**Title Page**

**Full Title:** Seasonal influenza vaccination during pregnancy and the risk of major congenital malformations in live-born infants: A 2010-2016 historical cohort study.

**Running title:** Safety of maternal influenza vaccination

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**Key point:** In this UK-based historical cohort study, there was no evidence to suggest that seasonal influenza vaccine was associated with major malformations when given in the first trimester or subsequently in pregnancy.

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**Abstract**

**Background:** Available evidence indicates that seasonal inactivated influenza vaccination during pregnancy protects both the mother and her newborn, and is safe. Nevertheless, ongoing safety assessments are important in sustaining vaccine uptake. Few studies have explored safety in relation to major congenital malformations, particularly in the first trimester when most organogenesis occurs.

**Methods:** Anonymised UK primary care data (the Clinical Practice Research Datalink), including a recently developed Pregnancy Register, were used to identify live-born singletons delivered between 2010 and 2016. Maternal influenza vaccination was determined using primary care records and stratified by trimester. Ascertainment of major malformations from infant primary care records was maximized by linkage to hospitalization data and death certificates. The relationship between vaccination and major malformations recorded in the year after delivery and in early childhood was then assessed using multivariable Cox regression.

**Results:** A total of 78,150 live-birth pregnancies were identified: 6,872 (8.8%) were vaccinated in the first trimester, 11,678 (14.9%) in the second and 12,931 (16.5%) in the third. Overall, 5,707 live-births resulted in an infant with a major malformation recorded in the year after delivery and the adjusted hazard ratio when comparing first-trimester vaccination to no vaccination was **1.06 (99%CI, 0.94-1.19; p=0.2)**. Results were similar for second and third-trimester vaccination and for analyses considering major malformations recorded beyond the first birthday.

**Conclusions:** In this large, population-based historical cohort study there was no evidence to suggest that seasonal influenza vaccine was associated with major malformations when given in the first trimester or subsequently in pregnancy.

**Manuscript Text**

**Introduction**

Pregnant women and newborn infants are at increased risk of complications following influenza infection.1,2 Seasonal influenza vaccination (SIV) during pregnancy has been shown to provide good protection to both groups.3-5 The World Health Organization recommended SIV for all pregnant women, regardless of trimester, in 2012.6 Women, however, are concerned about the safety of vaccination during pregnancy for their child.7,8

Several studies have demonstrated the safety of the 2009/10 monovalent pandemic vaccine with respect to major congenital malformations (MCMs), but few have assessed SIV.9-12 Those that have examined SIV, and have stratified by trimester of vaccination, have generally been limited by low numbers of first-trimester vaccinations and inadequate infant follow-up which could result in underascertainment of MCMs.9-12 To date, there has only been one large US study of first-trimester vaccination with adequate infant follow-up.13

We examined the association between SIV administered in the first and subsequent trimesters, and the risk of MCMs in a different setting by using a large UK cohort, with ascertainment of malformations using long-term follow-up in linked primary care, hospitalisation and mortality datasets.

**Methods
Data Sources**

This study utilized the Clinical Practice Research Datalink (CPRD), the CPRD/London School of Hygiene and Tropical Medicine (LSHTM) Pregnancy Register, hospital admissions data from the Hospital Episode Statistics database (HES), Office for National Statistics (ONS) death certificate data, deprivation quintiles linked to household post-codes, and data on influenza activity from the Royal College of General Practitioners (RCGP) Research and Surveillance Centre.

The CPRD contains anonymised, electronic primary care records for 7% of the UK population registered at a general practice. It includes diagnoses and procedures recorded using Read codes, vaccination records and prescriptions.14 The CPRD has been shown to be broadly representative of the UK population and diagnostic validity is high.14,15

The Pregnancy Register lists all pregnancies identified in CPRD, for women aged 11-49 years.16 It includes pregnancy outcomes and estimates of pregnancy timings derived from all available pregnancy data in CPRD including estimated delivery dates, last menstrual period dates, ultrasound dating scans and prematurity records. The first, second and third trimesters are defined as the pregnancy start through week 13, week 14 through 26 and week 27 through the pregnancy end, respectively. Live-birth deliveries are linked to records of infants registered at the same practice as their mother. Validation of the Pregnancy Register against linked electronic maternity records in HES has indicated overall good agreement, suggesting most pregnancies are well-captured in the Register.16

Patient data in CPRD can be linked to the HES and ONS data for 75% of English practices.14 Linked HES data include information on diagnoses and procedures, recorded using the International Classification of Diseases (ICD-10) and the Classification of Surgical Operations and Procedures (OPCS-4), respectively. ONS death certificate data include primary and contributory causes of death recorded using ICD-10. Deprivation quintiles are derived from the 2015 Index of Multiple Deprivation (IMD) for Lower Super Output Areas.17

Weekly general practice consultation rates for influenza-like illness from the RCGP were used to identify periods of influenza circulation above baseline levels for each season. The validity of these data has been confirmed through microbiological surveillance.18

This study received approval from the Independent Scientific Advisory Committee of the Medicines & Healthcare Products Regulatory Agency (reference 17\_040RA); the approved protocol was made available to the reviewers. Approval was also received from LSHTM’s ethics committee (reference 13720).

