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Effectiveness of maternal immunization with trivalent inactivated influenza vaccine in pregnant women and their infants

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Abstract

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Background. In randomized trials, it has been found that maternal influenza vaccination reduces influenza infections in both women and their infants. However, these trials have been performed in low-resource settings, and evidence from high-resource settings is limited.

Methods. Nested within a register-based cohort of all women giving birth in Denmark between 2010 and 2016 (n = 357,810 births), we conducted two case–control studies using a test-negative design of all pregnant women and their infants, respectively, tested for influenza virus with reverse transcriptase-polymerase chain reaction. Influenza virus-positive cases were matched (1:1) with influenza virus-negative controls for calendar time and (gestational or infant) age at testing. The effectiveness of maternal immunization with trivalent inactivated influenza vaccine was estimated from the odds ratios of vaccination among cases versus controls using logistic regression with adjustment for potential confounders.

Results. Among 313 pregnant women positive for influenza virus, 16 (5.1%) were vaccinated; by comparison, 34 (10.9%) pregnant women were vaccinated among 313 matched influenza virus-negative controls. The effectiveness of vaccination against laboratory-confirmed influenza infection in pregnant women was 63.9% (95% confidence interval [CI], 29.1 to 81.6). Among 460 infants positive for influenza virus, 23 (5.0%) were offspring of women vaccinated during pregnancy; by comparison, 52 (11.3%) infants were the offspring of women vaccinated during pregnancy among 460 matched influenza virus-negative controls. The effectiveness of maternal vaccination against laboratory-confirmed influenza infection in infants younger than 6 months of age was 56.8% (95% CI, 25.0 to 75.1).

Conclusions. Seasonal trivalent inactivated influenza vaccination in pregnancy was associated with a statistically significant reduced risk of laboratory-confirmed influenza infections in pregnant women and their infants in a high-resource setting.

Keywords: human influenza, pregnancy, treatment effectiveness, trivalent influenza vaccines.

Introduction

Pregnant women are considered at increased risk of influenza-related complications, and young children have a high burden of seasonal influenza [1, 2]. The World Health Organization recommends seasonal influenza vaccination during pregnancy to reduce infections in both women and their infants, as no vaccines are licensed for use before the age of 6 months [3]. However, there are gaps in the evidence supporting maternal immunization for the protection of women and their infants against influenza infections.

Immunomodulation that occurs during pregnancy with attenuation of cell-mediated immunity [4] may lead to a decreased immune response to influenza vaccines in pregnant women. In randomized controlled trials,
trivalent inactivated influenza vaccines during pregnancy reduced laboratory-confirmed influenza infections in women and their infants; vaccine efficacy ranged from 50% to 77% [5, 6] and from 30% to 63% [5-8], but how this translates into real-world settings is not clear. Additionally, these trials were conducted in Bangladesh, Nepal, Mali and South Africa, thus primarily in low-resource tropical climate settings, where the influenza seasonality and overall health may limit the generalizability of the results to high-resource temperate climate settings in Europe and North America.

Few observational studies, primarily from different areas of the USA, have assessed the effectiveness of seasonal trivalent influenza vaccination during pregnancy in preventing laboratory-confirmed influenza infections in women and their infants [9-14]. In an observational study from the USA from 2010 to 2012, maternal vaccination effectiveness was 44% in pregnant women [13]. In a multinational study (including four countries) from 2010 to 2016, vaccination effectiveness was 40% against influenza-associated hospitalization in pregnant women [14]. In four observational studies, all from the USA and mainly based on data from before the 2009 influenza A(pdm09H1N1) pandemic, the effectiveness of maternal vaccination ranged from 41% to 92% in their infants [9-12].

We conducted a nationwide observational study of maternal influenza vaccination effectiveness in a high-resource temperate climate setting. Nested in a cohort of all women giving birth in Denmark during the period 2010–2016, we evaluated the effectiveness of trivalent inactivated influenza vaccines administered during pregnancy against laboratory-confirmed influenza infections in pregnant women and their infants during consecutive influenza seasons from 2010 to 2017.

Methods

Nationwide cohort

The Medical Birth Registry contains records of all Danish births, including the unique personal identification numbers of the mother and infant, date of delivery and gestational age in days [15]. We identified a cohort of all women giving birth in Denmark from 1 October 2010 to 31 December 2016, excluding births (i) with missing or implausible record of gestational age, (ii) where mothers had not resided in Denmark throughout pregnancy (for complete capture of vaccination status), (iii) with stillborn offspring and (iv) with multiple offspring (Fig. 1). Pregnancy onset was estimated by subtracting gestational age, primarily based on ultrasonography [16], from the date of delivery. Information on maternal influenza vaccination was obtained from the Danish National Health Service Registry, which includes reimbursement reports of
influenza vaccines administered by general practitioners with date of vaccine administration [17]. Information on selected potential confounders was obtained from The Central Person Registry [18], the National Patient Registry [19], the National Prescription Registry [20], Statistics Denmark, the Medical Birth Registry [15] and the Danish National Health Service Registry [17] (see Supplementary Material for further details).

