Virology **303,** 1–8 (2002)
doi:10.1006/viro.2002.1719

RAPID COMMUNICATION

Reappearance and Global Spread of Variants of Influenza B/Victoria/2/87 Lineage Viruses in the 2000–2001 and 2001–2002 Seasons

Michael W. Shaw,*[1] Xiyan Xu,* Yan Li,† Susan Normand,† Robert T. Ueki,‡ Gail Y. Kunimoto,‡ Henrietta Hall,* Alexander Klimov,* Nancy J. Cox,* and Kanta Subbarao*

*Influenza Branch, Centers for Disease Control and Prevention, Atlanta, Georgia 30333; †National Microbiology Laboratory, Canadian Science Center for Human and Animal Health, Winnipeg, Manitoba, Canada R3E 3R2; and ‡State of Hawaii Department of Health, State Laboratories Division, 2725 Waimano Home Road, Pearl City, Hawaii 96782

Received April 22, 2002; returned to author for revision May 9, 2002; accepted July 18, 2002

Two antigenically and genetically distinct lineages of influenza B viruses, represented by the reference strains B/Victoria/2/1987 and B/Yamagata/16/1988, have cocirculated in humans since at least 1983. Between 1992 and 2000, Victoria lineage viruses were detected only in eastern Asia. From March to September of 2001 and during the 2001–2002 influenza season, Victoria lineage viruses were detected for the first time in a decade in several countries including Canada, USA, Italy, Netherlands, Norway, Philippines, India, and Oman. Phylogenetic analysis of the hemagglutinin (HA) gene of these viruses revealed that the viruses fell into two distinct clades: one group, represented by the reference strain B/Hong Kong/330/2001, contained viruses sharing three signature amino acids, Arg116, Asn121, and Glu164, while the other group of viruses, represented by B/Oman/16296/2001, shared Thr121 compared to the previous reference strain, B/Shandong/7/97. A number of the viruses in the latter group have been found to be reassortants having a Victoria lineage HA and a Yamagata lineage NA. In the current 2001–2002 season, Victoria-like viruses have now been associated with outbreaks in Asia, Europe, and North America. The reemergence of these Victoria lineage viruses worldwide, the fact that the majority of the B/Victoria-like isolates have poor cross-reactivity to B/Sichuan/379/99-like viruses in current vaccines, and the lack of exposure of young children in many areas of the world to these viruses has resulted in a World Health Organization Northern Hemisphere recommendation for the inclusion of a B/Victoria-like strain in vaccines for the 2002–2003 influenza season.

Key Words: influenza B virus; hemagglutinin; neuraminidase; evolution; reassortment; reemerging diseases.

Introduction. Two antigenically and genetically distinct lineages of influenza B viruses have cocirculated and caused disease in humans since at least 1988 (*1, 2*). These two phylogenetic groups, represented by the reference strains B/Victoria/2/1987 (Victoria) and B/Yamagata/16/1988 (Yamagata), are so different antigenically as to offer little or no postinfection cross-neutralizing antibody response in ferrets (*3*). Furthermore, in immunologically unprimed children, vaccination with a Yamagata-like vaccine strain did not induce detectable hemagglutination-inhibiting (HI) or neutralizing antibody to Victoria-like viruses (*4*). This lack of antigenic cross-reactivity has made the designation of a type B vaccine strain problematic in seasons when viruses of both lineages circulate since influenza virus vaccines are currently formulated to include only a single strain of influenza B virus; the WHO influenza vaccine recommendations for the Northern Hemisphere 1999–2000 season and the Southern Hemisphere 2000 season proposed either a Yamagata-like or a Victoria-like type B component, depending on local conditions (*5, 6*).

Influenza viruses of the Victoria lineage were the predominant type B strains circulating worldwide in the 1980s with the Yamagata lineage becoming the dominant type B virus in the early 1990s (*3, 7*). Since 1991, Victoria lineage viruses have been isolated infrequently and been limited almost entirely to eastern Asia (*5, 8–11*). In this article we describe the reappearance outside of East Asia of Victoria lineage viruses in the spring and summer of 2001 and the 2001–2002 season as two distinct phylogenetic groups based on the nucleotide sequence of the HA1 portion of the genes encoding the hemagglutinin (HA) surface antigens. Both genetic groups, similar to their predecessors, lack antigenic cross-reactivity with cocirculating Yamagata lineage viruses as represented by the current B/Sichuan/379/99-like vaccine strains.

Results and Discussion. The first Victoria-like isolates associated with the recent increase in activity were reported from Hong Kong SAR, China in January and Feb-

[1] To whom correspondence and reprint requests should be addressed. Fax: (404) 639-2334. E-mail: mws2@cdc.gov.

