Author’s response to reviews

Title: Influenza virus infection among pediatric patients reporting diarrhea and influenza-like illness

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Version: 7 Date: 23 December 2009

Author’s response to reviews: see over
Dear Dr. Alam,

We greatly appreciate and have addressed Dr. Chan’s comments to better clarify samples selected for viral culture. In addition, references were reformatted according to the guidelines given on the Biomed Central Website. Please see below for a point-by-point description of the modification made to the manuscript. Please consider the revised manuscript “Influenza virus infection among pediatric patients reporting diarrhea and influenza-like illness” for publication as a research article.

If additional information is required. Please contact the corresponding author.

respectfully submitted,

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Reviewer's report

Title: Influenza virus infection among pediatric patients reporting diarrhea and influenza-like illness

Version: 2 Date: 2 November 2009

Reviewer: Martin C. W. Chan

Reviewer's report:

Minor essential revisions

1. The selection scheme the authors used and the number of specimens they sent for MDCK culture remain unclear. For instance, in “Methods”->“Laboratory testing” section, the authors wrote “Based on RT-PCR results, clinical specimens that were positive were sent for viral isolation; a random sample of negative specimens was sent for viral culture as well.” I interpret that the authors sent all the flu viral RNA positive respiratory (n=85) and fecal (n=21) specimens for culture. In “Results” section, however, the authors said only 13 positive stool specimens were tested, and the number of positive respiratory specimens tested was not shown. The authors should state that a subset of positive specimens were randomly selected and should specify the exact number of specimens of each type tested.

   RESPONSE: We have edited the methods and results section to explain the selection scheme of samples sent for MDCK culture. In addition, in the results section we include how many of each sample (positive or negative) was selected for viral culture.

   In the “Methods” -> “Laboratory Testing” section, the description of samples sent for viral culture was re-written to state “Based on RT-PCR results, a random sample of positive clinical specimens was selected for viral isolation; a random sample of negative specimens was sent for viral culture as well.”

   In the “Results” section, the number of positive and negative respiratory specimens tested was included. Specifically, the manuscript now states “…a subset of randomly selected PCR positive and PCR negative samples (18 and 38, respectively) were submitted for viral culture…”

2. “References” section still need polishing. For example, journal New England Journal of Medicine was shown in two different styles in refs. 26 and 27.

   RESPONSE: The references were reformatted according to the guidelines given on the Biomed Central Website.
All other comments have been dealt with satisfactorily.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests.
Dear Editors,

We greatly appreciate and have addressed all of the reviewers' comments and suggestions. Please see below for a point-by-point description of the modification made to the manuscript. Please consider the revised manuscript “Influenza virus infection among pediatric patients reporting diarrhea and influenza-like illness” for publication as a research article.

If additional information is required. Please contact the corresponding author.

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Reviewer's report

Title: Influenza virus infection among pediatric patients reporting diarrhea and influenza-like illness

Version: 1 Date: 28 August 2009
Reviewer: Stephen Lambert

Reviewer's report:

Major Compulsory Revisions

1. The authors should provide figures for virus identification comparing throat swabs and nose swabs. It would be of particular interest if nose (site?) swabs were as sensitive as throat swabs.
   
   RESPONSE: We compared the agreement between nasal and throat swabs. The Kappa was 0.45 (very low agreement) on the identification of influenza virus. Based on this finding we wanted to maximize influenza positivity; therefore we decided to define upper respiratory tract infection as nasal OR throat positive. In addition, the focus of this study was to assess the identification of influenza virus among children presenting with diarrhea and influenza-like illness. We feel that by adding the comparison between two upper respiratory sites would take away from the primary objective.

2. Details of the nasal swab need to be provided: were specimens collected from just inside the nares or was the swab pushed into the mid-turbinate region.
   
   RESPONSE: Specimens were collected from the mid-turbinate region. This information has been included in the manuscript.

3. Details of the Indonesian Pediatric Diarrhea Network need to be provided: number of sites, number of sites that contributed specimens to the study, spread of sites (do they geographically cover and represent all of Indonesia?).
RESPONSE: The study sites included hospitals and community health centers in Jakarta, Yogyakarta, Denpasar, Mataram, Makassar and Medan. We have included this information in the manuscript.