The study was approved by the Danish Data Protection Agency and representatives of the board of the Danish Microbiology Database. Informed consent is not required for register-based research in Denmark.

Influenza virus testing and definition of cases and controls

The Danish Microbiology Database contains nationwide records from January 2010 on all microbiological diagnostic tests, including influenza virus tests requested by general practitioners or hospital physicians, with the sample date [21]. The Danish national guidelines for influenza testing recommend that patients belonging to high-risk groups (including pregnant women) presenting with influenza-like illness at general practice or hospital have a throat swab and are tested for influenza virus during the influenza season. Recommendations for testing further include all hospitalized patients with lower respiratory tract infections.

We identified all women in the cohort tested for influenza A or B virus during pregnancy and all live born singleton infants tested for influenza A or B virus. All swab specimens were tested for influenza virus with the use of real-time reverse transcriptase polymerase chain reaction. For some samples, subtype of influenza virus A was also identified. We included only one influenza test for each woman and for each infant; either the first positive influenza test or the first negative influenza test for those with more than one test and no positive test results. Pregnant women or their infants who tested positive for influenza A or B were designated as cases and those who tested negative were designated as controls.

Seasonal influenza vaccination

The Danish Health Authority originally recommended seasonal influenza vaccination for pregnant women with selected high-risk chronic diseases in any trimester; since 2010, vaccination is additionally recommended for all pregnant women in the second and third trimesters. The vaccination programme runs from 1 October to 28 February the following year for pregnant women and vaccination is free of charge.

The study period included consecutive influenza seasons from 2010 to 2017 where only trivalent inactivated split influenza virus vaccines containing two influenza A and one B strain (as recommended by the World Health Organization) were administered (Supplementary Table 1 shows the virus strains contained in the influenza vaccines).
Maternal vaccination was defined by receipt of influenza vaccine during pregnancy. Women were considered vaccinated 14 days after the vaccination date to allow for an immune response to occur; otherwise mothers were not considered vaccinated. Infants born to mothers in the 14-day period following vaccination were not considered vaccinated.

**Statistical analysis**

The fact that circulation of influenza viruses and uptake of influenza vaccines are both strongly seasonal (and in our study vaccine uptake among pregnant women also depends on gestational age) has implications for the design and analyses of a test-negative study [22]. Therefore, we took several precautions to limit bias due to these circumstances when estimating vaccine effectiveness in pregnant women and their infants. First, we excluded pregnant women and infants tested outside the influenza season (defined as calendar week 40 to 20 the following year). Secondly, among pregnant women, we matched virus-positive cases with virus-negative controls 1:1 for trimester of pregnancy at the time of testing and the closest sample date within the same season. We used the optimal pair matching function in R version 3.5.0 [23], which matches pairs minimizing the mean pairwise distance between sample dates for cases and controls. Likewise, among infants, we matched cases with controls 1:1 for infant age in 2-month intervals at the time of testing and the closest sample date within the same season. Among pregnant women, there were fewer controls than cases in one season, and we randomly selected a subset of cases to ensure at least one control for each case. Furthermore, in two seasons with insufficient numbers of controls tested in each trimester of pregnancy, we did not match for trimester at testing.