**Study design**

This historical cohort study compared live-birth pregnancies that received SIV, stratified by trimester of vaccination, to those unvaccinated. The primary outcome was the presence of any MCMs among infants in the year after delivery. Secondary outcomes examined any MCMs, major limb malformations and congenital heart defects recorded after delivery and anytime in the study period between September 1, 2010 and March 31, 2016 (the latest date for which all linked data were available).

**Study population**

Pregnancies resulting in a live-born singleton during the study period were identified from the Pregnancy Register. Pregnant women had to be registered at an up-to-standard practice (a quality standard set by CPRD to indicate continuous recording of data within the practice)14 for at least 6 months before the start of pregnancy to enable the ascertainment of pre-conception exposures. Live-born infants had to be eligible for HES and ONS linkage. Finally, pregnancies were required to overlap with a period of influenza vaccine availability (1st September to 31st March, annually) by at least one week.

**Identifying vaccinations**

In the UK, pregnant women are offered SIV in any trimester.19 The earliest vaccination record in each influenza season was identified in CPRD from immunisation records, prescriptions or Read codes, and used to determine the trimester of vaccination. Pregnancies were excluded if the timing or nature of vaccination was uncertain (e.g. if there was a possibility that SIV was received outside of the practice at an unknown time or a possibility that pandemic vaccine was received) (**Figure 1**).

**Identifying MCMs**

Code-lists for MCMs were developed with a consultant neonatologist (SK), following EUROCAT guidelines.20 MCMs were then ascertained from infant records in CPRD, HES data for diagnoses and procedures, and ONS. Pregnancies were excluded if there was an antenatal or infant record indicating a chromosomal or heritable anomaly, a malformation due to a known teratogen (e.g. foetal alcohol syndrome), or a congenital infection associated with malformations. Infants were followed-up from delivery for a year or until the end of the study period. Follow-up ended earlier if they died, left the practice or the practice stopped collecting data for CPRD.

**Potential confounders**

We considered *a priori* confounders to be maternal age and ethnicity, geographical region (due to variation in vaccine uptake and MCM ascertainment) and the earliest influenza season a pregnancy overlapped with. Other potential confounders included: household deprivation quintile (IMD), number of children in the household, maternal smoking, hazardous drinking, extreme body mass index (BMI) of <18 or ≥35, belonging to another clinical risk group for which vaccination was recommended during the study period,19 non-pregnancy related chronic hypertension, exposure to teratogenic drugs or live vaccines, and number of weeks the first trimester overlapped with influenza activity above baseline levels (see  **Supplementary Table 1** for details of how these were derived).

**Statistical analyses**

Baseline pregnancy characteristics were described by vaccination status. Logistic regression was used initially to model the univariable relationship between vaccination and MCMs recorded in the year after delivery and assess confounding. After *a priori* confounders, remaining potential confounders were added individually to the logistic regression model and assessed for a ≥5% change in the odds ratios between first-trimester vaccination or vaccination anytime and MCMs. Multicollinearity was monitored between IMD, ethnicity and region and between the number of children in the household and maternal age. Finally, random effects models were used to assess clustering by mother and practice. Once confounders had been identified using logistic regression models, all final analyses were conducted using Cox proportional hazards models to account for improved ascertainment of the outcome among infants with longer follow-up time. Results were compared to those from logistic regression. To account for multiple analyses, 99% confidence intervals (CIs) were calculated. All models were complete case analyses.

Three sensitivity analyses were conducted. First, we included pregnancies that received SIV in the 4 weeks prior to their start to account for any imprecision in the estimated pregnancy start dates. Second, we included MCMs recorded in HES or ONS after follow-up in CPRD had ended because the infant left the practice or the practice ended data collection. In the main analyses, pregnancies of women with unknown BMI or a BMI between 18 and 34 were combined in a single category as they had comparable associations with MCMs. The third sensitivity analysis excluded pregnancies of women with unknown BMI.

STATA version 14.2 was used for all analyses.

**Results**

**Characteristics of the eligible study cohort**

We identified 103,742 potentially eligible pregnancies resulting in live-born singletons during the study period. After exclusions were applied, the final cohort included 78,150 pregnancies among 71,124 women (**Figure 1**). Most pregnancies were of white women (85.5%), aged 25-34 (58.9%) years (**Table 1**).

Vaccine uptake was 40.3% (n=31,481): 8.8% (n=6,872) in the first trimester, 14.9% (n=11,678) in the second and 16.5% (n=12,931) in the third. Vaccination in the first trimester or anytime in pregnancy was less likely if the woman was: young, of Black ethnicity, living in a more deprived area, not part of a clinical risk group for which vaccination was recommended,19 unexposed to teratogenic medications and/or live vaccines, a current smoker or part of a household with children (**Table 1; Supplementary Table 2**). Vaccination also varied by region and the earliest influenza season the pregnancy overlapped with.

Of the 78,150 pregnancies, 7.3% (n=5,707) resulted in an infant with a MCM recorded in the year after delivery, whilst 7.7% (n=6,029) had a MCM recorded after delivery and anytime in the study period. Most MCMs were recorded early in life, with 51% recorded at delivery and 87.2% in the following three months (**Supplementary Figure 1**). Most infants had at least one year of follow-up (73.5%) and almost half had at least two (48.9%) (**Supplementary Table 3**).

**Primary analyses**

The univariable Cox regression analysis showed evidence for a crude association between vaccination anytime in pregnancy and MCMs recorded in the year after delivery; results were similar for first and second-trimester vaccination (**Table 2**). However, all associations were eliminated following adjustment for *a priori* confounders: maternal age and ethnicity, region and the earliest influenza season a pregnancy overlapped with (**Table 2**). The most important of these appeared to be region and season; hazard ratios (HRs) remained similar upon the addition of age and ethnicity **(Supplementary Tables 4-5)**. Both region and season were associated with age and ethnicity (χ2 p<0.001), suggesting that adjustment for the former likely resulted in partial adjustment for the latter.

Of the remaining potential confounders, only maternal IMD and number of children in the household were associated with both vaccination and MCMs in univariable analyses (**Supplementary Tables 2 and 6**). However, upon addition to the model, neither these nor any others altered HRs by ≥5% (**Supplementary Tables 4 and 5**). Fully-adjusted models showed no evidence of an association between vaccination anytime (HR, 1.02; 99% CI, 0.94-1.10; p=0.54), vaccination in the first trimester (HR, 1.06; 99% CI, 0.94-1.19; p=0.23) or the second (HR, 1.02; 99% CI, 0.92-1.13; p=0.63) and MCMs recorded in the year after delivery (**Table 2**). The logistic regression models used to investigate confounding gave very similar results to our final Cox regression models (**Supplementary Tables 4 and 5**).

**Secondary analyses**

Results from analyses in which follow-up was extended to include any MCMs ascertained from delivery until the end of the study period were almost identical (**Table 2**). Unadjusted models examining major limb malformations showed a crude association with vaccination in all trimesters (**Table 3**). However, adjusting for *a priori* or all potential confounders removed any associations. For congenital heart defects, no association was seen with vaccination in any model.

**Sensitivity analyses**

Sensitivity analyses that included 216 additional pregnancies for which vaccination occurred four weeks prior to their estimated start, or allowed for follow-up in HES and ONS data to continue after follow-up in CPRD had ended, or excluded 8,093 pregnancies of women with unknown BMI did not differ substantially from main analyses (**Table 4**).

**Discussion**

This UK-based historical cohort study examined the association between SIV during pregnancy and MCMs in live-born infants, between the 2010/11 and 2015/16 influenza seasons. Based on 6,872 pregnancies vaccinated in the first trimester, there was no evidence for an association with MCMs recorded in the year after delivery (adjusted HR, 1.06; 99% CI, 0.94-1.19; p=0.2). No evidence of an association was seen in analyses assessing subsequent trimesters or pregnancy overall, or analyses including MCMs recorded after delivery and anytime in the study period. Analyses of major limb and congenital heart defects adjusted for confounding also showed no evidence for an association with first-trimester or later vaccination.

**Strengths**

Reviews examining the safety of influenza vaccination with respect to MCMs have highlighted the limited number of studies examining first-trimester vaccination with SIV. Among the few such studies, further limitations such as the low number of pregnancies vaccinated in the first trimester and short follow-up time of infants have prompted calls for further safety evidence.9-12

The utilization of the Pregnancy Register, which includes information on trimester dates, allowed for the identification of a large number of pregnancies vaccinated in the first trimester. Follow-up in most studies has been limited to the immediate period around delivery.21-23 Whilst a few studies have attempted follow-up for the year after delivery,13,24 extending follow-up beyond a year has been shown to still increase the prevalence of recorded MCMs in CPRD.25-27 The majority of infants in our cohort had at least one year of follow-up and almost half had at least two. The value of longer follow-up is demonstrated by the fact that 12.8% of MCMs in our cohort were identified after 3 months and 5.3% after a year.

A further strength of this study was the linkage of CPRD data to HES and ONS to maximize MCM ascertainment. Previous research suggests that reliance on sole data-sources can lead to significant underascertainment of conditions.28 This may be particularly true for MCMs, many of which are likely to be identified in hospital and communicated in letters not available to researchers in the electronic primary care record unless encoded, which may be incomplete or delayed. Linkage to ONS further serves to ascertain those cases that may have been detected following the infant’s death. For completeness, we also examined MCM recordings made in HES or ONS after follow-up in CPRD had ended, but this made minimal difference.

