

Point-by-point Response to the reviewers' comments

Editors

Dear Georg Røggla and the BMJ,
Thank you for allowing us to revise our paper. The constructive advice of the peer reviewers has substantially improved our paper.
Attached are our detailed responses to their comments.

Please note that our revised manuscript contains slightly different HRs for stillbirth among siblings in the revised manuscript. In the original manuscript we defined stillbirth as stillbirth from gestational week 22 or later. That is correct for the population-based analysis but not for siblings born until 2008 (when only stillbirths from gestational week 28 or later were registered). We have re-calculated our data and they are similar but not identical to those of the initial manuscript (they do not change the interpretation of the paper). We apologize for this mistake, which has been corrected in the current version of our manuscript.

All changes in the manuscript have been marked with yellow colour.
On the behalf of the authors
Yours sincerely,

Prof Jonas F Ludvigsson
Corresponding author

Editor: What does the sibling analysis add?

Authors: The sibling analysis reduces intrafamilial confounding. By comparing offspring of the same mother discordant for vaccination exposure, we by design adjust for genetic and environmental factors shared by the siblings. This has been explained in the manuscript (introduction: page 5 and methods: page).

In our earlier manuscript on neurological and immunological diseases following H1N1 disease, we found that all-cause mortality was slightly lower in those undergoing vaccination (HR=0.95, 95%CI=0.94-0.96)(Ref: Persson et al. J Intern Med (2014) vol. 275 (2) pp. 172-90). If health aware individuals tend to seek vaccination more often than the general population it is likely to decrease the risk of adverse outcomes. Of note the earlier paper on stillbirth in H1N1 vaccinated women (Pasternak et al. BMJ (2012) vol. 344 pp. e2794) published by the BMJ also found 21% decreased risk of stillbirth in women exposed to H1N1 vaccine during pregnancy. Likewise we found a negative association between H1N1 vaccination during pregnancy and stillbirth (HR=0.83), but this association became weaker in the sibling analysis (HR=0.88) signalling the existence of residual confounding in the first analysis.

Potentially confounding could also work in the other direction. Assuming that women with comorbidity (and increased risk of stillbirth) to a higher extent would undergo vaccination, a

Ludvigsson et al. BMJ.2015.027974 entitled "Maternal vaccination against H1N1 influenza and offspring mortality – population based cohort study and sibling design

population-based study design would likely overestimate the risk of stillbirth. Hence, our sibling design adds to this paper.

Editor: How much does your paper add to a recent metaanalysis on this research question (BJOG. 2015 Jan;122(1):17-26)?

Authors: Our paper adds to the meta-analysis in BJOG in several ways.

First of all the paper by Fell et al does not at all address infant mortality beyond the neonatal period.

Second, ours is (to our knowledge) the first study, using a sibling design to tackle intrafamilial confounding.

Third, we add numbers and precision. On page 20 in their review Fell et al write, with regards to cohort studies on fetal death after H1N1 vaccination during pregnancy: *“Despite the similarity in the three studies with respect to the strain contained in the influenza vaccine and in the study design, the assessment of statistical heterogeneity was highly uncertain ($I^2 = 0\%$; 95% CI, 0–68%) and we were unable to explore the reasons for heterogeneity owing to the small number of studies. We opted not to compute a pooled summary estimate....”*

Our study included more pregnant women than did the studies by Beau, Håberg and Pasternak combined. This resulted in tight confidence intervals.

Editor: Isn't this primarily of historical interest?

Authors: As stated by one of the peer reviewers, Professor Stoltenberg, “It [the finding of the current study] is relevant for preparedness and health system governance, for policy makers, health workers, patient organizations and media.”

Hence, we believe our data are of great importance for future health care policy. We also believe our neutral data are comforting and likely to have an effect on the willingness of the public to undergo vaccinations when faced with similar epidemics.

Editor: Did any of the unvaccinated women get pandemic flu?

Authors: Unfortunately we have no reliable data on pandemic flu.

