Reduced cross-protection against influenza A(H3N2) subgroup 3C.2a and 3C.3a viruses among Finnish healthcare workers vaccinated with 2013/14 seasonal influenza vaccine

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Virus strains in the seasonal influenza vaccine for the 2014/15 northern hemisphere season remained unchanged from those in 2013/14. During spring 2014, drifted influenza A(H3N2) viruses, subgroup 3C.3a, were detected in Finland; another subgroup, 3C.2a, emerged in the 2014/15 season and has predominated. We monitored antibody responses against vaccine and epidemic strains (2013/14 and 2014/15) among Finnish healthcare workers after influenza vaccination with the 2013/14 vaccine. The data suggest reduced cross-protection towards both subgroups of drifted A(H3N2) viruses.

Early in the 2014/15 influenza season, drifted influenza A(H3N2) viruses have predominated in Europe [1,2]. In Finland, the season started earlier in the year than the previous season did (December 2014 as opposed to January 2014). We characterised a subset of circulating 2013/14 and 2014/15 influenza A(H3N2) viruses genetically to monitor changes in the circulating strains. On the basis of the genetic changes identified, representatives of these strains were selected for serological study. The strains recommended by the World Health Organization (WHO) for inclusion in the 2014/15 trivalent influenza vaccine (TIV) for the northern hemisphere [3] are the same as those in 2013/14 [4]: A/California/07/2009, A/Texas/50/2012 and B/Massachusetts/02/2012. By exploring the antibody responses to the 2013/14 TIV in Finnish healthcare workers (HCWs), we evaluated the seroprotection level against viruses included in TIV and compared it with vaccine-induced cross-protection towards selected epidemic virus strains from the 2013/14 and 2014/15 seasons. Our findings suggest reduced cross-protection towards the two subgroups of drifted A(H3N2) viruses detected in Finland (genetic subgroups 3C.3a and 3C.2).

Genetic characterisation of influenza A(H3N2) viruses in Finland in 2013/14 and 2014/15

As part of virological surveillance of influenza in Finland, a subset of influenza A(H3N2)-positive samples from sites in a sentinel influenza surveillance network and non-sentinel sites are selected throughout the season for genetic characterisation on the basis of their geographical origin and temporal distribution.

The sentinel network consists of healthcare centres collecting specimens from patients with influenza-like illness or acute respiratory infection and most also report clinical data. Healthcare centres of garrisons, also included in the network, only collect specimens. While intensive-care units are also part of the network, collecting specimens only, they are not considered as sentinel sites, as their participation is not agreed in advance (unlike that of healthcare centres). Other non-sentinel sites include clinical microbiology laboratories, for example.

Phylogenetic analysis of the haemagglutinin gene was performed as described previously [5]. Reference influenza A(H3N2) virus sequences for the phylogenetic tree were obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 1).

During the 2013/14 season, a total of 27 influenza A(H3N2) viruses were analysed: 25 belonged to group 3C.3 and two to group 3C.2 (Figure 1, Table 1). Of the

Table 1A

Origin of the haemagglutinin sequence information of influenza A(H3N2) viruses included in the phylogenetic analysis

