

Low vaccine effectiveness against influenza A(H3N2) virus among elderly people in denmark in 2012/13 – a rapid epidemiological and virological assessment

K Bragstad^{1,2}, H D Emborg^{2,3}, T K Fischer¹, M Voldstedlund³, S Gubbels³, B Andersen¹, K Mølbak³, T G Krause (TGV@ssi.dk)³

1. National Influenza Centre, Department of Microbiological Diagnostics and Virology, Statens Serum Institut, Copenhagen

2. These authors contributed equally to the work and share first authorship

3. Department of Infectious Disease Epidemiology, Statens Serum Institut, Copenhagen

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In Denmark, the 2012/13 influenza season has been dominated by influenza A(H3N2). We estimated the vaccine effectiveness (VE) of the trivalent influenza vaccine by linking national registers in a test-negative case-control study of patients tested for influenza aged ≥ 65 years. The adjusted VE against laboratory-confirmed influenza A and B was -11% (95% CI: -41 to 14) and 69% (95% CI: 26 to 87), respectively. Genetic characterisation of the influenza A(H3N2) viruses indicated genetic drift, with seven substitutions at key antigenic sites.

Background

In Denmark, consultation rates for influenza-like illness and the number of patients testing positive for influenza increased in week 51 (starting 17 December) and peaked in week 52 (starting 24 December) 2012. Compared with the previous three influenza seasons, the activity was particularly high among people aged ≥ 65 years. From week 51, excess mortality was observed in this age group [1]. Influenza A(H3N2) virus has to date been the dominant subtype.

Trivalent influenza vaccine (TIV) was offered free of charge to Danish citizens aged >65 years between weeks 40 and 52. We took advantage of unique national registries to obtain a rapid within-season estimate of influenza vaccine effectiveness (VE) in people of this age group. Furthermore, we describe the vaccination coverage among influenza A patients in intensive care units (ICUs), and characterised circulating influenza A(H3N2) viruses genetically.

Estimating vaccine effectiveness against laboratory-confirmed influenza

Information on patients aged ≥ 65 years tested for influenza A and B virus by PCR was obtained from the Danish Microbiology Database; information on administered TIV from weeks 39 to 48 was obtained from the Danish vaccination register [2,3]. The study period ran from week 40 (1 October 2012) to week 4 (27 January 2013).

The Danish Microbiology Database comprises real-time data on microbiological diagnostic test results for the entire Danish population. The Danish healthcare sector is public and all influenza testing is done according to national guidelines.

The VE against influenza A and B was estimated in a test-negative case-control design, using the formula $(1 - \text{odds ratio}) \times 100\%$.

Cases were defined as patients who tested positive for influenza A or B virus. Controls were patients who tested negative for both influenza A and B viruses. As our study is based on results of diagnostic samples, we do not have information on symptoms or indications for testing.

Influenza cases were included when they first tested positive. Patients were considered vaccinated if they received the TIV at least two weeks before the sample was taken; otherwise they were unvaccinated. In sensitivity analyses, the cut-off was changed to three weeks.

Data were analysed in SAS using PROC LOGISTIC. Adjustment was made for age group (65–69, 70–74, 75–80, ≥ 80 years) and place of sampling (general practitioner vs hospital).

Estimating vaccination coverage

Vaccination coverage among influenza A patients aged ≥ 65 years in all Danish ICUs was estimated by linking data from the national influenza ICU surveillance system [4] with that in the vaccination register.

Virus characterisation

Nucleic acid was extracted from 200 μl of clinical samples (sentinel, surveillance and diagnostic samples), full-length haemagglutinin (HA) genes were sequenced and analysed as described previously [5]. All samples

were initially screened for influenza A in a real-time reverse transcription (RT)-PCR reaction targeting the matrix gene [6]. If up to five samples were positive for influenza A(H3) within one week, all samples were set up for sequencing. If more than 5 samples were positive within one week, one sample from each of the five regions of Denmark with an influenza A real-time RT-PCR cycle threshold <35 was picked blindly for sequencing, if available.

Vaccine effectiveness results

A total of 1,443 patients aged ≥65 years were tested during the study period for influenza: 364 and 35 tested positive for influenza A and B, respectively (Table 1). Those who tested negative were considered as controls. Some 95% (1,374/1,443) of the patients were sampled at hospitals. Vaccination coverage increased with age among influenza A cases and controls, and the proportion of patients who tested positive for influenza A also generally increased with age (Table 1).

