Author's response to reviews

Title: Natural Prevalence of Antibodies to Spike Proteins of Four Non-SARS Human Coronaviruses Among a General Population in Beijing

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Version: 4 Date: 12 February 2013

Author's response to reviews: see over
Dear Editors,

Thank you very much for your consideration of our manuscript and request for a reversion.

Please find our point-by-point response to reviewers’ comments in “Responses to Reviewers’ Comments” below. As you will see, we have made every attempt to incorporate these suggestions as thoroughly as possible. Please note that the most of changes in the manuscript are correlated with the responses to reviewers’ comments as listed.

Sincerely,

Wenjie Tan, PhD, MD
Responses to Reviewers’ Comments

Reviewer #1

The manuscript by Zhou et al describes IgG and IgM directed to the 4 non-SARS-HCoVs in children and adults. An IFA assay was used to measure antibodies to the Spike protein of HCoV-HKU1, HCoV-NL63, HCoV-229E, and HCoV-OC43. The message that IgM is only present in children is noteworthy and has not been described before. It shows that for all viruses first infection takes place during childhood (age < 14 years), which is an important message. The levels of IgG and whether they are detected single/or double is of less interest to the reader, and should not be the focus of the manuscript, it is the IgM measurements which are worth while to be published. The manuscript needs some rewriting to make this message clearer.

Major compulsory revisions

1. High (detectable) levels of IgG antibodies are a sign of reinfection by the virus, and should not be regarded as seroconversions (with seroconversions you have an IgM and IgG response).

A: We have addressed the comment by changing the “seroconversion” to “seropositivity”
2. It does not have much value to show the numbers for single/double/etc positive for the various HCoVs. A double positive is regarded by the authors as a double infection (discussion third paragraph), which is not true, it just shows that prior to serum sampling the person had been infected, which might have been one virus 3 months ago, the other 6 months ago.

A: We addressed the comment in the third paragraph of discussion.

3. I suggest a title like: “First infections by all four non-SARS human coronaviruses takes place during childhood” (or something similar)

A: We revised the title following this suggestion.

4. Table 2 and the text about single double or triple can be deleted

A: We deleted Table 2 and related text.

5. P-values on frequency differences are not of great value. It is known that there is large variety in HCoV-infection rates from year to year. This may very well explain the different frequencies, and the differences found with the other literature. P-values based on the group <1 year can not be significant since there are only 8 samples.

A: We revised the statement as “The anti-S IgM positivity rate of HCoV-HKU1 was significantly higher than that of the other three HCoVs among the group < 3 years (Fig. 4)” (page 12 line229-231)
6. Table 3 and 4 can be deleted. Figure 3 and Figure 4 show all the interesting data nicely.
A: We deleted Table 3 and 4.

7. Figure 4, no need to show the dotted line.
A: We deleted the dotted line in the Fig 4

8. There are no page numbers and no line numbers; it is not possible to make specific
   comments or suggestions on the text.
A: We added the page and line numbers

Reviewer #2

There are some minor comments that should be addressed to improve the manuscript as
follows.

1. Introduction: The S protein is not as highly expressed as the CoV M and N Proteins. Please
   rephrase the sentence "The S and N proteins are most abundantly expressed..."; Instead,
   the authors may add that the spike protein is the most immunodominant CoV protein.
A: We revised in line 69 following this suggestion.

2. Figure 1 and related results section. Many conclusions and interpretations are based on the
   assumption that cross-reactivity in the presented spike-based IFA assay can be neglected.
   The authors state that "No cross-staining was observed when other HCoV antiserum was
used as the primary antibody (data not shown). The authors should provide evidence/data for this statement, specifically concerning virus pairs of 229E/NL63 (alphacoronaviruses) and OC43/HKU1 (betacoronaviruses). This is important, since traditionally CoVs were grouped according to serogroups (cross-reactivity of antisera). If cross-reactivity is observed, the authors should discuss this in the context of serum samples reacting against multiple spike proteins.

A: We revised in line 180-184 as “No cross-staining was observed when other HCoV antiserum was used as the primary antibody, specifically concerning virus-antibody pairs of subgroup 229E/NL63 (alphacoronaviruses) or OC43/HKU1 (betacoronaviruses). Furthermore, no false positive/negative result was observed in our study (data not shown).” and we have a statement in the discussion as “comparison of the S protein sequences of four HCoVs revealed that these proteins share <35% similarity [1,10]. We hypothesized that the difference in the amino acid sequence is sufficiently high to ensure the usefulness of S protein as a specific antigen for antibody detection.” (line 254-255)

3. Please add more details concerning the assessment of staining results. When were samples considered positive or negative; please comment about the reproducibility of the assay, are there samples (and if yes how many) that were positive and negative in repetitions ("false positives/negatives")?

A: We added a statement in the line 153-155 as “Serum samples that reacted with HCoV S protein at a dilution of >1:20 were considered positive for anti-S antibodies when duplicate
or triple test was consistent.” ;and we have a statement in final paragraph as “ further research is needed to determine the false-positivity and -negativity rates associated with this anti-S IFA by determining antibody titres during the acute and convalescent phases after primary HCoV infection. Comparison of serological methods and antigen preparations as well as sample exchange will facilitate validation of the assays for individual HCoV antibody determination.”

4. Please comment about the used spike sequences. Depending on the published sequence, the spike sequences date back to viruses that circulated decades ago. Is there any influence concerning possible genetic drift expected?

A: Thanks! We have a statement in the line 253 to 254 as “Previous studies of SARS-CoV serology have successfully used the S protein in enzyme immunoassays, immunoblots, and IFA [23-27]”; currently, we have no experimental evidence on the influence concerning possible genetic drift expected.

**Editorial requirement: **Copyediting:

After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further.
We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

A: We asked Dr. Lyna Zhang who works at CDC in the USA help to improve the quality of written English in revision. We also revised the abstract based on your comments.