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Reappearance of influenza B/Victoria/2/87-lineage viruses: epidemic activity, genetic diversity and vaccination efficacy in the Finnish Defence Forces

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SUMMARY

A new B/Shangdong/7/97-like influenza virus (Victoria/2/87 lineage) predominated during the 2002/2003 epidemic season in Finland and was estimated to account for 2246 of the 13 496 feverish upper respiratory tract infections (URIs) occurring among conscripts in the Finnish army. The incidence (1716/10 000 conscripts) was indicative of moderate epidemic activity at most. Analysis of the cross-reactive antibodies induced in 1988 suggests that the basis of the protection was probably established during the childhood of the conscripts. Vaccination in autumn 2002 prevented 42% of the URIs during the influenza B outbreak and 71% (95% CI 42–85) of infections interpreted as influenza B. Despite the low genetic variability of the Shangdong/7/97-like viruses, breakages of a potential glycosylation site in haemagglutinin (HA1, position 197) were frequent; their biological significance is discussed. The Shangdong/7/97-like strains were HA1/NA reassortants, as were also the less abundant strains that for HA1 belonged to the B/Yamagata/16/88 lineage. A further reassortment, which probably emerged during the outbreak in one of the garrisons, supports our hypothesis that circumstances in these settings may especially favour the emergence of diversity by reassortment.

INTRODUCTION

During the epidemic season of 2001/2002, two influenza B virus variants that had evolved from a common ancestor in the 1970s [1, 2] circulated in Europe [3–6]. The B/Sichuan/379/99-like viruses belonged to the B/Yamagata/16/88 lineage and were closely related to the viruses of the previous epidemic seasons. The B/Hong Kong/330/2001-like viruses belonged to the B/Victoria/2/87 lineage. This lineage had disappeared from Europe in the early 1990s and was then replaced by the Yamagata lineage. The last intense outbreak of Victoria-lineage virus in Finland occurred during 1987/1988 [7]. After low-intensity local outbreaks due to these viruses in 1989/1990 [8], the first outbreak of Yamagata-lineage virus occurred in Finland in 1990/1991 [9]. The global re-emergence of the Victoria-lineage viruses from East Asia in 2001/2002 resulted in a change in the recommended composition of influenza virus vaccines for use in autumn 2002, when a B/Hong Kong/330/2001-like virus (B/Shangdong/7/97) was substituted for the B/Sichuan/379/99-like vaccine virus [10]. In spring 2002, the B/Hong Kong/330/2001-like viruses predominated in many parts of the world [3] but were not isolated in Finland [11] (Ikonen et al., unpublished data).

The re-expansion of the Victoria-lineage viruses was epidemiologically an interesting new situation. One could expect that the B/Hong Kong/330/2001-like viruses would be capable of infecting children with no

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antigenic experience of the Victoria-lineage viruses, but the ability of the virus to infect older age groups was less axiomatic. Our study focuses on conscripts in the Finnish Defence Forces who had been between 5 and 7 years of age during the last intense outbreak of Victoria-lineage viruses during 1987/1988. We investigated to what extent Victoria- and Yamagata-lineage viruses were responsible for upper respiratory tract infections (URIs) in the Finnish Defence Forces during 2002/2003 as well as the genetic diversity of these viruses in garrisons scattered throughout Finland. The efficacy of the updated influenza virus vaccine against influenza B, estimated with the patients sampled and laboratory tests, and the effectiveness of the vaccine in reducing URIs was investigated in one of the garrisons.

MATERIALS AND METHODS

Study population and clinical surveillance

The monthly mean number of men who served in the Finnish Defence Forces in garrisons scattered throughout Finland varied from 17445 to 20113 conscripts during the study period from November 2002 to April 2003. The conscripts, whose enlistments were from 9 or 12 months, had entered the service at a mean age of 19·5 years. A minority had entered in January 2002, most in July 2002 or in January 2003, when the study was in progress. Vaccinations against influenza were administered in autumn 2002 in two garrisons, where a total of 428 conscripts received the vaccine. The occurrence of URI among the conscripts was monitored in all garrisons. Case definition was feverishness and one or more of the following symptoms: cough, sore throat and/or myalgia. The first patient contact with a physician during each episode was recorded and the monthly case numbers were reported to the Research Institute of Military Medicine, following established practice [12].