Vaccination coverage among the cases was 45%, which was similar to the coverage among the controls (41%), as well as the estimated national coverage among people aged ≥65 years (44%, data not shown). Most of the vaccinated study participants received TIV between weeks 40 to 44; cases of influenza A mainly occurred from week 52 onwards (Figure 1).

In the study population, VE against laboratory-confirmed influenza A was -11% (95% CI: -41 to 14), whereas VE against laboratory-confirmed influenza B was 69% (95% CI: 26 to 87) (Table 2).

In the main analysis, patients were considered vaccinated if they received the TIV at least two weeks before

FIGURE 1

Trivalent influenza vaccines received in week 39 (24 September 2012)–week 47 (25 November 2012) and laboratory-confirmed influenza A cases in week 40 (1 October 2012)–week 4 (27 January 2013) among 1,443 patients aged ≥65 years tested for influenza, Denmark

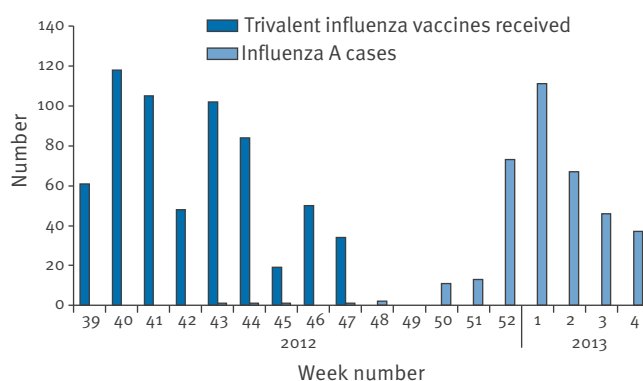


TABLE 1

Laboratory-confirmed influenza A and B cases (n=399) and influenza A and B test-negative controls (n=1,044) aged ≥65 years by trivalent influenza vaccination status, age group and sex, and vaccination coverage among influenza cases and controls by age group and sex, Denmark, week 40 (1 October 2012)–week 4 (27 January 2013)

Characteristic	Influenza A			Influenza B			Controls		
	Vaccinated (n)	Not vaccinated (n)	Vaccination coverage (%)	Vaccinated (n)	Not vaccinated (n)	Vaccination coverage (%)	Vaccinated (n)	Not vaccinated (n)	Vaccination coverage (%)
Age group									
65–69	24	51	32.0	2	11	15.4	117	211	35.7
70–74	26	44	37.1	2	4	33.3	92	153	37.6
75–79	43	41	51.2	1	4	20.0	90	111	44.8
≥80	72	63	53.3	2	9	18.2	129	141	47.8
Sex									
Male	81	108	42.9	3	16	15.8	231	326	41.5
Female	84	91	48.0	4	12	25.0	197	290	40.5
Total	165	199	45.3	7	28	20.0	428	616	41.0

^a Information on trivalent influenza vaccination was obtained for week 39 (24 September 2012) to week 47 (25 November 2012). Patients were considered vaccinated if they received the vaccine at least two weeks before the sample was taken; otherwise they were considered unvaccinated.

TABLE 2

Crude and adjusted odds ratios and adjusted vaccine effectiveness estimates against laboratory-confirmed influenza A and B among 1,443 patients aged ≥65 years and tested for influenza, Denmark, week 40 (1 October 2012)–week 4 (27 January 2013)

Influenza type	Crude odds ratio (95% CI)	Adjusted odds ratio ^a (95% CI)	Adjusted vaccine effectiveness ^a (95% CI)
A	1.19 (0.94 to 1.52)	1.11 (0.86 to 1.41)	-11% (-41 to 14)
B	0.36 (0.16 to 0.83)	0.31 (0.13 to 0.74)	69% (26 to 87)

^a Adjusted for age and place of sampling (general practitioner vs hospital).

the sample was taken. In sensitivity analyses, the cut-off was changed to three weeks, which did not change the estimates.

Vaccination coverage among intensive care unit patients

A total of 53 influenza A patients in ICUs were reported during the study period, of whom at least 22 were vaccinated with TIV at least two weeks before admission. Of the 53 ICU patients, 33 were aged ≥ 65 years. Samples from 16 of these elderly patients were subtyped: all contained influenza A(H3N2) virus. Of 32 elderly patients with known vaccination status, 15 were vaccinated at least two weeks before admission (47%). Nine were aged 65–69 years, seven were 70–74 years, six were 75–79 years and 10 were ≥ 80 years.