Influenza vaccine effectiveness was estimated in two case–control studies (one for pregnant women and one for infants) using a test-negative design, which compared the odds of vaccination among influenza virus-positive cases to the odds of vaccination among influenza virus-negative controls; the odds ratio was estimated using unconditional logistic regression. Vaccine effectiveness, expressed as a percentage, was calculated using the formula $(1-\text{odds ratio}) \times 100$ and reflects the relative difference in influenza risk among vaccinated compared to unvaccinated individuals. The regression models included the influenza season of test, the period of the influenza season (early, middle or late) of test, trimester of pregnancy (in the study of pregnant women)/infant age at testing (in the study of infants) and maternal characteristics (selected a priori and based on information available in registries and the potential for association with both vaccination and influenza infection). The maternal characteristics included calendar time at pregnancy onset, age, country of birth, region of residence, marital status, parity, educational level, household income, body mass index, smoking status, primary and secondary healthcare utilization, number of prescription drugs and medical conditions that increase the risk of severe influenza disease (Supplementary...
Table 2). SAS version 9.4 was used for all analyses with the exception of optimal matching which was conducted in R. Odds ratios were considered statistically significant when the 95% confidence intervals (CIs) did not overlap 1.0 and \( P < 0.05 \) (two-sided test). In the primary analyses, we estimated vaccine effectiveness for any influenza disease (A and B) during pregnancy and from birth and until 6 months of age in infants. In secondary analyses, we estimated vaccine effectiveness according to influenza subtype. We also estimated vaccine effectiveness separately for each of the influenza seasons in the study period. We conducted a number of preplanned sensitivity analyses. We estimated vaccine effectiveness (i) including only hospitalized cases (using the case definition of influenza-associated hospitalization according to the Center for Disease Control and Prevention: hospital admission within 4 days before the date of a positive test and until 2 weeks after [24]), (ii) including women tested postpartum (within the first 6 months after delivery), (iii) including infants tested after 6 months of age in the analyses of infants, (iv) excluding women vaccinated within 14 days of the sample date (analyses of pregnant women) or infants of mothers vaccinated within 14 days of delivery (infant analyses), (v) without matching influenza-negative controls, (vi) with case–control matching for influenza season of test, period of the season (early, middle or late) of test and (gestational or infant) age in days at testing, (vii) with no adjustment for maternal characteristics and (viii) with logistic regression conditional on the matched case–control pairs as strata (with no adjustment for matching criteria).

Results

In this nationwide study cohort of 350,888 women giving birth to 357,810 infants (35,577 women were vaccinated during pregnancy [10.1%]), 954 (0.3%) of the women were tested for influenza virus during pregnancy; 89 women were tested outside the influenza season and were excluded from analyses. Among 342,785 singleton livebirths, 7674 (2.2%) infants were tested for influenza virus in the first 6 months of life; 886 were tested outside the influenza season and were excluded from analyses (Fig. 1). The mean (±SD) gestational age at testing for pregnant women was 177 (±73.6) and 171 (±76.5) days for cases and matched controls, respectively. In addition, the mean infant (±SD) age at testing was 80 (±44.0) and 78 (±44.6) days for cases and matched controls, respectively (Table 1, Supplementary Table 3 and Supplementary Table 4). All infants tested during an influenza season were born to mothers who received vaccination within the same season.

Pregnant women

A total of 313 women tested positive for influenza A or B virus, 16 (5.1%) of whom were vaccinated; by comparison, 34 (10.9%) women were vaccinated among the 313 matched controls negative for influenza A
and B. After adjustment for potential confounders, the estimated vaccine effectiveness against laboratory-confirmed influenza infection in pregnancy was 63.9% (95% CI, 29.1 to 81.6; Fig. 2). Analyses according to virus subtype reduced the statistical power and statistically significant vaccine effectiveness was observed for influenza virus subtypes A (vaccine effectiveness, 72.1%; 95% CI, 33.9 to 88.2) and A(H1N1) (84.6%; 95% CI, 28.6 to 96.7), but larger sample sizes are required to demonstrate lower vaccine effectiveness for subtypes A(H3N2) (8.3%; 95% CI, -177.8 to 69.7) and B (42.5%; 95% CI, -43.7 to 77.0). Likewise, stratifying estimates by season also reduced the statistical power and statistically significant vaccine effectiveness was only observed in the 2010–2011 season (Supplementary Fig. 1). The analysis restricted to hospitalized cases (124 of 313) was based on small numbers and the estimated vaccine effectiveness was 29.6% (95% CI, -112.6 to 76.7). All other sensitivity analyses yielded robust effectiveness estimates comparable to the main analysis (Fig. 3).

