Reviewer’s report

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

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Reviewer: Martin C.W. Chan

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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.

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?

   - 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.

   - 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?

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 have 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.

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.

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.

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.
   - “Viable” influenza B virus was isolated from the stool specimen of one case.
2. “Discussion” section: Please define abbreviation “ILI” at its first mention.
3. “References” section: Please double-check the consistency of text formatting, especially that of abbreviated journal titles.
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.
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.

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.
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.