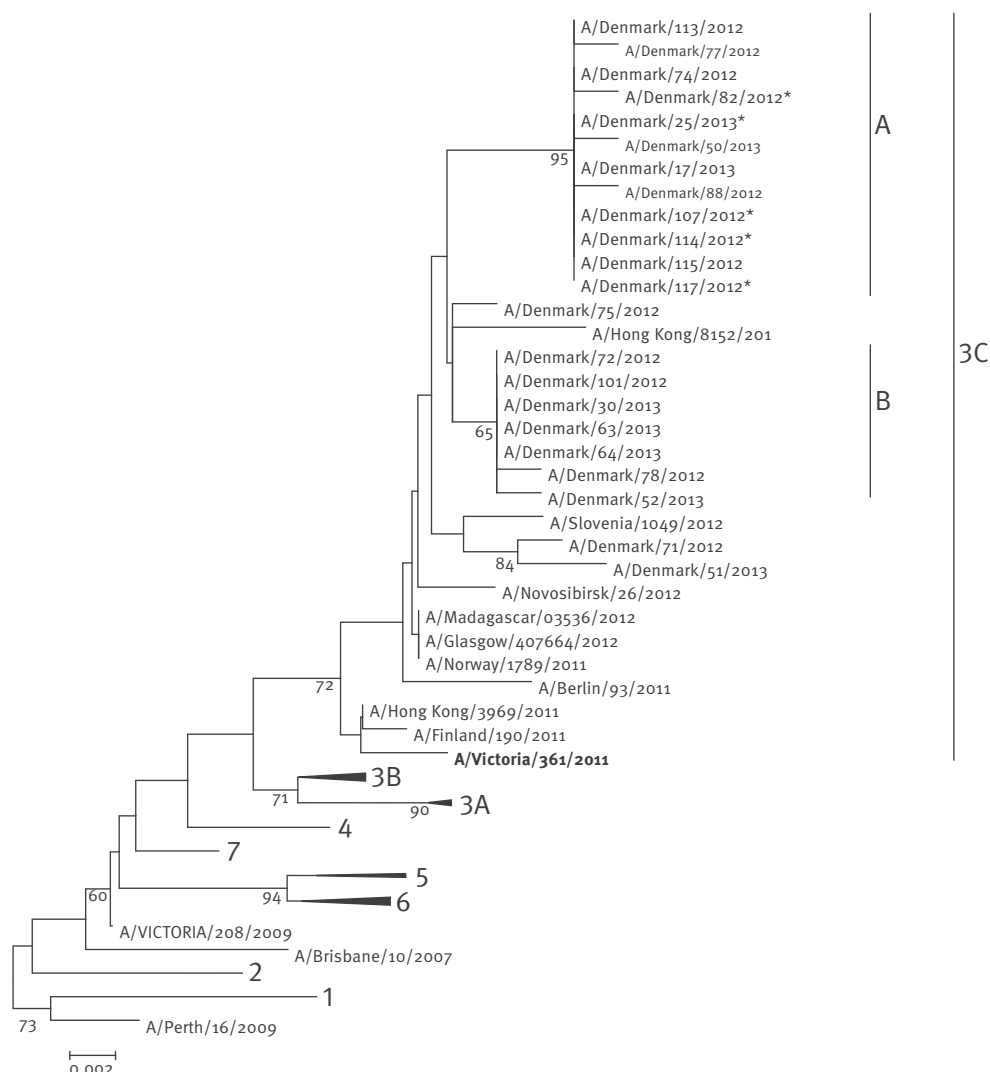
Information on comorbidity was available for 22 of the elderly patients: the two most common comorbidities were cardiovascular and chronic pulmonary disease, reported for 17 of the 22 patients.

Virus characterisation results

The National Influenza Centre in Denmark, Statens Serum Institut, subtyped 487 clinical specimens (those positive for influenza A or B) from week 40 (1 October 2012) to week 4 (27 January 2013). Of these, 395 (81%) were A(H3N2) viruses, 59 (12%) B-Yamagata viruses, 30 (6%) A(H1N1)pdm09 and 3 (<1%) B-Victoria. Based on HA amino acid sequence analysis, the Danish A(H3N2) viruses formed two main clades, A and B (Figure 2). Clade B is within the defined 3C A(H3) phylogenetic clade, where the 2012/13 A(H3N2) vaccine component

FIGURE 2

Phylogenetic tree of 22 influenza A(H3N2) virus sequences coding for 523 amino acids of the viral haemagglutinin, Denmark, week 46 (14 November 2012)–week 2 (13 January 2013)



A distance-based neighbor-joining phylogenetic tree was generated using Molecular Evolutionary Genetics Analysis (MEGA) software v.5 [14] with 1,000 bootstrap replicates (values ≥ 60 shown on branch) and rooted to A/Perth/16/2009. The Danish isolates were collected countrywide. Reference A(H3) sequences (Table 3) were included for comparison.

