**Mortality Associated with Seasonal and Pandemic Influenza among Pregnant and Non-Pregnant Women of Childbearing Age in a High HIV Prevalence Setting – South Africa, 1999-2009 (Supplementary Material)**

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**METHODS**

**Stage-1 model: national estimates of influenza-associated mortality**

To estimate the seasonal and pandemic influenza-associated mortality, we fitted Generalized Linear Models (GLM) with a Poisson distribution and an identity link to the number of monthly deaths among pregnant and non-pregnant women as previously described [[[1]](#endnote-1),[[2]](#endnote-2)]. The identity link was selected because it is considered the most biologically plausible link to model the impact of pathogen circulation on mortality [[[3]](#endnote-3),[[4]](#endnote-4),[[5]](#endnote-5),[[6]](#endnote-6)]. The full model (Model 1) included covariates for time trends and seasonal variation as well as proxies for viral circulation as follows:



 (1)

*E(Yi,t)* represents the number of deaths in pregnancy group *i* (pregnant or non-pregnant) and month *t*; *β0,i* is the pregnancy group-specific model constant; *β1,i* to *β4,i* are pregnancy group-specific coefficients associated with time trends (linear to quartic polynomial terms); *β5,i* and *β6,i* are pregnancy group-specific coefficients associated with harmonic terms accounting for annual background seasonal variations; *β7,i* to *β8,i* are pregnancy group-specific coefficients representing the contribution of influenza to mortality (seasonal influenza (*β7,i*): including A(H1N1), A(H3N2) and B; and pandemic influenza (*β8,i*): A(H1N1)pdm09); and *εi* is the pregnancy group-specific error term. *Seasonal\_influenza(t)* and *A(H1N1)pdm09(t)* are proxies for monthly viral activity, estimated as the monthly number of specimen testing positive for influenza over the annual number of specimens tested for the specific pathogen. We used standardization by the annual total of all specimens tested for the specific pathogen, to reduce possible bias associated with differences in specimen sampling and laboratory methods over time [1,2,[[7]](#endnote-7)]. Models were fitted separately for each age group (for non-pregnant women only) and cause of death evaluated in this study.

Through model selection procedures, we assessed the fit of models including higher order polynomials to represent more subtle time trends (1st to 6th degree) and additional harmonic terms representing annual and semi-annual periodicity (*sin(2tiπ/12)* and *cos(2tiπ/12)*; *sin(4tiπ/12)* and *cos(4tiπ/12)*). The final model (Model 1) was that for which the Akaike value (AIC) was minimized, that is, the model that provided best fit to the data whilst maintaining parsimony. Furthermore, we assessed the model fit using two different proxies for monthly viral activities. Proxy 1 was obtained by dividing the monthly number of specimen testing positive for influenza to the annual number of specimens tested for the specific pathogen (model presented in equation 1). Proxy 2 was not standardized by the annual number of specimens tested. The estimates from models using proxies 1 or 2 were comparable; however the models fitted using proxy 2 yielded consistently higher AIC values compared to the models fitted using proxy 1. In addition, we implemented a sensitivity analysis where we compared the estimates from our count model (equation 1) to those obtained from a rate model. The estimates obtained from the rate models remained within 4% of their main-analysis values (count model) across the analysis implemented over the different causes of death evaluated in the study. Modeling counts or rates in equation 1 gives similar results given slow trends in population sizes. Given the minimal difference between the estimates from the count and rates models, we chose to model death counts instead of death rates in line with previously developed methodologies [1,2,[[8]](#endnote-8),[[9]](#endnote-9)]. We also considered b-splines (1 knot per month was best) instead of polynomial terms to model background seasonality, but polynomial terms provided the best fit to the South African data, perhaps because of the relatively crude monthly resolution of the data; however this hypothesis could not be tested.

