Author's response to reviews

Title: Viral aetiology of acute respiratory infections among children and associated meteorological factors in southern China

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Author's response to reviews: see over
Response to Reviewers

Reviewer 1:

Reviewers report
Title: Viral aetiology of acute respiratory infections among children and associated meteorological indicators in southern China
Version: 1 Date: 9 September 2014
Reviewer: Jesse Papenburg

Reviewers report:
Major Compulsory Revisions
1. The authors explore associations between meteorological factors and detection of respiratory viruses in children 0-16 years old with signs / symptoms of acute respiratory infection in Shantou, China. Results of multiple comparisons and logistic regression models are presented. A greater emphasis on the exploratory nature of these analyses must be presented. Multiple comparisons increase the risk of false-positive associations. Even more importantly, regarding meteorological factors and respiratory virus circulation, there are inherent autocorrelation structures in the data. In other words, the number of viruses detected during a week is related to the counts from the previous weeks and future weeks. The same is true for temperature, humidity and wind velocity. Because of the autocorrelation within each individual time series, the correlation of two unrelated time series can be spuriously but significantly high due to chance alone or due to the confounding effect of an unmeasured seasonal covariate. Since the authors did not account for this autocorrelation (for example, through the use of time series analysis methods), the observed associations must be interpreted cautiously.

   Thank you. As per your advice, we have put more emphasis on the exploratory nature of our analysis and described the caveat you described and cautious interpretation in the manuscript (page 11, line 7-9).

2. It is very surprising to see such high rates of positivity for EV and HRV, and especially such a high rate of co-detection of EV-HRV. 215 of 558 infections (39%) were dual infections of EV-HRV, and HRV-EV were co-detected in 307 samples!!! This is shocking. The authors mention in the Discussion that because of the genetic relatedness of EVs and HRVs, PCR can sometimes not distinguish between the two targets and will generate a signal for both even though only one of the two is present. Their analysis of differences in Cp between the two targets is insufficient, in my opinion, to exclude the possibility that many EV-HRV co-detections were, in truth, infection with one of those two picornaviruses. Because the primary outcome of their study is co-infections, this is a major problem. I believe that this must be addressed by sequencing (of at least a proportion of HRV-HRV co-detections) to determine the validity of these results.

   Following your advice, we have retested all the previous HRV-EV co-positive samples (n=307), by using a new and more specific (published) set of HRV primers/3 probes in real-time PCR, which was described by the developers of the original PCR to overcome the problem of cross-reactivity [1]. Using this PCR, 32% (96/298) of co-detections were confirmed as HRV-EV and 68% (202/298) as single EV cases. Accordingly, we have redone our analyses and revised the Methods (page 5, line 14-16), Results (page 7, line 3-5), and Discussion (page 11, line 16-21).
Minor Essential Revisions

1. Last sentence of the Background. Please be more specific when stating your objectives. Some information regarding time, place and patient population should be included here. Also, the term "seasonal factors" is quite vague; I suggest using "meteorological factors" instead.
   
   It has been revised (page 4, line 10-12).

2. It is unclear if the study setting is a walk-in clinic, emergency department or some other outpatient facility. Please specify.
   
   They are walk-in clinics. It has been revised accordingly (page 4, line 16).

3. Please mention if any patients were admitted to hospital.
   
   Since we did not follow-up the patients, we did not know. We have revised it as per your advice (page 12, line 3-4).

4. Statistical analysis (p. 6). Chi-square tests compare the differences in the distributions of categorical variables (or differences in proportions), not simply any difference.
   
   Thank you. It has been revised (page 5, line 26).

5. Please describe in the Methods how the regression models were built, i.e., how decisions were made regarding which variables should be included in the final model. Also, please define what the outcomes of the models were.
   
   It has been revised (page 6, line 2-6).

6. Please explain (in the Methods) how the results in Table 3 were obtained. What are you comparing using Chi-square (as mentioned in the notes for Table 3)? What does "most favourable" mean? "Favourable" is not a statistical outcome.
   
   We have revised Table 4 (formerly Table 3) and explained our methods in the legend.

7. “Favorable” has been corrected as “optimal” according to your advice.

8. In the first sentence of the Discussion, change "seasonality" to "meteorological factors".
   
   It has been revised (page 8, line 3).

9. Please complete Reference # 12
   
   It has been revised (Ref. 14 currently, page 15).

10. Table 4 (notes). "adjusted for age" and "all variables with p<0.05..." should be in separate sentences; they are not related.
    
    Thank you. It has been revised in Table 5 (previous Table 4).

11. The last two sentences ("These findings...") of the first paragraph of p. 4 are not the way that most experts in respiratory virus epidemiology interpret the literature. The importance of interactions between viruses is not understood at this point, and the majority of acute viral respiratory illnesses are likely due to the effect of one primary pathogen. Such a vague mention of interactions between other classes of microorganisms (bacteria, fungi and parasites) seems beyond the scope of this article, unless a specific point is brought up that could be used as an analogy for respiratory viruses.
    