4. More detail of what upper respiratory tract swabs were used, and how they were handled after collection, is required. Was viral transport medium used? How were specimens transported to the laboratory?

RESPONSE: More detailed information has now been included in the manuscript. Nasal swab and throat swab were used as respiratory tract specimens. Collected respiratory specimens were placed into viral transport media and shipped to the laboratory via air or ground courier, within 24 hours after collection.

5. Was a central laboratory used for PCR and viral culture, or were specimens tested at a number of regional (how many?) laboratories using the same protocol? How were specimens transported to the laboratory/laboratories? The discussion raises transport time as a potential issue for cell culture negativity, but no detail is provided in the Results for the delay from collection to testing for either PCR or culture? Where specimens stored prior to testing – if so, how?

RESPONSE: Influenza identification was conducted at a central laboratory (NAMRU2 - Jakarta) using RT-PCR method and cultured we have included this information in the manuscript. We have also included information on how specimens were shipped and stored upon arrival in the manuscript.

6. The results don’t specify if any PCR negative stool specimens were positive by cell culture.

RESPONSE: There were no samples that were PCR negative / culture positive in this study. We have included this information in the manuscript.

7. Detail or reference needs to be provided in the methods as to how A/B and H/N typing was performed.
RESPONSE: We have provided the reference to how we conducted influenza virus type and subtype identification in the method. The reference has been included in the manuscript (p. 4, last paragraph).

Minor Essential Revisions
1. There is no reference to Table 2 in the text.
   RESPONSE: Table 2 has been deleted.

2. Given two methods are used to identify influenza viruses (PCR, cell culture), sentences in the Results, such as the one following, need to clearly state the method of identification referred to (in this case, PCR): Both influenza A and B viruses were detected in stool specimens, including one A (H1N1), three A (H3N2), and 17 B viruses.
   RESPONSE: We have modified this sentence in the manuscript to explain these results were by PCR.

Discretionary Revisions
1. The title for Table 1 and Table 2 are the same, and data from Table 1 are repeated in Table 2. Table 1 and Table 2 should be combined for simplicity.
   RESPONSE: Manuscript has been revised based on the reviewer’s suggestions. There is now one Table in the manuscript.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests.
Reviewer's report

Title: Influenza virus infection among pediatric patients reporting diarrhea and influenza-like illness

Version: 1 Date: 7 September 2009

Reviewer: Martin C.W. Chan

Reviewer's report:

Overview of Manuscript

This study by Dilantika et al. describes the detection of seasonal influenza viruses in stool of pediatric patients (<6 years of age) with concurrent diarrhea and influenza-like illness. The authors suggest that seasonal influenza viruses may be associated with diarrhea in pediatrics and that gastrointestinal tract may serve as an alternative route of influenza transmission. As pointed out by the authors, gastrointestinal manifestation of seasonal influenza has long been known in infants, young children and adolescents, but the fecal shedding of the viruses in these age groups remains largely unexplored. Thus, the findings of this manuscript are important for influenza researchers to better evaluate the enteric role of seasonal influenza viruses. Overall, the manuscript is well written but the data will benefit from additional explanation and controls.

Major Compulsory Revisions

1. The authors should emphasize that they recruited participants with “concurrent” diarrhea and influenza-like illness at presentation.

RESPONSE: We have modified the manuscript to state that participants had “concurrent” diarrhea and Influenza like illness at presentation.

2. “Methods” section: Methodological details on the processing of stool specimens are largely omitted. Since fecal detection of influenza virus is not a routine procedure in a majority of laboratory settings, provision of technical details will definitely help readers interpret data and compare findings with other similar studies. In particular, but not limited to:

- How did the authors extract viral RNA from stool specimens? Did they start from a 10% stool suspension in PBS or viral transport medium or from something
else?

RESPONSE: Additional information on specimen collection has been included in the manuscript. Prior to RNA extraction, we prepared 10% of stool solution in PBS and stored left-over stool at 70º C. The solution was used for viral extraction using QIAamp viral RNA minikit. This information has been included in the manuscript.

- How and how long did they store stool specimens before MDCK virus culture? Repeated freeze-thaw cycle will have substantial detrimental effect on the viability of influenza virus, which also may account among many other factors for the low positive rate by MDCK culture compared with RT-PCR as observed by the authors.