Isolate name	Segment ID	Country	Collection date	Originating laboratory	Submitting laboratory	Authors
A/Alabama/05/2010	EPI278808	United States	2010-Jul-13	US Air Force School of Aerospace Medicine	Centers for Disease Control and Prevention	
A/Athens GR/112/2012	EPI358885	Greece	2012-Feb-01	Hellenic Pasteur Institute	National Institute for Medical Research	
A/Hong Kong/146/2013	EPI426061	Hong Kong (SAR)	2013-Jan-11	Government Virus Unit	National Institute for Medical Research	
A/Hong Kong/5738/2014	EPI539806	Hong Kong (SAR)	2014-Apr-30	Government Virus Unit	National Institute for Medical Research	
A/Iowa/19/2010	EPI335923	United States	2010-Dec-30	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
A/Ireland/M28426/2013	EPI467996	Ireland	2013-Apr-02	National Virus Reference Laboratory	National Institute for Medical Research	
A/Johannesburg/3495/2012	EPI405940	South Africa	2012-Jul-04	Sandringham, National Institute for Communicable D	National Institute for Medical Research	
A/Madagascar/0648/2011	EPI319276	Madagascar	2011-Feb-21	Institut Pasteur de Madagascar	National Institute for Medical Research	
A/Nebraska/4/2014	EPI539619	United States	2014-Mar-11	Centers for Disease Control and Prevention	National Institute for Medical Research	
A/Norway/1186/2011	EPI326137	Norway	2011-Mar-16	Norwegian Institute of Public Health	National Institute for Medical Research	
A/Norway/1330/2010	EPI302231	Norway	2010-Dec-03	WHO National Influenza Centre	National Institute for Medical Research	
A/Norway/1903/2014	EPI539623	Norway	2014-May–20	WHO National Influenza Centre	National Institute for Medical Research	
A/Perth/16/2009	EPI211334	Australia	2009	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention	
A/Samara/73/2013	EPI460558	Russian Federation	2013-Mar-12	WHO National Influenza Centre Russian Federation	National Institute for Medical Research	
A/Stockholm/18/2011	EPI326139	Sweden	2011-Mar-28	Swedish Institute for Infectious Disease Control	National Institute for Medical Research	
A/Switzerland/9715293/2013	EPI540526	Switzerland	2013-Dec-06	National Institute for Medical Research	Centers for Disease Control and Prevention	
A/Texas/50/2012	EPI391247	United States	2012-Apr-15	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention	
A/Victoria/361/2011	EPI349106	Australia	2011-Oct-24	Melbourne Pathology	WHO Collaborating Centre for Reference and Research on Influenza	Deng, Y-M; Caldwell, N; Iannello, P; Komadina, N.
A/Finland/385/2013	EPI502957	Finland	2013-Dec-11	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/404/2014	EPI556921	Finland	2014-Feb-06	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/410/2014	EPI556922	Finland	2014-Feb-26	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/428/2014	EPI556939	Finland	2014-Feb-17	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/433/2014	EPI557055	Finland	2014-Feb-07	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/437/2014	EPI557056	Finland	2014-Mar-24	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/438/2014	EPI557057	Finland	2014-Apr-03	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/439/2014	EPI557058	Finland	2014-Apr-23	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M

Table 1B

Origin of the haemagglutinin sequence information of influenza A(H3N2) viruses included in the phylogenetic analysis

Isolate name	Segment ID	Country	Collection date	Originating laboratory	Submitting laboratory	Authors
A/Finland/440/2014	EPI557059	Finland	2014-Apr-28	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/461/2014	EPI557060	Finland	2014-Oct-22	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/462/2014	EPI557061	Finland	2014-Oct-08	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/463/2014	EPI557062	Finland	2014-Nov-20	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/464/2014	EPI557063	Finland	2014-Nov-24	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/465/2014	EPI557064	Finland	2014-Dec-01	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/466/2014	EPI557065	Finland	2014-Nov-21	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/467/2014	EPI557066	Finland	2014-Nov-24	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/468/2014	EPI557067	Finland	2014-Nov-27	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M
A/Finland/469/2014	EPI557068	Finland	2014-Nov-27	National Institute for Health and Welfare	National Institute for Health and Welfare	Ikonen, N; Haanpää, M

All reference and Finnish haemagglutinin sequences are available from the Global Initiative on Sharing Avian Influenza Data (GISAID) EpiFlu database.

25 group 3C.3 viruses, six represented a new drifted A(H3N2) type, group 3C.3a viruses. In Finland, these viruses were first detected in February 2013.

At the beginning of the 2014/15 season, all nine influenza A(H3N2) viruses analysed belonged to another drifted group, 3C.2a.

Monitoring antibody response after influenza vaccination in a cohort of healthcare workers

A total of 79 clinically healthy HCWs (12 men, 67 women), median age 46 years (range: 22–66), were recruited on a voluntary basis during autumn 2013 from the personnel of the Department of Medicine at the Helsinki University Hospital and the Viral Infections Unit at the National Institute for Health and Welfare, Helsinki. The employer vaccinated each participant with the 2013/14 seasonal influenza vaccine, which was trivalent, non-adjuvanted, containing the three WHO-recommended influenza virus strains. One dose was administered intramuscularly. Serum samples were collected before vaccination (day 0) and three weeks and six months after vaccination.

