Author's response to reviews

Title: Seasonal Effects of Influenza on Mortality in a Subtropical City

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Author's response to reviews: see over
Dear Dr Alam,

Re: Seasonal Effects of Influenza on Mortality in a Subtropical City
Manuscript # 4404430412243797

Thank you very much for your e-mail dated 6 April, 2009, informing me that our above-captioned manuscript is potentially suitable for publication and requesting us to respond to the reviewer’s comments and to revise the manuscript accordingly. We have now written a response to each individual comment of the reviewer’s point by point as shown below. The manuscript is now revised and resubmitted as in the document ‘Seasonal_MBCID_revise_clean.doc’.

In addition to changes in response to the reviewer’s comments, we have made some minor changes to the texts to improve the readability of the paper. A file ‘Seasonal_MBCID_revise.doc’ with track changes is also submitted.

I should be grateful for your consideration for publication of our manuscript in the BMC Infectious Diseases.

Your sincerely,

Chit Ming Wong
Corresponding Author

Seasonal Effects of Influenza on Mortality in a Subtropical City

Response (Res) to Referee’s comments:

Referee 1:
Reviewer’s report This interesting paper explores seasonal variation in the severity of influenza viruses in Hong-Kong, using a variety of mortality outcomes. In other words, the authors test the hypothesis that a given level of virus activity will have a different mortality impact depending on the season. Hong-Kong is the perfect location to test this kind of hypothesis due to the pattern of near year-round influenza activity there. The authors conclude that for most (but not all) disease
categories, the influenza impact is most pronounced in winter and late spring/early summer.

I have a few comments that may help improve the interpretation of results, including differences in seasonal effect by disease category, and refine the proposed mechanism responsible for such effects.

Major Compulsory Revisions

- As the authors mention in the discussion, a confounding factor in their study is virus subtype, since different influenza subtypes are known to be associated with different mortality impact. If I understand correctly, influenza A/H3N2 is more predominant in summer in Hong Kong (p.15), which could explain summer peaks in “excess risk” in some disease categories. Since the authors appear to have some data on the weekly proportion of H3N2, could they include this information in their model and test whether seasonal effects remain for H3N2 viruses?

Res: We applied the time-varying coefficient Poisson models to the weekly death numbers from 1998 to 2002 when the subtype data were available in Hong Kong as described in the Methods section. This time we added the variables for weekly proportions of subtype H3N2 and for the product of two sinusoidal pairs with H3N2 proportions to measure the seasonal effects of H3N2, while the proportions of H1N1 and type B were also entered into the model. The excess risks associated with H3N2 showed similar patterns as the combined seasonal effects of three types/subtypes of influenza viruses, suggesting that the H3N2 accounted for the majority of seasonal effects of influenza (Appendix 1). We also repeated the above analyses for H1N1 and type B viruses, respectively and found that none of them had significant seasonal variations. However, we are aware that the lack of seasonal effects in H1N1 and B could be the result of mild activities of these two types/subtypes during our study period. The fifth paragraph of Discussion section is now revised into:

“As H3N2 is believed to cause more mortality and to spread more efficiently than H1N1 and B [34,35], we hypothesize that seasonal effects of influenza could be a result of different subtypes dominating over different seasons. Since the virus subtype data during the study period were not available from QMH, we obtained from the Government Virus Unit of the Department of Health in Hong Kong the weekly proportions of specimens respectively positive for influenza H3N2, H1N1 and B from 1998 to 2002. Overall, the proportions of H3N2 isolates in all influenza virus positive specimens show a similar two-peak pattern to the seasonal variation of influenza effects from winter to summer, with a higher peak in the summer than in the winter (See Additional file 5). We then applied the time-varying coefficient Poisson model to the weekly death numbers from 1998 to 2002 when the subtype data were available in Hong Kong, with the variables for weekly proportions of subtype H3N2 and for the product of two sinusoidal pairs and H3N2 proportions added into the model to measure the seasonal effects of H3N2. The proportions of H1N1 and type B were also entered into the model. The results suggested that H3N2 contributed to the majority of seasonal effects of influenza, as the excess risks associated with H3N2 showed similar patterns as those associated with the combined
proportions of three types/subtypes of influenza viruses (data not shown). We repeated the above analyses separately for H1N1 and type B viruses and found that none of them has significant seasonal variations. However, the lack of seasonal effects in H1N1 and B could be the result of mild activities of these two types/subtypes during our study period. Nevertheless, we could not rule out that changing dominance of different virus subtypes could also contribute to seasonal effects of influenza.”