RESPONSE: Stool specimens were stored at -70º C and specimens for viral culture were based on RT-PCR results. Specimens went through one freeze-thaw cycle prior to MDCK virus culture.

- The authors mention they obtained both nasal and throat swabs. Did they pool these swabs together before testing? If not, how did they define “upper respiratory” positive cases when only either nasal or throat swab was tested positive?

RESPONSE: Swab samples were not pooled, we tested stool, nasal swab, and throat swab of each study participant individually. We defined “upper respiratory” positive influenza cases when either the nasal swab and/or throat swab was tested positive. More detail on specimen testing and results by specimen site has been included in the manuscript.

3. “Results” section: It is rather difficult to follow the virus detection rate by MDCK culture. For example, the authors state that “a sample of PCR positive and negative specimens were submitted for viral culture with a total of 12 (24.0%) from upper respiratory and one (4.0%) positive from stool.”. Based on the numbers provided, one would expected the authors subjected 25 (1/0.04) stool specimens for MDCK culture. But it remains unclear how they selected these
stool specimens as only 21 stool specimens were tested positive by RT-PCR. 
RESPONSE: We conducted MDCK culture on a random selection of positive and negative stool samples to include 13 samples that were positive by PCR and 12 that were negative. We have included this information in the manuscript.

4. “Discussion” section: The authors mention influenza virus was the only (enteric) pathogen detected in some of their cases. It is interesting to know the proportion of flu virus RNA positive stool specimens that are co-infected with other diarrheal pathogens. Moreover, I would suggest they test their stool specimens for other leading pediatric diarrheal pathogens including rotavirus and norovirus, if not included in their original panel of pathogens tested, to exclude potential co-infections. Ruling out other diarrheal causes will substantially strengthen their speculation that seasonal influenza viruses may be associated with diarrhea in the studied population.
RESPONSE: Extensive work has been done to characterize these samples to include testing of bacterial, parasitic and other viral pathogens. Manuscripts are in preparation to provide further information on this study and we hope to submit in the near future.

5. “Discussion” section: It is of great interest that 15 patients were tested positive for seasonal influenza virus in stool specimens but not in respiratory specimens. One may argue did the authors collect parallel stool and respiratory specimens? If yes, their observation may have important implications in the challenge facing influenza diagnosis, at least in children <6 years of age. The authors should provide more details on how they collected specimens and discuss their observation in wilder context.
RESPONSE: We collected stool and respiratory specimens at the same time, when patients visited hospital or community health center. More detail on specimen collection has been provided in the manuscript.

Minor Essential Revisions
1. “Abstract” section:
- Abbreviation “UTI” may be misinterpreted as urinary tract infection. Choosing more commonly used abbreviations such as “URTI” is desired.
RESPONSE: We have changed “UTI” to “URTI” in the manuscript.
- “Viable” influenza B virus was isolated from the stool specimen of one case.
RESPONSE: This edit has been made to the manuscript.
2. “Discussion” section: Please define abbreviation “ILI” at its first mention.
RESPONSE: The manuscript has been edited and the abbreviation “ILI” has been defined in the background.
3. “References” section: Please double-check the consistency of text formatting, especially that of abbreviated journal titles.
RESPONSE: Journal titles have been formatted to the BMC requirements based on the endnote program.
4. “Table 1”: One patient in the “Upper Respiratory” category and 4 patients in the “Neither” category did not have fever. This appears contradict with their participant inclusion criteria. A table footnote may be needed to explain these cases.
RESPONSE: The tables have been edited to correct this error.
5. “Table 2”: This table is not mentioned in the main text of the manuscript. In fact, I personally think this table does not add much as comparing influenza A and B infections is not the focus of this manuscript.
RESPONSE: Table 2 has been deleted.

Discretionary Revisions
1. “Discussion” section: The authors suggest investigating the fecal shedding of swine-origin influenza virus causing the current pandemic. To better put readers into context, I would suggest adding references that implicate the enteric involvement of the current pandemic H1N1 virus.
RESPONSE: An additional reference has been included describing the clinical description of patients with swine-origin influenza virus.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.