    Thanks. We have removed that statement.

12. I do not understand the last sentence of the first paragraph of the Background. The viruses studied (not just novel coronaviruses) are clearly the causative agents of primary infections. I do not understand what is meant by "secondary" and "bystander".
    
    It has been removed.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.
 Declaration of competing interests: I declare that I have no competing interests

Reviewer 2:

Reviewer's report
Title: Viral aetiology of acute respiratory infections among children and associated meteorological indicators in southern China
Version: 1 Date: 23 September 2014
Reviewer: Shobha Broor
Reviewer's report: the comments are enclosed in the file attached
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Abstract
1. Line 19 and 24: it should be overlapping seasonal trends rather than overlapped trends
   1 Thank you. It has been revised in the Abstract (page 2, line 19), Results (page 7, line 8), and Conclusions (page 12, line 15).

Background:
1. The statement that children for ARI visit clinic at least 12 times a year may not be true for all regions and all age groups it is very general statement quoted in one study should be removed.
   1 It has been rephrased (page 3, line 8-9).

Methods:
1. The major flaw of this study is that only children attending outpatients have been enrolled, which means only mild and moderate disease has been captured, children with severe respiratory disease which are the major concern for burden of disease.
   1 Thank you, we have added it as our limitation in the Discussion (page 12, line 6-7).
2. Difficulty in breathing and increased respiratory rate have not been included as criteria of ARI and ARI has not been classified in to mild severe or very severe based on respiratory rates as per WHO criteria.
   1 We used modified WHO standard case definition of ARIs [2]; therefore, we did not include difficulty in breathing and increased respiratory rate. Please see our revision under Methods (page 4, line 22).
3. Why only probes have been given for hPeV and internal control what about primer pairs?
   1 Following your suggestion, the sequence information of all 14 viruses has been attached in Additional File 1.
   1 It has been rephrased (page 5, line 17-20).

Results:
1. The temperature to define fever has been recorded as oral or underarm temperature as for temperature fever is defined as > 38 C. Thus the row under fever with <38 C, what does it represent?
   1 Thanks for pointing it out; we have revised it as fever of >38C (underarm temperature) in the Table 1 and its legend.
2. Table 1: No odds ratios have been determined for virus positive and negative children
It has been revised as per your advice in Table 1.

Enterviruses and rhinovirus are also commonly found in asymptomatic or control children and as no controls were enrolled in this study it is difficult to assess the role of these viruses in etiology of ARI.

It has been added as our limitation in the Discussion (page 12, line 7-9).

Table 3 can be simplified or else this information can be given in text and table can be deleted.

We have made it simpler; please see Table 4 (formerly Table 3).

Combined virus – virus and metrological factor analysis does not make much sense. I think combining metrological indicators with co-virus infections in the same table does not make much sense, the two variables are very different. The frequency of co-infections with different viruses also depends on the prevalence of the virus in clinical samples, viruses which are present at a higher frequency will have more chances of co-infections with other viruses.

Thank you for your suggestion. We have redone the multivariate logistic regression analysis by using individual viruses as dependent variables and meteorological factors as independent variables, or using viruses as dependent variables and the other viruses as independent variables (no combination of viruses and meteorological factors as independent variables simultaneously this time). Please see our revision under Methods (page 6, line 2-6). In order to save space, we still keep these results into the same table but put a divider between meteorological factors and viruses. (Please see the revised Table 5).

Undoubtedly the viruses with higher frequency have more chances to be co-detected with others. However, there are not only positive but also negative associations between them. For example, EV, HRV, and PIV3 were the viruses of highest detection rates (Table 3), but they were all negatively associated with each other (Table 5 and Figure 2). Therefore, our findings are useful to understand the associations between viruses.

Figure 2 is very small to discern all the different lines should be given in a bigger scale

We had submitted all the figures with high resolutions and as big as possible, but they look very small in PDF document. Please download the original figures for review. Thank you.

Discussion:
The reason for co-detection of EV and HRV being higher in this study is because these viruses are commonly not included in the panel in other studies.

EV and HRV were included in the panels of many studies globally, and EV-HRV pair has been the commonest pair among outpatient children with ARIs from Finland (19.6% of co-detections) [3] and infants with acute bronchiolitis from Brazil [4]. Co-detected rate of EV-HRV in this study is similar to that in Finland [3] (please see our revision in page 8, line 20-24).

The study has some merits as it has used stateart technology for detection of multiple viruses in children with ARI, however it has certain disadvantages that children with severe ARI were not enrolled, this is only a study in outpatients. The definition and classification of ARI used is not standard as per WHO.

Please see our explanations above and revisions under Methods (page 4, line 22), and Discussion (page 12, line 6-7).

The frequency of detection of EV and HRV in no-ARI patients has not been discussed.

It has been addressed as a limitation under Discussion (page 12, line 7-9).

Manuscript can be approved after answers to various comments are received.

We are grateful to both reviewers for your suggestions, which are justified, helpful, and have greatly improved our manuscript.

References:

