Author’s response to reviews

Title: Detection of a divergent Parainfluenza 4 virus in an adult patient with influenza like illness using next-generation sequencing

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Editor:

Personally, I suggest to extent the title from ?Detection of a divergent Parainfluenza 4 ?? to ?Detection of a divergent Parainfluenza 4 virus ?? as NGS revealed a divergent VIRUS and not a disease.

The title has been adjusted to reflect the study more accurately, as the editor has kindly suggested.

Reviewer 1:

1. The review would like to see a map of how 32 reads located on the reference genomes, or a table of such detail information.

A supplementary file (Additional File 2) containing a table listing the mapped positions of the 32 reads relative to PIV4b isolate SKPIV4 has been included and noted in the Results section.

2. As the nucleotide sequence coding gene V was contained in gene P, the authors used the ORF of P only, or ORFs of P and V in phylogenetic analysis by concatenating ORFs? Please indicate clearly.

The description of the phylogenetic analyses has been clarified if the Figure 1 legend:

“Maximum likelihood analyses of concatenated NP, P, M, F, HN and L open reading frames of PIV4 isolates AB543337, AB543336, KF483663, JQ241176, EU627591, EU627591, KF878965, KF908238 using the Tamura-Nei substitution model with 1000 bootstrap replicates.”
3. Other 5 PIV4a/b genomes were used in phylogenetic analysis. Why not added the genome sequence of PIV4a (KF878965), as it was sequenced by the same institute of the authors?

The genomic sequence of KF878965 was not available at the time of manuscript submission. The genome has now been included in the phylogenetic and recombination analyses.

4. Were the sequences of PCR fragments confirmed by a second Sanger sequencing? Re-sequencing was strongly recommended.

Yes, all sanger sequencing was performed bidirectionally. Additional text in the “Genome sequencing & assembly” subsection has been inserted to address this question:

“Sanger sequencing was performed on the overlapping amplified cDNA bidirectionally.”

5. The format of the citation should be re-written. For example: “... particularly in children. [1–5] Four serotypes...” should be written as “... particularly in children [1–5]. Four serotypes...” in Background, paragraph one.

The citation format has been adjusted as per reviewer suggestion.

Reviewer 2:

The term parainfluenza virus should have a space between both words throughout the document.

The term “parainfluenzavirus” has been adjusted to “parainfluenza virus” throughout the manuscript.

Discretionary Revisions:

1. Conclusion of the Abstract: I would suggest eliminating the sentence stating that “These findings suggests a possible role for Parainfluenza 4 in the aetiology of adult respiratory disease within the community setting”. While this study further supports this understanding, studies dating back as far as 2009 have shown that PIV is associated with upper and lower respiratory tract infections in otherwise healthy adults in both the inpatient and community setting.

It was our aim to highlight the presence and possible association of PIV (specifically PIV4) with disease in the otherwise healthy community, however we acknowledge the additive nature of our data, and as such have adjusted the sentence in question to reflect the spirit of point 1:

“These findings further support a possible role for Parainfluenza 4 in the aetiology of adult respiratory disease within the community setting, and highlight the caution needed to be used in designing PCR assays from limited sequence information or in using proprietary commercial PCR assays.”
2. Background: It would be helpful to briefly comment on the known differences between PIV4 a and PIV4 b serotypes.

Additional details about the two PIV4 subtypes has now been added to the Background:

“Two antigenically distinct PIV4 subtypes, PIV4a and PIV4b, exist [6]. Functionally and epidemiologically, little is known about the two PIV4 subtypes, however both are capable of co-circulating within the same population [7].”

3. Methods: First paragraph. It would be helpful to mention the total number of samples collected from which 299 used in the study were obtained.

The total number of samples tested for respiratory viruses in the previous study have now been included:

“In total, 643 samples were screened for adenovirus, human metapneumovirus, parainfluenza viruses 1, 2 & 3, respiratory syncytial virus, influenza A and B, picornaviruses, bocavirus, coronaviruses (OC43, 229E, NL63 and HKU1) and WU and KI polyomaviruses using real-time PCR [8].”

4. Results: Was this the only PIV4 virus identified out of the 299 samples? If not, the total number identified, along with their genotypes if known, should be provided. It would also be useful to state how many of the 299 had additional pathogen(s) vs. no pathogen detected using this method.

One additional PIV4 was detected. This, along with its genotype has now been included in the results:

“During the study period, one other PIV4 detection was observed within the study population.”

and

“Phylogenetic analyses confirmed QLD-01 was a divergent member of the 4b subtype clade (Figure 1), while the second PIV4 clustered within the 4a subtype (Figure 1, KF878965).”

Other viruses were also detected in this study population using the highlighted virus discovery pipeline, however their analyses is ongoing and will be the subject of future publications.

5. Discussion paragraph 3: There are several studies that have shown upper respiratory illness, lower respiratory tract infection, and influenza like illness in otherwise healthy adults in both inpatient and community settings. It would be more correct to state that this finding contributes to this body of literature rather than stating that PIV4 “may be capable of causing upper respiratory tract infections in otherwise healthy adults within the community setting.

The reviewer is correct that there are numerous studies describing upper/lower RTIs and ILI in the community setting. However, to our knowledge, there are few
if any that specifically describe PIV4’s possible role in ILI within the community (most studies have focused on hospital/health care settings, which may be assumed to bias towards more severe disease presentations). It was our aim to highlight PIV4’s particular presence in the otherwise healthy community setting. However, to help clarify the issue, we have modified the sentence to address the reviewer’s concerns:

“Recent studies have reported PIV4 infections associated with both lower and upper respiratory tract symptoms within the hospital setting [1–6]. This study provides further evidence of PIV4’s possible involvement in upper respiratory tract infections in otherwise healthy adults within the community setting.”