Influenza vaccine effectiveness in adults 65 years and older, Denmark, 2015/16 – a rapid epidemiological and virological assessment

H Emborg 1 , TG Krause 1 , L Nielsen 2 , MK Thomsen 3 , CB Christiansen 4 , MN Skov 5 , XC Nielsen 6 , LS Weinreich 7 , TK Fischer 8 , J Rønn 8 , R Trebbien 8

1. Department of Infectious Disease Epidemiology, Statens Serum Institut, Copenhagen, Denmark
2. Department of Clinical Microbiology, Herlev Hospital, Herlev, Denmark
3. Department of Clinical Microbiology, Aarhus University Hospital, Aarhus, Denmark
4. Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark
5. Department of Clinical Microbiology, Odense University Hospital, Odense C, Denmark
6. Department of Clinical Microbiology, Slagelse Hospital, Slagelse, Denmark
7. Department of Clinical Microbiology, Aalborg University Hospital, Aalborg, Denmark
8. Department of Microbiological Diagnostics and Virology, National Influenza Center, Statens Serum Institut, Copenhagen, Denmark

Correspondence: Hanne-Dorthe Emborg (hde@ssi.dk)


In Denmark, both influenza A(H1N1)pdm09 and influenza B co-circulated in the 2015/16 season. We estimated the vaccine effectiveness (VE) of the trivalent influenza vaccine in patients 65 years and older using the test-negative case–control design. The adjusted VE against influenza A(H1N1)pdm09 was 35.0% (95% confidence interval (CI): 11.1–52.4) and against influenza B 4.1% (95% CI: −22.0 to 24.7). The majority of influenza A(H1N1)pdm09 circulating in 2015/16 belonged to the new genetic subgroup subclade 6B.1.

In Denmark, both influenza A(H1N1)pdm09 and influenza B co-circulated in the 2015/16 season. The trivalent influenza vaccine (TIV) did not include the circulating influenza B Victoria lineage and there is evidence in Europe for genetic evolution of the circulating influenza A(H1N1)pdm09 virus [1]. We estimated the influenza vaccine effectiveness (VE) in people aged 65 years and older. In addition, we describe the genetic and antigenic characteristics of the influenza A(H1N1)pdm09 variant and the influenza B strain circulating in Denmark.

In Denmark, both influenza A(H1N1)pdm09 and influenza B co-circulated in the 2015/16 season. The trivalent influenza vaccine (TIV) did not include the circulating influenza B Victoria lineage and there is evidence in Europe for genetic evolution of the circulating influenza A(H1N1)pdm09 virus [1]. We estimated the influenza vaccine effectiveness (VE) in people aged 65 years and older. In addition, we describe the genetic and antigenic characteristics of the influenza A(H1N1)pdm09 variant and the influenza B strain circulating in Denmark.

Data for vaccine effectiveness estimation

In the Danish Microbiology Database, all patients swabbed at the general practitioner's (GP) or at hospital and tested for influenza A and B viruses by PCR are registered in real time [2]. During the influenza season, national guidelines recommend that patients belonging to risk groups, including the elderly who present with influenza symptoms at GPs and hospitals are swabbed and tested for influenza. At hospitals, all patients with lower respiratory infections are also recommended to be swabbed. All diagnostic influenza tests from patients aged 65 years and older were included in this study.

Influenza symptoms were defined as sudden onset of fever, muscle ache and upper airway symptoms. The trivalent influenza vaccine (TIV) is offered free of charge to Danish citizens 65 and older between week 40 and week 53, and date of vaccination is registered in the Danish Vaccination Register [3]. In The Danish National Hospital Register, data on all hospital admissions are collected [4]. Comorbidities that can lead to severe influenza disease and were diagnosed between October 2010 and October 2015 were extracted from the Danish National Hospital Register.

Data from the Danish Microbiology Database, the Danish Vaccination Register and the Danish National Hospital Register were linked using unique identifiers.

Case definitions and statistical analysis

Cases were defined as patients who tested positive for influenza A(H1N1)pdm09 or influenza B, and a patient was only included the first time a test was positive for either type. Controls were patients who tested negative for both influenza A and B. Patients were considered vaccinated if they had received the TIV at least two weeks before the sample was taken. A logistic regression model was used to estimate VE against influenza A(H1N1)pdm09 and influenza B using the test-negative case–control design (1-OR) × 100%. The
estimates were adjusted for sex and co-morbidities diagnosed within a five-year period before the 2015/16 influenza season. Among 195 subtyped influenza A isolates from patients aged 65 years and older, less than 10% (n = 18) were A(H3N2) and VE against this subtype was not estimated.

The statistical programme SAS version 9.4 was used for the descriptive and statistical analyses (SAS Institute, Cary, United States).

