Author's response to reviews

Title: Molecular characterization of influenza viruses collected from young children in Uberlandia, Brazil - from 2001 to 2010: presence of reassortment between 2002 strains

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Author's response to reviews:

Cover letter 1
01/14/2015
Dear Reviewer: Shufang Fan,
I am pleased to resubmit for publication the revised version of MS: 1491494613140433 “Molecular characterization of influenza viruses collected from young children in Uberlandia, Brazil - from 2001 to 2010: presence of reassortment between 2002 strains” I appreciated your constructive criticisms and I addressed each of your concerns as outlined below:

- The conclusion in the title “presence of reassortment between 2002 strain” was confusing, since it is hard to be understood based on the data presented in this study.

The conclusion in the title “presence of reassortment between 2002 strain” was excluded.

1. In line 142, the description of “among these 376” is confusing –

The numbers were changed and the description now specifically states that I was referring to the 376 samples.

2. In line 180, “table 2” should be “Figure 2”

The term “table 2” was excluded.

3. In line 185-228, the description was hard to be understood, since whole virus names of vaccine were used in text and only vaccine numbers were used in Figure 1 and Figure 2. I suggested that the authors should use consistent names in whole manuscript.

The whole virus names of vaccine were placed in the Figure 1 and Figure 2.

4. The discussion is unnecessarily long and should be shortened.

The discussion is shortened.
The manuscript was copyedited and the length of the discussion was reduced.

5. In table one, the subtitles of column 7 and 8 were not suitable.
Regarding to the table one, would not be the table 2? It was removed from the main text and placed as an additional table S1. I hope the subtitles of column 7 and 8 is suitable now.

6. In figure 1, “444 (2002)” should be “444 (2006)”.  
Done.

7. In figure 2, the vaccine name should be consistent with the description in text.
Done.

Sincerely,
Thelma Fátima de Mattos Silva Oliveira

Cover letter 2
01/14/2015
Dear Reviewer: Paul Horwood,
I am pleased to resubmit for publication the revised version of MS: 1491494613140433 “Molecular characterization of influenza viruses collected from young children in Uberlandia, Brazil - from 2001 to 2010: presence of reassortment between 2002 strains” I appreciated your constructive criticisms and I addressed each of your concerns as outlined below:

Major compulsory revisions
1) Why was IFA used for the identification of viruses? There is no description of the methods used for cell culture isolation before the use of IFA for virus detection.

IFA was used as the most accessible and fast technique for respiratory viruses we had available at that time, since tests with rapid results performed within a few hours of onset of symptoms, might be useful for primary care clinician.

2) How were samples selected for molecular methods? Only the positives? This is not clear in the methods.

This is briefly alluded to at the end of the specimens.

3) The most interesting part of this study was the detection of H1N2 from 17.9% (albeit only 5 samples) from the surveillance. It would be interesting to conduct more indepth sequence analysis of these strains.

I would like to thank you for the suggestion. These sequences are further analyzed.
Minor essential revisions

Abstract:
4) Line 29: though antigenic drift
The word through was substituted by the word “by”.
5) Line 30: continue research
The manuscript was copyedited and the term was excluded.
6) Line 32: presenting with acute
Done.
7) Line 42: sequences from vaccine strains
Done.
Background:
8) Line 59: to public health
Done.
9) Line 59-60: due to annual epidemics and the potential of pandemics
Done.
10) Line 60: of the Orthomyxoviridae
Done.
11) Line 61: types A, B and C
Done.
12) Line 61: can be further divided
Done
13) Line 64: -- H1N1 and H3N2 do not just "predominate", they are the only strains with sustainable transmission in humans
The phrase “Subtypes H1N1 and H3N2 currently predominate in the human population [3] was substituted by …” with subtypes H1N1 and H3N2 characterized by the sustained transmission in the human population [3]”
14) Line 66: through antigenic drift
Done
15) Line 66: -- "every year" -- not actually true - new influenza strains that can evade the immune system or vaccination do not emerge every year.
The term "every year" was eliminated.
16) Line 77: strain information
Done.
17) Line 81: presenting with acute
Done.
Methods:
18) Line 89: presenting with acute
Done.
19) Line 90-91: There needs to be much better description of the surveillance
procedures that were used in the collection of samples: What was the case
definition? Were samples collected from inpatients or outpatients?
The description of the collection of clinical samples was clarified in method as
described by Costa et al [9].
20) Line 93: Were the samples added to cell culture before IFA?
No, the samples were not inoculated in cell cultures.
Results:
21) The results section is much too long and could be substantially reduced.
The manuscript was copyedited and the length of the results was reduced.
22) Line 138-145: The figures in this section are very confusing. It needs to be
made much more clearer how samples were selected for molecular analysis.
I reorganized paragraphs and figures to highlight results presented in this paper.
The selection of samples is briefly alluded to at the end of the specimens.
23) Why was there not a breakdown of the detection of other viruses by IFA? If
this component of the study is not important then I suggest it is removed completely
The question is not clear to the authors. Besides all samples were tested for
respiratory viruses by IFA, except hMPV and HRV, because there were no
antibodies available.
24) Line 192: "and in special A" -- not clear what is meant here
The text has been rewritten to address the criticisms outlined above.
25) Line 197: "extra potential ' -- not clear what is meant here.
The word " extra" has been substituted by “additional”.
Discussion:
26) Line 256-257: Many other studies reported much higher rates of influenza
detection - why have you only mentioned the ones with lower rate of detection:
These studies mentioned were exclusively conducted in Brazil by using children
samples. I tried to make more clearly the sentence.
27) Line 262-264: The authors are making statements about severity of illness
based on hospitalization, but not information has been provided about the
selection of cases. Considering the small number of cases and lack of
information that has been provided they cannot make the conclusion that
influenza is not a major cause of severe respiratory disease in children
This paragraph was removed from the text.
28) Line 282-283: This is a strange reference -- influenza viruses commonly
co-circulate with other strains. It is not clear why the authors have referenced this
occurring in South Korea (which is a very long way from Brazil)
This reference was removed and the paragraph was rewritten at the beginning.
29) Line 287: "Probably a fourth viral variant" -- it unclear what the authors are referring to here. Are they suggesting the detection of seasonal H1N1?
The word “variant” was substituted by “subtype”.
30) Line 293-294: "these isolates did not have pandemic potential" -- on what basis was this conclusion reached? The authors only provide limited sequence data on these strains which is not enough to reach this conclusion.
The sentence “These isolates did not have pandemic potential ….would provide protection against the H1N2 isolates [37].” has been removed from the text.
31) Line 321: annual
Done.
32) Table 2 - This Table should be provided as a supplementary file.
Done.
33) Table 3 - This Table is unnecessary and the data should be described in the Text
Done.

Sincerely,
Thelma Fátima de Mattos Silva Oliveira