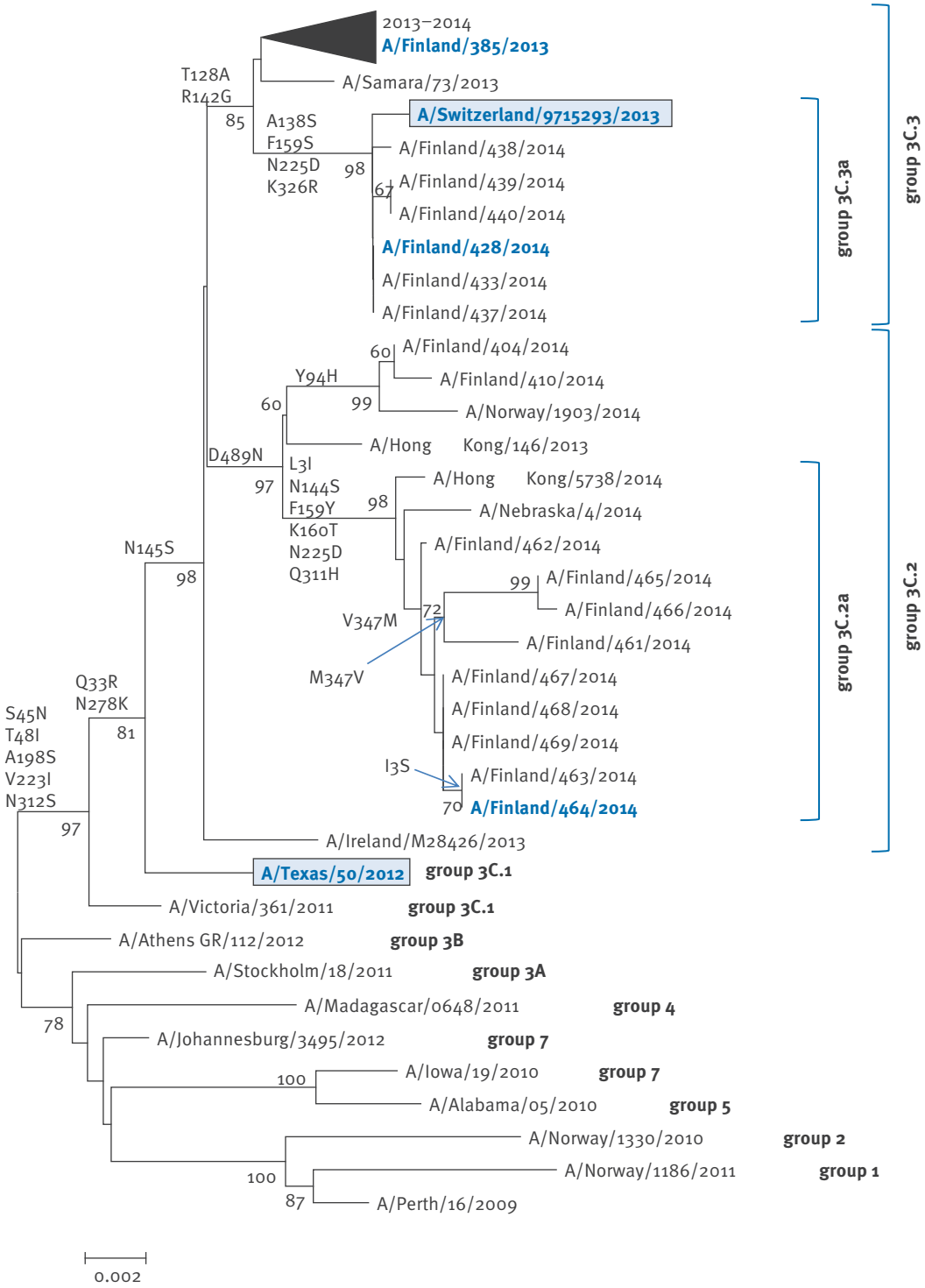
The study protocol was approved by the Ethic Committee of the Department of Medicine, Helsinki University Hospital (298/13/03/00/12) and the Finnish Medicines Agency (EudraCT 2012–003727–38). Written informed consent was provided by all participants.