We estimated the excess mortality associated with influenza each month by subtracting an expected baseline from the monthly mortality predictions of Model 1. The baseline was obtained by setting the relevant influenza covariate to 0 (i.e., to obtain a baseline for seasonal influenza, we set the seasonal influenza proxy to 0). Annual excess mortality was estimated as the sum of the monthly excess mortality for each year. We obtained the 95% confidence interval (CI) for the estimated excess mortality using bootstrap resampling on blocks of calendar years (12-month block resampling with replacement) over 1000 replications [1,2,[[10]](#endnote-10)]. For each resampled dataset we refitted the regression model and the 95% CI were obtained from the 2.5th and 97.5th percentiles of the estimated influenza-associated mortality from the 1000 resampled datasets.

**Stage-2 model: estimates of influenza-associated mortality by HIV status**

In South Africa, a diagnosis of AIDS is rarely coded on the death certificate [[[11]](#endnote-11)], hindering direct estimation of influenza-associated excess mortality by HIV status. Instead, we used a two-stage regression approach that builds on the annual national excess mortality estimates provided by Model 1 as previously described [1,2]. The rationale for the annual regression relies on using the increasing trend in HIV prevalence over time to estimate the fraction of national excess deaths attributed to HIV-positive and HIV-negative individuals (in particular, if HIV was not a risk factor for influenza-related mortality, then influenza-related mortality would not increase over time, and influenza-related deaths would occur among HIV-infected and HIV-uninfected populations proportionally to their group sizes). The annual regression further accounts for increasing HAART coverage (which tends to decrease influenza-related excess mortality among HIV-infected individuals over time [8]) and circulation of more severe influenza subtypes (A(H3N2) vs A(H1N1) or B). Through model selection procedures, we assessed the fit of models including higher order polynomials (1st to 3rd degree) to represent time trends of health indicators unrelated to influenza, HIV or HAART as well as the removal of the influenza, HIV or HAART covariate from the models. The model with 2nd degree polynomial time trends and influenza, HIV and HAART covariates had the best fit to the data. The model accounts for the combined effect of varying HIV prevalence in the population over the years as well as different HIV interventions, including the prevention of HIV infection and the effect of HAART on HIV infected individuals.

We fitted separate multivariate GLM for each group (pregnant and non-pregnant women) and cause of death, considering a Poisson distribution and an identity link (Model 2) as previously described [1,2]. The following model was used for seasonal influenza:

 (2)

Where *E(Yi,t)* represents the number of seasonal influenza-associated excess deaths for pregnancy group *i* (pregnant or non-pregnant) and year *t* (as obtained from the stage-1 approach); *αi* is an offset representing the population size of pregnancy group *i*; *β0,i* is the pregnancy group-specific intercept; *β1,i* and *β2,i* are pregnancy group-specific coefficients associated with time trends (linear and quadratic) included to account for potential variations of health indicators unrelated to influenza, HIV prevalence or HAART coverage in the population; *β3,i* is the pregnancy group-specific coefficient associated with dominant seasonal influenza type/subtype each year (categorical variable with A(H3N2)-dominant years as reference group vs. A(H1N1) or B) [7,[[12]](#endnote-12)]; *β4,i* is the coefficient associated with the HIV prevalence in the population in pregnancy group *i* and year *t*; *β5,i* is the coefficient associated with HAART coverage among HIV-infected individuals in the population in pregnancy group *i* and year *t*; and *εi* is the pregnancy group-specific error term. Models were fitted separately for each age group (for non-pregnant women only) and cause of death evaluated in this study. The estimated model parameters are provided in Table S1.

Mortality rates by HIV status were obtained by dividing the estimated influenza-associated deaths by HIV status from Model 2 by the mid-year population estimates within each category (i.e. HIV-infected and –uninfected pregnant and non-pregnant women). The model was not fitted to mortality estimates for influenza A(H1N1)pdm09 as the approach requires several years of circulation of a specific virus to partition excess deaths by HIV status.

**Standardization of influenza-associated mortality rates**

Indirect standardization was used because the low number of deaths among pregnant women hindered our ability to obtain accurate estimates of influenza-associated mortality by age group. Annual age standardized rates of influenza-associated mortality among pregnant women were obtained as follows [[[13]](#endnote-13)]:

 (3)

*SRatePW*is the annual age standardized influenza-associated mortality rate among pregnant women; *DeathPW* is the annual crude number of estimated influenza-associated deaths among pregnant women (15-49 years of age); *RateNPW* is the annual estimated influenza-associated mortality rate among non-pregnant women in age group *i* (the age groups used for age standardization were: 15-21, 22-28, 29-35, 36-42 and 43-49 years); *PopPW* is the annual number of pregnant women in age group *i*; and *RateNPW* is the annual crude estimated influenza-associated mortality rate among non-pregnant women (15-49 years of age).