**Limitations**

Whilst our study had a number of strengths, there were also limitations. Coding algorithms to identify MCMs were developed in accordance with EUROCAT guidelines and with a consultant neonatologist. The few studies that have assessed the positive predictive value of MCMs recorded in CPRD have found this to be good overall (78-86%), with results for congenital heart defects being above 90%.29-32 However, validation of diagnoses in HES have not been undertaken.

The estimate of gestation at the time of vaccination is based on the Pregnancy Register’s use of a wide range of information recorded in primary care which is thought to give rise to increased accuracy. However, any imprecision in the estimated pregnancy start date could result in misclassification of the timing of vaccination during pregnancy. Sensitivity analyses including pregnancies that received SIV in the 4 weeks prior to their start went some way in addressing this and did not reveal evidence for an association with MCMs. In addition to the above, whilst general practitioners are required to document vaccinations received outside of the surgery and the maternal influenza vaccination programme was delivered almost entirely through general practices over the study period, misclassification of vaccination could potentially occur if women were vaccinated elsewhere and practitioners were not notified.33

We adjusted for a number of potential confounders but were not always able to determine maternal smoking, hazardous drinking or BMI at the start of pregnancy and sometimes had to rely on the most proximate record. Although in our main analyses women with unknown BMI were categorized as not having any evidence of extreme BMI, our sensitivity analyses excluding these pregnancies yielded similar results. We cannot discount the possibility of residual confounding from other risk factors for MCMs that may also be associated with vaccine uptake in pregnancy and that are likely to be poorly recorded in CPRD, such as religion.34

This study only examined live-birth pregnancies with linked infant records, excluding 10.6% of pregnancies because they lacked linkage. There are many reasons for non-linkage, including the practice stopping contributing to CPRD or mothers moving away. It is possible that severe malformations resulting in the death or prolonged hospitalization of neonates could also prevent linkage, but it seems unlikely that this incomplete ascertainment would depend on maternal vaccination status. This study also did not explore any potential role of malformations on the causal pathway between vaccination and pregnancy losses. However, studies thus far have found no evidence for an association between vaccination and such outcomes.10,35,36

**Comparison with other studies**

Our results are consistent with those from other studies that have examined SIV receipt during pregnancy and have shown no association with MCMs; this includes analyses of first-trimester vaccination for which point estimates from other studies ranged between 0.67 and 1.91, with confidence intervals including the null.13,22-24 Reassuringly, our point estimates for MCMs following first-trimester vaccination are in line with those from the largest study to date, which examined SIV receipt between 2004-2013 in the US (adjusted prevalence ratio, 1.02; 95% CI, 0.94-1.10; p=0.55).13 Ours is the next largest study and provides further evidence on the safety of SIV during pregnancy in another setting and for subsequent years, using a recently-developed Pregnancy Register that considers all available data in CPRD to estimate gestation at the time of vaccination as well as maximizing ascertainment of MCMs through long-term follow-up in linked data.

The lack of an association between first-trimester vaccination and congenital heart defects in our study was consistent with results from two other studies, including the large US study.13,23 Whilst other studies have examined limb malformations and not found any association with vaccination, they have grouped these with defects in other organ systems or examined a limited selection of particular diagnoses such as talipes equinovarus (clubfoot).13,23 This study assessed all major limb malformations as a stand-alone subgroup and confirmed the lack of association with SIV.

**Conclusions**

The findings from this large cohort study, in which the majority of infants were followed-up for at least one year, provide further evidence on the safety profile of influenza vaccination in pregnancy. There was no evidence for an association between first-trimester vaccination and MCMs, limb malformations or congenital heart defects after controlling for confounding.This study shows ongoing monitoring of the safety of first-trimester vaccination is possible using CPRD and could usefully include additional MCM subgroups when sufficient numbers become available.

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**Drafting the manuscript:** MP, PM, CM, HM

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**Table 1 - Characteristics of eligible pregnancies included in analyses, by vaccination status.**