The serum samples were tested by the haemagglutination inhibition (HI) test for the presence of antibodies against TIV vaccine viruses and Finnish influenza virus isolates from the 2013/14 and 2014/15 seasons. TIV strains for the northern hemisphere 2013/14 and 2014/15 seasons were A/California/07/2009 (group 1), A/Texas/50/2012 (group 3C.1) and B/Massachusetts/02/2012 (clade 2). For comparison, we also included B/Wisconsin/01/2010 (clade 3), the vaccine strain for the northern hemisphere 2012/13 season, and A/Switzerland/9715293/2013 (H3N2) (group 3C.3a), the vaccine strain for the southern hemisphere 2015 season. We also included A/Finland/420/2014 (group 6B), which represented the Finnish A(H1N1) pdm09 strain that circulated in 2013/14. In addition, A/Finland/385/2013 (2013/14), A/Finland/428/2014 (2013/14) and A/Finland/464/2014 (2014/15) were selected as representative of circulating Finnish A(H3N2) viruses for groups 3C.3, 3C.3a and 3C.2a, respectively.

The assay was performed as previously described [6] using erythrocytes from turkeys for A(H1N1)pdm09 viruses and guinea pigs for A(H3N2) and influenza B viruses. A(H3N2) viruses were assayed in the presence of 20 nM oseltamivir carboxylate (Roche). For statistical analyses, serum specimens with HI titres <10 were assigned a titre value of 5. We calculated the geometric mean titres (GMT) with 95% confidence intervals and presumable seroprotection rate (using the commonly accepted European Medicines Agency criteria [7]: HI titre $\geq 1:40$) for each virus. Statistical significance of

Figure 1

Phylogenetic analysis of the haemagglutinin sequences of influenza A(H3N2) viruses from Finnish surveillance data during two epidemic seasons, 2013/14 and 2014/15



All sequences included in the phylogenetic tree were 1,650 nucleotides long. The tree was constructed using the neighbour-joining method with Mega software version 5.1 and with 1,000 bootstrap replicates. Bootstrapping values ≥ 60 are shown. The viruses used in serological tests are in bold blue. In addition, the vaccine viruses for northern and southern hemisphere are shown in boxes. Arrowed lines represent the location of amino acid substitutions.

Table 2

Geometric mean titres against influenza A(H1N1)pdm09, A(H3N2) and B viral strains measured by haemagglutination inhibition test before and after vaccination of 79 healthcare workers with 2013/14 trivalent influenza vaccine, Finland

Influenza virus strain	Group	Geometric mean titres (95% CIs)		
		Day 0 n=79	Day 21 n=77	Day 180 n=72
A(H1N1)pdm09				
A/California/07/2009[a]	1	31.5 (26.0–40.4)	63.9 (51.9–74.5)	38.8 (32.3–48.6)
A/Finland/420/2014	6B	34.8 (29.6–50.4)	85.3 (68.8–105.5)	45.9 (37.4–61.7)
A(H3N2)[b]				
A/Texas/50/2012[a]	3C.1	33.2 (26.5–41.0)	70.3 (57.8–86.0)	50.7 (39.7–60.4)
A/Finland/385/2013	3C.3	25.3 (20.7–31.0)	46.5 (38.1–56.0)	26.8 (21.6–32.4)
A/Switzerland/9715293/2013[c]	3C.3a	11.4 (8.9–14.3)	19.5 (15.1–26.4)	12.1 (9.6–16.0)
A/Finland/428/2014	3C.3a	8.6 (7.3–10.0)	13.7 (11.7–17.8)	8.7 (7.5–10.2)
A/Finland/464/2014	3C.2a	7.6 (6.7–8.7)	12.3 (10.8–15.0)	9.3 (8.1–10.7)
B (Yamagata)				
B/Massachusetts/02/2012[a]	Clade 2	19.8 (16.3–24.5)	37.1 (31.2–44.3)	30.3 (24.7–37.1)
B/Wisconsin/01/2010[d]	Clade 3	19.1 (15.8–23.2)	34.0 (28.4–40.7)	30.0 (25.1–35.7)

CI: confidence interval.