Infants

A total of 460 infants tested positive for influenza A or B virus, of whom 23 (5.0%) were the offspring of women vaccinated during pregnancy; by comparison, 52 (11.3%) infants were the offspring of women vaccinated during pregnancy among the 460 matched controls negative for influenza A and B. After adjustment for potential confounders, the estimated maternal vaccine effectiveness against laboratory-confirmed influenza infection in infants younger than 6 months of age was 56.8% (95% CI, 25.0 to 75.1; Fig. 2). Statistically significant vaccine effectiveness was observed for influenza virus subtypes A (vaccine effectiveness, 45.9; 95% CI, 3.6 to 69.7), A(H1N1) (95.2%; 95% CI, 60.1 to 99.4) and B (86.6%; 95% CI, 35.5 to 96.8), but analyses according to virus subtype reduced the statistical power and a larger sample size is required to demonstrate lower vaccine effectiveness for virus subtype A(H3N2) (-11.3%; 95% CI, -150.8 to 50.7). Likewise, stratifying estimates by season reduced the statistical power and significant vaccine effectiveness was only observed in the 2015–2016 season (Supplementary Fig. 2). When the analysis was restricted to hospitalized cases (357 of 460), vaccine effectiveness was 61.4% (95% CI, 26.0 to 79.9). In the analyses including infants older than 6 months of age, we observed no statistically significant vaccine effectiveness against laboratory-confirmed influenza infection among infants aged 6 to 12 months (vaccine effectiveness, -16.8%; 95% CI, -117.0 to 37.2) and 12 to 18 months (-5.6%; 95% CI, -110.7 to 47.1). All other sensitivity analyses yielded robust effectiveness estimates comparable to the main analysis (Fig. 4).
Discussion

Administration of seasonal trivalent inactivated influenza vaccines during pregnancy was associated with a statistically significant reduced risk of laboratory-confirmed influenza infections in both pregnant women and their infants younger than 6 months of age in Denmark between 2010 and 2017.

Our finding of vaccine effectiveness among pregnant women of 63.9% (95% CI, 29.1 to 81.6) confirms, but is somewhat higher than, the results of the two previous case–control studies of the effectiveness of seasonal influenza vaccinations against laboratory-confirmed infections in pregnant women [13,14]. In a prospective test-negative design study from 2010 to 2012 in the USA, the estimated effectiveness was 44% (95% CI, 13 to 67) based on 100 influenza-positive cases [13]. In a recent multi-country (Australia, Canada, Israel and the USA) test-negative design study, including 598 hospitalized pregnant women with laboratory-confirmed influenza infection between 2010 and 2016, the effectiveness was 40% (95% CI, 12 to 59) against influenza-associated hospitalization during pregnancy [14]. Our estimate is also within the range of the results of two clinical trials of seasonal influenza vaccination among pregnant women in low-resource settings in South Africa (vaccine efficacy, 50.4%; 95% CI, 14.5 to 71.2) [5] and Mali (76.6%; 95% CI, 28.4 to 94.3) [6]. However, the statistical precision of the estimates in all these studies is limited by the relatively small numbers of pregnant women positive for influenza virus, and the confidence intervals are all wide and overlapping.

Four observational studies from different areas of the USA and one small observational study from England have assessed the effectiveness of maternal influenza vaccination against laboratory-confirmed influenza infections in their infants [9-12, 25]. Our finding of vaccine effectiveness among infants of 56.8% (95% CI, 25.0 to 75.1) confirms, but is somewhat lower than, the results of the two most recent studies [12,25]. A retrospective cohort study from the Intermountain Region in the USA, conducted in 2005–2014, reported an overall vaccine effectiveness of 70% (95% CI, 54 to 81) based on 658 influenza virus-positive infants, but ascertainment of influenza vaccination during pregnancy was based on mothers’ self-report at the time of delivery which may be subject to recall bias [12]. A small English study, conducted from 2013 to 2014, reported a vaccine effectiveness of 71% (95% CI, 24 to 89), but used a screening method that compared maternal vaccination status (collected through the general practitioner) among 37 influenza virus-positive cases to maternal vaccine coverage in the population [25]. Likewise, our finding of vaccine effectiveness against influenza-associated hospitalization in infants of 61.4% is lower than the estimated effectiveness of 81% in the study from the Intermountain Region [12]. In a prospective study of Native American Indians in Southwest USA, conducted between 2002 and 2005, a modest effectiveness of 41% (95% CI, 7 to 63) was reported based on 83 laboratory-confirmed influenza-infected infants [10]. In a hospital-based study of 91
influenza virus-positive infants, a high effectiveness of 91.5% (95% CI, 61.7 to 98.1) was observed during 2000 to 2009 [9]. In another hospital-based study of 151 influenza virus-positive infants, a modest effectiveness of 48% (95% CI, 9 to 70) was observed during a similar period (2002–2009) [11]. Estimates of effectiveness among infants in most observational studies (including ours) are slightly higher than the results of four clinical trials in low-resource settings in South Africa, Mali, Nepal and Bangladesh (pooled vaccine efficacy, 36%; 95% CI, 22% to 48%) [26]. Different health status as well as other factors could explain this discrepancy; again, all studies were based on relatively limited numbers of infants testing positive for influenza virus, with wide and often overlapping CI values.