0042-6822/02 $35.00

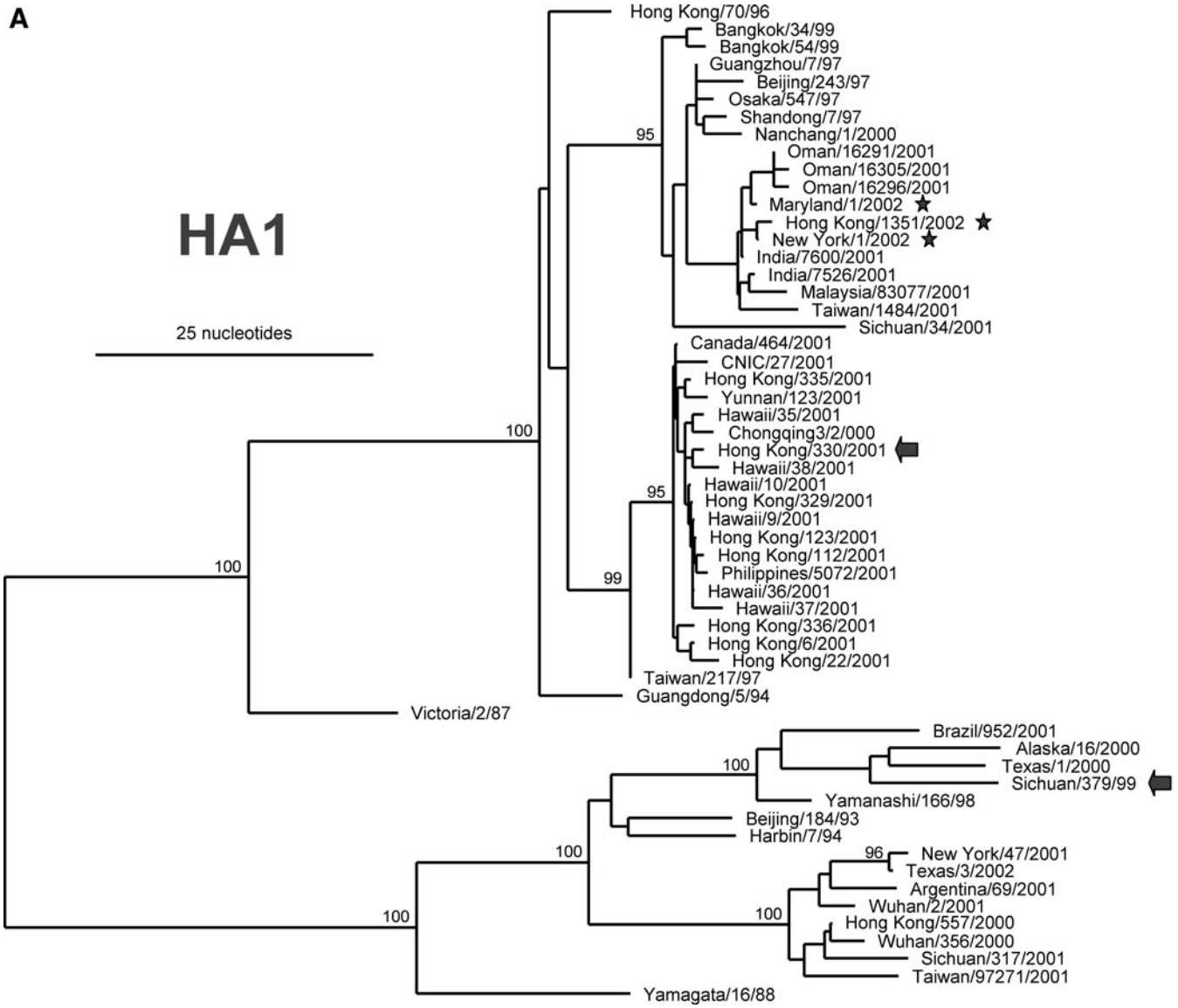

FIG. 1. Dendrograms showing the phylogenetic relationships of the hemagglutinin (HA1) and neuraminidase (NA) genes from recent human type B influenza isolates. The nucleotide sequences were compared to other type B influenza sequences available from GenBank using the sequence analysis software of the University of Wisconsin Genetic Computer Group (*19*) and mapped into a phylogenetic tree using the Phylogeny Inference Package, version 3.5 (*20*). Bootstrap values for nodes occurring in 95% or more of multiple bootstrap replicates are given immediately to the left of the node. (A) Dendrogram for the HA1 fragment of the HA gene. (B) Dendrogram for the NA gene. Arrows denote the Northern Hemisphere vaccine strains recommended by WHO for the 2001–2002 and 2002–2003 influenza seasons (B/Sichuan/379/1999 and B/Hong Kong/330/2001, respectively). The stars mark the isolates having a Victoria-lineage HA and a Yamagata lineage NA. GenBank Accession Nos. for the sequences used are AF532525–AF523566 and AY139033–AY139081.