Virological surveillance

We recommended that health-care personnel of the garrisons collected nasopharyngeal aspirates (NPAs) from 5 to 10 URI patients during the rising phase of every URI outbreak and some additional specimens at a later phase. In addition, scattered samples were taken from sporadic cases. More extensive NPAs were collected as part of vaccine investigations in the Pori Brigade, where these specimens were taken from 59 of the 522 URI patients (11·3%) noted during the continuous 15-week observation period (weeks 2–16, 2003). With these measures a total of 299 NPAs were collected during the 6-month study period in 2002/2003 from 13 garrisons and one military hospital (referred to as military units hereafter). Acute and convalescent sera were collected from 213 patients and the total number of patients who donated either NPAs or paired sera or both was 302. The case definition was the same as that applied for monitoring of URIs. We recommended that the NPAs be taken within 3 days of the onset of illness, as had been carried out in Finland during previous epidemic seasons [12]. In practice, 73% of the NPAs were collected within 3 days and 92% within 5 days.

Most of the NPAs (90%) were inoculated within 48 h, and the rest after a storage period of at most 1 week at $-60\,^{\circ}C$ onto Madin–Darby canine kidney (MDCK) cell cultures for isolation of influenza viruses. With a few exceptions, all the cultures that did not show haemagglutinating activity within 3–5 days after inoculation had a second passage in MDCK cells; otherwise the cultivation procedure was as described previously [13]. Typing of the influenza isolates was performed with a haemagglutination inhibition (HI) assay as described previously [14] and supplemented afterwards [13] using rat antisera produced against B/Hong Kong/330/01 (Victoria lineage), B/Fin/141/2002 (Yamagata lineage), A/New Caledonia/20/99(H1N1) and A/Panama/2007/99(H3N2). A time-resolved fluoroimmunoassay was applied [15] when the NPAs were examined for the presence of several microbial antigens: influenza A and B, respiratory syncytial virus, the adenovirus group and parainfluenza virus types 1, 2 and 3. The paired sera were studied with a standard complement fixation technique [16] for the presence of antibodies to the same viruses as above. Of the agents examined serologically and in antigen detection, the results for influenza B (and A) viruses are shown. The roles played by the other agents studied are of less concern here and, with the exception of the situation in the Pori Brigade where the vaccination campaign was executed, will be discussed elsewhere (Kleemola et al., unpublished observations).

Sequencing and phylogenetic analysis

Of the 94 influenza B viral strains isolated from the conscripts in 2002/2003, 28 strains together with three strains isolated from civilian patients in late 2002,

when the garrison outbreaks had not yet begun, were analysed with nucleotide sequencing of the HA1 domain of the viral haemagglutinin (accession numbers: AY744303 to AY744329 for Victoria lineage and AY744334 to AY744337 for Yamagata lineage). Of the 28 strains, 13 were analysed for partial nucleotide sequencies of viral neuraminidase (NA; accession numbers AY744290 to AY744302). RNA extraction, cDNA synthesis, amplification procedures with polymerase chain reaction and sequencing were performed in our laboratory as previously described [13]. Prior to RNA extraction and sequencing, the viruses were propagated through 2–4 passages in MDCK cell cultures. The sequences of the primers used for amplification and sequencing of NA and HA1 are available on request. The DNAML program of the Phylip software package (Phylogenetic Interference Package 3.4, J. Felsenstein, University of Washington, Seattle) was used to generate phylogenetic trees.

Vaccination and vaccinees

A trivalent commercial influenza vaccine (Influvac®, Solvay Pharmaceuticals BV, Olst, The Netherlands; Shangdong/7/97 served as the influenza B virus component) was offered on a voluntary basis to male conscripts (born during 1981–1983) who served in the Pori Brigade (Southwest Finland) and had entered the army in July 2002. A total of 333 men accepted the offer, of whom 296 were vaccinated in November 2002 and the rest in December. Depending on the week, 230–309 vaccinated men and 234–343 unvaccinated men from the July 2002 intake, as well as 0–1143 unvaccinated men from the January 2003 intake served in the garrison during the 15-week study period. The efficacy of the vaccine and its effectiveness in reducing URIs were calculated as outlined previously [13].

