Author’s response to reviews

Title: Epidemiological and clinical profiles of respiratory syncytial virus infection in hospitalized neonates in Suzhou, China

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Author’s response to reviews:

Thanks for your advices again. We would like to express our sincere gratitude to the section editor and reviewers for their constructive and positive comments.

Section Editor's comments:

Using DFA for virus detection is still acceptable and practiced around the world, but it can be significantly less sensitive than PCR testing. The authors need to mention this in the Discussion since bocavirus and Mycoplasma are being detected by PCR, but the 'seven common viruses' are being detected by DFA - so a comparison of the proportional incidences between these pathogens may not be entirely accurate.

Response: In present study, human bocavirus, metapneumovirus, and Mycoplasma pneumoniae were detected by PCRs and seven other viruses (RSV, IV-A, IV-B, PIV-1,2,3, and ADV) were detected by DFA. Generally speaking, DFA was significantly less sensitive than PCR testing. However, the sensitivity of DFA in comparison to rt-RT-PCR was highest (86%) during the first 3 days of symptoms onset and decreased gradually till it reached 65% after the first week. The
specificity of DFA in comparison to rt-RT-PCR ranged between 99 and 100% irrespective of the date of collection. [*]. We added this information in the Text and quoted the following reference. [*] Shafik CF, Mohareb EW, Youssef FG. Comparison of direct fluorescence assay and real-time RT-PCR as diagnostics for respiratory syncytial virus in young children. J Trop Med. 2011;2011:781919.

The Figure legends and the annotations on the figures need to be more detailed - each figure and legend should stand alone without having to refer to the main text, e.g. Figure 1: Total cases of what? Incidence of what? Figure 2: If 'Mean velocity' means 'Mean wind velocity' then the authors need to make this clear, similarly for 'Total rainfall'. Is it mean relative humidity or are these individual relative humidity points? These parameters need to be stated clearly in the legend, and any abbreviations used in the figures should also be explained.

Response: Yes, we have modified the figures and legends. Thanks. See in Figure legend and Figures.

Reviewer #1: Comments:

This study examined the epidemiology of RSV infections among neonates who were admitted to a large children's hospital in Soochow. The data presented provide useful insight for a relatively understudied population. The manuscript requires significant rewriting to improve its clarity.

Abstract:
1. Please provide total number of hospitalised neonates with LRI in Results.

Response: Yes, the total number of 1803 cases has been added in the results.

Main text:

Methods:
1. Neonates and newborn are used interchangeably in the manuscript but has the potential to mean different age to different people. For clarity, please provide age definition (=<28 days, if this was the case for the study).

Response: In China, Newborns = Neonates. However, these have the potential to mean different age to different people in other country. Consequently, all “Newborns” in text has changed into “Neonates” and in section of “Study population”, we provide age definition of ≤ 28 days as neonates.

2. Please remove "Of all the hospitalized neonates with LRTs, 374 cases were confirmed with RSV." from the Methods section as it is (more appropriately) mentioned in the Results section.

Response: Yes, this sentence has been moved to the section of Results. See in text.

3. Please provide ethics approval reference number.
Response: The reference number of ethics is 2015008 approved by the Ethics Committee of Children’s Hospital of Soochow University.

4. Please provide the reference for the bocavirus and mycoplasma PCR methods.

Response: In fact, human bocavirus, human metapneumovirus and Mycoplasma pneumoniae were detected by PCRs as previously described. See in reference [6].

Results:
1. Please clarify if 'non RSV-positive neonates' referred to neonates infected with viruses other than RSV, or did it include those tested negative for any virus/pathogen as well?

Response: In present study, “non RSV-positive neonates” referred to neonates infected with viruses other than RSV. All “non RSV-positive neonates” has been changed to “other virus positive neonates”. See in text and table 3.

2. Please give the male to female ratio for the 'non RSV-positive neonates' as well.

Response: The male to female ratio of other virus infected cases is 1.67:1, and it has been added into the text.

3. Please summarise the results for all positive NPA (including other pathogens) in a Table.

Response: We summarise the results for all positive NPAs in section of Results. See in text.

4. It is difficult to read Figure 2 in its present form. Suggest stacking the line chart on top of the bar chart. The y-axis is too wide for certain parameters and they are compressed too much for meaningful interpretation.

Response: Yes, I changed the Figure 2. But I think stacking the line of mean temperature on the bar chart is better than other climate factors on top of the bar chart because only temperature is associated with number of RSV positive cases using Multivariate regression analysis.

5. Under "Clinical and laboratory characteristics in neonates with RSV infection", please change kilograms to grams.

Response: Yes, we have changed the “kilograms” to “grams”.

6. In the same section, when you mention "non-RSV (other virus positive) neonates", did you mean single infection with other respiratory virus or other co-infections without RSV? From the n =32, it seemed to suggest the latter case. I would be surprised if there had been only 32 non-RSV-related infections for the study cohort.
Response: In this section, non-RSV neonate means other virus positive neonate. Here 32 cases with single other virus infection were just detected in 2014. Therefore, we reanalysed the data including other virus positive neonates during 4 years (2010-2014) in Table 3 and a total of 131 cases were confirmed with other virus infection.

7. Table 3. Please specify what were the underlying conditions collected for the purpose of this study in the Methods section. Not all underlying conditions may be relevant to RSV infection and the inclusion of them may be misleading.

Response: Yes, underlying conditions included “congenital heart disease; bronchopulmonary dysplasia; bronchopulmonary malformation and Down Syndrome” which was mentioned in Table 3. See table 3. This is also changed in section of “Data collection”.

8. Were the laboratory parameters performed at presentation or any time during admission? Similarly, were the symptoms/signs collected at presentation or any time during admission?

Response: In present study, laboratory parameters and clinical symptoms were performed and collected with 24 hour on admission.

9. What is the significance of AST and CK-MB in patients with RSV infections?

Response: Here are ALT and CK-MB increases which represent as liver function and cardiac muscle impairment and ALT and CK-MB might be positive associated with severity of RSV-LRIs.

10. Since disease severity was not otherwise described in detail in the Methods section, please change "disease severity" with "NICU stay”.

Response: Yes, It has been changed. See in text.

11. Please keep percentages to single decimal place.

Response: Yes, we have unified the percentages all over the manuscript.

12. Please describe the climate conditions during which RSV activities peaked.

Response: We added “RSV activity was always peaked in winter season which has the lowest temperature whole year” in Results section.

13. Please clarify who interpreted the chest radiographs.

Response: Chest radiography was performed using standard equipment and radiographic techniques, and reviewed by the radiologists in digital format. We have clarified in text of “Study population”.
Discussion

1. Please discuss in greater details the difference between your study and the Tunisian [ref 10] and Netherland [ref 11] studies. E.g. age of neonates, inclusion/exclusion criteria, underlying condition, climate factors etc.

Response: Yes. We discussed difference between our study and Ref 10, Ref 11 according to age of neonates, inclusion/exclusion criteria, underlying condition, climate factors throughout the manuscript. See in text.

2. How often was mycoplasma detected alone without RSV? Should not generalise the finding of co-infection of RSV and mycoplasma (8/1803, 0.4% of total study population) as it being an important cause of lower respiratory tract infections among neonates.

Response: In present study, a total of 45 cases were detected with Mycoplasma pneumoniae, accounting for 2.5% of all LRI cases and 17.8% (8/45) cases with Mycoplasma pneumoniae were coinfected with RSV. Our recent study suggested that Mycoplasma pneumoniae also was a common pathogen in bronchiolitis in Suzhou [Ref. 6]. These indicated that Mycoplasma pneumoniae may be an important pathogen in infants with LRIs.

3. Another factor why older neonates may have increased risk of RSV infection is the waning of passively derived maternal antibodies.

Response: Yes. I really agree with it. Thanks for your good suggestion.

4. Both temperate (Discussion, para 2, second last sentence) and subtropical climates (Discussion, para 3, third last sentence) have been mentioned for Suzhou, please clarify which is the correct description.

Response: Thanks. We have clarified it as subtropical climate in Suzhou for both paragraphs.

Reviewer #2:

This manuscript reviews the epidemiology and clinical parameters of RSV infection in Suzhou China. The data is not different from previous studies but it does encompass a large number of subjects and over several year's time span.

I have a few specific comments:

1. Although quite obvious visually with raw data, would the authors consider calculating seasonability/time trends for RSV infection and meteorological data. Comparisons can then also be made graphically.

Response: Thanks. It is distinct for RSV seasonality if reanalysing the raw data according to different seasons. But differences in months cannot be described clearly. Based on
your advice, we added the season partitioning in legend of Figure 2 for better understanding.

2. Mycoplasma pneumoniae was detected as a co-infection. Did these subjects require treatment with macrolides or did the subjects recover without antibiotic therapy.

Response: Only a few severe cases were treated with macrolides such as oral azithromycin in view of drug security. In addition, mycoplasma pneumoniae infection is self-limited in most cases. What’s more, 91% cases have macrolide resistance in Suzhou according to 23S rRNA gene detection (2063 A→G mutation) (data not published). Taken together, we do not frequently use macrolides to treatment mycoplasma pneumoniae in neonates.

3. The observation that the incidence occurred mainly in late neonatal period might be reconsidered. Based on Figure 1, it appears that the incidence peaked at 2 weeks? The reduced number in the first week could be due to stay for the first few days in nursery after birth?

Response: Yes. The reduced number in the first week could be due to stay for the first few days in nursery after birth. Therefore, I re-discussed this issue of Figure 1 in section of Discussion. See in text. Thanks.

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Editorial Requests
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Please note that all submissions to BMC Infectious Diseases must comply with our editorial policies. Please read the following information and revise your manuscript as necessary. If your manuscript does not adhere to our editorial requirements this will cause a delay whilst the issue is addressed. Failure to adhere to our policies may result in rejection of your manuscript.

Ethics:

If your study involves humans, human data or animals, then your article should contain an ethics statement which includes the name of the committee that approved your study. If ethics was not required for your study, then this should be clearly stated and a rationale provided.

Consent:

If your article is a prospective study involving human participants then your article should include a statement detailing consent for participation. If individual clinical data is presented in your article, then you must clarify whether consent for publication of these data was obtained.

Response: We have clarified this issue in section of Materials and Methods. “This study was approved by the Ethics Committee of Children’s Hospital of Soochow University (Ref. No.2015008), and consent forms were obtained from the guardians for all children enrolled.”
Availability of supporting data:

BioMed Central strongly encourages all data sets on which the conclusions of the paper rely be either deposited in publicly available repositories (where available and appropriate) or presented in the main papers or additional supporting files, in machine-readable format whenever possible. Authors must include an Availability of Data and Materials section in their article detailing where the data supporting their findings can be found. The Accession Numbers of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript must be provided and include the corresponding database name.

Response: I can send the original data to BMC if necessary.

Authors Contributions:
Your 'Authors Contributions' section must detail the individual contribution for each individual author listed on your manuscript.

Response: Yes. We have detailed in manuscript.