Author's response to reviews

Title: Characterising the Aetiology of Severe Acute Gastroenteritis Among Patients Visiting a Hospital in Qatar using Real-time Polymerase Chain Reaction

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Author's response to reviews: see over
Dear Dr Esposito,

Regarding the following manuscript: **Characterising the Aetiology of Severe Acute Gastroenteritis Among Patients Visiting a Hospital in Qatar using Real-time Polymerase Chain Reaction**

On behalf of my co-authors, I would like to re-submit this manuscript to be considered for publication in *BMC Infectious Disease*. Comments from the first and second review are addressed below. Please note that we were requested to make track changes in the manuscript and re-submit it. Tracking some of the changes was difficult as the previous version was deleted from our system so we have highlighted the amends made in red and uploaded the document showing the amends as a supporting document. The main document is submitted as a clean version.

This article reports the frequency and aetiology of viral infectious Acute Gastroenteritis in children and adults in Qatar. The study aims to identify common gastroenteritis viruses affecting the population in Qatar and, to our knowledge; no one has attempted such a study previously in the Middle East.

We confirm that this manuscript has not been submitted to any other journal.

Thank you in advance for considering this work for publication in *BMC Infectious Diseases*. I look forward to hearing from you.

Yours sincerely,

Asma Althani
**FIRST REVIEW**

**Response to reviewers**

**Reviewer 1**

1. Abstract. The statement ‘Viruses, especially noroviruses, play a major role as agents of severe diarrhoea in children and adults in Qatar’ indicates a causal relationship between the observed pathogens and AGE. We can only say there is an association between the presence of certain pathogens and AGE. Change the word causal.

   **Done**

2. Page 4. ‘The patients were enrolled in the study between June 2009 and November 2009.’ Why were samples only collected over a 6m period. If there is significant seasonality of any or all the viruses then your estimates of prevalence are biased. This represents a serious epidemiological design flaw.

   Unfortunately our protocol did not account for the seasonality of all the viruses and hence we have outlined this flaw in our discussion section:

   “Future studies should be carried out in samples collected throughout the year to assess the impact of winter vs. summer infections and international travel.”

   In addition, please note that the climate is relatively constant in Qatar throughout the year so even though we acknowledge that we need to carry out a study with samples collected throughout the year, we are relatively confident that our collected samples provide a good overview of the prevalence of the viruses.

3. Page 4. ‘Whole stool samples were collected from each subject during the hospital visit. The samples were obtained only once from each patient’. Did you collect samples within a certain number of hours post admission in order to avoid sampling children who might have an in-hospital after a longer in-stay? The methods should be revised to include this and if you did collect later than 48hr you should include this as a study limitation.

   The samples were collected within 24 hours – the article has been amended to reflect this.

4. Page 6. ‘and astrovirus was detected in 0.30% (1 out of 288).’ The observation of only one astrovirus positive is a little perplexing. Can you be sure your assay was working? What controls were in place?

   Both positive and negative control for all viruses were used though as always there are errors.

5. Co-infection. Did you identify concurrent enteric viral infections? Please describe the coinfection proportion and which were the main pathogens involved.

   We did not detect any co-infection - see amend in article (Results section)

6. Page 6. Epidemiology. Please indicate the prevalence of each virus over time which might give an indication of the seasonal pattern of occurrence.
As described previously, we don't believe that we can draw definitive conclusions re seasonal patterns from our study as the samples were collected over 6 months. We can provide a table demonstrating virus type by month the sample was extracted in but we do not feel that it will be helpful to the reader. Another minor point to add is that the samples were collected once so we cannot accurately show the occurrence of viruses in the same patients over the 6 months.

This is an initial study to provide an overview of the prevalence of these viruses in Qatar in order to show a need for more studies in the country and in the region. We are hoping to carry out seasonal studies with larger samples over time which will help answer these questions.

7. Page 8. ‘Finally, the lack of healthy controls limits conclusions about the relative importance of each pathogen’. Given that you recognise this as a significant limitation can you justify why you did not collect data from such controls?

The authors would not “justify” not collecting data from controls per se. Generally data collection in a controlled case setting from “normal” individuals is quite difficult in our hospital for a number of bureaucratic reasons, causing long delays. Hence we decided to run a small study with these samples and are applying to run a study with larger samples, including controls, in the future. As per our specific regulations in Qatar, we have to show a need for case control studies before we get permissions to work with “healthy”/“control” subjects.

8. Page 2. ‘These include three RNA viruses (rotavirus, norovirus, and astrovirus) and one DNA virus (enteric adenovirus) [3,4].’ Is ref 4 appropriate ie a study in Germany?