|  | **No. pregnancies (%)****N=78,150** | **No. pregnancies unvaccinated (%)****n=46,669** | **No. pregnancies vaccinated in trimester 1 (%)****n=6,872** | **No. pregnancies vaccinated anytime (%)****n=31,481** |
| --- | --- | --- | --- | --- |
| **Maternal age (years)** |
| <18 | 719 (0.9) | 458 (63.7) | 33 (4.6) | 261 (36.3) |
| 18-24 | 13,243 (17) | 8,451 (63.8) | 982 (7.4) | 4,792 (36.2) |
| 25-34 | 46,030 (58.9) | 27,138 (59) | 4,150 (9) | 18,892 (41) |
| ≥35 | 18,158 (23.2) | 10,622 (58.5) | 1,707 (9.4) | 7,536 (41.5) |
| **Maternal ethnicity** |
| White | 66,849 (85.5) | 39,618 (59.3) | 5,939 (8.9) | 27,231 (40.7) |
| South Asian | 5,501 (7) | 3,272 (59.5) | 507 (9.2) | 2,229 (40.5) |
| Black | 2,881 (3.7) | 1,953 (67.8) | 196 (6.8) | 928 (32.2) |
| Other | 1,850 (2.4) | 1,171 (63.3) | 146 (7.9) | 679 (36.7) |
| Mixed | 1,069 (1.4) | 655 (61.3) | 84 (7.9) | 414 (38.7) |
| **Maternal IMD statusa** |
| 1=least deprived | 15,847 (20.3) | 8,730 (55.1) | 1,579 (10) | 7,117 (44.9) |
| 2 | 14,905 (19.1) | 8,569 (57.5) | 1,345 (9) | 6,336 (42.5) |
| 3 | 15,144 (19.4) | 8,880 (58.6) | 1,406 (9.3) | 6,264 (41.4) |
| 4 | 16,064 (20.6) | 10,015 (62.3) | 1,304 (8.1) | 6,049 (37.7) |
| 5=most deprived | 16,190 (20.7) | 10,475 (64.7) | 1,238 (7.7) | 5,715 (35.3) |
| **Region** |
| London | 12,922 (16.5) | 8,295 (64.2) | 991 (7.7) | 4,627 (35.8) |
| North East | 1,811 (2.3) | 1,203 (66.4) | 113 (6.2) | 608 (33.6) |
| North West | 11,636 (14.9) | 6,771 (58.2) | 1,133 (9.7) | 4,865 (41.8) |
| Yorkshire & The Humber | 1,453 (1.9) | 922 (63.5) | 123 (8.5) | 531 (36.6) |
| East Midlands | 780 (1) | 549 (70.4) | 45 (5.8) | 231 (29.6) |
| West Midlands | 8,545 (10.9) | 4,561 (53.4) | 997 (11.7) | 3,984 (46.6) |
| East of England | 7,862 (10.1) | 4,463 (56.8) | 741 (9.4) | 3,399 (43.2) |
| South West | 9,974 (12.8) | 5,936 (59.5) | 777 (7.8) | 4,038 (40.5) |
| South Central | 11,670 (14.9) | 6,710 (57.5) | 1,157 (9.9) | 4,960 (42.5) |
| South East Coast | 11,497 (14.7) | 7,259 (63.1) | 795 (6.9) | 4,238 (36.9) |
| **Mother was part of a clinical risk groupb**  |
| No | 73,804 (94.4) | 44,513 (60.3) | 6,230 (8.4) | 29,291 (39.7) |
| Yes | 4,346 (5.6) | 2,156 (49.6) | 642 (14.8) | 2,190 (50.4) |
| **Maternal smoking status** |
| Non | 41,081 (52.6) | 23,922 (58.2) | 3,729 (9.1) | 17,159 (41.8) |
| Current | 17,687 (22.6) | 11,630 (65.8) | 1,278 (7.2) | 6,057 (34.3) |
| Ex | 19,382 (24.8) | 11,117 (57.4) | 1,865 (9.6) | 8,265 (42.6) |
| **Maternal hazardous drinking** |
| No | 77,502 (99.2) | 46,308 (59.8) | 6,811 (8.8) | 31,194 (40.3) |
| Yes | 648 (0.8) | 361 (55.7) | 61 (9.4) | 287 (44.3) |
| **Extreme maternal BMI** |
| No | 71,335 (91.3) | 42,560 (59.7) | 6,235 (8.7) | 28,775 (40.3) |
| Underweight (<18) | 1,656 (2.1) | 1,042 (62.9) | 147 (8.9) | 614 (37.1) |
| Obese (≥35) | 5,159 (6.6) | 3,067 (59.5) | 490 (9.5) | 2,092 (40.6) |
| **Maternal chronic hypertension (non-pregnancy related)** |
| No | 77,097 (98.7) | 46,074 (59.8) | 6,760 (8.8) | 31,023 (40.2) |
| Yes | 1,053 (1.4) | 595 (56.5) | 112 (10.6) | 458 (43.5) |
| **Maternal exposure to teratogenic medication(s)c or live vaccinesd**  |
| No | 73,370 (93.9) | 43,928 (59.9) | 6,386 (8.7) | 29,442 (40.1) |
| Yes | 4,780 (6.1) | 2,741 (57.3) | 486 (10.2) | 2,039 (42.7) |
| **Earliest influenza season a pregnancy overlapped with** |
| 2009/10 | 5,234 (6.7) | 5,171 (98.8) | 0 (0) | 63 (1.2) |
| 2010/11 | 13,040 (16.7) | 10,135 (77.7) | 425 (3.3) | 2,905 (22.3) |
| 2011/12 | 18,468 (23.6) | 11,254 (60.9) | 1,607 (8.7) | 7,214 (39.1) |
| 2012/13 | 15,910 (20.4) | 7,833 (49.2) | 2,067 (13) | 8,077 (50.8) |
| 2013/14 | 13,383 (17.1) | 6,906 (51.6) | 1,503 (11.2) | 6,477 (48.4) |
| 2014/15 | 9,987 (12.8) | 4,715 (47.2) | 1,251 (12.5) | 5,272 (52.8) |
| 2015/16 | 2,128 (2.7) | 655 (30.8) | 19 (0.9) | 1,473 (69.2) |
| **No. of weeks the first trimester overlapped with influenza activity above baseline levels** |
| None | 63,145 (80.8) | 35,467 (56.2) | 4,813 (7.6) | 27,678 (43.8) |
| 0-2 | 6,556 (8.4) | 4,649 (70.9) | 1,091 (16.6) | 1,907 (29.1) |
| 2-4 | 1,668 (2.1) | 1,200 (71.9) | 154 (9.2) | 468 (28.1) |
| 4-6 | 2,059 (2.6) | 1,525 (74.1) | 260 (12.6) | 534 (25.9) |
| 6-8 | 2,954 (3.8) | 2,377 (80.5) | 346 (11.7) | 577 (19.5) |
| 8-10 | 703 (0.9) | 541 (77) | 88 (12.5) | 162 (23) |
| 10-12 | 1,065 (1.4) | 910 (85.5) | 120 (11.3) | 155 (14.6) |
| **No. of children in the maternal household** |
| None | 27,868 (35.7) | 15,211 (54.6)  | 2,833 (10.2) | 12,657 (45.4) |
| 1-2 | 42,911 (54.9) | 26,419 (61.6) | 3,565 (8.3) | 16,492 (38.4) |
| ≥3 | 7,371 (9.4) | 5,039 (68.4) | 474 (6.4) | 2,332 (31.6) |