HI antibody screening

A total of 19 acute- and convalescent-phase paired sera collected during the 2002/2003 epidemic season from conscripts whose illness was shown, based on virus isolation, to be caused by the B/Hong Kong/330/01-like predominant virus variant (Victoria lineage), were studied for the presence of HI antibodies to B/Fin/159/2002 (a representative of the predominant variant) and B/Fin/202/2003 (a representative of the less abundant variant; Yamagata lineage). Another set of paired sera ($n=32$) collected from influenza B patients during the 1987/88 outbreak in the Finnish Defence Forces and stored at $-20\,^{\circ}C$, were available and studied for the presence of HI antibodies to B/Fin/159/2002 and B/Fin/56/88. The latter is a Victoria-lineage field strain isolated in 1988. The strains used as antigens in the HI tests were cultivated exclusively in MDCK cell cultures. The HI tests were performed with standard microtitre techniques [14, 17], using goose instead of hen erythrocytes. The sera were pretreated with *Vibrio cholerae* filtrate to remove non-specific inhibitors, and with packed goose erythrocytes to remove cell agglutinins, which are sometimes present in sera at low titres.

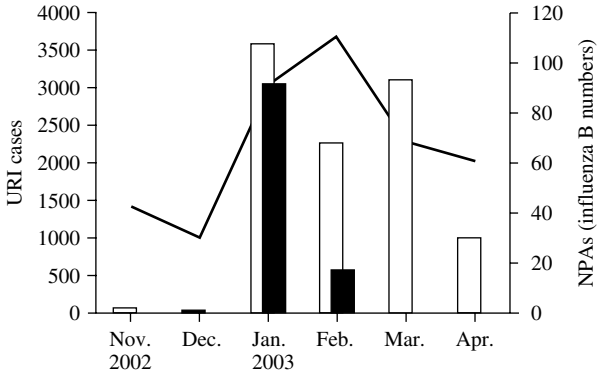

Fig. 1. Monthly numbers of upper respiratory tract infections (URI, ——) among conscripts in the Finnish Defence Forces during the observation period from November 2002 to April 2003 and monthly numbers of sampled patients (□) and their infections with influenza B virus (■).

RESULTS

Epidemic activity

A total of 13496 URI cases were reported among conscripts in the Finnish Defence Forces during the study period (November 2002 to April 2003); the monthly figures are given in Figure 1. In total there were 0·7 infections per man for the 6-month study period as counted from monthly mean strength and monthly incidences. The 302 URI patients from whom samples were collected covered 2·2% of the URI cases. The three diagnostic tests used (virus isolation, antigen detection and antibody determination) yielded a total of 109 infections with influenza B and 26 infections with influenza A, i.e. 36·1% and 8·6% respectively, of the sampled patients. A total of 94 influenza B strains were isolated, 89 of which were shown to be B/Hong Kong/330/2001-like viruses antigenically (Victoria lineage; isolated in 14 military units from January to February) and five of which were more

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Anti-B/Fin/141/02 (Yamagata lineage)	Anti-B/Hong Kong/330/01 (Victoria lineage)							
	<10	10	20	40	80	160	320	640
<10					26	10	14	5
10					7	3	2	5
20					7	5	1	2
40						2		
80								
160								
320								
640			2					
1280	1	1	1					

Fig. 2. Influenza B strains ($n=94$) isolated during the 2002/2003 epidemic season from conscripts in Finland: reactivity in HI assay with two antisera capable of differentiating Victoria-lineage and Yamagata-lineage strains.

closely related to the virus that had been circulating in Finland during the 2001/2002 epidemic season (Yamagata lineage; isolated in January 2003 in two military units) (Fig. 2). Based on the influenza B-positive cases among the 302 sampled patients and the proportions of Victoria-lineage and Yamagata-lineage viruses isolated in January and February, the former predominant virus accounted for 3346 of the 13 496 URI cases, indicating an incidence of 1716 infections per 10 000 conscripts in the Finnish Defence Forces during the 2002/2003 epidemic season.

Molecular epidemiology

The 27 representative B/Hong Kong/330/2001-like strains that were investigated for the presence of sequences coding for HA1 belonged to the Victoria lineage and were most closely related to B/Shangdong/7/97 and some recent isolates, such as B/New York/1/2002, B/Maryland/1/2002 and B/Hong Kong/1351/2002 (Fig. 3). Hereafter, these strains are referred to as B/Shangdong/7/97-like viruses. The intra-epidemic HA1 heterogeneity of these viruses was very low (Fig. 3); 17 of the 27 strains formed group A in which eight strains differed from the consensus, five of them by 1 and three by 2–4 bases if the co-dominant mixtures are not taken into account. The group A viruses were isolated from seven localities scattered throughout Finland. Six synonymous signature substitutions were characteristic of group B ($n=10$), in which only two strains differed from the consensus by 1 base. The group B viruses were isolated from seven localities, two of which (Säkylä and Sodankylä) were the same as in group A. A striking characteristic of the Shangdong-like strains was the amino-acid variation at positions 197 and 199. This variation resulted in the loss of a potential glycosylation site by three different pathways (NEI, SET, NEN) and in

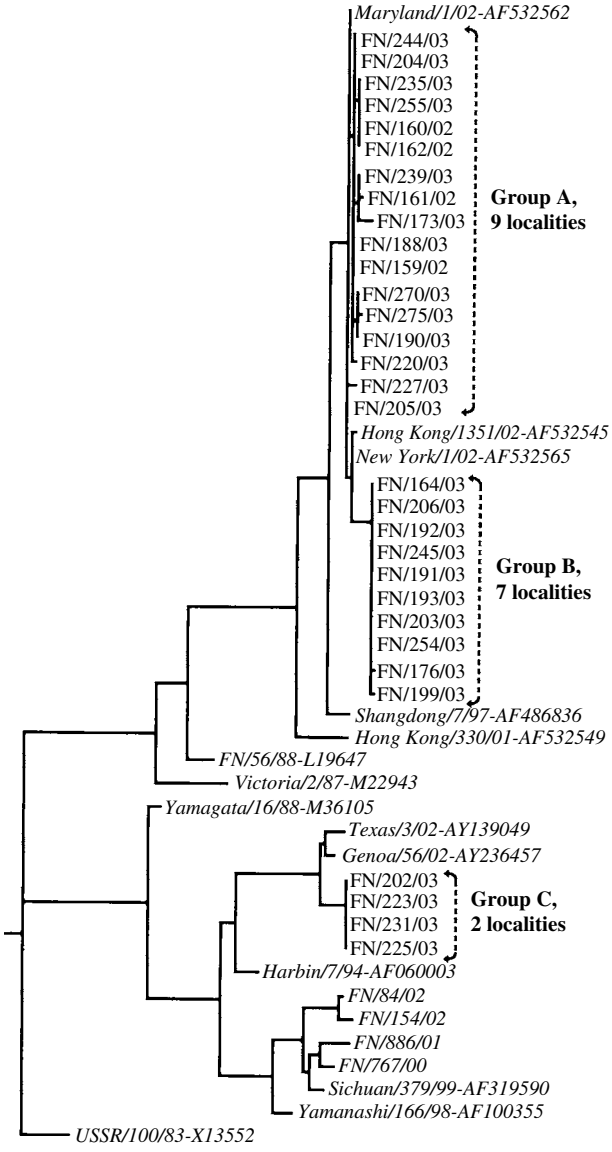

Fig. 3. Phylogenetic tree of the HA1 sequences of 31 influenza B viruses isolated during the 2002/2003 epidemic season in Finland (with capitals) and 18 other viruses included for comparison (in italics). The tree grows to the right. The horizontal lines are proportional to the number of nucleotide changes. Finland is abbreviated to FN. Accession numbers for the 2002/2003 strains are given in the Materials and Methods section.

the frequent appearance of co-dominant amino-acid mixtures.

All of the 11 strains in groups A and B, analysed for the partial sequence encoding NA, proved to be reassortants that shared the B/Sichuan/379/99-lineage NA (Fig. 4). Similar reassortants had been circulating in the world during the previous epidemic seasons (B/Hong Kong/1351/2002, B/New York/1/2002 and B/Maryland/1/2002). The NA sequences of our strains

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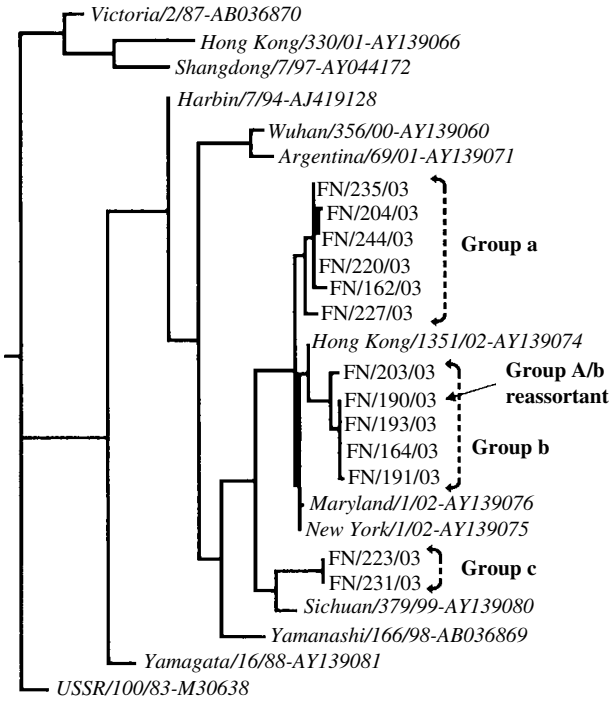

Fig. 4. Phylogenetic tree of the partial NA sequences of 13 influenza B viruses isolated during the 2002/2003 epidemic season in Finland and 13 other viruses included for comparison. Structure of the tree and other details as given in Figure 3 legend. Accession numbers for the 2002/2003 strains are given in the Materials and Methods section.

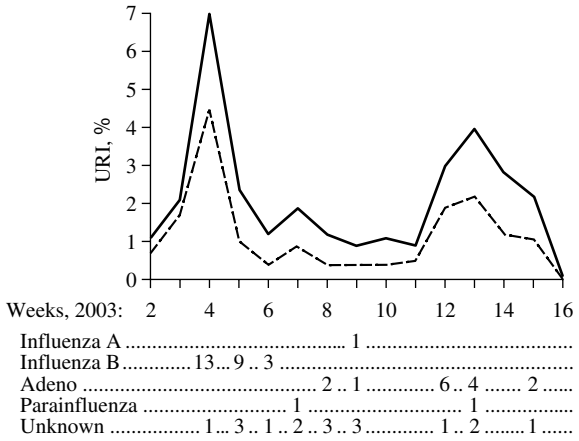

Fig. 5. Incidence of upper respiratory tract infections (URI) in the Pori Brigade, where a number of the conscripts were vaccinated against influenza in autumn 2002. ——, Weekly percentages of all URI cases; – – –, cases who exhibited fever of $\geqslant 38\ ^{\circ}C$. Weekly numbers of nasopharyngeal aspirates collected and laboratory findings are enumerated.

fell into groups a and b, similar to the HA1 sequences in groups A and B, but with one exception, B/Fin/190/2003 was an apparent A/b reassortant. It was isolated in one of the two garrisons (Sodankylä) where the two HA1 groups and two NA groups were simultaneously detected.

The four strains that antigenically resembled the recent Yamagata-lineage viruses (Fig. 2) and that were examined for the presence of HA1 sequences were grouped in this lineage with B/Harbin/7/94 and some recent strains, such as B/Finland/Wv5/02 [11], B/Texas/3/2002 and B/Genoa/56/2002 (Fig. 3, group C). They shared the change from asparagine at site 126 to aspartic acid, which appeared in 2000 and was suggested as being responsible for altered antigenicity [18]. During the 2001/2002 season, B/Sichuan/379/99-like strains had been isolated in Finland (Fig. 3) in addition to a strain (B/Fin/141/2002) that exhibited Harbin-lineage HA1 and NA [3]. In contrast, the strains occurring in 2002/2003 with Harbin-lineage HA1 were reassortants that exhibited NA sequences closely related to those of B/Sichuan/379/99 (Fig. 4, group c).

Vaccine efficacy

Two URI outbreaks occurred during the 15-week observation period in the Pori Brigade where the vaccination efficacy and clinical effectiveness were investigated. The first of the outbreaks (weeks 2–6) was caused by influenza B, while the later outbreak (weeks 12–15) was caused by other agents, mainly adenoviruses (Fig. 5). A total of 24 influenza B viral strains were isolated from the NPAs collected from 59 URI patients. All these strains were antigenically B/Hong Kong/330/2001-like. Seven of the strains were examined for the presence of HA1 sequences and were closely related to B/Shangdong/7/97 (the vaccine virus). The number of men and vaccination status in the two intakes of conscripts are given in the Material and Methods section (Vaccination and Vaccinees). The incidence of URIs, as indicated by intakes and vaccination status during the influenza B virus outbreak and the rest of the observation period, are given in the Table. During the influenza B virus outbreak, vaccination prevented 42% (95% CI 0–66) of URIs and 40% (95% CI −32 to 73%) of URIs with fever of $\geqslant 38\ ^{\circ}C$ in the July 2002 intake, but was ineffective during the rest of the observation period, when adenoviruses were shown to be circulating in the garrison. The calculated efficacy for preventing virologically indicated influenza B infections was 71% (95% CI 42–85) during the influenza B virus outbreak and 81% (95% CI 52–93) for preventing these infections with a fever of $\geqslant 38\ ^{\circ}C$.

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Table. *Upper respiratory tract infections (URI) (%) among unvaccinated (V−) and vaccinated (V+) conscripts in the Pori Brigade during weeks 2–6 when the outbreak was caused by influenza B, and weeks 7–16 when influenza B was not detected*

		Weeks 2–6		Weeks 7–16	
Intake	Vaccination status	URI total	Fever ⩾38 °C	URI total	Fever ⩾38 °C
Jan. 2003	V−	15·5%	9·7%	20·1%	10·5%
July 2002	V−	11·1%	5·4%	13·8%	6·1%
July 2002	V+	6·5%	3·2%	16·7%	7·7%

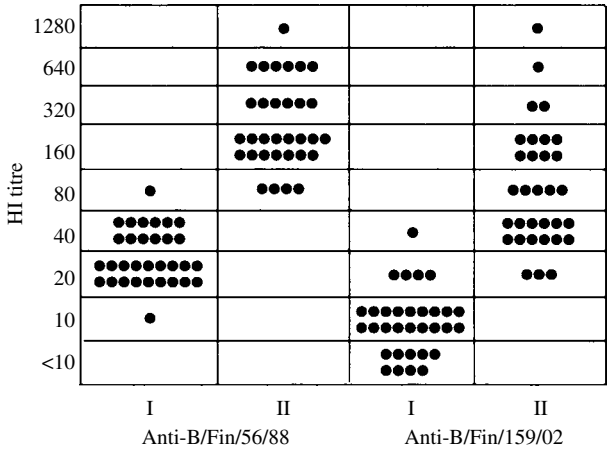

Fig. 6. HI antibodies to an antecedent (B/Fin/56/88) and the recent (B/Fin/159/2002) Victoria-lineage drift variants in acute- (I) and convalescent-phase (II) sera ($n=32$) in infections with B/Victoria/2/87-like virus during the outbreak of 1987/1988.