ruary of 2001, as represented by the B/Hong Kong/330/2001 (HK330) prototype strain. An isolate previously obtained from Chongqing on the Chinese mainland in December of 2000 was retrospectively determined to also be HK330-like. In March of 2001, a single isolate obtained in Canada had the same antigenic characteristics as the Asian Victoria lineage isolates. Upon investigation, this virus, designated B/Canada/464/2001, was deemed to be travel-related with infection originally contracted in China. In the absence of other cases in North America, it was considered an isolated case. However, in April and May 2001, an outbreak of respiratory illness caused by this new variant was identified in Hawaii, marking the first documented sustained circulation of the Victoria lineage outside of east Asia since the early 1990s. Similar viruses continued to be isolated in Hawaii through June 2001, but were not detected elsewhere in the U.S. or Canada during this time. Of a total of 149 influenza B virus isolates characterized antigenically at CDC from April to September of 2001, 27 (18%) were HK330-like. Table 1 shows the results of HI testing of representative Victoria-like and Yamagata-like isolates

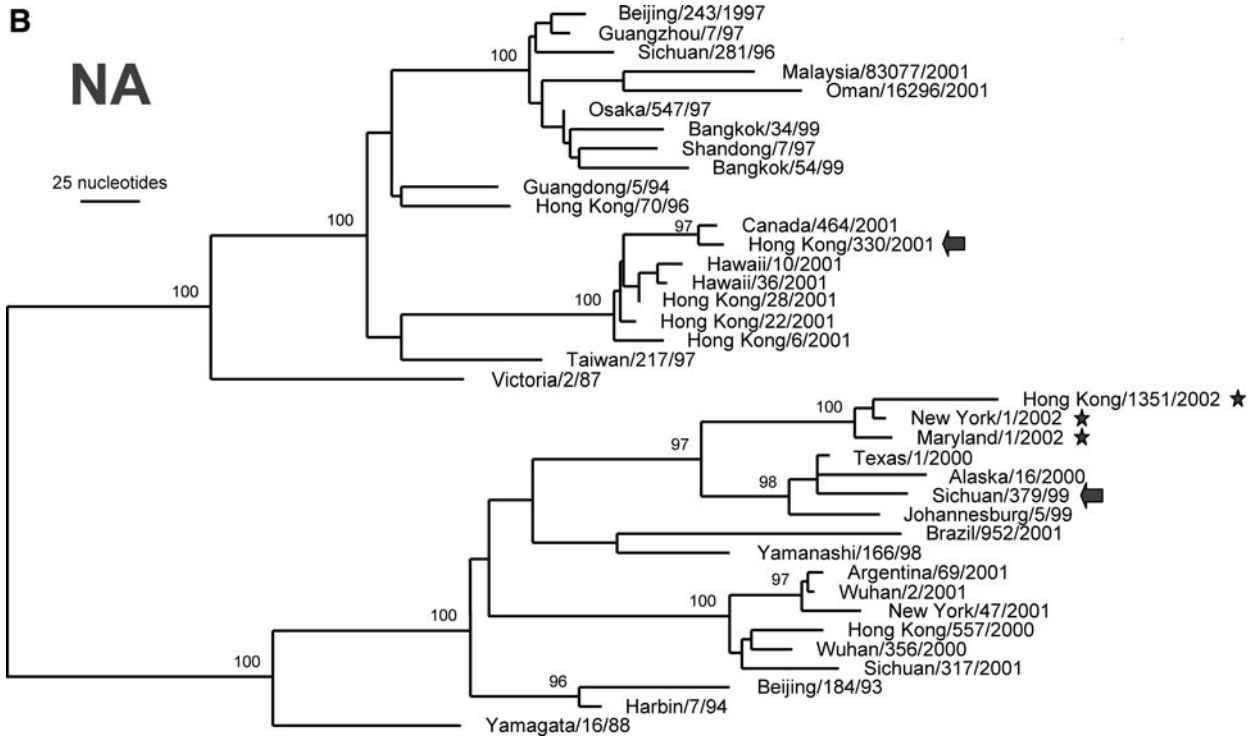

FIG. 1—*Continued*

TABLE 1

Hemagglutination Inhibition Reactions of Influenza Type B Viruses of the Yamagata and Victoria Lineages[a]

