Author's response to reviews

Title: Epidemiology and clinical presentation of the four human parainfluenza virus types

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Author's response to reviews: see over
Dear James:

Thank you for making hard efforts to review and arrange review of our MS-1543378597271418. As you can see from the following “Listed Responses to the Reviewers”, we have revised the MS, and addressed the reviewers’ concerns and made appropriate changes to improve the quality of this MS.

Some changes in the MS have been highlighted with colors; I hope that you will find the revised MS now acceptable for publication by “BMC Infectious Diseases”.

Thank you very much indeed for your time and efforts to arrange all of these and I look forward to hearing a positive reply from you very soon.

Best regards

Sincerely Yours

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Listed Responses to the Reviewers

Reviewer: Nan-Chang Chiu

Reviewer's report:

Discretionary Revisions

Clarifying the following questions would make this paper more valuable.

1. The typical clinical presentation of parainfluenza virus infection is croup that will cause hoarseness in patients frequently. However, only 4% of HPIV-positive patients in this report were recorded to have this presentation. How to explain that?

Response:

Clinical symptoms of patients were progressive, many factors would interfere this procedure. So the percentage of certain symptoms would change or fluctuate according to different situation. Although the ratio in our study is not as high as other reports, the positive ratio of hoarseness in HPIV-positive patients is significantly higher than HPIV-negative patients.

2. Diarrhea is a common associated symptom in children with respiratory infectious diseases. More detail evaluation of age distribution between HPIV-positive and HPIV-negative patients with diarrhea would be helpful.

Response:

In this study, 16 patients presented with Diarrhea (Diarrhoea), and the age distribution of these patients had no special characteristic comparing to other symptoms, so we did not figure out specially, but we give the age distributions of the 16 patients here to make it clear.

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<thead>
<tr>
<th>Age groups</th>
<th>Patients no.</th>
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<td>0–3 mon</td>
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3. Comparison with regional parainfluenza virus infection data is important for interpretation the epidemiology results. Some of the references are not suitable. Actually, several parainfluenza virus infection papers have been published in recent few years that are worth quoted.

Response:

Thank you for giving us a related regional HIPV epidemiologic paper, that was very helpful to compare with each other, and we have quoted the paper in the MS (reference 26).
Reviewer: Robin Brittain-Long

Reviewer's report:
As molecular methods detecting genetic material from pathogens have become the mainstream of respiratory viral diagnostics in many laboratories around the world, large quantity data derived from these molecular tests is accumulating. Because sensitivity and specificity is markedly increased with these molecular diagnostic methods, as well as adding new viruses to the panel, epidemiological studies are important and justified in the field. Liu et al. have conducted a retrospective descriptive epidemiological study, based on respiratory samples collected during 26 consecutive months in Guangzhou, southern China. The study design is however not clear in the current manuscript and needs to be clarified (see below). The study aims to explore epidemiological features, as well as clinical manifestations, of human parainfluenza virus infections in both children and adults from patients with acute respiratory tract illness. The research question of exploring the epidemiology of HPIV and its four types using multiplex PCR diagnostic methods is important to the field and the study is partly well conducted. However, it suffers from some methodological errors, which need to be corrected. My main concern is the analysis of clinical manifestations as described below, which will in my mind need further analysis.

Major Compulsory Revisions

1. The methods section needs to be expanded (the suggestions below could be regarded as ‘minor essential revisions’ but are essential in order for the reader to be able to validate the findings, and have hence been placed under Major Compulsory Revision). Please include the following:
a) Please clarify the chosen study design

b) Please state how the study population was selected. If the population was selected based on available samples in the laboratory and retrospectively analysed please make this clear to the reader, and if not please clarify. Were some samples from the study period not analysed, and if so why not? This is very important as this may bias the results. If a potential bias is identified this should be mentioned in the discussion.

Response to a) and b):

Firstly, Thanks for your efforts to help revise this MS.