**Influenza virus characterisation**

All influenza samples received at The National Influenza Center in Denmark (NIC) were screened for influenza virus by an in-house multiplex real-time reverse-transcriptase PCR (qRT-PCR), with primers and probes detecting influenza A and B virus as well as subtypes of H3 haemagglutinin (HA) and N1pdm09 neuraminidase. Subtyping of influenza B virus is also performed by an in-house duplex qRT-PCR which differentiates between the Yamagata and Victoria lineage on a fragment of the HA gene.

Sequencing of the HA gene of influenza A(H1N1)pdm09 and influenza B viruses was performed on extracted viral RNA from 62 and 20 samples, respectively. Total nucleic acid was extracted using 200 µl of sample material and the MagNA Pure LC Total Nucleic Acid Isolation Kit on the MagNa Pure 96/32 (Roche). RT-PCR of the complete HA gene was performed using in-house primers and an in-house one-step RT-PCR programme on a TRIO cycler (Biometra). Sequencing was performed by using Big Dye chemistry on an ABI3500 capillary sequencer (Thermo Fisher). Assembly of contigs was done in Bionumerics version 6.6 (Applied maths) and alignment and phylogenetic analysis were conducted with MEGA version 6 [5]. For alignment, the Muscle algorithm was used and phylogenetic trees were created by the maximum likelihood method using 1,000 bootstrap replicates. Sequences were also analysed by BLAST at NCBI GenBank, the Global Initiative on Sharing All Influenza Data (GISAID) and at the FLUSERVER [6]. The authors gratefully acknowledge the 59 originating and submitting laboratories who contributed sequences used in the phylogenetic analysis to GISAID (www.gisaid.org).

Virus isolation was successful for 32 influenza A(H1N1)pdm09 and 13 influenza B samples by standard procedures in confluent monolayers of MDCK and/or MDCK-SIAT cells [7]. Several samples were shipped in E-swab medium which is cytotoxic and therefore is challenging for virus isolation [8]. Antigenic characterisation was performed by HA inhibition (HAI) test [7] using reference ferret antiserum against A/California/07/2009 (H1N1pdm09), B/Brisbane/60/2008 (Victoria lineage) and B/Phuket/3073/2013 (Yamagata lineage) provided

---

**Figure 1**

Trivalent influenza vaccines received (n = 1,831) and laboratory-confirmed influenza A(H1N1)pdm09 and B cases among tested patients ≥ 65 years (n = 468), Denmark, 28 September 2015–9 March 2016

Influenza vaccines are given free of charge to the elderly 65 years and older from 1 October to 31 December. Due to delay in registration of vaccinations, data from week 53 were not available at the time this analysis was performed.

In weeks 40 to 53, between 0 and two influenza A(H1N1) and B cases were registered per week (not visible at presented range of the y-axis).
**Figure 2**

Phylogenetic tree of the haemagglutinin gene with reference viruses for the different phylogenetic clades of H1N1pdm09 influenza A viruses (n = 40)

The Danish viruses are indicated with a black circle. A subclade formed by viruses with the amino acid substitutions S101N, S179N and I233T, subclade 6B.1, is indicated as well as the subclade formed by viruses with the V560T, V605L, E508G and D518E substitutions, subclade 6B.2. The authors gratefully acknowledge the 59 originating and submitting laboratories who contributed sequences used in the phylogenetic analysis to GISAID (www.gisaid.org).

**Vaccine effectiveness results**

By 9 March 2016, 3,831 patients 65 years and older were tested for influenza A(H1N1)pdm09 and B, and 65% of them were swabbed at a hospital. In total, 177 patients were positive for influenza A(H1N1)pdm09 and 291 for influenza B. In total, 1,505 (82%) of 1,831 study participants had received the TIV before 2 November in 2015 (Figure 1).

Vaccine coverage in cases diagnosed with influenza A(H1N1)pdm09 was 37.8%, which is lower than the coverage in controls (48.3%), cases diagnosed with influenza B (46.4%) (Table) and the estimated national coverage of 44% (data not shown). The coverage, for both cases and controls, was higher among patients with comorbidities compared with patients without comorbidities (Table).

Adjusted interim VE among those aged 65 years and older against influenza A(H1N1)pdm09 was 35.0% (95% confidence interval (CI): 11.1–52.4) and against influenza B 4.1% (95% CI: −22.0 to 24.7).

**Virus characterisation results**

Full gene sequencing of the HA gene from 62 influenza A(H1N1)pdm09 samples revealed in 46 of them an amino acid substitution at position 179 (H1 complete open reading frame numbering) from serine to asparagine, which leads to a potential glycosylation site formed by positions 179–181 with the amino acid motif asparagine–glutamine–serine (NQS) (Table).

