Reviewer's report

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

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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 it’s 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.

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

d) How were respiratory samples stored before analysis?
e) Were all samples analysed by the same laboratory?

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

g) How was data regarding diagnosis collected?

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 categorise 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 deduce 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

Minor Essential Revisions

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

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

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.

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.

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)

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

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

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

9. Spelling of Chlamydia throughout the text/tables/figures. Please write Chlamydophila pneumonia

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

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)

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

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

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

Level of interest: An article of importance in its field

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.

Declaration of competing interests:

I declare that I have no competing interests