aFor 46 (0.06%) pregnancies, maternal household IMD was unavailable and practice-level IMD was used. bChronic respiratory, heart, kidney, liver or neurological disease, diabetes, immunosuppression due to disease or treatment, asplenia or dysfunction of the spleen. **c**Exposure from six months before pregnancy start until the end of the first trimester. **d**Exposure from three months before pregnancy start until the end of the first trimester. Abbreviations: IMD, Index of Multiple Deprivation; BMI, body mass index.

**Table 2 – Examining the association between vaccination and MCMs.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Vaccination****(No. pregnancies)** | **No. MCMs/person-years (rate per 100 person-years)** | **HR, unadjusted** **(99% CI)** | ***P* value** | **HR, adjusted for *a priori* confounders (99% CI)** | ***P* value** | **HR, adjusted for all potential confounders** **(99% CI)** | ***P* value** |
| **Models including MCMs ascertained in the year after delivery (N=5,707 MCMs)** |
| **Never** (46,669) | 3,289/38,898 (8.5) | 1.00 |  | 1.00 |  | 1.00 |  |
| **Any trimester** (31,481) | 2,418/24,827 (9.7) | 1.10 (1.03-1.18) | <0.001 | 1.03 (0.96-1.11) | 0.33 | 1.02 (0.94-1.10) | 0.54 |
| **Trimester 1** (6,872) | 565/5,560 (10.2) | 1.17 (1.04-1.32) | <0.001 | 1.08 (0.96-1.22) | 0.11 | 1.06 (0.94-1.19) | 0.23 |
| **Trimester 2** (11,678) | 902/9,153 (9.9) | 1.11 (1.01-1.22) | 0.006 | 1.03 (0.93-1.14) | 0.45 | 1.02 (0.92-1.13) | 0.63 |
| **Trimester 3** (12,931) | 951/10,115 (9.4) | 1.05 (0.96-1.16) | 0.17 | 1.00 (0.91-1.10) | >0.99 | 0.99 (0.90-1.10) | 0.86 |
| **Models including MCMs ascertained after delivery and anytime in the study period (N=6,029 MCMs)** |
| **Never** (46,669) | 3,505/102,311 (3.4) | 1.00 |  | 1.00 |  | 1.00 |  |
| **Any trimester** (31,481) | 2,524/54,389 (4.6) | 1.09 (1.02-1.17) | 0.001 | 1.03 (0.96-1.10) | 0.36 | 1.02 (0.94-1.09) | 0.56 |
| **Trimester 1** (6,872) | 594/11,648 (5.1) | 1.18 (1.05-1.32) | <0.001 | 1.09 (0.97-1.22) | 0.07 | 1.07 (0.95-1.20) | 0.16 |
| **Trimester 2** (11,678) | 941/20,203 (4.7) | 1.10 (1.00-1.21) | 0.008 | 1.03 (0.93-1.13) | 0.48 | 1.02 (0.92-1.13) | 0.65 |
| **Trimester 3** (12,931) | 989/22,539 (4.4) | 1.04 (0.95-1.14) | 0.27 | 0.99 (0.90-1.09) | 0.85 | 0.99 (0.90-1.09) | 0.73 |

A priori confounders were maternal age, maternal ethnicity, region and the earliest influenza season a pregnancy overlapped with. Other potential confounders included the number of weeks the first trimester overlapped with a period of influenza activity above baseline levels as well as the following maternal factors: IMD, number of children in the household, smoking status, hazardous drinking, extreme BMI, clinical risk group, chronic hypertension and exposure to teratogenic drugs and/or live vaccines. Abbreviations: MCM, major congenital malformations; IMD, Index of Multiple Deprivation; BMI, body mass index; HR, Hazard Ratio; CI, Confidence Interval.

**Table 3 - Examining the association between vaccination, major limb malformations and congenital heart defects.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Vaccination****(No. pregnancies)** | **No. MCMs/person-years (rate per 100 person-years)** | **HR, unadjusted** **(99% CI)** | ***P*****value** | **HR, adjusted for** ***a priori* confounders** **(99% CI)** | ***P*** **value** | **HR, adjusted for all potential confounders** **(99% CI)** | ***P*****value** |
| **Models including limb malformations ascertained after delivery and anytime in the study period (N=2,425 limb malformations)** |
| **Never** (46,669) | 1,350/107,080 (1.3) | 1.00 |  | 1.00 |  | 1.00 |  |
| **Any trimester** (31,481) | 1,075/56,940 (1.9) | 1.20 (1.08-1.33) | <0.001 | 1.10 (0.99-1.23) | 0.03 | 1.07 (0.96-1.21) | 0.11 |
| **Trimester 1** (6,872) | 235/12,259 (1.9) | 1.20 (1.00-1.44) | 0.01 | 1.07 (0.89-1.29) | 0.34 | 1.03 (0.86-1.25) | 0.66 |
| **Trimester 2** (11,678) | 405/21,145 (1.9) | 1.22 (1.06-1.41) | <0.001 | 1.11 (0.96-1.29) | 0.07 | 1.09 (0.93-1.27) | 0.17 |
| **Trimester 3** (12,931) | 435/23,536 (1.9) | 1.18 (1.02-1.36) | 0.003 | 1.11 (0.96-1.28) | 0.07 | 1.09 (0.94-1.26) | 0.14 |
|  **Models including congenital heart defects ascertained after delivery and anytime in the study period (N=789 heart defects)** |
| **Never** (46,669) | 479/109,133 (0.4) | 1.00 |  | 1.00 |  | 1.00 |  |
| **Any trimester** (31,481) | 310/58,303 (0.5) | 0.99 (0.82-1.20) | 0.90 | 0.96 (0.79-1.17) | 0.58 | 0.93 (0.76-1.15) | 0.39 |
| **Trimester 1** (6,872) | 67/12,568 (0.5) | 0.97 (0.69-1.36) | 0.82 | 0.93 (0.66-1.31) | 0.58 | 0.91 (0.64-1.29) | 0.49 |
| **Trimester 2** (11,678) | 129/21,621 (0.6) | 1.12 (0.87-1.44) | 0.26 | 1.08 (0.83-1.41) | 0.45 | 1.04 (0.79-1.37) | 0.68 |
| **Trimester 3** (12,931) | 114/24,114 (0.5) | 0.89 (0.68-1.16) | 0.25 | 0.87 (0.66-1.14) | 0.18 | 0.85 (0.65-1.13) | 0.14 |