Strain designation			Reference antisera							
Antigens	Date collected[b]	Lineage[c]	B/Sichuan/379/1999[d]	B/Shizuoka/15/2001[d]	B/Johannesburg/69/2001[d]	B/Beijing/243/1997[d,e]	B/Hong Kong/22/2001[d]	B/Hong Kong/330/2001[d]	B/Sichuan/379/1999[f]	B/Hong Kong/22/2001[f]
B/Sichuan/379/99	12/02/99	Y	640	1280	320	20	5	20	320	10
B/Shizuoka/15/2001	01/17/01	Y	320	1280	640	10	5	10	320	10
B/Johannesburg/69/2001	06/12/01	Y	320	1280	320	10	5	10	160	10
B/Beijing/243/97	01/09/97	V	20	10	5	320	160	1280	10	80
B/Hong Kong/22/2001	01/18/01	V	10	10	5	160	640	640	5	640
B/Hong Kong/330/2001	04/09/01	V	10	5	5	160	160	640	5	160
B/Taiwan/97271/2001	12/10/01	Y	160	640	320	5	5	5	320	5
B/New York/47/2001	12/31/01	Y	320	640	320	10	5	5	320	5
B/Texas/3/2002	01/29/02	Y	640	1280	320	10	20	10	640	5
B/Oman/16296/2001	10/03/01	V	10	20	10	160	160	320	5	160
B/Philippines/5072/2001	10/26/01	V	5	5	5	40	80	160	5	40
B/Malaysia/83077/2001	10/30/01	V	5	5	5	80	80	160	5	160
B/India/7600/2001	12/05/01	V	20	10	10	80	80	160	5	160
B/Hong Kong/1351/2002	01/17/02	V	5	5	5	160	160	320	5	80
B/New York/1/2002	01/28/02	V	10	10	10	80	160	160	5	160

[a] HI tests conducted as described in Kendal *et al.* (18). Titers are presented as reciprocals of the highest dilution showing complete hemagglutination inhibition. Homologous titers (reference antiserum vs. the homologous antigen) are underlined.

[b] Month/Day/Year.

[c] Y = Yamagata/16/88-like; V = Victoria/2/87-like.

[d] Ferret postinfection antisera.

[e] Antigenically equivalent to B/Shandong/7/1997.

[f] Sheep antisera.

TABLE 2

Amino Acid Differences in Influenza B HA1 Molecules Compared to B/Shandong/7/97 Virus

AA number	Shandong/7/97	Victoria/2/87	India/7569/2001	India/7605/2001	Taiwan/1484/2001	India/7526/2001	India/7600/2001	Hong Kong/1351/2002	New York/1/2002	India/77276/2001	Maryland/1/2002	Oman/16296/2001	Oman/16305/2001	Oman/16291/2001	Oman/16299/2001	Malaysia/83077/2001	Taiwan/217/97	Hong Kong/335/2001	Yunnan/123/2001	Canada/464/2001	Hong Kong/336/2001	Hong Kong/6/2001	Hong Kong/22/2001	CNIC/27/2001	Hong Kong/330/2001	Hawaii/38/2001	Hawaii/35/2001	Hawaii/10/2000-egg	Chongqing/3/2000	Philippines/93079/2001	Philippines/5072/2001	Hawaii/10/2001	Hawaii/26/2001	Hawaii/9/2001	Hong Kong/123/2001	Hong Kong/112/2001	Hawaii/37/2001	Hawaii/36/2001	Hong Kong/329/2001	Hong Kong/497/2001	Hong Kong/1434/2002	Hong Kong/666/2001	Hong Kong/1115/2002	AA number
2	R									G																																	2
36	P																						L																				36
68	G	A																																									68
73	T	M																																									73
75	N	T																																									75
88	R	K																																									88
116	H																R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	116
121	I	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	121
129	K	T																																						E	E	E	129
137	I	V														V																												137
150	G																																					K					150
163	N												S																		D												163
164	D																E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	164
172	S	P																																									172
175	I	V																																									175
197	N	S			K			K																	S	S														S		197
199	N	T	T	T	T	T	T	T	T		T	T				T	T	A	P	T	T	T/N	T	T	T	T	I	I	I		T	T	T	T	T	T	T	T	T	T	I	199
202	A	V																					V																				202
230	G																				D																						230
235	T	A																																									235
252	V				M																																						252

illustrating the poor antigenic cross-reactivity between the two lineages.

In September, October, and November of 2001, B/Victoria lineage isolates obtained from the Philippines, Oman, and India were characterized at CDC. Those from the Philippines (represented by B/Philippines/5072/2001) were HK330-like, while those from Oman and India (represented by B/Oman/16296/2001 and B/India/7600/2001) more closely resembled the antigenically equivalent reference strains B/Beijing/243/97 and B/Shandong/7/97 (Table 1). Since the beginning of 2002, Victoria-like viruses have been reported from North America and Europe (*9*), clearly demonstrating that the Victoria lineage has begun to circulate worldwide.

To further characterize the newly reemergent Victoria-like influenza B isolates at the molecular level, representative viruses of the Victoria lineage isolated in 2000–2002, including seven from the Spring 2001 Hawaii outbreak, viruses from Canada and the United States mainland, and viruses from South and East Asia, were chosen for nucleotide sequence analysis. For comparison, representative contemporary viruses of the Yamagata lineage were also included. Figure 1 shows the phylogenetic relationships of the hemagglutinin and neuraminidase genes from these viruses. For both the HA1 and NA genes, the isolates associated with the first reported outbreaks in 2001, as represented by B/Hong Kong/330/2001, fell into a distinct clade most closely related to the 1997 isolate, B/Taiwan/217/97. This clade was distinct from the clade containing the majority of previously isolated Victoria-like viruses, including the reference strains B/Beijing/243/97 and B/Shandong/7/97. However, it should be noted that a second group of recent Victoria lineage isolates characterized for the present study, B/Sichuan/34/2001, B/Taiwan/1484/2001, and viruses from Oman, India, Malaysia, and the United States, fell within the previously dominant clade defined by the majority of isolates from 1997 to 2000, demonstrating that these viruses have also begun to circulate outside of their previous East Asia confines. Viruses from these two clades were phylogenetically distinct (Fig. 1) but were antigenically similar (Table 1). Victoria lineage type B viruses of both clades continue to be isolated with neither appearing at present to become the dominant group.