- It is somewhat surprising seasonal effects vary by mortality outcome, not only in amplitude, but also in seasonal patterns. For instance, there is no evidence of seasonal effect for P&I, which is supposed to be the most specific indicator of influenza-related mortality. Also, there is a very high winter maximum of excess risk for COPD; while peaks in other disease categories are most pronounced in spring/summer (Fig 2). Is there a biological mechanism related to how influenza virus infection triggers death, directly or indirectly, which could explain these differences by disease category? Also, the fact that there is no seasonal effect on P&I is worrying – I understand that P&I provides an underestimate of influenza burden, however it is very specific and should reflect the same seasonal variations as the other death categories (unless we assume a seasonal bias in coding of deaths).

Res: It is possible that preexisting chronic conditions are more likely recorded as underlying cause of deaths even though they died directly from P&I. It is reasonable to assume that people who have been suffering from chronic diseases are more vulnerable to infections and would die quickly after infection. But they are likely recorded as dying from their preexisting conditions. By contrast, those who have been relatively healthy could stand a longer time before death and be classified as P&I deaths eventually. Given these circumstances, more significant seasonal effects of influenza on chronic diseases could be explained by the harvesting effects on the fragile population which brought temporal variations in the composition of susceptibility pool, thereby resulting in a big turnover in the mortality risks for chronic diseases associated with influenza, but a less clear seasonal variation for P&I deaths. To clarify this point, we have now revised the sixth paragraph of Discussion section as follows:

“The seasonal effects are significant for chronic conditions but not for the mortality of pneumonia and influenza which is considered as the most specific endpoint of influenza infection [1,10]. We think this could be due to the fact that we used the underlying cause of death, which probably results in underreporting of pneumonia and influenza cases and subsequently underestimating the influenza associated mortality for pneumonia and influenza. The effects of influenza on chronic conditions, especially COPD, decrease promptly after reaching the peaks, thereby exhibiting more pronounced seasonal variations than the seasonal effects on pneumonia and influenza. We speculate that there is a harvesting effect for influenza associated mortality [37]. The vulnerable people, who had been suffering from chronic diseases, would die soon after infection and their causes of deaths would likely be recorded as their preexisting conditions. At the beginning of an epidemic, influenza infections probably claim the most vulnerable people to empty the susceptibility pool, leading to a subsequent decline in the influenza effects. Such a
temporal change in the susceptibility pool may not be evident for those who had been previously healthy and were grouped as pneumonia and influenza deaths.”

- I think generally the mechanistic explanations proposed in the discussion lack precision.
  o For instance, it is assumed that since influenza excess risk peaks in winter and spring/summer in cardio-respiratory diseases, there may be a synergistic interaction between influenza virus infection and very hot or very cold temperatures (end of p13). However what are the average temperatures in winter, late spring and early summer in Hong-Kong, and how different are these temperatures from temperatures in autumn, a season in which a clear dip in excess risk is found (Table 2)?

Res: We agree that our discussion part cannot be more precise, largely because to date the mechanisms for influenza seasonality and seasonal variations in host susceptibility remain unclear. The average temperatures for autumn, winter, spring and summer are 25.6°C (range 19.3-31.7°C), 17.6°C (8.9-22.7°C), 22.9°C (14.6-28.1°C) and 28.6°C (25.3-30.3°C), respectively (Table 1). The temperature ranged from 8.8°C to 32°C in Hong Kong during our study period. The plots of weekly numbers of all-cause and P&I deaths and weekly average temperature are shown in Appendix 2. We can observe the clear mortality seasonality in Hong Kong. In general the least death numbers were recorded in autumn and one big winter spike usually appeared weeks after the day with lowest temperature and a small spike also appeared soon after the day with the highest temperature recorded in summer. This similar mortality seasonality was also reported in Taiwan which has a similar subtropical climate as Hong Kong, suggesting that in the subtropics the temperature effects on mortality exhibited a U-shape pattern with higher mortality risks in cold winter and hot summer [1]. Similarly, influenza viruses tend to be active in winter and late spring. Overlapping in timing of peaks between influenza activity and the temperature effects on mortality, plus their similar biological effects, suggest that the synergistic interaction between influenza and temperature is highly plausible. To further clarify this in our paper, we have now added the following sentences into the second paragraph of Discussion section:

“Exposure to low and high temperature can both increase blood viscosity and trigger cardiovascular events [22]. Influenza infections have similar prothrombotic and proinflammatory effects [23]. Numerous studies in the temperate regions showed that exacerbations of COPD frequently occur in cold temperature when influenza viruses tend to be active, as a result of stimulated airway inflammation [24,25]. Similar to the seasonal effects of influenza, the mortality counts of all-cause deaths also exhibited a two-peak pattern. Usually one big winter spike usually appearing weeks after when the lowest temperature was recorded and a small spike also appeared soon after the week when the highest temperature recorded in summer (data not shown). The least death numbers recorded in autumn. This mortality seasonality was also reported in other subtropical cities [22]. Furthermore, a multiplicity study in the United States demonstrated that the temperate effects on mortality exhibited a U-shape pattern with a turning point around
26°C for the southern cities [26]. Similarly, influenza viruses tend to be active in winter and early summer. Overlapping in timing of peaks between influenza activity and the temperature effects on mortality, plus the similar biological effects of viruses and extreme temperate, suggest that the synergistic interaction between influenza and temperature is highly plausible.”

The authors propose that virulence could vary over the course of one season, as viruses drift antigenically. They cite phylogenetic evidence from New York state data (ref 26), however all New York state analyses so far have revealed that there is indeed *no* evidence of local evolution within a season – in fact that is one of the major reasons for suspecting that the Tropics could be a source of new influenza viruses. Obviously the situation in Hong-Kong could be different from that of NY, but the NY data are probably not the right data to cite here in support of local evolution within a season.

Res: We agree with the Referee’s comments. Some large-scale phylogenetic studies did show that several lineages of influenza viruses could co-circulate within the same season, but in agreement with the Referee’s comments they also stated that there is “little evidence for antigenic drift at the scale of individual seasonal epidemics in those temperate populations studies so far” [2]. It has been proposed that the subtropics and tropics, especially East and Southeast Asia, could be the virus reservoirs where the reassortment of co-circulating lineages could occur and novel virus strains could evolve and later spread to the temperate regions [3]. If this hypothesis is true, we have a strong reason to believe that virulence of influenza viruses may not be consistent throughout the year, in view of the frequent emergence of novel strains in the subtropics and tropics. We now revise the second paragraph of p.15 as follows:

“……We proposed that the seasonal changes of influenza virulence might reflect the frequent antigenic shifts of viruses within an influenza epidemic season Several large-scale phylogenetic studies could not find evidence of local evolution of virus strains in the temperate regions and proposed that the subtropics and tropics, especially East and Southeast Asia, are more likely the virus reservoirs where the reassortment of co-circulating lineages could occur to result in emerging novel virus strains that later spread to the temperate regions [31-33]. The better understanding to the mechanisms of influenza virus evolution must be based on the more comprehensive surveillance networks in the tropics and subtropics.”

I think the authors should distinguish between probability of infection (which may be related to antigenic drift and population immunity, and which is not what their excess risk measures) and probability of death given infection (which is related to virus pathogenicity, and is perhaps more related to what this excess risk analysis gets at, and could be mediated by internal genes and/or interaction between influenza virus and other pathogens responsible for lethal superinfections).
Res: We agree with the Referee. Now we clarify the above points in the first paragraph of Discussion to make it clearer for readers:

“……The excess risk is also different from the virus activity: the former measure the severity of diseases given influenza infections, but the latter probability of infections. The virus activity is controlled by transmission efficiency of viruses as well as by the herd immunity, but the probability to develop into a severe disease is more likely affected by the pathogenicity of viruses and individual immunity levels against respiratory pathogens including influenza virus and pneumococcus bacteria, the latter of which often cause secondary pneumonia. ……”

o Isn’t the analysis of morbidity the natural next step to attempt to distinguish between severity of disease and clinical infection?