One dose of non-adjuvanted trivalent 2013/14 seasonal influenza vaccine was administered intramuscularly to Finnish healthcare workers. Day 0 refers to serum samples collected before vaccination.

[a] Vaccine strain, northern hemisphere season 2013/14 and 2014/15.

[b] Haemagglutination inhibition test with 20nM oseltamivir carboxylate (Roche).

[c] Vaccine strain, southern hemisphere season 2015.

[d] Vaccine strain, northern hemisphere season 2012/13.

differences was estimated using Student's t-test (paired, two-tailed), with a significance level of $p < 0.05$.

For all virus strains tested, there was a significant ($p < 0.01$) increase in the GMTs of the antibody response three weeks after TIV vaccination (Table 2). At six months, the GMTs decreased by 39.4–46.2%, 24.4–42.3% and 11.9–18.4% for influenza A(H1N1)pdm09, A(H3N2) and B viruses, respectively. The decrease was significant ($p < 0.05$ to $p < 0.001$) for both types influenza A viruses.

The baseline seroprotection rate for A(H1N1)pdm09 viruses was 57.0–58.2% (Figure 2). Three weeks after vaccination, the GMTs were higher for the recently circulating A/Finland/420/2014 strain than for the vaccine strain ($p < 0.05$). Post-vaccination seroprotection rates were 89.6% and 85.7% for A/Finland/420/2014 and A/California/07/2009 viruses, respectively.

The seroprotection rate for A(H3N2) vaccine virus A/Texas/50/2012 was 60.8% before vaccination and 87.0% three weeks after it. Three weeks post-vaccination, the GMTs were somewhat weaker for A/Finland/385/2013, a Finnish representative of group 3C.3 virus strains, than to the vaccine strain ($p < 0.01$). Significantly lower GMTs were detected for the group 3C.3a strain A/Switzerland/9715293/2013 as well as the Finnish group 3C.3a strain A/Finland/428/2014 and recently circulating group 3C.2a strain A/Finland/464/2014 compared with the vaccine strain ($p < 0.0001$).

For drifted Finnish 3C.3a and 3C.2a viruses, baseline seroprotection rates were low (8.9% and 1.3%, respectively) and fivefold reductions in GMTs (for both) were detected three weeks after vaccination, compared with the vaccine strain. The reduction in GMTs for A/Finland/428/2014 (group 3C.3a) was in line with recently reported HI and neutralisation levels [8,9].