HI antibody responses

Young military conscripts infected with B/Victoria/2/87-like virus during the epidemic season of 1987/1988 in Finland were shown to respond with HI antibodies not only to the epidemic virus at that time (B/Fin/56/88) but in lower titres, still mostly extending the protective level 1:40, to B/Fin/159/2002 representing the new epidemic drift variant of the Victoria lineage (Fig. 6). Infections of conscripts with this new variant during the 2002/2003 epidemic season frequently resulted in a significant increase in HI antibodies to B/Fin/202/2003, a representative from the Yamagata lineage (Fig. 7).

DISCUSSION

The new Shangdong/7/97-like Victoria-lineage (HA1) virus was shown to be the predominant influenza

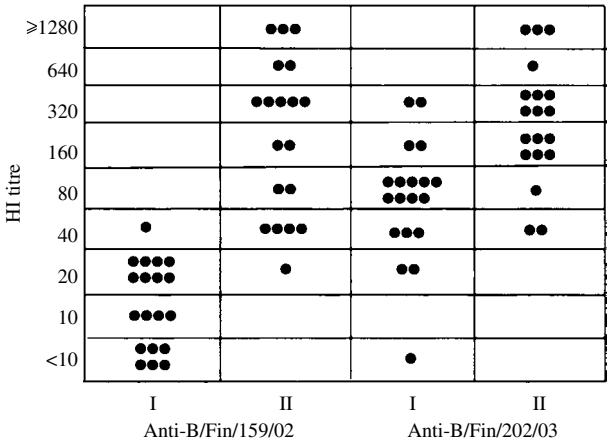

Fig. 7. HI antibodies to the predominant Victoria-lineage virus (B/Fin/159/2002) and the co-circulating less abundant Yamagata-lineage virus (B/Fin/202/2003) in acute- (I) and convalescent-phase (II) sera ($n=19$) in infections with the predominant virus during the outbreak of 2002/2003.

virus and a Yamagata-lineage virus less abundant in the Finnish Defence Forces during the 2002/2003 epidemic season. The epidemic activity of influenza B had been rather low in Finland during the 2001/2002 season [19]. A somewhat higher level of activity was noted during the 2000/2001 season [19], when an incidence of 1640/10 000 in the Finnish Defence Forces [20] was recorded. This is the same order of magnitude as the 1716/10 000 for the predominant influenza B virus during 2002/2003 in the present study. Similar investigations were also conducted in the Finnish Defence Forces in the early 1990s, when a much higher incidence of influenza B was detected (2737/10 000) during 1992/1993 [12]. Given that the predominant influenza B virus during 2002/2003 was a new drift variant, its incidence in the Finnish Defence Forces was rather low, especially when compared with the incidence of influenza A during many previous seasons [12, 20]. High feverish infections presumably were over-represented among the sampled patients in our investigations, and the diagnostic methods used were not sufficiently sensitive to reveal all influenza infections. However, differences in these biases from one epidemic season to another are unlikely.

The comparably low incidence may have resulted from many of the conscripts being primed by Victoria-lineage viruses during their childhood. In the present study, 90% of young patients who contracted influenza B in 1988 responded with HI antibody to the present Victoria-lineage virus at a titre (1:40)

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considered to be protective. Low incidence of influenza B might also be peculiar to the elderly in Finland in 2002/2003, as indicated by laboratory findings on influenza from the National Infectious Disease Register [20, 21]. Infections with Shangdong/7/97-like virus during the 2002/2003 epidemic season were shown to induce the formation of HI antibodies to B/Fin/202/2003, a Yamagata-lineage virus. Consequently, the 2002/2003 outbreak may diminish in the years following the epidemic activity not only of B/Shangdong/7/97-like viruses but, due to anamnestic antibody responses, the epidemic activity of the recent Yamagata-lineage viruses.

The intra-epidemic genetic variability of the Shangdong/7/97-like viruses in the Finnish Defence Forces during 2002/2003 in garrisons scattered throughout Finland was rather low and restricted almost completely to synonymous mutations. A high degree of sequence homology, which is a frequent short-term phenomenon after the emergence of a novel epidemic variant [22], was also characteristic of these viruses during the 2001/2002 season in northern Italy [6] and in Israel [11]. Transmission of the virus mainly among small children, who had no previous antigenic experience with Victoria-lineage viruses and presumably exhibited low antibody-mediated selection pressure, may have contributed to the sequence homogeneity.