Res: We have done the analysis for hospital admissions in Hong Kong. We found similar two-peak seasonal patterns for the influenza effects on hospitalization. However, we also notice that there may be potential biases introduced during referral practice and hospital bed shortages in some seasons. Therefore the results need more careful interpretation.

- Separate analyses are presented for all ages and 65 yrs and over, but it appears as the results for all ages are driven by the 65+. Could the authors perform the same analyses for children (eg, under 5 ?) It would be interesting to see whether seasonal mortality effects varied by age.

Res: Unfortunately, the death rate of children in Hong Kong is very low in Hong Kong. The annual total number of deaths in children under 5 is around 150, which is too small to allow us to perform a Poisson regression on the weekly counts.

- One of the sensitivity analyses considers similar seasonal effects for RSV, as I understand (p 10). Was there evidence of seasonality in excess risk for RSV as well?

Res: The excess risks associated with RSV showed a significant seasonal variation for all-cause, CRD, COPD and IHD, but no significant seasonal effects were found for P&I and cerebrovascular diseases. The age difference in excess risks was found for all-cause deaths, with seasonal effects significant in the all-ages group but not in the elderly. Appendix 3 shows the excess risks of mortality associated with per inter-quartile range increase of RSV activity (10.2% in our study). Different from the seasonal effects of influenza, only one peak is observed for RSV effects. For most disease categories, this peak occurred in the winter, whereas COPD mortality has a clear summer peak.

Discretionary Revisions
- Did the authors consider lags for RSV and the other viruses?
Res: We did not consider the lags for RSV and other viruses in our analysis. We revise the second paragraph of p.7 as follows:

“The weekly proportions of specimens positive for influenza viruses were then entered into the core model, along with the proportions of RSV, adenovirus, PIV-1, PIV-2 and PIV-3 at the current week, to calculate the effects of influenza with adjustment for co-circulation of other respiratory viruses…..”

- What do negative excess risk estimate mean (fig 2)? Is influenza virus infection protective against mortality in fall (Table 2)?

Res: Negative estimates do not necessarily suggest a protective effect in autumn. We think they were probably the results of overestimated baseline mortality in our models. Even though we first built core models to take account of the variations due to seasonality of mortality and confounders and then carefully evaluated the adequacy of these models, it is still possible that part of influenza effects (probably synergistic interaction effects with temperature) were over adjusted by the core models. The problem in evaluating the extent to which the confounders should be adjusted for is also often encountered in other disease burden models [4-7] and warrants further studies.

Level of interest An article whose findings are important to those with closely related research interests
Quality of written English Acceptable
Statistical review Yes, and I have assessed the statistics in my report.
Declaration of competing interests 'I declare that I have no competing interests'

Res: We thank for the Referee’s positive comments.

Comments from the Associate Editor:
The reviewer found the paper potentially suitable for publication but raised important questions. Additional efforts should be made in the interpretation of results and additional analyses could strengthen the study.

Please, also check the formulas (e.g. pages 8 and 9, indices differed) and the bibliography (ref 28 and 30 are duplicates)

Res: We have now revised according to the Editor’s comments.

References in cover letter


Appendix 1. Excess risks (%) associated with per IQR increase in proportions of positive H3N2 specimens. (A) All-ages
Appendix 1 (Continued).
(B) 65+ age group

![Graph showing excess risk (%) for different causes over weeks in year]

- All-cause 65+ years
- CRD 65+ years
- P&I 65+ years
- COPD 65+ years
- Cerebrovascular 65+ years
- IHD 65+ years
Appendix 2. The time series plot of weekly number of all-cause, P&I deaths and mean temperature in Hong Kong, 1996-2002.
Appendix 3. Excess risks (%) associated with per IQR increase in proportions of positive RSV specimens.

(A) All-ages age group
Appendix 3 (Continued).
(B) 65+ age group

![Graph with excess risk (%)](image-url)