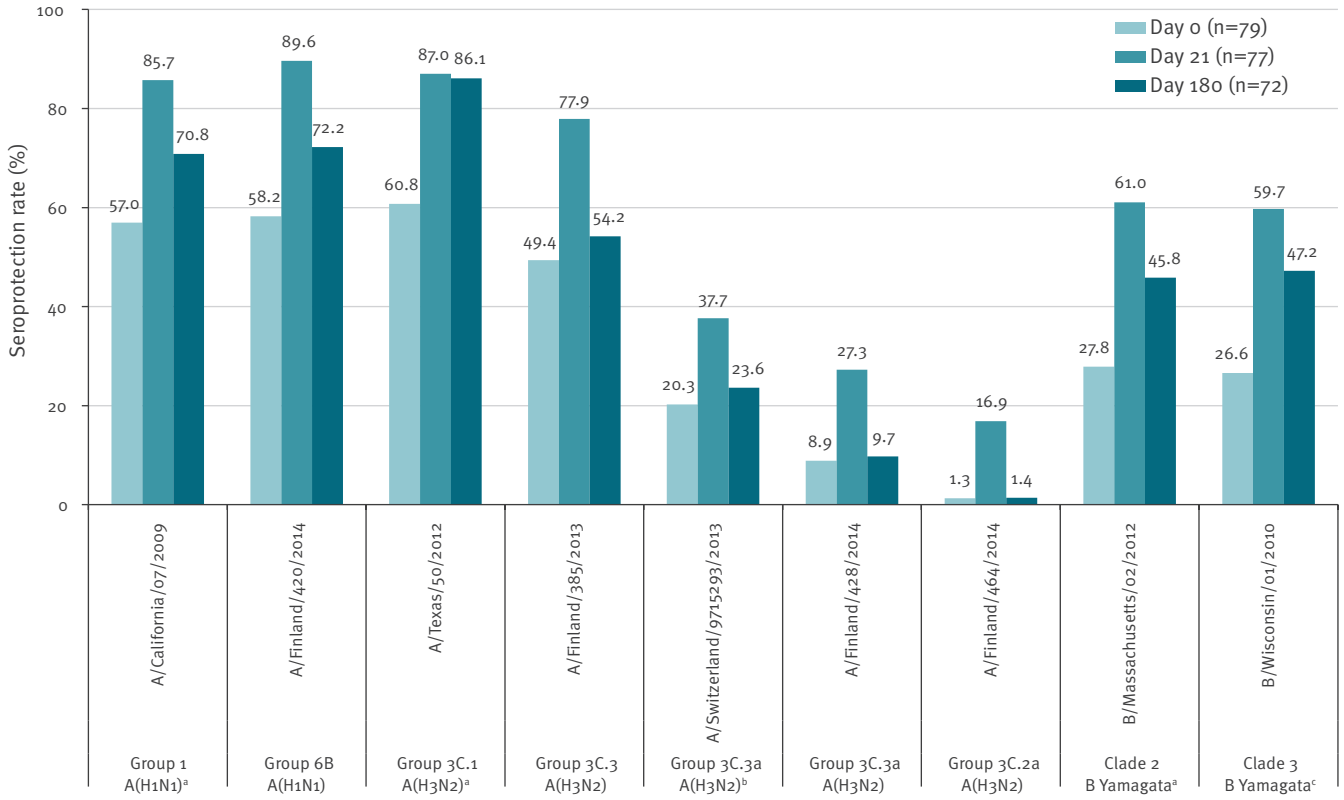
The baseline seroprotection rate for influenza B viruses was 26.6–27.8%. Three weeks after vaccination, very similar seroprotection rates were seen for vaccine strains B/Massachusetts/2/12 (61.0%) and B/Wisconsin/01/2010 (59.7%), which both represent the Yamagata-lineage viruses but belong to different clades.

Discussion

During the 2013/14 season, the relative prevalence of A(H1N1)pdm09, A(H3N2) and B influenza viruses varied from one European country to another [10]. In Finland, A(H1N1)pdm09 viruses predominated but A(H3N2) viruses were also detected. Most of the circulating A(H1N1)pdm09 and A(H3N2) viruses corresponded well with those included in the seasonal TIV vaccine for that season. Viruses from patients requiring intensive care

Figure 2

Seroprotection rates determined by haemagglutination inhibition test before and after vaccination of 79 healthcare workers with 2013/14 trivalent influenza vaccine, Finland



Seroprotection rate was defined as the percentage of participants with a haemagglutination inhibition titre ≥ 40. Day 0 refers to serum samples collected before vaccination.

[a] Vaccine strain, northern hemisphere season 2013/14 and 2014/15.

[b] Vaccine strain, southern hemisphere season 2015.

[c] Vaccine strain, northern hemisphere season 2012/13.

were not proven genetically different from other circulating viruses [11].

Representatives of influenza A(H3N2) groups 3C.2 and 3C.3 were found in Europe in the 2013/14 season and since February 2014, two new genetic subgroups, 3C.2a and 3C.3a, emerged in these clusters [8,10]. Both of these genetic subgroups contain viruses that show antigenic drift from the vaccine virus [1]. In Finland, infections caused by A(H3N2) genetic subgroup 3C.3a viruses were detected between February and April 2014. Genetic subgroup 3C.2a viruses, in contrast, did not circulate in Finland during the 2013/14 season but only emerged in the 2014/15 season.

Drifted influenza A(H3N2) viruses have been circulating in the countries of the European Union and European Economic Area in the 2014/15 season. The majority of genetically characterised viruses belong to group 3C.2a although 3C.3a viruses have also been detected [1]. In the United States (US), the Centers for Disease Control and Prevention has issued a health advisory notice regarding the circulation of drifted influenza A(H3N2) viruses in the US [12]. Early estimates of the current seasonal influenza vaccine effectiveness from the US and Canada suggest low effectiveness against circulating A(H3N2) viruses [13,14].