Table 2 shows the differences in HA1 amino acids among the type B viruses compared to the sequence of the B/Shandong/7/97 reference strain. The phylogenetic clade containing the Hong Kong/330/2001-like (HK-like) viruses is distinguished by three signature amino acids, Arg116, Asn121, and Glu164, when compared to previ-

TABLE 3

Amino Acid Differences in Victoria Lineage Influenza B NA Molecules Compared to B/Shandong/7/97 Virus

Amino acid	Shandong/7/97	Beijing/243/97	Sichuan/52/96	Sichuan/281/96	Guangzhou/7/97	Osaka/547/97	Bangkok/34/99	Bangkok/54/99	Sichuan/40/99	Hong Kong/70/96	Malysia/83077/2001	Oman/16296/2001	Taiwan/217/97	Hong Kong/6/2001	Hong Kong/22/2001	Hong Kong/28/2001	Hong Kong/330/2001	Canada/464/2001	Hawaii/10/2001
10	T												I						
45	V									I			I	I	I	I	I	I	I
50	M													T	T	T	T	T	T
54	C										Y	Y		S	S	S	S	S	S
60	V													F	F	F	F	F	F
69	E												K	K	K	K	K	K	K
128	K			I															
141	G						E												
186	R																		K
250	R									K			K	K	K	K	K	K	K
262	I							L									V	V	
271	V		I																
290	A			V															
304	K							Q											
320	E												K						
345	N									S		S	S	S	S	S	S	S	S
358	A											T							
360	R									K			K	K	K	K	K	K	K
378	E							D											
395	A																	D	
403	I	M	M	M	M	M	M	M	M	M	M	M							
404	E													K					
406	P													S					
436	K													T	T	T	T	T	T
459	V																I	I	

TABLE 4

Amino Acid Differences in Victoria Lineage Influenza B NB Molecules Compared to B/Shandong/7/97 Virus

Amino acid	Shandong/7/97	Beijing/243/97	Sichuan/52/96	Sichuan/281/96	Guangzhou/7/97	Osaka/547/97	Bangkok/34/99	Bangkok/54/99	Sichuan/40/99	Hong Kong/70/96	Malysia/83077/2001	Oman/16296/2001	Taiwan/217/97	Hong Kong/6/2001	Hong Kong/22/2001	Hong Kong/28/2001	Hong Kong/330/2001	Canada/464/2001	Hawaii/10/2001
21	T									V		A	V	V	V	V	V	V	V
22	V	I			I		I	I	I	I	I	I	I	I	I	I	I	I	I
43	F			L															
47	S									N	N	N	N	N	N	N	N	N	N
62	C													F	F	F	F	F	F
65	C			I									R	R	R	R	R	R	R
67	S	P	P	P	P								P		P	P	P	P	P
71	R												K	K	K	K	K	K	K
77	S							P											
96	E													K	K	K	K	K	K
99	P		I								L	L							