The Swedish Institute for Infectious Disease Control (now Public Health Agency of Sweden) has estimated that about 1 500 000 persons, whereof about 600 000 symptomatic, were infected with Influenza A(H1N1) in 2009/2010. This is equivalent to about 15% and 6% respectively, however given that the proportion of pregnant women with Influenza A has not been estimated in Sweden we have chosen not to cite any figure in the revised paper.

During the second season, 2010/2011 the estimated number of persons with symptomatic A(H1N1) was 308 500 (the number of persons infected without symptoms has never been estimated).

(Reference: Vaccinationskampanjen mot influensa A(H1N1) 2009. Utvärdering av hälsoeffekter säsongerna 2009/2010 och 2010/2011. Smittskyddsinstitutet (Swedish Institute for Infectious Disease Control), Juni, 2013. Artikelnummer: 2013-101-13

(<http://www.folkhalsomyndigheten.se/page/files/12869/vaccinationskampanjen-influensa-a-h1n1-2009.pdf>, accessed on September 15, 2015)

In the revised paper we have added the following sentence (page 15):

“Secondly, were unable to ascertain which mothers had pandemic flu during pregnancy.”

Editor: From a statistical point of view we would like to know more about the second comparison (within families). In particular the order of the pregnancies. If this was random or always the "index" was later than the "control". The flow chart could have some extra information about the pregnancies (with and without vaccinations) added. Mostly it is extra data needed for clarity but the analysis seems adequate.

Authors: In the sibling analyses, most but not all unexposed pregnancies took place before the index pregnancy (vaccination).

The proportions were as follows: Pregnancies *before*: 80.1%; Pregnancies *after*: 19.9% (peter, please add data).

In the revised paper we have added the following sentence to the background results of our paper (page 12): "Among the unexposed pregnancies in siblings (n=39,314), 31,496 (80.1%) took place before the pregnancy exposed to Pandemrix, and 7,818 (19.9%) took place after the index pregnancy."

Reviewer 1: Pedro Moro

Reviewer:

This manuscript describes a prospective cohort study in seven healthcare regions of Sweden that assessed the risk of offspring mortality (stillbirth, early neonatal period, late infant) following exposure in utero to AS03-adjuvanted H1N1 influenza vaccine.I would recommend its publication in the BMJ.

Authors: We would like to thank epidemiologist Moro for his kind comments.

I do have some comments regarding some of the wording used by the authors in the introduction

Reviewer:

In lines 20-25 under "With few exceptions (e.g. increased risk of preterm birth[24] and non-significant increased risk of stillbirth[11]), research suggests that H1N1 vaccination has few adverse effects on pregnancy outcomes"

This sentence seem to imply that preterm birth and stillbirth are real risks following influenza vaccination in pregnancy. The study the authors cite for preterm birth did find an increased relative risk but it was one finding inconsistent with most other studies. The other mention of non-significant increased risk of still birth should be taken out. The sentence should be changed. It could be changed to something like: "Most research suggests that H1N1 vaccination has few adverse effects on pregnancy outcomes"

Authors: The sentence in question has been changed in accordance with the suggestion of the peer reviewer (page 5).

Reviewer:

Ludvigsson et al. BMJ.2015.027974 entitled "Maternal vaccination against H1N1 influenza and offspring mortality – population based cohort study and sibling design

Also in the introduction, in lines 46-55, the authors state: “Maternal vaccinations could potentially influence offspring mortality through several mechanisms. Offspring mortality could increase if fetal reactions to viral or non-viral components of the vaccine [26] have long-term consequences, or through an excess of congenital malformations as indicated by some research [21]”. These statements are very speculative. The references provided (21 and 26) don’t really provide solid reasons to back-up these statements.