New antigenic A(H3N2) clusters appear on average every 3.3 years [15]. Seven amino acid locations have been shown to be responsible for the major antigenic changes in A(H3N2) viruses [16]. Subgroup 3C.2a and 3C.3a viruses carry specific amino acid substitutions that drifted from the corresponding main groups. Both subgroups have a substitution at position 159, which has shown to be one of seven positions responsible for the major antigenic changes between 1968 and 2003 A(H3N2) viruses [16].

In our analysis of antibody response, GMTs against the circulating A/Finland/428/2014 virus (a group 3C.3a A(H3N2) virus) were found to be significantly lower

than GMTs against the homologous A/Texas/50/2012 vaccine virus. These results are in line with those from Finnish A(H3N2) variant strains tested in WHO Collaborating Centre for Reference and Research on Influenza, in London, United Kingdom, using HI and virus neutralisation assays [9] and previous serological studies [8]. The pre-vaccination seroprotection rate of the HCWs we tested for this virus variant was only less than 10%. Even at three weeks after vaccination, the cross-protection rate was only less than 30% and decreased to less than 10% within 6 months.

The GMTs were found to be significantly lower against the currently circulating subgroup 3C.2a A(H3N2) virus A/Finland/464/2014 than against the homologous A/Texas/50/2012 vaccine virus. Only one of the 79 HCWs tested had pre-existing seroprotective antibody levels against this virus variant. Three weeks after vaccination, the cross-protection rate was 16.9% and decreased to less than 2% within six months. Subgroup 3C.2a viruses have also shown to have poor reactivity with post-infection ferret antisera against vaccine virus A/Texas/50/2012 [17].

Although influenza A(H1N1)pdm09 viruses have undergone genetic changes from the A/California/07/2009 strains present in the vaccine, the majority of epidemic viruses in Europe have been antigenically similar to the vaccine virus [2,10]. Our serological results indicate a strong vaccine-induced seroprotection rate against A(H1N1)pdm09 viruses. Consistent with this, more than half of the Finnish HCWs tested had pre-existing immunity against A(H1N1)pdm09 viruses. This may be due to the history of sequential TIV vaccinations in the study group or natural infections by A(H1N1)pdm09 viruses.

We acknowledge at least a few limitations in our serological analysis. First, the number of HCWs included in the study was limited. Secondly, the HCWs we tested did not represent all age groups: thus the results do not necessarily apply to children or elderly individuals. Antibody responses to influenza A(H1N1)pdm09 vaccination are age dependent [18] and low vaccine effectiveness against A(H3N2) has been reported among elderly persons [19]. For influenza B viruses, the overall impact of lineage-level mismatch between vaccine and circulating strains has been shown to be considerable, especially among children and adolescents [20]. Thirdly, HCWs are often vaccinated more regularly than others (in Finland, influenza vaccination is recommended for all HCWs who come in contact with patients) and they are also at higher risk of contracting influenza virus. The impact of repeated vaccination on vaccine effectiveness against influenza is still under investigation and discussion [21,22].

In conclusion, our serological data suggest that although the 2013/14 and 2014/15 TIV would protect against A(H1N1)pdm09 viruses, the protection against influenza A(H3N2) 3C.2a and 3C.3a virus variants would be suboptimal. The current epidemic situation in the northern hemisphere underlines the need to change the A(H3N2) component of the 2015/16 vaccine to a virus that represents one of the drifted groups. With minimal pre-existing immunity and a limited cross-protective effect from the TIV, the population in the northern hemisphere may be more susceptible to the new influenza A(H3N2) virus variants during the current 2014/15 season. However, influenza vaccination is strongly encouraged for HCWs, as well as for persons in risk groups, to reduce influenza disease burden and the spread of the epidemics.

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Conflicts of interest

None declared.

Authors' contributions

All authors have contributed to, seen and approved the manuscript. AH performed the serological data analysis and wrote the draft manuscript. NI made the genetic characterisations and participated in the writing of the manuscript. IJ, ER, AK and VJA were involved in the design of TIV vaccination study and sera collections. OL and HN provided their comments and participated in discussions. CSK was responsible for the viral laboratory facility and participated in the writing of the manuscript.

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