506/2004*	15.0	28.3	21	50
		B/Brisbane/32/2002*	18.3	37.7	29	54

* B/Victoria/2/87 lineage.

† B/Yamagata/16/88 lineage.

Australian influenza vaccine composition and circulating B viruses

Table 3 shows the comparison of the vaccine strains recommended by the Australian Influenza Vaccine Committee (AIVC) with the predominant circulating B-lineage. The AIVC is the committee in Australia, which advises the TGA on the appropriate strains which should be included into the Australian influenza vaccine each year. This decision is made in October each year some 9–10 months prior to the next influenza season. The vaccine component was well matched with the circulating strain in two of the three years in which there was clearly a predominant lineage (2000–2002). In the following three years mixed lineages were seen in Australia, and while the B/Yamagata lineage viruses were in the majority in these years the vaccine contained a B/Yamagata lineage virus in only one of these years (2005). The decision to include a B/Victoria-lineage virus in the 2003 vaccine was due to the predominance of B/Victoria-like viruses in Australia and elsewhere in 2002. The same lineage was selected for the 2004 vaccine as the B/Victoria-like viruses still predominated worldwide in 2002–03 and Australia only had a handful of B viruses in 2003 that were from both lineages.

Discussion

A significant amount of the impact of influenza is due to the influenza B viruses.[12] While influenza B infections are usually associated with a lower mortality than influenza A infections, occasional deaths can occur. Influenza B infections are often in children who are generally unvaccinated, as was the case in New Zealand in 2005 where two children and one adolescent died following influenza B infection.[3] Two of these cases developed *Staphylococcus aureus* pneumonia and septicemia and in the other case the subject was on aspirin for another condition and developed Reye's Syndrome.[3] Childhood deaths from influenza B infections are rare but do occur,[13] however, they are far more common following influenza A outbreaks as was evidenced in the 2003–04 influenza season in the United States of America where 153 deaths were reported in children under 18 years of age.[14] Influenza B outbreaks can also occur in schools,[15,16] on cruise ships[17] and in nursing homes,[18,19] causing significant morbidity. This makes the matching of the B vaccine strain to the circulating strain an important part of minimising the effects of the virus.

Influenza B viruses, unlike influenza A viruses, have multiple evolutionary lineages which can co-exist for considerable periods of time.[20] This has occurred since the early 1980s when a new lineage (B/Yamanashi/16/88-like) appeared to evolve from B/USSR/100/83-like viruses[4] and from then on has co-circulated with the existing virus lineage (B/Victoria/2/87-like).[4,5] During this time, the patterns of circulation have changed periodically and over the last six years both lineages have predominated in particular countries in particular years, until recently when both lineages have co-circulated in the same countries at the same time. Interestingly, sera from naive ferrets that are generated by infections with a single virus (and have not been exposed to other human influenza viruses), show little or no cross-reactivity between the two B lineages. In contrast, sera from vaccinated humans (adults and elderly) do show some cross-boosting when vaccinated with virus from one lineage against the other lineage *in vitro*, although this cross-boosting is at a much lower level than the boosting obtained with viruses from the same lineage. Presumably this is due to a combination of prior exposure or vaccine priming but may also be in part due to differences in the type of immune responses generated with the killed viral vaccines used in humans as opposed to the live virus given to ferrets.

Phylogenetically both lineages have shown modest antigenic drift over the last six years. In the last 2–3 years the B/Victoria lineage viruses have shown more drift than seen previously, resulting in a change of vaccine recommendation for 2006 to

Table 3. The annual vaccine recommendations by the Australian Influenza Vaccine Committee (AIVC) and the predominant B virus lineage that circulated in Australia during that year

Year	AIVC recommended B strain	B vaccine lineage	Circulating B lineage (Australia)
2000	B/Yamanashi/166/98	Yamagata†	Yamagata
2001	B/Sichuan/379/99	Yamagata	Yamagata
2002	B/Sichuan/379/99	Yamagata	Victoria
2003	B/Shangdong/7/97 or B/Brisbane/32/2002	Victoria*	Mixed
2004	B/Shangdong/7/97 or B/Brisbane/32/2002	Victoria	Mixed
2005	B/Jiangsu/10/2003	Yamagata	Mixed

* B/Victoria/2/87 lineage.

† B/Yamagata/16/88 lineage.

B/Malaysia/2506/2004 from B/Brisbane/32/2002 (or B/Shangdong/7/97), as recommended for the 2004 Australian influenza B vaccine component. In the last six years there have been three changes in the Yamagata lineage derived vaccines with the most recent change being made in the 2005 Australian vaccine where B/Jiangsu/10/2003 was used.

It is unknown why B/Victoria lineage viruses that were limited to East Asia in 2000 and for most of the previous decade, have re-emerged but a similar phenomenon was seen with the A(H1N1) strain A/Bayern/262/95. These strains circulated worldwide in 1995–1998, while the A/New Caledonia/20/99-like strains were limited to Asia during this period. Subsequently the A/Bayern-like viruses were completely replaced by the A/New Caledonia-like viruses, which are still circulating.[6] Interestingly, since 2001 the B/Victoria viruses have also undergone reassortment with B/Yamagata viruses and now practically all B viruses contain a B/Victoria-lineage haemagglutinin and a B/Yamagata-lineage neuraminidase.[6,9] This reassortment has occurred previously with B viruses[21] and may represent a further evolutionary strategy that influenza B viruses[22] have to evade the immune system and prolong co-circulation of dual lineages.

The continued co-circulation of two influenza B lineages makes selection of the best matched influenza B virus for the annual influenza vaccine difficult, especially as this decision has to be made some 9–10 months before the peak of the upcoming influenza season. This lag is required to allow manufacturers to produce sufficient vaccine and for regulators to produce reference reagents and to licence the vaccines. In recent years only the Japanese manufacturers have included two B viruses in their influenza vaccine making it a quadrivalent vaccine (with an A(H1N1) and an A(H3N2) virus). WHO, the manufacturer and regulators in other countries have not embraced this approach due to its impact on production capacity, cost, and the lack of time to produce reagents. Indeed, the Japanese manufacturers now also only produce a trivalent influenza vaccine with a single B component. It is worth noting that in many sera from post-vaccinated adults and the elderly, modest but useful levels of antibody were produced against viruses from the alternative B lineage not present in the vaccine. This partial cross-reactivity reduces the need for an additional B virus lineage to be added to the vaccine currently. However, if the two lineages continue to drift apart (and co-circulate), ultimately the only way of ensuring optimal vaccine coverage against viruses of both lineages may be to include both lineages in the influenza vaccine. Alternatively, other types of vaccines such as the live attenuated influenza vaccine (Flumist®, MedImmune Vaccines Inc., USA) may offer some advantage in terms of breadth of protection against co-circulating lineages over the conventional killed influenza vaccines.[23]

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