In this study, we analyzed all the patients collected from the three hospitals which meet the ARTI standard as described in the section of Methods, Paragraph 2. And 4447 of 4755 (93.5%) patients’ presentations were analyzed, while other 308 patients’ record were not fully recorded, so were not enrolled in the further presentation analysis.

c) What type of respiratory samples was collected (nasopharyngeal swabs, nasopharyngeal aspirates, nasal washes, throat swabs, sputum, endotracheal tube aspirates, broncho-alveolar lavage samples)?

Response:

Sample type had been described in the section of Methods, Paragraph 2 as: “Throat swab samples were collected from patients with ARTI…”

d) How were respiratory samples stored before analysis?

Response:

We have revised the MS in the section of Methods, Paragraph 2 as: “The samples were refrigerated at 2 to 8°C and transported on ice to State Key Laboratory of Respiratory Diseases and analysed every working day or stored at -80°C before testing”.

e) Were all samples analyzed by the same laboratory?

Response:
Yes, all samples were analyzed in State Key Laboratory of Respiratory Diseases as described above.

f) How was data regarding symptoms collected? From medical notes or databases retrospectively?

Response:
The symptoms were collected according to the patients’ medical records, and we revised the MS in the section of Methods, Paragraph 3 as “Clinical presentations were collected and categorized into the following six groups from the patients’ medical records using designed presentation cards.”

g) How was data regarding diagnosis collected?

Response:
The sentence has been revised as “Patients with pneumonia or bronchitis which diagnosed by chest radiography…” to make it clear in section of Methods, Paragraph 3.

2. The second aim of this study was to explore Clinical manifestations of parainfluenza virus infection. The fact that the authors are trying to shed some light on the symptoms of viral respiratory infections, and attempt to find clues to distinguish between HPIV and other pathogens as well as between different types of HPIV, is admirable. The methodology used to achieve this aim was to categorize symptoms and diagnosis into six syndromes (URTI, LRTI, influenza-like symptoms, GI-illness, convulsions and Others including rash) and then compare two groups of patients; (1) the group of HPIV positive patients with (2) all remaining patients, i.e. both patients negative for any pathogen in the
PCR test as well as a mixture of patients positive for any of the other 12 pathogens investigated. This methodology is problematic for several reasons. One is that the reader doesn’t really know what the group of HPIV positive patients is compared against. It is impossible to deduct from which tested pathogen the symptom might come from. Another is that some of the symptoms in both groups could potentially be derived from a bacterial infection not tested for in this study, as common bacterial respiratory infections cause similar symptoms. Thirdly we know very little about the underlying clinical characteristics of patients in each group that was compared, and hence there are potential confounding factors that might influence the results. If an attempt to distinguish clinical features of HPIV infection from other respiratory infections is to be made the following is suggested;

a) A well defined study population

b) Carrying out a multivariate analysis (possibly with the help of a statistician) for each of the pathogens tested for, to see if any symptom remain predictive of HPIV infection

c) An alternative could be to compare two well defined groups of patients, e.g. one group with Influenza A or B and one group with HPIV (since this might have a clinical implication for choosing to prescribe antiviral medication to the latter group)

d) If the latter analysis, or similar, is chosen then creating a baseline characteristics table (for table 1) depicting demographics, coexisting illness, clinical parameters (such as vital signs, duration of illness), hospital stay etc. would be advised

Response:

We know that respiratory illness is very complex. In this work, our focus was to explore the basic features of the HPIV infection comparing to non-HPIV infection in the patients
with ARTI, but not comparing to any certain pathogen. We think that make non-HPIV infection as a integrate group is valid and helpful to the purpose. So we hope to keep the result. However, you give us very valuable advice to our further study, especially in the field of co-infection and co-existing illness which is very complex to analyze.

Minor Essential Revisions

1. Please state sex ratio for the studied population, and not just for those patients that were positive for HPIV

Response:

The male to female ratio of total studied population was 2898:1857 and had been described in contrast way as “The male to female ratio was 139:39 in HPIV-positive patients and 2759:1818 in HPIV-negative patients (p<0.001)” in section of Results, Paragraph 2.

2. Please state age distribution (median age, range and possibly age groups) for the studied population

Response:

We have revised the MS as “The mean age of the patients was 16.0±20.1 years, and ranged from one day to 91 years” in section of Results, Paragraph 1.