Collapsed branches are given for all clades except for clade 3C, for simplicity. Clade designations are given at collapsed branch nodes or on the side of the clade for clade 3C. The influenza A(H3N2) vaccine component for the northern hemisphere influenza season 2012/13 is shown in bold. Sequences from samples from patients vaccinated before week 47 2012 are marked with an asterisk.

TABLE 3

Haemagglutinin sequences of influenza A(H3) virus isolates included in the phylogenetic analysis^a

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI165489	Australia	2007-Jan-01	A/Brisbane/10/2007		Other Database Import	
EPI272062	Australia	2009-Jun-02	A/VICTORIA/208/2009	Victorian Infectious Diseases Reference Laboratory	WHO Collaborating Centre for Reference and Research on Influenza	Deng Y-M, Iannello P, Caldwell N, Leang S-K, Komadina N
EPI379585	Australia	2012-May-09	A/VICTORIA/802/2012	Austin Health	WHO Collaborating Centre for Reference and Research on Influenza	Deng Y-M, Iannello P, Caldwell N, Jelley L, Komadina N
EPI349106	Australia	2011-Oct-24	A/Victoria/361/2011	Melbourne Pathology	WHO Collaborating Centre for Reference and Research on Influenza	Deng Y-M, Caldwell N, Iannello P, Komadina N
EPI377369	Russian Federation	2012-Apr-09	A/Novosibirsk/26/2012	WHO National Influenza Centre	National Institute for Medical Research	
EPI377291	Russian Federation	2012-Apr-19	A/Ekaterinburg/4/2012	WHO National Influenza Centre	National Institute for Medical Research	
EPI326139	Sweden	2011-Mar-28	A/Stockholm/18/2011	Swedish Institute for Infectious Disease Control	National Institute for Medical Research	
EPI302231	Norway	2010-Dec-03	A/Norway/1330/2010	WHO National Influenza Centre	National Institute for Medical Research	
EPI346595	Finland	2011-Nov-25	A/Finland/190/2011	National Institute for Health and Welfare	National Institute for Medical Research	
EPI392250	Ghana	2012-Jun-20	A/Ghana/ARI 1494/2012	University of Ghana	National Institute for Medical Research	
EPI392307	France	2012-Mar-08	A/Paris/972/2012	CRR virus Influenza region Sud	National Institute for Medical Research	
EPI392294	Madagascar	2012-Jun-12	A/Madagascar/03619/2012	Institut Pasteur de Madagascar	National Institute for Medical Research	
EPI392292	Madagascar	2012-Jun-04	A/Madagascar/03536/2012	Institut Pasteur de Madagascar	National Institute for Medical Research	
EPI319276	Madagascar	2011-Feb-21	A/Madagascar/0648/2011	Institut Pasteur de Madagascar	National Institute for Medical Research	
EPI377311	Estonia	2012-Mar-09	A/Estonia/66234/2012	Health Protection Inspectorate	National Institute for Medical Research	
EPI392272	Hong Kong (SAR)	2012-Jun-26	A/Hong Kong/8152/2012	Government Virus Unit	National Institute for Medical Research	
EPI331093	Hong Kong (SAR)	2011-May-19	A/Hong Kong/3969/2011	Government Virus Unit	National Institute for Medical Research	
EPI377386	Slovenia	2012-Apr-02	A/Slovenia/1049/2012	Laboratory for Virology, National Institute of Public Health	National Institute for Medical Research	
EPI379788	Iceland	2012-Apr-14	A/Iceland/24/2012	Landspítali - University Hospital	National Institute for Medical Research	
EPI358885	Greece	2012-Feb-01	A/Athens GR/112/2012	Institut Pasteur Hellenique	National Institute for Medical Research	
EPI379845	Ukraine	2012-Apr-01	A/Ukraine/5398/2012	Ministry of Health of Ukraine	National Institute for Medical Research	
EPI335714	Norway	2011-Aug-02	A/Norway/1789/2011	Norwegian Institute of Public Health	National Institute for Medical Research	
EPI326137	Norway	2011-Mar-16	A/Norway/1186/2011	Norwegian Institute of Public Health	National Institute for Medical Research	
EPI377330	United Kingdom	2012-Apr-03	A/Glasgow/407664/2012	Gart Naval General Hospital	National Institute for Medical Research	
EPI379766	Denmark	2012-Apr-21	A/Denmark/42/2012	Statens Serum Institut	National Institute for Medical Research	
EPI360950	Germany	2011-Jul-03	A/Berlin/93/2011	National Institute for Medical Research	Centers for Disease Control and Prevention	