Additional substitutions were revealed at amino acid position S101N and I233T in the 46 samples having the S179N. Two of the patient samples had an additional substitution at H155Y. Nine samples had a different amino acid motif with substitutions at positions V169T, V190I, E508G and D518E.

Phylogenetic analysis revealed that all 62 sequenced HA genes of A(H1N1)pdm09 viruses belonged to genetic clade 6B (Figure 2), however, the 46 viruses with the S101N, S179N, and I233T substitutions formed their own subclade which now is categorised by the WHO as subclade 6B.1. In addition, the nine V169T, V190I, E508G and D518E viruses clustered together with the A/Minnesota/32/2015(H1N1pdm09) virus (Figure 2) and are now categorised as subclade 6B.2.

Of the 32 A(H1N1)pdm09 viruses isolated in cell culture, 25 belonged to subclade 6B.1, three belonged to subclade 6B.2, and four belonged to clade 6B. Antigenic characterisation showed all 32 virus isolates to be equally inhibited or inhibited to a lesser extent (two- to fourfold decrease in HAI titre), by ferret antisera against A/California/07/2009 (H1N1pdm09) compared with the A/California/07/2009 (H1N1pdm09) reference virus HAI titres.

Of 447 influenza B virus samples from all age groups received for the national influenza surveillance programme at NIC Denmark by mid-March 2016, 350 were subtyped; 307 (88%) belonged to the B-Victoria lineage and 43 (12%) belonged to the B-Yamagata lineage. The HA genes of 15 B-Victoria viruses were sequenced and all belonged to clade 1A, corresponding to the

**Table**

Laboratory-confirmed influenza A(H1N1)pdm09 and B cases (n = 468) and influenza A and B test-negative controls (n = 3,363) 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, 28 September 2015–9 March 2016

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Influenza A(H1N1)pdm09</th>
<th>Influenza B</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinated (n)</td>
<td>Not vaccinated (n)</td>
<td>Vaccination coverage (%)</td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65–69</td>
<td>16</td>
<td>42</td>
<td>27.6</td>
</tr>
<tr>
<td>70–74</td>
<td>20</td>
<td>29</td>
<td>40.8</td>
</tr>
<tr>
<td>75–79</td>
<td>18</td>
<td>22</td>
<td>45.0</td>
</tr>
<tr>
<td>≥80</td>
<td>13</td>
<td>17</td>
<td>43.3</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>34</td>
<td>30.6</td>
</tr>
<tr>
<td>Yes</td>
<td>52</td>
<td>76</td>
<td>40.6</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>45</td>
<td>38.4</td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>65</td>
<td>37.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>67</td>
<td>110</td>
<td>37.8</td>
</tr>
</tbody>
</table>

* Sex was not known for one person.
discuss the limitations of the present study.

Results

Influenza A(H1N1)pdm09

We estimated similar VE against influenza A(H1N1)pdm09 in season 2014/15 and 2015/16 in those aged 65 years and older in spite of the occurrence of the new subclade 6B.1. This is reassuring as the WHO recommendations for the influenza A(H1N1)pdm09 component in the 2016/17 vaccine for the northern hemisphere remained the same as in previous years, while the influenza B component changed from Yamagata to Victoria [16].

Acknowledgement

Test results for influenza virus were obtained from the Danish Microbiology Database (MiBa, http://miba.ssi.dk), which contains all electronic reports from departments of clinical microbiology in Denmark since 2010, and we acknowledge the collaboration with the MiBa Board of Representatives.

The authors gratefully acknowledge the 59 originating and submitting laboratories who contributed sequences used in the phylogenetic analysis to GISAID (www.gisaid.org).

We also acknowledge for the laboratory work, Dennis Jelsbak Schmidt and Bente Andersen, National Influenza Center Denmark, Statens Serum Institut, Copenhagen, Denmark.

Statens Serum Institut would also like to acknowledge the participation in the I-MOVE+ (Integrated Monitoring of Vaccines in Europe) project that has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement Nº 634446.

Conflict of interest

None declared.

Authors’ contributions

Hanne-Dorthe Emborg led the writing of the paper. Ramona Trebbien was responsible for the virological characterisation and Jesper Rønn for the laboratory work. Lene Nielsen, Marianne Kragh Thomsen, Claus Bohn Christiansen, Marianne Nielsine Skov, Xiaohui Chen Nielsen and Lenette Sandborg Weinreich performed the initial diagnostics of influenza positive samples. Tyra Grove Krause and Thea Kelsen Fischer conceptualised the study together with Hanne-Dorthe Emborg and Ramona Trebbien and discussed
the data and perspectives. All authors provided contributions to the paper and approved the final version.

References


License and copyright

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC BY 4.0) Licence. You may share and adapt the material, but must give appropriate credit to the source, provide a link to the licence, and indicate if changes were made.

This article is copyright of the authors, 2016.