The analyses according to virus subtype were based on a low number of vaccinated cases and interpretation is limited by a lack of statistical precision. However, our findings of variation in vaccine effectiveness across influenza subtypes are consistent with a meta-analysis of test-negative design studies among non-pregnant individuals where the pooled vaccine effectiveness was 61% for A(H1N1)pdm09, 54% for type B and 33% for type A(H3N2) [27].

This study has a number of strengths. First, our study of maternal influenza vaccination provides estimates of vaccine effectiveness for both pregnant women and their infants in the same setting and study period. Furthermore these estimates of vaccine effectiveness are from a high-resource setting in Europe where previously no data have been reported on pregnant women and only one small study of a single influenza season in England provides data from infants [25]. Secondly, we have provided data on recent influenza seasons (2010–2017) with seasonal manifestations of the A(H1N1)pdm09 influenza virus strain in contrast to several previous studies, which were primarily based on seasons before 2009. Finally, we took advantage of the unique Danish health registries for unbiased and independent ascertainment of nationwide records of vaccination and laboratory-confirmed influenza infections.

Several limitations should be considered. First, we cannot entirely exclude the possibility of bias and confounding. However, our study results proved robust in a wide range of sensitivity analyses in which different selection criteria for the influenza-negative controls were used (matching on other covariates or no matching) and different regression models were applied (excluding maternal covariates or conditional on the matched case–control pairs). Secondly, information on employer-paid influenza vaccination was not available in our study, but we expect few pregnant women to be vaccinated in this setting, as vaccination by general practitioners is free of charge (general practitioners also manage the recommended medical consultations throughout pregnancy in gestational weeks 6–10, 25 and 32). Thirdly, information on clinical symptoms is not available in the Danish national registries and we could not restrict our study population to individuals tested within 5 days of symptom onset (the average duration of virus shedding in influenza-
Infected individuals tested after 5 days of symptom onset may be falsely negative and, if vaccination is effective, this would reduce our estimates of vaccine effectiveness. However, all influenza tests are requested by physicians and according to national guidelines (with awareness of testing high-risk groups with influenza-like illness during influenza seasons), and we assume that pregnant women and infants are tested soon after symptom onset. Fourthly, we used laboratory-confirmed influenza that reduces misclassification of influenza status, but this outcome measure is strictly defined as many individuals with influenza illness are only cared for at home. Although the test-negative study design reduces selection biases from differences in healthcare-seeking behaviours by including only those patients tested [29], selection bias may still be present if testing is associated with both the risk of influenza disease (as per design) and influenza vaccination. However, vaccination coverage was 10.9% among the pregnant women in the influenza virus-negative control group compared to 10.1% among the pregnant women giving birth in the entire study cohort. The low influenza vaccine coverage among pregnant women in the control group and the entire study cohort in our study is similar to results from many other European countries despite the official recommendations (the median vaccine coverage was less than 10% in a European survey) [30], whereas coverage is substantially higher in the USA (50% in a survey) [31]. Fifthly, it is possible that vaccination may not prevent influenza infection for some individuals but may reduce disease severity and thereby such individuals are probably less likely to seek medical care and be tested. In this case, the test-negative design study only estimates effectiveness against medically attended influenza infection (which is still important from a public health perspective). However, a randomized controlled efficacy trial of the trivalent inactivated influenza vaccine in non-pregnant adults showed that vaccination did not attenuate symptoms among influenza virus-positive cases [32]. Lastly, among the 350,888 women giving birth in our cohort, only 954 (0.3%) of the pregnant women and 7674 (2.2%) of their infants were tested for influenza. Therefore, our study population includes a small proportion of all influenza-infected individuals, but the presumed ‘all or nothing effect’ of influenza vaccination against influenza disease provides some reassurance that our results can be generalized to pregnant women and infants not tested (i.e. external validity).

In conclusion, the results of our observational study showed that trivalent inactivated influenza vaccination in pregnancy was associated with a significant reduction in risk of laboratory-confirmed influenza infections among both women and their infants. Our study provides real-world evidence from a high-resource temperate climate setting supporting maternal influenza vaccination as an effective method in preventing influenza infection in both mothers and their infants, two groups at high-risk of influenza-related complications.
Authors’ contributions

Ditte Mølgaard-Nielsen and Anders Hviid conceived and designed the study. Ditte Mølgaard-Nielsen conducted the statistical analysis and drafted the manuscript. Thea Kølsen Fischer, Tyra Grove Krause and Anders Hviid revised the manuscript critically for important intellectual content. Anders Hviid obtained funding and supervised the study. Ditte Mølgaard-Nielsen had full access to all data in the study and guarantees the integrity of the data and accuracy of the data analysis. All authors participated in the acquisition of data, contributed substantially to interpretation of the results and approved the final version submitted for publication.