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI211334	Australia	2009-Jan-01	A/Perth/16/2009	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention	
EPI278808	United States	2010-Jul-13	A/Alabama/05/2010	U.S. Air Force School of Aerospace Medicine	Centers for Disease Control and Prevention	
EPI335923	United States	2010-Dec-30	A/Iowa/19/2010	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
EPI376512	United States	2012-May-03	A/Minnesota/10/2012	Minnesota Department of Health	Centers for Disease Control and Prevention	
EPI416258	Denmark	2012-Dec-11	A/Denmark/82/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416257	Denmark	2012-Dec-01	A/Denmark/77/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416256	Denmark	2012-Nov-23	A/Denmark/74/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416255	Denmark	2013-Jan-03	A/Denmark/30/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416254	Denmark	2012-Dec-15	A/Denmark/115/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416253	Denmark	2012-Dec-28	A/Denmark/113/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416251	Denmark	2012-Dec-31	A/Denmark/88/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416250	Denmark	2012-Dec-05	A/Denmark/78/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416249	Denmark	2012-Nov-22	A/Denmark/75/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416248	Denmark	2013-Jan-02	A/Denmark/52/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416247	Denmark	2013-Jan-08	A/Denmark/51/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416246	Denmark	2013-Jan-13	A/Denmark/50/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416245	Denmark	2013-Jan-02	A/Denmark/25/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416244	Denmark	2013-Jan-01	A/Denmark/17/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416243	Denmark	2012-Dec-27	A/Denmark/117/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416242	Denmark	2012-Dec-19	A/Denmark/114/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416241	Denmark	2012-Dec-26	A/Denmark/107/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416240	Denmark	2012-Dec-30	A/Denmark/101/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI406237	Denmark	2012-Nov-08	A/Denmark/71/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI406235	Denmark	2012-Nov-14	A/Denmark/72/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K

^a From the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID).

(A/Victoria/361/2011) is assigned, but the Danish clade A should probably be assigned a new A(H3) clade. Five of the 22 Danish HA sequences included in the phylogenetic analysis, collected from week 46 (11 December 2012) to week 1 (2 January 2013), were from patients who had been vaccinated before week 47: all five were located in clade A (Figure 2). Altogether, seven amino acid substitutions in the HA defined the Danish clade A, compared with egg-grown/tissue grown A/Victoria/361/2011 – at antigenic site B: T128A, R/Q156H and V186G; site A: R142G and N145S; site C: N278K; and site D: S/Y219S. Additional substitutions were Q33R, E/D190D and V347M. The clade B viruses did not possess the substitutions at positions 128, 142 and 347. The clade A viruses have lost the 126 N-linked glycosylation seen in A/Victoria/361/2011 and clade B (predicted by NetNGlyc 1.0 server [7]). For comparison, we included reference HA sequences from influenza A(H3) viruses, obtained from the EpiFlu database of

the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 3).

Discussion

We took advantage of unique newly established national registries to obtain a rapid within-season VE estimate. In the current season, which to date has been dominated in Denmark by influenza A(H3N2) virus, we calculated an adjusted VE point estimate of –11% (95% CI: –41 to 14) against laboratory-confirmed influenza A in patients aged ≥65 years tested for influenza. Given the confidence interval, we cannot exclude a VE of up to 14%, which is still considerably lower than expected. By contrast, vaccination did protect against influenza B (adjusted VE point estimate of 69%). As the National Influenza Centre has subtyped 93% of the influenza A viruses in Denmark as influenza A (H3N2), we expect the poor VE against influenza A to be associated with this subtype. The poor VE against influenza A was supported by a comparable vaccination coverage among

ICU patients with influenza A and among Danish people in general in the same age group (≥ 65 years). We also identified genetic drift of circulating influenza A(H3N2) viruses, which may explain the low VE in elderly people (aged ≥ 65 years).

The study was observational, with the biases this study type may entail. We were unable to adjust for comorbidity, but we adjusted VE for age group – which was associated with both vaccination uptake and testing positive for influenza A – and place of sampling, which are both likely to be correlated with comorbidity. Although other test-negative VE studies have shown that adjustment for comorbidity only resulted in minor changes in the VE estimates [8], we cannot exclude the possibility that some residual confounding with comorbidity does exist; however, it is unlikely that the effect of confounding is of a magnitude that would dramatically change the estimates. Furthermore, the VE against influenza B, estimated on only a few cases, was comparable to early estimates from the United States Centers for Disease Control and Prevention [9]. It is possible that some cases and controls classified as unvaccinated had in fact been vaccinated after week 47. However, by way of comparison, in 2011, 95% of the vaccinations were given before week 48.

Our study design has a number of advantages. It was cost-effective because data were obtained from existing sources, and information on vaccination was registered prospectively and independently of outcome. The specificity of the outcome was high and independent of patient recall. The patients tested for influenza may differ from the general population of elderly people but the test-negative design reduces the risk of selection bias, since cases and controls presumably share the same health-seeking behaviours. As 95% of the samples were taken in a hospital setting, the study may indicate a poor protection against severe outcomes of influenza (requiring hospitalisation).

Elderly people who are tested for influenza may represent a more vulnerable group, who have a weaker response to vaccination compared with the elderly population in general; however, if this would explain the findings, we would also expect a low VE against influenza B.

Genetic characterisation revealed that the Danish clade A viruses may be the cause of the low VE observed in elderly people. Four or more substitutions in two or more antibody binding sites are predicted to give an antigenically different virus [10] – thus the seven differences described for the Danish isolates are a cause for concern. Key substitutions causing the genetic drift are at positions 128 and 142, which are rare substitutions. The A128 substitution causes loss of an N-linked glycosylation site and amino acid changes in the 140–146 region of HA antigenic site A is characteristic for antigenically distinct viruses of epidemic significance [11]. These two substitutions should therefore be

considered carefully when antigenic drift is investigated further.

In conclusion, our epidemiological data suggest a low VE against influenza A(H3N2) among elderly people in Denmark; this is in contrast with early VE estimates in the range of 45–55% reported from other studies including all age groups [8,9,12].

Our molecular investigations indicate genetic drift; however, antigenic data on the clade A viruses are needed to document antigenic drift. Therefore, virus neutralisation and haemagglutination inhibition assays are in progress, along with an investigation of the role of the neuraminidase, as the neuraminidase also plays an important role in the genetic and antigenic drift of influenza viruses [13].

Matching of the influenza A(H3N2) vaccine virus with circulating influenza strains needs further investigation but should be taken into consideration before the coming selection of influenza vaccine strains for the next season, 2013/14. Influenza vaccination is still considered useful among Danish citizens this season due to the high protection against influenza B.

Acknowledgements

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Conflict of interest

None declared.

Authors' contributions

K Bragstad: Conceived the idea for the study and made the genetic and antigenic characterisations, she drafted the first version of the paper, made revisions, and approved the final version of the paper. H-D Emborg: Cleaned the data from the microbiology database and the vaccination database and made the statistical analysis of VE. She contributed to the interpretation of the data and reviewed the first draft of the paper and approved the final version of the paper. M Voldstedlund: Has established the microbiology database and retrieved the influenza data from the database. She assisted in the data cleaning and interpretation of the results,

she reviewed the first draft of the paper and approved the final version of the paper. T K Fischer: Contributed with the interpretation of the virological and epidemiological data and revised the first draft of the paper critically and approved the final version of the paper. S Gubbels: Is in charge of the surveillance of influenza at ICUs in Denmark and contributed with the data on vaccination coverage among ICU patients. She contributed to the interpretation of the results and reviewed and approved the final draft of the paper. B Andersen: Contributed considerably in the laboratory, she approved the final version of the paper. K Mølbak: Has established the microbiology database, he conceived the idea and the design of the VE study and contributed to the interpretation of the results. He revised the first draft of the paper critically and approved the final version of the paper. T G Krause: Conceived the idea and the design of the VE study, she was involved in the data analysis and the interpretation of the results. She wrote the first draft of the paper together with K Bragstad, revised the paper and made the final draft of the paper.

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