TABLE 5

Influenza B Virus Strains Used in the Present Study

Strain	Isolation date	Passage history[a]	GenBank sequence Accession Number	
			HA	NA
B/Hong Kong/70/96	06/18/96	C4	AF532556	AY139050
B/Sichuan/281/96	11/29/96	E2	AY139033	AY139051
B/Guangzhou/7/97	01/16/97	C3	AF532533	AY139052
B/Taiwan/217/97	02/17/97	C4	AY139035	AY139053
B/Osaka/547/97	unk.	C2	AY139036	AY139054
B/Bangkok/54/99	02/01/99	C5	AF532528	AY139055
B/Bangkok/34/99	02/21/99	C5	AF532527	AY139056
B/Nanchang/1/2000	01/17/00	E3	AF532564	ND
B/Texas/1/2000	02/10/00	C2	AY139037	AY139058
B/Hong Kong/557/2000	10/04/00	orig.	AF532553	AY139057
B/Alaska/16/2000	11/22/00	E2	AF532526	AY139059
B/Wuhan/356/2000	12/13/00	Ex	AY139038	AY139060
B/Chongqing/3/2000	12/26/00	C1E2	AF532530	ND
B/Wuhan/2/2001	01/01/01	E3	AY139039	AY139061
B/Hong Kong/6/2001	01/08/01	C3	AF532554	AY139062
B/Hong Kong/22/2001	01/18/01	C3	AF532547	AY139063
B/Hong Kong/28/2001	01/22/01	C3	ND	AY139064
B/CNIC/27/2001	02/05/01	C2	AF532532	ND
B/Hong Kong/112/2001	02/20/01	C3	AF532543	ND
B/Hong Kong/123/2001	02/23/01	C2	AF532544	ND
B/Canada/464/2001	03/05/01	C2	AF532531	AY139065
B/Hong Kong/329/2001	04/09/01	C3	AF532548	ND
B/Hong Kong/330/2001	04/09/01	E2	AF532549	AY139066
B/Hong Kong/335/2001	04/11/01	C3	AF532550	ND
B/Hong Kong/336/2001	04/11/01	C3	AF532551	ND
B/Yunnan/123/2001	04/18/01	E4	AY139040	ND
B/Hawaii/9/2001	04/19/01	C3	AF532541	ND
B/Hawaii/10/2001	05/09/01	C3	AF532535	AY139067
B/Hawaii/10e/2001	05/09/01	E2	AF532534	ND
B/Hawaii/35/2001	05/19/01	C3	AF532537	ND
B/Sichuan/317/2001	05/24/01	E3	AY139034	AY139068
B/Taiwan/1484/2001	05/25/01	C3	AY139041	ND
B/Hawaii/37/2001	06/01/01	Cx	AF532539	ND
B/Hawaii/36/2001	06/03/01	C3	AF532538	AY139069
B/Hawaii/38/2001	06/11/01	Cx	AF532540	ND
B/Hong Kong/497/2001	06/11/01	C3	AF532552	ND
B/Hawaii/26/2001	06/26/01	C3	AF532536	ND
B/Brazil/952/2001	07/05/01	Ex	AF532529	AY139070
B/Argentina/69/2001	07/13/01	C3	AF532525	AY139071
B/Hong Kong/666/2001	08/29/01	C3	AF532555	ND
B/Oman/16299/2001	09/22/01	C3	AY139042	ND
B/Oman/16291/2001	09/26/01	C3	AF532566	ND
B/Oman/16305/2001	09/26/01	C3	AY139043	ND
B/Oman/16296/2001	10/03/01	C3	AY139044	AY139072
B/Philippines/93079/2001	10/15/01	Cx	AY139045	ND
B/India/772767/2001	10/23/01	Cx	AF532561	ND
B/Philippines/5072/2001	10/26/01	Cx	AY139046	ND
B/Malaysia/83077/2001	10/30/01	Cx	AF532563	AY139073
B/India/7569/2001	11/07/01	Cx	AF532558	ND
B/India/7526/2001	11/10/01	Cx	AF532557	ND
B/India/7605/2001	11/10/01	Cx	AF532560	ND
B/India/7600/2001	12/05/01	Cx	AF532559	ND
B/Taiwan/97271/2001	12/10/01	Cx	AY139047	ND
B/New York/47/2001	12/31/01	Cx	AY139048	ND
B/Hong Kong/1115/2002	01/15/02	E5	AF532542	ND
B/Hong Kong/1351/2002	01/17/02	E1	AF532545	AY139074
B/Hong Kong/1434/2002	01/18/02	E3	AF532546	ND
B/New York/1/2002	01/28/02	Cx	AF532565	AY139075
B/Maryland/1/2002	unk.	Cx	AF532562	AY139076

[a] C = tissue culture; E = Embryonated hens' eggs.

ously circulating Victoria lineage isolates. Amino acid 164 has been demonstrated to be within an epitope critical to the antigenicity of Victoria lineage viruses which is absent in viruses of the Yamagata lineage (*12*). The second phylogenetic clade containing the Oman/16296/2001-like (Oman-like) viruses is distinguished by Thr121 compared to B/Shandong/7/97, a change which is shared with the prototype B/Victoria/2/87 virus. Variation in the amino acids defining the potential N-linked glycosylation site at amino acids 197–199 was apparent in viruses from both the HK-like and the Oman-like clades (Table 2). The presence or absence of this site, which is near the putative receptor-binding region, is affected by the host cell in which the virus is grown, with viruses cultivated in embryonated eggs usually lacking the site (*13*). The results obtained from these recent Victoria-lineage viruses confirm that observation.

Table 3 shows the deduced amino acid sequence differences among the NA genes from isolates of the Victoria-like viruses compared to the B/Shandong/7/97 sequence. As shown in Fig. 1, similar to the HA1 molecules, the neuraminidase genes could be separated into two phylogenetic clades. Viruses belonging to the HK-like clade are defined by five distinct amino acids (Table 3). Four of these residues, Thr50, Ser54, Phe60, and Lys69, are in the stalk region of the molecule; the only residue of the five present in the globular head is Thr436. Three other residues, Lys250, Ser345, and Lys360, are also seen in the sequence of the phylogenetically distinct B/Hong Kong/70/96. The Oman-like viruses have an NA gene more closely related to those of the previously dominant Victoria-like strains circulating in East Asia being different only at position 54 (Cys to Tyr).

The deduced amino acid sequences of the Victoria-lineage NB glycoproteins vary in a manner similar to the NA glycoproteins (Table 4). The NB of the recent HK-like isolates can be distinguished by amino acids Phe62 and Lys96; an additional three signature residues are shared with Taiwan/217/97 and/or Hong Kong/70/96 (Val21, Arg65, and Lys71). The NB of the two Oman-like viruses sequenced are distinguished by a leucine at position 99 but because of the small sample size it should only be considered a tentative feature at present. Asn47 is common to recent isolates of both clades and is shared with Hong Kong/70/96 and Taiwan/217/97 (Table 4). Because NB is much less well characterized than the HA and NA glycoproteins, it is not practical at present to speculate on the possible significance of the changes noted.

Analysis of the NA genes of the current isolates revealed that the B/New York/1/2002 isolate, which clearly has a Victoria-like HA (Fig. 1A and Table 2), has a neuraminidase gene of the Yamagata lineage (Fig. 1B). This has also been seen in other 2002 isolates such as Maryland/1/2002 and Hong Kong/1351/2002 (Fig. 1). Such reassortment between Yamagata- and Victoria-lineage type B viruses has been reported before (*14, 15*) and similar reassortment has been seen between cocirculating clades of type A H3N2 viruses (*16*). More isolates will need to be characterized before the relative frequency of this apparent reassortment event and its effects on antigenicity can be determined.

It is not known why the Victoria lineage viruses were confined to East Asia for the past 10 years or why they have now begun to spread beyond. A similar phenomenon was observed within the influenza type A H1N1 viruses where A/New Caledonia/20/99 lineage viruses, which are currently the dominant strain, were limited to Asia for four years while A/Bayern/262/95 lineage viruses circulated worldwide (*17*; CDC, unpublished data).

The appearance of new genetic variants of the Victoria lineage of type B influenza virus with a demonstrated ability to spread beyond Asia suggests that these viruses may have the capacity to become the dominant sublineage of influenza B viruses worldwide. The fact that the influenza B/Victoria-lineage isolates examined from the 2000–2001 and 2001–2002 seasons, similar to all viruses of the Victoria lineage, show extremely poor cross-reactivity to recent B/Sichuan/379/99-like vaccine strains and the spread of these viruses led the WHO to recommend the inclusion of a representative of this new antigenic group in the vaccine (*9*). Influenza B viruses are clearly deserving of continued intensive surveillance in coming seasons to monitor the spread and impact of these Victoria lineage variants in areas where populations are susceptible. The demonstrated ability of these two major antigenic groups to undergo reassortment of their surface antigens complicates selection of an influenza B vaccine strain and necessitates more rigorous monitoring of both HA and NA genes to determine the dominant genotype(s) in different locations around the world.

Materials and Methods. Viruses. Influenza B viruses used in this study, their isolation dates, and passage history are listed in Table 5.

Hemagglutination inhibition tests. HI tests were performed as described in Kendal *et al.* (*18*).

Nucleotide sequence analysis. Viral RNA was extracted and purified from culture supernates using either the QIAmp Viral RNA mini kit (Qiagen, Santa Clara, CA) or an automated MagNAPure LC instrument using the MagNAPure LC Total Nucleic Acid Isolation kit (Roche Diagnostics, Indianapolis, IN). RT-PCR was conducted using the Access RT-PCR System kit (Promega Corp., Madison, WI) and the cDNA products purified using the QIAquick PCR purification kit (Qiagen, Santa Clara, CA). Sequencing reactions were performed using the ABI BigDye terminator cycle sequencing kit with reaction products resolved on either an ABI 3100 Genetic Analyzer or an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA). Sequences of the primers used for amplification and sequencing are available upon request.

DNA sequence analysis was performed using the sequence analysis software of the University of Wisconsin Genetic Computer Group (*19*). Bootstrap confidence values were determined using 100 replicates before determining phylogenetic distances which were mapped into neighbor-joining trees using the Phylogeny Inference Package, version 3.5 (*20*).

ACKNOWLEDGMENTS

The authors thank Dr. Andre Dascal at the Jewish General Hospital of Quebec for providing the virus B/Canada/464/2001 and the World Health Organization National Influenza Centers, Communicable Diseases Surveillance and Response, World Health Organization, Geneva, Switzerland for virus isolates and epidemiologic information used in this study.