Conflict of interest statement

None of the authors has any conflicts of interest to report.

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*Fig. 1* Flowchart of the study participants.
Matched for trimester of pregnancy at testing and the closest sample date within the same season.

Matched for infant age in 2-month intervals at testing and the closest sample date within the same season.

**Fig. 2** Adjusted estimates of maternal influenza vaccine effectiveness among pregnant women and infants, overall and according to virus subtype.
<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Influenza Virus-Positive Cases</th>
<th>Influenza Virus-Negative Matched Controls</th>
<th>Adjusted Vaccine Effectiveness* % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>16/313</td>
<td>34/313</td>
<td>63.9 (29.1-81.6)</td>
</tr>
<tr>
<td>Virus Subtypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A</td>
<td>9/199</td>
<td>34/313</td>
<td>72.1 (33.9-88.2)</td>
</tr>
<tr>
<td>H1N1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5/93</td>
<td>34/313</td>
<td>84.6 (28.6-96.7)</td>
</tr>
<tr>
<td>H3N2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5/36</td>
<td>34/313</td>
<td>8.3 (-177.8-69.7)</td>
</tr>
<tr>
<td>Influenza B&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7/114</td>
<td>34/313</td>
<td>42.5 (-43.7-77.0)</td>
</tr>
<tr>
<td>Infants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>23/460</td>
<td>52/460</td>
<td>56.8 (25.0-75.1)</td>
</tr>
<tr>
<td>Virus Subtypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A</td>
<td>21/367</td>
<td>52/460</td>
<td>45.9 (3.6-69.7)</td>
</tr>
<tr>
<td>H1N1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;5/103</td>
<td>52/460</td>
<td>95.2 (60.1-99.4)</td>
</tr>
<tr>
<td>H3N2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10/104</td>
<td>52/460</td>
<td>-11.3 (-150.8-50.7)</td>
</tr>
<tr>
<td>Influenza B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;5/92</td>
<td>52/460</td>
<td>86.6 (35.5-96.8)</td>
</tr>
</tbody>
</table>

* Adjusted for influenza season of test, period of influenza season (early, middle or late) of test, trimester of pregnancy at testing in pregnant women/infant age (2-month intervals) at testing in infants and maternal characteristics including calendar time at pregnancy onset, age, country of birth, region of residence, marital status, parity, educational level, household income, pre-pregnancy BMI, smoking status, days of hospitalization in previous year, outpatient hospital contacts in previous year, number of filled prescriptions in previous year, contacts with general practitioner in previous year, contacts with specialist in private practice in previous year and medical conditions increasing the risk of severe influenza disease.

<sup>b</sup> Not adjusted for influenza season of test.

<sup>c</sup> Not adjusted for influenza season of test, period of influenza season (early, middle or late) of test, calendar time at pregnancy onset, country of birth, region of residence, pre-pregnancy BMI, days of hospitalization in previous year, outpatient hospital contacts in previous year, number of filled prescriptions in previous year, contacts with general practitioner in previous year and contacts with specialist in private practice in previous year.

**Fig. 3** Sensitivity analyses of vaccine effectiveness among pregnant women.
<table>
<thead>
<tr>
<th>Sensitivity Analyses</th>
<th>Influenza-Virus Positive Cases</th>
<th>Influenza-Virus Negative Controls</th>
<th>Adjusted Vaccine Effectiveness* % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. vaccinated / Total no.</td>
<td>No. vaccinated / Total no.</td>
<td></td>
</tr>
<tr>
<td>Influenza-associated hospitalization(^b)</td>
<td>8/124</td>
<td>10/124</td>
<td>29.6 (-112.6-76.7)</td>
</tr>
<tr>
<td>Including women tested postpartum(^c)</td>
<td>18/371</td>
<td>40/371</td>
<td>62.0 (29.2-79.6)</td>
</tr>
<tr>
<td>Excluding women vaccinated within 14 days of sample date(^d)</td>
<td>16/312</td>
<td>34/309</td>
<td>64.0 (29.2-81.7)</td>
</tr>
<tr>
<td>Unmatched test-negative design</td>
<td>16/367</td>
<td>45/498</td>
<td>62.2 (27.1-80.4)</td>
</tr>
<tr>
<td>Other matching criteria(^e)</td>
<td>16/313</td>
<td>30/313</td>
<td>59.7 (18.5-80.1)</td>
</tr>
<tr>
<td>Not adjusted for maternal characteristics(^f)</td>
<td>16/313</td>
<td>34/313</td>
<td>61.5 (27.3-79.6)</td>
</tr>
<tr>
<td>Conditional logistic regression(^f)</td>
<td>16/313</td>
<td>34/313</td>
<td>64.1 (26.3-82.6)</td>
</tr>
</tbody>
</table>

\(^a\) Adjusted for influenza season of test, period of influenza season (early, middle or late) of test, trimester of pregnancy at testing and maternal characteristics including calendar time at pregnancy onset, age, country of birth, region of residence, marital status, parity, educational level, household income, pre-pregnancy BMI, smoking status, days of hospitalization in previous year, outpatient hospital contacts in previous year, number of filled prescriptions in previous year, contacts with general practitioner in previous year, contacts with specialist in private practice in previous year and medical conditions increasing the risk of severe influenza disease.

\(^b\) Using case definition according to the Center for Disease Control and Prevention [22].

\(^c\) Within the first 6 months after birth.

\(^d\) Matching for influenza season of test, period of influenza season (early, middle or late) of test and gestational age (in days) at testing.

\(^e\) Maternal characteristics included calendar time at pregnancy onset, age, country of birth, region of residence, marital status, parity, educational level, household income, pre-pregnancy BMI, smoking status, days of hospitalization in previous year, outpatient hospital contacts in previous year, number of filled prescriptions in previous year, contacts with general practitioner in previous year, contacts with specialist in private practice in previous year and medical conditions increasing the risk of severe influenza disease.

\(^f\) With the matched case–control pairs as strata and no adjustment for matching criteria, only for maternal characteristics [5].

**Fig. 4** Sensitivity analyses of maternal vaccine effectiveness among infants.
Table 1 Selected characteristics of influenza virus-positive cases and influenza virus-negative matched controls, 2010–2016

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pregnant women tested for influenza, n (%)</th>
<th>Infants younger than 6 months of age tested for influenza, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influenza virus-positive cases (n = 313)</td>
<td>Influenza virus-negative matched controls (n = 313)</td>
</tr>
<tr>
<td></td>
<td>Influenza virus-positive cases (n = 460)</td>
<td>Influenza virus-negative matched controls (n = 460)</td>
</tr>
<tr>
<td>Influenza season of test, calendar year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period of influenza season of test</td>
<td>Early</td>
<td>Middle</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>Days</td>
<td>5 (1.6)</td>
<td>217 (69.3)</td>
</tr>
<tr>
<td>First trimester/0–1 month</td>
<td>12 (3.8)</td>
<td>197 (62.9)</td>
</tr>
<tr>
<td>Second trimester/2–3 months</td>
<td>33 (7.2)</td>
<td>331 (72.0)</td>
</tr>
<tr>
<td>Third trimester/4–5 months</td>
<td>33 (7.2)</td>
<td>330 (71.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestational/infant age at testing</th>
<th>Days</th>
<th>First trimester/0–1 month</th>
<th>Second trimester/2–3 months</th>
<th>Third trimester/4–5 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>177.4 ±73.6</td>
<td>170.6 ± 76.5</td>
<td>80.0 ±44.0</td>
<td>78.0 ±44.6</td>
</tr>
<tr>
<td>First trimester/0–1 month</td>
<td>43 (13.7)</td>
<td>54 (17.3)</td>
<td>185 (40.2)</td>
<td>185 (40.2)</td>
</tr>
<tr>
<td>Second trimester/2–3 months</td>
<td>87 (27.8)</td>
<td>87 (27.8)</td>
<td>185 (40.2)</td>
<td>185 (40.2)</td>
</tr>
<tr>
<td>Third trimester/4–5 months</td>
<td>183 (58.5)</td>
<td>172 (55.0)</td>
<td>90 (19.6)</td>
<td>90 (19.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calendar time at pregnancy onset</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>38 (12.1)</td>
<td>48 (15.3)</td>
<td>135 (43.1)</td>
<td>92 (29.4)</td>
</tr>
<tr>
<td>First trimester/0–1 month</td>
<td>40 (12.8)</td>
<td>46 (14.7)</td>
<td>118 (37.7)</td>
<td>109 (34.8)</td>
</tr>
<tr>
<td>Second trimester/2–3 months</td>
<td>236 (51.3)</td>
<td>184 (40.0)</td>
<td>7 (1.5)</td>
<td>33 (7.2)</td>
</tr>
<tr>
<td>Third trimester/4–5 months</td>
<td>214 (46.5)</td>
<td>199 (43.3)</td>
<td>12 (2.6)</td>
<td>35 (7.6)</td>
</tr>
</tbody>
</table>

| Maternal age at pregnancy onset, years | 30.9 ±5.3 | 30.7 ±5.0 | 30.5 ±5.2 | 30.2 ±5.0 |
| Mother born in Denmark | 243 (77.6) | 244 (78.0) | 351 (76.3) | 349 (75.9) |
| Married or living with a partner | 258 (82.4) | 260 (83.1) | 381 (82.8) | 375 (81.5) |
| Bachelor degree or higher educational level | 138 (44.1) | 143 (45.7) | 177 (38.5) | 190 (41.3) |
| Household income in second tertile | 116 (37.1) | 106 (33.9) | 163 (35.4) | 168 (36.5) |
| Pre-pregnancy BMI of 18.5–24.9 kg/m² | 186 (59.4) | 195 (62.3) | 254 (55.2) | 286 (62.2) |
| Smoking during pregnancy | 33 (10.5) | 31 (9.9) | 68 (14.8) | 48 (10.4) |

<table>
<thead>
<tr>
<th>Days of hospitalization in previous year</th>
<th>0</th>
<th>1–2</th>
<th>≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>230 (73.5)</td>
<td>34 (10.9)</td>
<td>49 (15.7)</td>
</tr>
<tr>
<td>First trimester/0–1 month</td>
<td>244 (78.0)</td>
<td>25 (8.0)</td>
<td>44 (14.1)</td>
</tr>
<tr>
<td>Second trimester/2–3 months</td>
<td>348 (75.7)</td>
<td>57 (12.4)</td>
<td>55 (12.0)</td>
</tr>
<tr>
<td>Third trimester/4–5 months</td>
<td>343 (74.6)</td>
<td>43 (9.4)</td>
<td>74 (16.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contacts with general practitioner in previous year</th>
<th>0</th>
<th>1–4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>9 (2.9)</td>
<td>97 (31.0)</td>
</tr>
<tr>
<td>First trimester/0–1 month</td>
<td>14 (4.5)</td>
<td>94 (30.0)</td>
</tr>
<tr>
<td>Second trimester/2–3 months</td>
<td>11 (2.4)</td>
<td>149 (49.2)</td>
</tr>
<tr>
<td>Third trimester/4–5 months</td>
<td>16 (3.5)</td>
<td>132 (28.7)</td>
</tr>
<tr>
<td>Medical conditions increasing the risk of severe influenza disease&lt;sup&gt;a&lt;/sup&gt;</td>
<td>207 (66.1)</td>
<td>205 (65.5)</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>≥5</td>
<td>59 (18.9)</td>
<td>67 (21.4)</td>
</tr>
</tbody>
</table>

Values are presented as n (%) or means ±SD. Total percentages may not equal 100 because of rounding. For additional characteristics, see Supplementary Tables 3 and 4.

<sup>a</sup> Diagnosis in the previous year of respiratory, cardiovascular, haematological, neurological, liver and kidney, rheumatic and inflammatory bowel diseases, or filled prescriptions in the previous year of beta-2 agonist inhalants, corticosteroid inhalants, cardiovascular drugs, oral antidiabetic drugs, insulin, intestinal anti-inflammatory agents, immunosuppressants or systemic corticosteroids or obesity defined as maternal pre-pregnancy BMI of ≥35 kg/m².
370,698 birth records between October 1, 2010-December 31, 2016

4,972 records were excluded due to 4,776 missing gestational age 196 implausible gestational age (<22 wk or >45wk)

365,996 eligible births

357,810 births among 350,888 pregnant women
356,504 live born infants (342,785 singletons) 1,306 stillborn infants (1,156 singletons)

8,186 births excluded from women not having lived in Denmark continuously during pregnancy

350,888 eligible pregnant women

349,934 pregnant women were not included

89 pregnant women not tested for influenza virus during influenza season (calendar week 40 to 20 the following year)

349,934 pregnant women were not included

954 pregnant women tested for influenza virus A and B.

367 women positive for influenza A or B

54 cases excluded due to no match

313 women positive for influenza A or B

505 matchedb infants not included

498 women negative for influenza A and B

185 controls not included

313 matcheda women negative for influenza A and B

146 matchedb infants not included

342,785 eligible singleton live born infants

7,674 infants younger than 6 months of age tested for influenza virus A and B

886 infants not tested for influenza virus during influenza season (calendar week 40 to 20 the following year)

335,111 infants were not included

15,025 infants were excluded due to 1,306 stillbirths 13,719 multiple live births

7,674 infants younger than 6 months of age tested for influenza virus A and B

6328 infants negative for influenza A and B

5868 infants not included

460 women positive for influenza A or B

460 matchedb infants not included

498 women negative for influenza A and B

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