A priori confounders were maternal age, maternal ethnicity, region and the earliest influenza season a pregnancy overlapped with. Other potential confounders included the number of weeks the first trimester overlapped with a period of influenza activity above baseline levels as well as the following maternal factors: IMD, number of children in the household, smoking status, hazardous drinking, extreme BMI, clinical risk group, chronic hypertension and exposure to teratogenic drugs and/or live vaccines. Abbreviations: MCM, major congenital malformations; IMD, Index of Multiple Deprivation; BMI, body mass index; HR, Hazard Ratio; CI, Confidence Interval.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Models** | **HR, unadjusted (99% CI)** | ***P*****value** | **HR, adjusted for** ***a priori* confounders** **(99% CI)** | ***P*****value** | **HR, adjusted for all potential confounders** **(99% CI)** | ***P*****value** |
| **Models including MCMs diagnosed in the year after delivery** |
| **Main model** | **1.17 (1.04-1.32)** | **<0.001** | **1.08 (0.96-1.22)** | **0.11** | **1.06 (0.94-1.19)** | **0.23** |
| Including pregnancies vaccinated in the 4 weeks prior to the starta | 1.19 (1.06-1.33) | <0.001 | 1.09 (0.97-1.23) | 0.06 | 1.07 (0.95-1.21) | 0.14 |
| Including diagnoses made beyond truncation of follow-up in CPRDb | 1.17 (1.04-1.32) | <0.001 | 1.08 (0.96-1.22) | 0.11 | 1.06 (0.94-1.19) | 0.23 |
| Excluding pregnancies with unknown BMIc | 1.18 (1.05-1.33) | <0.001 | 1.09 (0.96-1.23) | 0.09 | 1.07 (0.94-1.21) | 0.19 |
| **Models including MCMs diagnosed after delivery and anytime in the study period** |
| **Main model** | **1.18 (1.05-1.32)** | **<0.001** | **1.09 (0.97-1.22)** | **0.07** | **1.07 (0.95-1.20)** | **0.16** |
| Including pregnancies vaccinated in the 4 weeks prior to the starta | 1.19 (1.07-1.33) | <0.001 | 1.10 (0.98-1.24) | 0.03 | 1.08 (0.96-1.21) | 0.09 |
| Including diagnoses made beyond truncation of follow-up in CPRDd | 1.17 (1.05-1.31) | <0.001 | 1.09 (0.97-1.22) | 0.06 | 1.07 (0.95-1.20) | 0.14 |
| Excluding pregnancies with unknown BMIc | 1.18 (1.05-1.33) | <0.001 | 1.09 (0.96-1.23) | 0.08 | 1.07 (0.95-1.21) | 0.16 |

**Table 4 - Examining the association between first-trimester vaccination and MCMs in sensitivity analyses.**

A priori confounders were maternal age, maternal ethnicity, region and the earliest influenza season a pregnancy overlapped with. Other potential confounders included the number of weeks the first trimester overlapped with a period of influenza activity above baseline levels as well as the following maternal factors: IMD, number of children in the household, smoking status, hazardous drinking, extreme BMI, clinical risk group, chronic hypertension and exposure to teratogenic drugs and/or live vaccines. **a**This model included an additional 216 pregnancies with a vaccination in the 4 weeks before the pregnancy start. **b**There were 22 infants with a MCM recorded in HES or ONS after follow-up in CPRD had ended. **c**This model excluded 8,093 pregnancies that belonged to women with unknown BMI. **d**There were 110 infants with a MCM recorded in HES or ONS after follow-up in CPRD had ended. Abbreviations: MCM, major congenital malformations; CPRD, Clinical Practice Research Datalink; HES, Hospital Episode Statistics; ONS, Office for National Statistics death certificate data; BMI, body mass index; IMD, Index of Multiple Deprivation; HR, Hazard Ratio; CI, Confidence Interval.

Potentially eligible pregnancies resulting in a live-born singleton infant between September 1, 2010 – March 31, 2016**a** **N=103,742**

**4,543 (4.4%)** Excluded based on uncertainty in vaccination timing:

 **47 (0.05%)** Concurrent evidence of vaccine receipt and non-receipt

 **2,253 (2.2%)** Vaccination received before or after the pregnancy period

 **846 (0.8%)** Pregnancy spanned two influenza seasons and a vaccination record was identified in both

 **1,119 (1.1%)** Evidence of vaccination outside the practice at an unknown time

 **276 (0.3%)** Vaccination between April and August each year (likely a historical recording of an earlier vaccination)

 **2 (0.002%)** Date of vaccination not recorded

**1,266 (1.2%)** Excluded based on congenital malformation criteria:

 **598 (0.6%)** Congenital infections associated with malformations**c**

 **668 (0.6%)** Congenital malformations with known causes**d**

**1,564 (1.5%)** Excluded pregnancies due to unknown maternal ethnicity or smoking status or uncertain clinical risk group status**e**

**N=78,150** pregnancies

**Figure 1 - Derivation of pregnancies used in analyses. a**At least one week of the pregnancy had to occur when influenza vaccine was available. All infants had to be eligible for linkage to HES and ONS data. **b**Pandemic vaccine was available alongside SIV in 2009/10 and 2010/11. Pregnant women could be offered the pandemic vaccine in 2010/11, or in 2009/10 if their pregnancy ended after September 1, 2010 but started in the prior influenza season. Pregnancies that received pandemic vaccine or an unspecified influenza vaccine in 2009/10 or 2010/11 were excluded. **c**Toxoplasmosis, Rubella, Cytomegalovirus, Herpes, Parvovirus, Varicella-zoster, Syphillis, HIV. **d**Chromosomal anomalies, heritable conditions, or malformations due to a known teratogen. **e**Amongst pregnancies for which linkage to the infant record was available, 720 had unknown maternal ethnicity, 403 had unknown maternal smoking status and 449 had an uncertain maternal clinical risk group status.

**7,269 (7.0%)** Excluded due to the possibility a pandemic vaccine was received**b**

**10,950 (10.6%)** Excluded pregnancies which were recorded as resulting in a live-birth but for which linkage to the infant’s records was unavailable (and therefore the outcome could not be ascertained)