The adults and children distribution had been described in the section of Results, Paragraph 1 in contrast way as “Pathogens were detected in 2439/4755 (51.3%) samples, and were detected in a higher proportion of samples from children (<14 years old) (1503/2793; 53.8%) than from adults (≥14 years old) (936/1962; 47.7%) (p<0.001)”.

The detail age distributions were analysed in the Figure 2.

3. The authors make a point in the 1st paragraph of the discussion about the lack of
(correctly so) studies evaluating parainfluenza infection in adults, and claim to address this issue. I think in its current text this study is misleading the reader in portraying a study that evaluates adults as much as children, since in fact 88.9% of patients that were positive for HPIV were under 5 years of age (and consequently the number of children of any age positive for HPIV will be even higher), and 97% of co-infected patients were children. Even though all ages are included in this study the vast majority is children and this needs to be commented on more clearly. As I understand the data provided of the HPIV positive patients 10 out of 178 patients (5.6%) were adults and the rest consequently children. Details of age of the entire study population (see comment 3 above) need to be elucidated. The finding that the clinical presentation of HPIV may differ by age is interesting and could be expanded with references to the literature, although the number of adults, i.e. 10, is small.

Response:

We have revised the MS in this section to make it clear as “Previous studies have predominantly focused on HPIV-1, HPIV-2 and HPIV-3 infection in children because of high positive rate and morbidity of three types of HPIV infection in children”. Details of age of the entire study population had been elucidated in the Figure 2 and in the section of Results, Paragraph 1.

The relative reference had been added in the fifth paragraph of the Discussion section.

4. Please comment in the discussion on the fact that the most common virus causing respiratory illness in both children and adults, i.e. Rhinovirus was not tested for.

Response:

We have commented in the last paragraph of the Discussion section.
5. The finding of significantly more diarrhoea among HPIV positive patients than HPIV negative patients does not ‘confirm pathogenic activity of HPIV gastrointestinal illness’ and should be altered (see comment 2 under Major revisions)

Response:

The description has been revised as “suggested pathogenic activity of HPIV in gastrointestinal illness”.

6. Please add a paragraph in the Discussion section of limitations of the study.

Response:

We have described in the last paragraph of the Discussion section.

7. Methods section, 2nd paragraph. Please rephrase the word ‘rhinobyon’, throughout the text. I presume nasal congestion is meant?

Response:

We wrote “nasal obstruction” instead of “rhinobyon” to make it clear.

8. Methods section, 2nd paragraph. Please be consistent in spelling the word ‘dyspnoea’ (UK spelling) or ‘dyspnea’ (American spelling).

Response:

We have revised and replaced “dyspnea” with “dyspnoea”.


Response:

Done

10. Methods section, Real-time PCR paragraph, second sentence. Please write ’13 other common respiratory pathogens…’ instead of ‘..other 13…..’
Response:

Done

11. Results section, 1st and 2nd paragraph. Please clarify if percentage of detected pathogens were calculated with ‘all detections’ as denominator (as stated for InfA, RSV and MP in the 1st paragraph), or calculated with ‘samples’ as the denominator (as stated in the 2nd paragraph for HPIV)

Response:

We have revised the description as “The pathogens identified most frequently were infA (833/4755; 17.5%), RSV (524/4755; 11.0%) and MP (274/4755; 5.8%)”.

12. Discussion section, 4th paragraph, ‘…(September 2009 to November 2009…)’ is written with a larger font than the rest of the text.

Response:

Done

13. Conclusion, 1st paragraph, 1st sentence. Please rephrase as sentence is missing words and grammatically incorrect.

Response:

The sentence has been revised as “HPIV infection led to a wide spectrum of symptoms, and LRTI was the significant presentation.”

Discretionary Revisions

1. The title accurately reflects the paper's content, although reference to the retrospective nature and use of PCR methods may benefit potential readers.

Response:

The title reflects the main content of the MS, and the method of this MS will be provided
as keywords.

2. Methods section, 2nd paragraph. Consider using the term ‘coryza’ instead of ‘snivel’ if that is what is meant.

Response:

Done

Thanks for your efforts to help revise this MS, we hope the MS now can meet your requirement.