Reassortment was recently shown [23] to be an important means of genetic variation in the evolution, not only of influenza A, but of influenza B viruses as well. The Victoria-lineage (HA1) variant and the less abundant Yamagata-lineage variant of influenza B in the present study were both HA1/NA reassortants that possessed dissimilar Yamagata-lineage NAs. The former reassortant also circulated elsewhere in the world in 2002/2003 [24] and was also detected in 2001/2002 [3, 5, 11, 25]. There is evidence of genetic reassortments of influenza B viruses not only between the Yamagata and Victoria lineages but also between genetically distinct sublineages [26]. In our study, an HA1/NA reassortment was detected that probably arose during the outbreak in one of our garrisons. Circumstances in the garrisons may greatly favour the emergence of diversity by reassortment. In Finland conscripts may visit their home districts an average of three times per month. Sometimes they are located far from the garrison in different parts of the country. Simultaneous transmission of different virus variants to garrisons crowded with military servicemen is expected to be a frequent phenomenon.

The presence of carbohydrate chains in the vicinity of the receptor-binding site (RBS) in HA1 may promote virus growth by masking antigenic sites, thus preventing epitope recognition by antibodies [27, 28]. For influenza B viruses loss of the glycosylation site at amino acid 197 in an area analogous to the antigenic site B of the H3N2-subtype influenza A virus HA1 occurs during egg adaptation and results in antigenic changes [29–33]. This loss has been characteristic, not only of egg-grown field strains, but occasionally also of strains isolated in MDCK cells [3, 6, 24, 33–35]. The evolutionary significance of these findings is still obscure. Despite the low overall genetic variability of the Shangdong/7/97-like viruses isolated and passaged exclusively in MDCK cells in our study, breakages of the glycosylation site 197 by three different pathways and a large number of co-dominant mixtures were recorded. This suggests that carbohydrates at position 197 may have become a burden on the virus at the present evolutionary juncture. A decline in the advantage of carbohydrates can be expected, if a virus variant has adapted to affect young people whose antibody status is poor. To a certain extent this seems to be characteristic of the present Victoria-lineage viruses. In these circumstances, loss of the glycosylation site would be advantageous, if the carbohydrates interfere with receptor binding, e.g. virus affinity to the receptor. There is some evidence of this interference in laboratory conditions [32, 33], but there is insufficient knowledge to understand what actually happens during human infection.

The influenza vaccine in use in autumn 2002 matched very well with the predominant influenza B virus. The calculated efficacy for preventing virologically diagnosed influenza B infections (71% among conscripts in the July 2002 intake) was rather good with regard to the high infection pressure in the garrisons and compared with the efficacy of 57% recorded in another Finnish garrison in 1998 when H3N2-subtype influenza A virus was circulating and antigenic and genetic matching to the vaccine virus was incomplete [13]. The efficacy against influenza B of the vaccine used is similar to the efficacies recorded decades ago among military recruits [36, 37] and more recently among healthy adults in influenza A outbreaks during epidemic seasons when there was also good antigenic matching [38]. Predictably, the vaccination was ineffective in our study in preventing non-influenza-related URIs. Vaccination was offered to conscripts who had entered the army in the preceding July. The incidence of URI was much higher among

unvaccinated conscripts who did not enter the army until January 2003 compared with the unvaccinated conscripts in the July 2002 intake. This was seen in the influenza B outbreak (weeks 2–6; RR 1·40, 95% CI 0·97–2·00, $P<0·064$) as well as the remaining observation period (weeks 7–16; RR 1·45, 95% CI 1·07–1·96, $P<0·014$). The differences in incidence may largely be explained by differences in service and accommodation circumstances, which presumably contribute to differences in exposure to infection. Comparative studies of the two intakes would be useful for analyses on factors behind the differences in incidence and would expand our knowledge of exposure-related vaccination efficacy.

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