For seasonal influenza age standardized rates were obtained by HIV status using the outputs from Model 2 and the appropriate denominators by HIV status. For influenza A(H1N1)pdm09 overall age standardized rates (irrespective of the HIV status) were obtained using the outputs from Model 1. In our study we could not estimate the influenza A(H1N1)pdm09-associated mortality by HIV status because our modeling method requires HIV prevalence data over several years of circulation of the pandemic virus.

**Estimation of relative risk**

To assess the relative risk (RR) for influenza-associated mortality related to pregnancy we compared the influenza-associated death rates (crude and age standardized) among pregnant and non-pregnant women using log-binomial regression. For seasonal influenza the overall RR for influenza-associated mortality related to pregnancy was adjusted by HIV status.

**RESULTS**

**Table S1:** Estimated parameters for the assessment of the number of HIV-infected individuals among seasonal influenza-associated deaths in pregnant and non-pregnant women (Model 2), South Africa, 1999-2009.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **Pregnant women** | | | | **Non-pregnant women** | | | |
| **All-causes** | **All-respiratory** | **All-circulatory** | **P&I** | **All-causes** | **All-respiratory** | **All-circulatory** | **P&I** |
| **Rate ratio**  **(95% CI)** | **Rate ratio**  **(95% CI)** | **Rate ratio**  **(95% CI)** | **Rate ratio**  **(95% CI)** | **Rate ratio**  **(95% CI)** | **Rate ratio**  **(95% CI)** | **Rate ratio**  **(95% CI)** | **Rate ratio**  **(95% CI)** |
| Time trend (linear) | 0.5 (0.3-0.7) | 0.4 (0.1-0.8) | 0.4 (0.2-0.7) | 0.3 (0.1-0.7) | 0.4 (0.2-0.7) | 0.2 (0.1-0.4) | 0.4 (0.1-0.8) | 0.3 (0.1-0.6) |
| Time trend (quadratic) | 1.1 (0.9-1.3) | 1.3 (1.1-1.5) | 1.2 (1.1-1.3) | 1.2 (1.0-1.4) | 1.3 (1.1-1.5) | 1.1 (0.9-1.5) | 1.2 (1.1-1.3) | 1.2 (1.0-1.4) |
| Dominant influenza subtype |  |  |  |  |  |  |  |  |
| A(H3N2) | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference |
| A(H1N1) | 0.9 (0.7-1.2) | 0.8 (0.6-1.0) | 0.7 (0.5-0.9) | 0.8 (0.7-0.9) | 0.8 (0.6-1.1) | 0.7 (0.5-0.9) | 0.9 (0.7-1.2) | 0.8 (0.5-1.2) |
| B | 0.7 (0.5-0.9) | 0.6 (0.3-0.9) | 0.7 (0.4-1.0) | 0.5 (0.2-0.8) | 0.6 (0.3-0.9) | 0.5 (0.2-0.8) | 0.7 (0.5-0.9) | 0.6 (0.3-0.9) |
| HIV | 1.5 (1.1-2.0) | 1.6 (1.3-1.9) | 1.5 (1.2-1.8) | 1.7 (1.3-2.1) | 1.6 (1.4-1.9) | 1.8 (1.5-2.1) | 1.5 (1.2-1.8) | 1.8 (1.4-2.2) |
| HAART | 0.9 (0.8-1.0) | 0.8 (0.7-0.9) | 0.9 (0.7-1.1) | 0.9 (0.8-1.0) | 0.8 (0.7-0.9) | 0.9 (0.8-1.0) | 0.8 (0.7-0.9) | 0.9 (0.7-1.1) |

Abbreviations: P&I: pneumonia and influenza; CI: confidence intervals; HIV: human immunodeficiency virus; HAART: highly active antiretroviral treatment.

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