Authors: In the revised paper we have omitted the following lines:

~~“Maternal vaccinations could potentially influence offspring mortality through several mechanisms. Offspring mortality could increase if fetal reactions to viral or non-viral components of the vaccine[26] have long-term consequences, or through an excess of congenital malformations as indicated by some research[21]. On the other hand, a lower prevalence of influenza in offspring of vaccinated mothers might decrease offspring mortality[27].”~~

Reviewer:

Table 1: under the variable status at end of follow-up, what is dead referring to? Later death? Please specify

Authors: This has been clarified in the revised paper.

We have added that “Dead” were divided into dead during
(*pregnancy*)
(*neonatal*)
(*childhood*) [*new*]

In the Table legend we have added that childhood death refers to the time from the 2nd week after birth and onwards.

Reviewer:

For the characteristic gestational age, mean (SD), is the number in parentheses the standard deviation or the proportion of vaccinated or not vaccinated? Please provide the gestational age in weeks

Authors: We apologize for not introducing the acronym. SD is for (one) standard deviation (and has been added to the text). We have changed the scale from days to weeks in Table 1.

Reviewer:

The flowchart is also explained well in the text so it could be deleted

Authors: The flowchart has been deleted in the revised version of the manuscript.

Reviewer 2: Camilla Stoltenberg

Reviewer:

This is a well written paper using excellent population based data from Sweden, a reliable and

Ludvigsson et al. BMJ.2015.027974 entitled "Maternal vaccination against H1N1 influenza and offspring mortality – population based cohort study and sibling design

original design, state of the art and novel methodological approaches, and sound and cautious interpretation. The study adds important new and relevant knowledge to the field. The study results suggest that maternal H1N1 vaccination during any trimester of pregnancy has no adverse effect on offspring mortality, during pregnancy, in the early neonatal period or in early childhood. I strongly recommend publication of this paper in BMJ, and the paper can be published as it is after minor editorial revision.

Authors: We would like to thank Professor Stoltenberg for her kind comments.

Minor specific comments

Reviewer:

Page 3, line 32-33 Data sharing. Why do the authors say that “No additional data will be made available”? What does this mean? I thought that linked Swedish routine registry data were available for any research group provided that one has ethical approval and purposes within the legal framework for such data. What happens if other researchers want to challenge the analyses in this study? Can they apply for access to the linked data prepared for this study?

Authors: We apologize for being unclear about this.

According to Swedish law, we are not allowed to share data with other researchers, but they can apply for data through the Swedish National Board of Health and Welfare. This has changed in the manuscript (page 2).

Reviewer:

Page 3, line 9. I believe that it should be written “source of bias” rather than “source for bias”.

Authors: Corrected - thank you.

Reviewer:

Page 8, line 47. I assume it is correct that only siblings discordant for both exposure and outcome contributed to the analyses but I (and probably other readers) need this to be explained in some more detail. Why is it not only discordance for exposure which is required?

Authors: Apologizes for not being precise enough. We agree it's more correct not to say discordant on outcome. However, since most families discordant on exposure have no event, they will fall out of the conditional (stratified) likelihood, so to present numbers that actually contribute to sibling analysis we changed the text to "only siblings discordant on exposure and with at least one event within the family (n=3,801, 1,130, and 2,190) contributed to the analyses of stillbirth, neonatal death and death in the subsequent period."

Reviewer:

Page 10, line 50. Should it be “If the mother was...” rather than “If the mother were..”?

Authors: Many people will write “if the mother was...” however, this sentence can also be regarded as a conjunctive/subjunctive and then “if the mother were...” may be used.

However, for convenience we have change to “*if the mother was...*”

Reviewer 3: Laura Price

Reviewer:

The study appears well constructed and clearly reported, and will provide useful information to women considering vaccination during pregnancy. I am, however, unsure of how the sibling control works for a study that includes perinatal death as an outcome measure, particularly as risk of stillbirth is increased in women >35 and then again in those >40. Could the authors add a few more sentences describing how the sibling control group adds reassurance to the study design?

Authors:

We acknowledge that further explanation of the advantages of the sibling model is needed. We refer to our response to the editor (beginning of this document) where we have outlined the reasons for using a sibling-approach.