REFERENCES

1. Rota, P., Wallis, T. R., Harmon, M. W., Rota, J. S., Kendal, A. P., and Nerome, K. (1990). Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. *Virology* **175,** 5–68.
2. Kanegae, Y, Sugita, S., Endo, A., Ishida, M., Senya, S., Osako, K., Nerome, K., and Oya, A. (1990). Evolutionary pattern of the hemagglutinin gene of influenza B viruses isolated in Japan: Cocirculating lineages in the same epidemic season. *J. Virol.* **64,** 2860–2865.
3. Rota, P. A., Hemphill, M. L., Whistler, T., Regnery, H. L., and Kendal, A. P. (1992). Antigenic and genetic characterization of the haemagglutinins of recent cocirculating strains of influenza B virus. *J. Gen. Virol.* **73,** 2737–2742.
4. Levandowski, R. A., Regnery, H. L., Staton, E., Burgess, B. G., Williams, M. S., and Groothuis, J. R. (1991). Antibody responses to influenza B viruses in immunologically unprimed children. *Pediatrics* **88,** 1031–1036.
5. WHO. (1999a). Recommended composition of influenza virus vaccines for use in the 1999–2000 season. *Wkly. Epidemiol. Rec.* **7,** 57–61.
6. WHO. (1999b). Recommended composition of influenza virus vaccines for use in 2000. *Wkly. Epidemiol. Rec.* **74,** 321–325.
7. Hemphill, M. L., Rota, P. A., Ivanova, V. T., Slepushkin, A. N., and Kendal, A. P. (1993). Antigenic and genetic analyses of influenza type B viruses isolated in Russia, 1987–91. *Epidemiol. Infect.* **111,** 539–546.
8. Nerome, R., Hiromoto, Y., Sugita, S., Tanabe, N., Ishida, M., Matsumoto, M., Lindstrom, S. E., Takahashi, T., and Nerome, K. (1998). Evolutionary characteristics of influenza B virus since its first isolation in 1940: Dynamic circulation of deletion and insertion mechanism. *Arch. Virol.* **143,** 1569–1583.
9. WHO. (2002). Recommended composition of influenza virus vaccines for use in the 2002–2003 season. *Wkly. Epidemiol. Rec.* **77,** 62–66.
10. Nakagawa, N., Kubota, R., Maeda, A., Nakagawa, T., and Okuno, Y. (2000). Heterogeneity of influenza B virus strains in one epidemic season differentiated by monoclonal antibodies and nucleotide sequences. *J. Clin. Microbiol.* **38,** 3467–3469.
11. CDC. (2001). Update: Influenza activity—United States and worldwide, May-September 2001. *MMWR Morb. Mortal. Wkly. Rep.* **50,** 822–825.
12. Nakagawa, N., Kubota, R., Nakagawa, T., and Okuno, Y. (2001). Antigenic variants with amino acid deletions clarify a neutralizing epitope specific for influenza B virus Victoria group strains. *J. Gen. Virol.* **82,** 2169–2172.
13. Robertson, J. S., Naeve, C. W., Webster, R. G., Bootman, J. S., Newman, R., and Schild, G. C. (1985). Alterations in the hemagglutinin associated with adaptation of influenza B virus to growth in eggs. *Virology* **143,** 166–174.
14. McCullers, J. A., Wang, G. C., He, S., Webster, R. G. (1999). Reassortment and insertion-deletion are strategies for the evolution of influenza B viruses in nature. *J. Virol.* **73,** 7343–7348.
15. Xu, X., Shaw, J., Smith, C. B., Cox, N. J., and Klimov, A. I. (2001). Multiple lineages co-circulation and genetic reassortment of the neuraminidase and hemagglutinin genes within influenza viruses of the same type/subtype. *In* "Options for the Control of Influenza IV" (A. Osterhaus, N. Cox, and A. Hampson, Eds.), pp. 383–387. Elsevier Science, New York.
16. Xu, X., Cox, N. J., Bender, C. A., Regnery, H. L., and Shaw, M. W. (1996). Genetic variation in neuraminidase genes of influenza A (H3N2) viruses. *Virology* **224,** 175–183.
17. Daum, L. T., Canas, L. C., Smith, C. B., Klimov, A., Huff, W., Barnes, W., and Lohman, K. L. (2002). Genetic and antigenic analysis of the first A/New Caledonia/20/99-like H1N1 influenza isolates reported in the Americas. *Emerg. Infect. Dis.* **8,** 408–412. [Available from: URL:http://www.cdc.gov/ncidod/EID/eid.htm.]
18. Kendal, A. P., Pereria, M. S., and Skehel, J. (1982). "Concepts and Procedures for Laboratory-Based Influenza Surveillance." World Health Organization, Geneva, Switzerland.
19. Devereaux, J., Haeberli, P., and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12,** 387–395.
20. Felsenstein, J. (1989). Phylip-phylogeny inference package (version 3.2). *Cladistics* **5,** 164–166.
21. Klimov, A. I., Cox, N. J., Yotov, W. V., Rocha, E., Alexandrova, G. I., and Kendal, A. P. (1992). Sequence changes in the live attenuated, cold-adapted variants of influenza A/Leningrad/134/57 (H2N2) virus. *Virology* **186,** 795–797.