New reference added

9. Page 2/ The statement ‘Saporovirus, Aichivirus and members of enterovirus group also cause AGE but are less common’ requires a reference.

Reference added

10. Page 4. ‘AGE was defined as #1 episode diarrhoea with abdominal discomfort with 24 hour period, with or without vomiting and passing of loose/watery/soft stool with or without mucus.’ This definition is a little unclear. If it is both with and without a particular symptom then that symptom is irrelevant and should be removed.

This has been re-worded and with/without symptoms removed.

11. Page 6. The statement ‘Parasitic infections were not investigated in this study.’ should be deleted as it is clear this is so from the methods.

Done

12. Page 6. ‘Viral agents were identified in 131/288 samples’ and ‘Bacterial agents were detected in 35 of 288 cases (12.2%),’. Both statements are repeats made earlier in the first sentence. Repetition should be avoided.

Done
13. Page 7. ‘No correlations of symptom severity and viral aetiology were made in this study. The causes of these diseases (e.g. recent travel, food, etc) were not investigated.’ These are not results and so should be included as limitations in the discussion.
Done

14. Page 7. ‘An aetiologic viral agent was identified in almost 50% of the patients (131/288).’ Remove aetiologic.
Done

15. Page 8. ‘Indeed, the lack of rotavirus infection among the elderly group does suggest that adults may have been infected through exposure from infected children.’ Please explain the logic of this statement. Since elderly individuals are less likely to be in a home environment with very young children. However, we have removed this sentence now as to allow for incorporation of all the other comments

Amended

17. Page 8. Conducting the study in the rotavirus vaccine era is a limitation and should be stated at that point of the discussion.
Done

18. Page 6. ‘Aetiologic viral agents were identified in 131 patients’. Strictly speaking this is not an aetiologic study. I suggest that aetiology be replaced.
Done

Reviewer 2:

1. This statement should be clarified......Viral and bacterial pathogens were detected in 131 (45.5%) and 34 (12.2%) of the 288 patients recruited (There is no data to support the detection of bacterial pathogens in the result section of the manuscript)

Please see Page 6 of results:

...A total of 288 stool samples were analysed, and viral agents were identified in 131 patients (45.5%), followed by bacterial infections (n=34; 12.2%). ...Salmonella was the most common bacterial species detected (n=23), followed by E.Coli (n=6); Shigella (n=3); and Campylobacter (n=3)....

2. The statement...The manufacturer’s instructions were followed to extract viral RNA (EZ1 Virus Mini Kit v2.0, catalog number 955134 include country and city where this kits is manufacture)

Done

2. Reviewer 2: all the reviewers’ editorial comments have been incorporated in the revised draft
SECOND REVIEW

1. A marked up version of the manuscript so that I can see exactly what changes have been made

A clean version has been submitted and a version with changes in red font has been added as supporting doc

(2) The number of samples tested, the number positive and proportion positive by month of study for norovirus, rotavirus and adenovirus.

Please see table of infections with viruses identified by month. The highest number of infections was detected in July and September (warm season – see below) though it is difficult to make accurate conclusions re seasonality because:

1. Low number of the samples
2. The samples were taken only once during the 6 month study period
3. The differences could be simply because more subjects were recruited during those two months

This information has not been added to the revised manuscript as we do not see a great value in it. However, we are happy to include if the reviewers think it is relevant.

<table>
<thead>
<tr>
<th>Month</th>
<th>Positive for rotavirus</th>
<th>Positive for adenovirus</th>
<th>Positive for norovirus</th>
<th>Positive for astrovirus</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>8</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>July</td>
<td>11</td>
<td>8</td>
<td>32</td>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>August</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>September</td>
<td>10</td>
<td>5</td>
<td>16</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>October</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

(3) For the calendar year (or years) of the study for each month (ie Jan-Dec) the average daily temperature, rainfall, relative humidity %. This will enable a judgement to be made on the response made on the possible importance of seasonality.

Date obtained from Qatar Met Office: The warm season lasts from May 10 to September 26 with an average daily high temperature above 37°C. The hottest day of the year is July 6, with an average high of 41°C and low of 31°C. During the warm season, which lasts from May 10 to September 26, there is a 1%
average chance that precipitation (rain, thunderstorms, etc) will be observed at some point during a given day.

The relative humidity typically ranges from 18% (dry) to 94% (very humid) over the course of the year, rarely dropping below 8% (very dry) and reaching as high as 100% (very humid). The air is driest around May 28, at which time the relative humidity drops below 21% (dry) three days out of four; it is most humid around January 1, exceeding 90% (very humid) three days out of four.

Relative humidity graph: