



RESEARCH ARTICLE

REVISED Molecular Detection of Sapovirus in Children Under Five Years with Acute Gastroenteritis in Mansoura, Egypt between January 2019 and February 2020 [version 4; peer review: 2 approved]

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Abstract

Background: Sapovirus has emerged as a viral cause of acute gastroenteritis. However, there is limited data on sapovirus in Egypt. The present study aimed to evaluate the presence of sapovirus in children with acute gastroenteritis <5 years in Mansoura, Egypt from January 2019 to February 2020 by reverse transcriptase-polymerase chain reaction (RT-PCR).

Methods: The cross-sectional study enrolled a 100 children <5 years who presented with acute gastroenteritis at an outpatient clinic in Mansoura, Egypt between January 2019 and February 2020. Clinical data, demographic data and a stool sample was collected from each child. Stools were screened by microscopy for parasites and culture methods for bacteria and excluded from the study if positive for either. Specimens were also screened for rotavirus by enzyme immune assays (EIA) and sapovirus by reverse transcription PCR.

Results: The most frequently detected virus was rotavirus by ELISA 25% (25/100). RT-PCR detected sapovirus in 7% (7/100) of the stool samples. The children with sapovirus were all from rural regions and presented mainly during the winter season in Egypt 42.9% (3/7). The main presenting symptoms were fever 71.4% (5/7) and vomiting 57.1% (4/7). None of the children with sapovirus had dehydration. Rotavirus was significantly associated with sapovirus infections in five samples (5/7), 71.4%, P=0.01.

Conclusion: The present study highlights the emergence of sapovirus as a frequent pathogen associated with acute gastroenteritis in

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Reviewer Status

Invited Reviewers

	1	2
version 4 (revision) 24 Nov 2021	 report	 report
version 3 (revision) 09 Nov 2021		 report
version 2 (revision) 24 May 2021	 report	 report
version 1 17 Feb 2021	 report	

1. **Marta Diez Valcarce** , Centers for Disease Control and Prevention, Atlanta, USA
2. **Nicola A. Page** , National Institute for Communicable Diseases (NICD), Johannesburg, South Africa

Any reports and responses or comments on the

children. There is a need for a national survey program for the study of sapovirus among other pathogens associated with acute gastroenteritis for better management of such infection.

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article can be found at the end of the article.

Keywords

sapovirus

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Author roles: **Zaki MES:** Conceptualization, Formal Analysis, Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; **Shrief R:** Data Curation, Methodology, Writing – Review & Editing; **Hassan RH:** Conceptualization, Data Curation, Methodology, Writing – Review & Editing

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REVISED Amendments from Version 3

Author Response to the reviewer

Thanks Sir for your effort for the revision.

Discussion, paragraph 1: Please rather use low-income, middle-income or high-income as these have been defined by the World Bank rather than underdeveloped which is not specific or well defined. This correction was noted in the previous review and has not been full corrected.

Corrected

Discussion, paragraph 2: Please delete the word hospital. The previous review indicated that either outpatient or hospital should be retained and the other word deleted as it was unclear where the study was conducted.

Deleted

Discussion, paragraph 3, line 3 states "A previous systematic review of sapovirus in African countries revealed that rotavirus..." Not sure why the authors are referring to a sapovirus paper for rotavirus prevalence and the reference used for the study is incorrect - should more likely be 4 or 24 and not 14. Please correct the sentence and the reference.

A reference was corrected

Any further responses from the reviewers can be found at the end of the article

Introduction

Sapovirus is a single strand non enveloped RNA virus that belongs to the *Caliciviridae* family¹⁻⁴. The length of its genome is 7.1 to 7.7 kb, and its polyadenylated 3' terminal is responsible for viral replication, while its 5' terminal is associated with viral translation through production of VPg^{5,6}. The viral genome consists of two to three open reading frames⁵. There are 15 genogroups of the virus with only four of them can infect humans, namely GI, GII, GIV and GV^{2,5,7,8}.

The laboratory methods for detection of sapovirus include enzyme-linked immunosorbent assay, electron microscopy, reverse transcription-polymerase chain reaction (RT-PCR) and next-generation sequencing³. The sensitivity and specificity of RT-PCR are good and can be used for routine identification of the virus³. The sensitivity and specificity of most RT-PCR detection assays for sapovirus are above 90%^{9,10}. The primers used for the amplification of the virus depend upon the use of a segment from capsid region^{3,11}. The classification of sapovirus depends upon complete sequence analysis of VPI^{6,12,13}.

Diarrhea represents the second common cause of death in children and is associated with about 525 000 deaths around the world¹⁴. Sapovirus gastroenteritis occurs via ingestion of contaminated food and water and also by direct contact with affected individuals¹³⁻¹⁶. The infection occurs both sporadically and as an outbreak^{2,11}. Treatment is symptomatic to prevent the aggravation of the disease^{3,11}, and prevention of the infection depends mainly upon access to clean drinking water and food and on good hygiene habits (WHO's five keys to food safety) and hand washing¹⁷.

There are data available about sapovirus infection in different countries such as Peru¹⁸ Iran¹⁹ and Ethiopia²⁰. There is limited data on sapovirus in Egypt. Therefore, the present study aimed to evaluate the presence of sapovirus in children with acute gastroenteritis by RT-PCR.

Methods

Study design and participants

The cross-sectional study enrolled 100 children <5 years who presented with acute gastroenteritis at an outpatient clinic in Mansoura, Egypt between January 2019 and February 2020. Clinical data, demographic data and a stool sample was collected from each child. Stools were screened by microscopy for parasites and culture methods for bacteria and excluded from the study if positive for either. Specimens were also screened for rotavirus by enzyme immune assays (EIA) and sapovirus by reverse transcription PCR.

The procedures followed were approved by the Mansoura Faculty of Medicine, Egypt ethical committee on human experimentation (R.20.11.1053) and were carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983. Written informed consent was obtained from the parents of the included children.

Each child was subjected to full medical history by asking the parents about the residence area and age of the child, which was then followed by clinical examination. A stool sample was collected from each child for laboratory examination.

Stool samples

Each stool sample was subjected to study by direct microscopic examination, study for rotavirus by ELISA Ridascreen® (R-Biopharm AG- An der Neuen Bergstraße 1764297 Darmstadt, Germany), and the remaining samples was subjected to RNA extraction and RT-PCR for sapovirus.

ELISA for rotavirus. The ELIA was completed according to the manufacturer's instructions with EIA plates read at 450nm on a Statfax Chromate 4300 (Unit No. 518, 5th Floor, MGF Metropolis Mall, MG Road, Gurgaon, Haryana-122002).

Sapovirus PCR

Extraction of RNA and complementary DNA preparation. The stool samples were subjected to the extraction of RNA of sapovirus using QIAamp Viral RNA kit (Qiagen). The extraction was performed according to the instructions supplied by the manufacturer.

Reverse transcriptase

A total volume of reaction mixture were prepared by adding 7.5 µl of extracted RNA to 2.05 µl 5× First-Strand Buffer (Invitrogen-USA), 0.75 µl of 10 mM dNTPs (Qiagen-USA), 0.375 µl (1 µg/ µl) of random primer (Qiagen-USA), 0.75 µl of 10 mM DTT (Invitrogen), 0.5 µl of RNase Inhibitor (Qiagen-USA), and 0.75 µl (200 U/ µl) of SuperScript Reverse Transcrip tase II (Thermofisher-USA). MilliQ water was added to give a total volume of 15.0 µl.

PCR for sapovirus. The amplification process was carried out using previously reported primers with nucleotide sequences of primers as follows: SLV5749 forward 5'-CGGRCYTCAA AVSTAC-CBCCCCA-3'; SLV5317 reverse 5'- CTCGCCACCTACRA-WGCBTGGTT-3'⁹.

The cDNA generated from the previous step was used as 2.5 µl and added to ready to use amplification mixture supplied from Qiagen with 0.4 µl of the used primers in total volume 25 µl. The amplification procedures were performed using the following conditions: denaturation at 94°C for 5 minutes, then 35 cycles composed of 94°C for 45 seconds- 55°C for 45 seconds and 72°C for 1 minute, then final extension of 7 minutes at 72°C (MiniAmp Thermal Cycler, Applied Biosystem).

PCR products were visualized under UV illumination after electrophoresis on a 1% agarose gel stained with ethidium bromide. The estimated amplified fragment size for sapovirus was 434 bp⁹.

Statistical analysis

Data were analysed with SPSS 22 (SPSS Inc, Chicago, Illinois, USA). Quantitative values were calculated as numbers and percentages. The use of the chi-square test performed comparisons, and the P-value was considered significant if it was <0.05.

Results

The study included 100 children with AGE manifested by diarrhoea associated predominately with fever (56%) 56/100, vomiting (47%) 47/100, abdominal pain (42%) 42/100. A minority had dehydration (11%) 11/100. Their mean age± SD was 53.33± 11.71 months. Most cases presented in the spring season (34%) 34/100 followed by winter (24%) 24/100. Demographics of the children are shown in [Table 1](#).

The most frequently detected virus was rotavirus by ELISA (25%) 25/100. RT-PCR detected sapovirus in seven samples 7% 7/100 of the stool samples.

The children with sapovirus were all from rural regions (Belkas, Dekrnes, Aga) and presented mainly during the winter season (22 December–19 March) in Egypt in three children (42.9%) 3/7. The main presenting symptoms were fever in five children (71.4%) 5/7 and vomiting in four children (57.1%) 4/7. None of the children with sapovirus had dehydration. Rotavirus was significantly associated with sapovirus infections in five children (71.4%, P=0.01) 5/7 ([Table 2](#)).

Discussion

Viral pathogens represent a significant aetiology for acute gastroenteritis. These infections are usually self-limited in high income countries while it may lead to mortality in low income countries, especially in children^{15,21}.

The present study including children <5 with diarrhea in an outpatient setting showed that the majority of cases experienced fever, vomiting and abdominal pain; common symptoms of viral gastroenteritis. These patients usually present to outpatient clinics and do not require hospital admission

Table 1. Demographic and clinical data of the studied children (n=100).

Parameter	
Age, months (mean± SD)	53.33± 11.71
Gender, n (%)	
Male	66 (66)
Female	34 (34)
Season, n (%)	
Summer (21 June-22 September)	20 (20)
Autumn (23 September-21 December)	22 (22)
Winter (22 December-19 March)	24 (24)
Spring (20 March-20 June)	34 (34)
Residence, n (%)	
Rural	68 (68)
Urban	32 (32)
Abdominal pain, n (%)	42 (42)
Fever, n (%)	56 (56)
Vomiting, n (%)	47 (47)
Dehydration, n (%)	11 (11)

except in rare instance of dehydration. The findings support the results noted in previous study²².

The proper management of children with AGE relies upon appropriate and robust diagnosis of the aetiology. In the present study, the most frequently detected virus was rotavirus by ELISA (25%). A previous systematic review in African countries revealed that rotavirus is associated with AGE in 31.5% of children and 25.7% in the general population¹⁶. Previous studies in Africa reported that the prevalence of rotavirus infections ranged from 22.73% up to 30% in children below 5 years^{23,24}. The study of rotavirus in Africa was carried out from 2006 to 2008 in 11 African countries and 2200 samples out of 5461 stool was positive for rotavirus²⁴. A previous study published 2018 from Abu El-reesh hospital in Cairo, Egypt reported that the prevalence of rotavirus was 31% among 119 hospitalized children below 5 years with AGE²⁵.

The study of sapovirus as an emerging pathogen associated with AGE has gained importance in recent years. Research has been facilitated by the emergence of the molecular techniques in laboratory diagnosis^{18,26,27}. In the present study, sapovirus was detected among 7% of children with AGE by RT-PCR. A previous meta-analysis study reported that the prevalence of sapovirus was 6.5% with a remarkable difference in the presence of sapovirus between low income and high-income countries²⁸. Another study reported a lower prevalence of sapovirus 4.6% (10/219)²⁸. The incidence of sapovirus infection in a study from Puru published in 2018 in the first and second years of life was 4.3 and 11.1 per 100 child-months, respectively¹⁸. In a case-control study from United states of America published 2019 in 300 children below 2 years with AGE versus 272 matched

Table 2. Comparison between sapovirus positive children and sapovirus negative children.

	Sapovirus positive (n=7)	Sapovirus negative (n=93)	Odds ratio	95%CI	P-value
	n (%)				
Gender					
Male	4 (57.1)	62 (66.7)	0.667	0.14-3.155	0.61
Female	3 (42.9)	31 (33.3)			
Age, months (mean± SD)	48.86± 9.40	51.52± 11.88			0.6 F=0.33
Rural residence	7 (100)	61 (65.6)	0.897	0.828-0.972	0.09
Season					
Summer (21 Eune-22 September)	0 (0)	20 (21.5)	1.55	0.32-7.315	0.41
Autumn (23 September-21 December)	2 (28.6)	20 (21.5)			
Winter (22 December-19 March)	3 (42.9)	21 (22.6)			
Spring (20 March-20 Eune)	2 (28.6)	32 (34.4)			
Vomiting	4 (57.1)	43 (46.2)	1.6	0.32-7.31	0.7
Abdominal pain	2 (28.6)	40 (43.01)	0.53	0.1-2.87	0.69
Fever	5 (71.4)	51 (54.8)	2.059	0.38-11.157	0.46
Dehydration	0 (0)	11 (11.8)	1.085	1.02-1.15	0.43
Rotavirus	5 (71.4)	20 (21.5)	9.12	1.646-50.594	0.01

healthy control the prevalence of sapovirus was 7.0% versus 3.0% ($P = .07$)²⁹. The variation of the prevalence rates reported may be due to the variation of the climate, environment, socio-economic factors, and cultural practices beside the difference of the used method of diagnoses.

The treatment of sapovirus depends mainly upon oral rehydration solution and zinc supplementation³⁰. The risk factors for sapovirus infection are not fully understood. The prevention of sapovirus infection depends mainly upon efficient hand hygiene practice, environmental disinfection, proper sewage disposal, and limited contact with ill individuals. There are conflicting data about the role of improvement of water sanitation in the prevention of sapovirus as it is a common pathogen in both high and low-income countries. However, as it is transmitted by contaminated water and food³¹, improving food safety and access to clean water and improved sanitation services will reduce the burden of the infection.

The use of new molecular technologies for sapovirus detection in different samples from patients, food and environment, is important to recognize the mode of sapovirus transmission. Infection at a young age may predispose to durable immunity. Therefore, the development of a vaccine toward this virus may reduce the burden of this infection³².

In the present study, there was no significant difference between the clinical presentation of sapovirus positive and sapovirus negative children. The clinical symptoms associated with AGE usually include diarrhoea, vomiting, and fever, making laboratory diagnosis essential for appropriate management. Therefore, there is a need for a national survey program to improve the monitoring of the circulation of enteric viruses including sapovirus alongside other pathogens associated with gastroenteritis to improve the control measures³².

Conclusions

The present study highlights the presence of sapovirus as a pathogen associated with AGE in children from Mansoura, Egypt during 2019 and 2020. There is a need for a national survey program for the study of sapovirus among other pathogens association with AGE for better management of such infection.

Data availability

Underlying data

Figshare: Molecular study of sapovirus in acute gastroenteritis in children: a cross-sectional study, <https://doi.org/10.6084/m9.figshare.13574933.v1>³³

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

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<http://www.doi.org/10.6084/m9.figshare.13574933.v1>

Open Peer Review

Current Peer Review Status:  

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Reviewer Report 29 November 2021

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Nicola A. Page 

Center for Enteric Diseases, National Institute for Communicable Diseases (NICD), Johannesburg, South Africa

All corrections were made.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Expertise includes epidemiology of enteric viruses

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 25 November 2021

<https://doi.org/10.5256/f1000research.79575.r101104>

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Marta Diez Valcarce 

Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

After the revisions I approve the article for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular epidemiology of gastroenteritis viruses

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 3

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Nicola A. Page 

Center for Enteric Diseases, National Institute for Communicable Diseases (NICD), Johannesburg, South Africa

The revised paper has been improved in the current version and describes the detection of sapovirus in children under five presenting to an outpatient clinic for the treatment of diarrhoea between January 2019 and February 2020 in Mansoura, Egypt.

The following minor errors were noted:

- Discussion, paragraph 1: Please rather use low-income, middle-income or high-income as these have been defined by the World Bank rather than underdeveloped which is not specific or well defined. This correction was noted in the previous review and has not been full corrected.
- Discussion, paragraph 2: Please delete the word hospital. The previous review indicated that either outpatient or hospital should be retained and the other word deleted as it was unclear where the study was conducted.
- Discussion, paragraph 3, line 3 states "A previous systematic review of sapovirus in African countries revealed that rotavirus..." Not sure why the authors are referring to a sapovirus paper for rotavirus prevalence and the reference used for the study is incorrect - should more likely be 4 or 24 and not 14. Please correct the sentence and the reference.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Expertise includes epidemiology of enteric viruses

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 16 Nov 2021

Maysaa El Zaki, Mansoura Faculty of Medicine, Mansoura, Egypt

Author Response to the reviewer

Thanks Sir for your effort for the revision.

- *Discussion, paragraph 1: Please rather use low-income, middle-income or high-income as these have been defined by the World Bank rather than underdeveloped which is not specific or well defined. This correction was noted in the previous review and has not been full corrected.*
- **Response:** Corrected.
- *Discussion, paragraph 2: Please delete the word hospital. The previous review indicated that either outpatient or hospital should be retained and the other word deleted as it was unclear where the study was conducted.*
- **Response:** Deleted.
- *Discussion, paragraph 3, line 3 states "A previous systematic review of sapovirus in African countries revealed that rotavirus..." Not sure why the authors are referring to a sapovirus paper for rotavirus prevalence and the reference used for the study is incorrect - should more likely be 4 or 24 and not 14. Please correct the sentence and the reference.*
- **Response:** The reference was corrected.

Competing Interests: No competing interests were disclosed.

Version 2

Reviewer Report 02 November 2021

<https://doi.org/10.5256/f1000research.56913.r85957>

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Marta Diez Valcarce 

Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

I accept the manuscript in this new revised form.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 14 September 2021

<https://doi.org/10.5256/f1000research.56913.r93206>

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Nicola A. Page 

Center for Enteric Diseases, National Institute for Communicable Diseases (NICD), Johannesburg, South Africa

The paper describes the EIA screening of rotavirus and RT-PCR detection of sapovirus in 100 children with acute diarrhoea enrolled from outpatient clinics in Mansoura, Egypt between January 2019 and February 2020. Children with bacterial or parasite infections were excluded from the study. Researchers collected data on symptoms, date of presentation and area of residence. While the paper has merit, there are a few major challenges with the paper in the current format:

- When reporting viral epidemiology, authors should include person, place and time. The title of the article is misleading in this sense as it does not indicate either place or time and is rather vague on the persons. The authors should consider changing it to “Molecular detection of sapovirus in children under five years with acute gastroenteritis in Mansoura, Egypt between January 2019 and February 2020”. This would include person, place and time – more appropriate for a study describing sapovirus epidemiology.
- Is the work clearly and accurately presented and does it cite the current literature? Some of the literature cited is not appropriate for the statement/argument being made. Problems with references have been detailed in the report below.
- Is the study design appropriate and does the work have academic merit? While the work may have merit, the study design is a little confusing as it was not clear when the parasite and bacterial screening was done and when the cases were excluded from the study due to detection of enteric parasites and bacteria. It was also not clear if cases were enrolled as outpatients or patients admitted to the Mansoura Hospital. This requires some clarification in the methods section.
- Are sufficient details of methods and analysis provided to allow replication by others? No, there were not sufficient details provided for the PCR reactions. In addition, one of the primers was incorrectly labelled. Corrections to these sections have been suggested in the detailed report below.
- If applicable, is the statistical analysis and its interpretation appropriate? Mostly. When reporting percentage, it is better to provide the numbers used to calculate the prevalence or percentage – make it clearer for the reader to interpret and evaluate the results.
- Are all the source data underlying the results available to ensure full reproducibility? Yes

- Are the conclusions drawn adequately supported by the results? Not all of the conclusions are supported. Sapovirus is not an emerging virus, it was always present as a cause of gastroenteritis in children. What has changed is our ability to detect it. The authors should always include person, place and time when discussing sapovirus prevalence from this or other studies.

Page 1:

- Abstract:
 - Background, line 1: Sapovirus has not recently emerged, it was always present but what has changed is our ability to detect it. Rather say that sapovirus has been shown to be an important viral cause of gastroenteritis in children under two years of age. This statement is supported by both the GEMS and MAL-ED studies that used molecular detection methods for sapovirus.
 - Background, line 2: The way that this sentence is written is misleading. There is data to support the circulation of sapovirus in children - see GEMS and MAL-ED which were done in multiple countries in Africa, South East Asia and Latin America. It might be more correct to say that there is limited data on sapovirus in Egypt or in Mansoura, Egypt.
 - Background, line 4: Please indicate place and time and be more specific for persons. i.e. children <5 years and include "...in Mansoura, Egypt from January 2019 to February 2020."
- Methods, line 7: This section is unclear, consider rewriting as follows: "The cross-sectional study enrolled a 100 children <5 years who presented with acute gastroenteritis at an outpatient clinic in Mansoura, Egypt between January 2019 and February 2020. Clinical data, demographic data and a stool sample was collected from each child. Stools were screened by microscopy for parasites and culture methods for bacteria and excluded from the study if positive for either. Specimens were also screened for rotavirus by enzyme immune assays (EIA) and sapovirus by reverse transcription PCR.
- Results: Please report prevalence as a percentage followed by the numbers used to calculate the percentage i.e. 10% (10/100). This needs to be corrected throughout the paper.
 - Results, line 20: Typically all numbers <10 should be written out. While this is no longer a recommendation by the International Committee of Medical Journal Editors, the authors should consider this for the current article under review.
 - Results, line 21-24: Delete this section from "There was an insignificant..." to the end of the section. Only the main findings should be reported in the abstract so if the results are not significant exclude from the abstract.

Page 3:

- Introduction, paragraph 2, line 5: This is very vague – please define good. Rather state that the sensitivity and specificity of most RT-PCR detection assays for sapovirus are above 90%.

Look at article from Yan et al., 2003 J Med Virol and Svraka et al., 2009 J Clin Micro.

- Introduction, paragraph 2, line 6: The primers used in this study amplify the capsid and not the VP1-RdRp junction. Please modify this sentence to reflect this fact.
- Introduction, paragraph 3, line 1: This is not an appropriate reference for this statement. Please insert an appropriate and original reference, try looking at the World Health Organization or similar global bodies that conduct these calculations.
- Introduction, paragraph 3, line 8: Prevention of sapovirus will include access to clean water and uncontaminated food but also on good hygiene habits (WHO's five keys to food safety) and hand washing. Please update sentence to reflect this.
- Methods, paragraph 5, line 5: This is unclear. Were the children admitted to the hospital or were they enrolled from the outpatient clinic? When was a stool specimen taken to exclude parasites and bacteria? Please also indicate if you mean children under five years of age or if you included slightly older children.
- Methods, paragraph 5, line 4: "...Mansoura, Egypt. The study was conducted from January 2019 to February 2020. All children provided a stool specimen which was screened for parasites by direct microscopy and bacterial aetiology by culture. Children positive for an enteric parasite or bacteria were excluded from the study."
- Methods, ELISA for rotavirus: This section is unnecessary. It is sufficient to say that the EIA was completed according to the manufacturer's instructions with EIA plates read at 450nm on a Statfax Chromate 4300 - Please provide manufacturers' details for the Statfax 4300.
- Methods, Extraction of RNA and cDNA amplification: Please supply enough detail so that someone else could replicate the assay if they wanted to – in the current format no detail was provided for primer concentration, buffer or kit used, manufacturer of the RNase inhibitor or where the dNTPs or RT enzyme were manufactured, concentration used etc. Please rewrite this section.

Page 4:

- Methods, PCR for sapovirus: Primer name incorrect - SLV5749 forward, please correct. Please also provide the concentration that the primers were used at or the reference of the method used in the lab with enough information to be replicated by the person reading it.
- These primers were also originally designed by Yan, H., F. Yagyu, S. Okitsu, O. Nishio, and H. Ushijima. 2003. Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. J. Virol. Methods 114:37-44. Please use the original reference and not Svraka.
- Results - Please report prevalence as a percentage followed by the numbers used to calculate the percentage i.e. 10% (10/100). This needs to be corrected throughout the paper.
- Discussion, line 1: Data from the GEMS and MAL-ED studies support this statement. Have a look at these studies.

- Discussion, line 2: Using the word developing, underdeveloped or developed is not appropriate to describe a country as it is not well defined i.e. all countries could technically be described as developing. Rather use the word low-income or high-income as this has been defined by the World Bank.
- Discussion, paragraph 2: This paragraph is a repetition of the results and not a discussion. Please rewrite this section.
- Consider "The present study including children <5 with diarrhoea in an outpatient/hospital (please indicate the correct setting as it is unclear from the article presented) setting showed that the majority of cases experienced fever, vomiting and abdominal pain; common symptoms of viral gastroenteritis. These patients usually present to outpatient clinics and do not require hospital admission except in rare instance of dehydration. The findings support the results noted in this study."
- Discussion, paragraph 3, line 4: "A previous study in Nigeria in 2014-2015 revealed..." Please also indicate if children were under 5 years and if by general population you mean everyone over five years or all ages.
- Discussion, paragraph 3, line 8: Please provide the time period and be more specific about the place and age of the cases - person, place and time.
- Discussion, paragraph 4, line 6: "A previous systematic review...of sapovirus in African countries..."
- Discussion, paragraph 4, line 9: Please provide the persons, place and time for the stated sapovirus prevalence?

Page 5:

- Discussion, paragraph 2, line 7: "...role of improvement of water and sanitation..."
- Discussion, paragraph 2 line 10: "...improving food safety and access to clean water and improved sanitation services..."
- Discussion, paragraph 3, line 3: Change mandatory.
- Discussion, paragraph 4, line 7: "...circulation of enteric viruses including sapovirus..."
- Conclusions – Please remove the word emergence and frequent as these are not appropriate given the data presented. Also include the place i.e. Mansoura, Egypt. In the current format this paper requires extensive reworking prior to indexing looking at the comments made above.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Expertise includes epidemiology of enteric viruses

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 Sep 2021

Maysaa El Zaki, Mansoura Faculty of Medicine, Mansoura, Egypt

Thanks for the reviewer comments and for the time to review the article. I have made the required changes

- *When reporting viral epidemiology, authors should include person, place and time. The title of the article is misleading in this sense as it does not indicate either place or time and is rather vague on the persons. The authors should consider changing it to "Molecular detection of sapovirus in children under five years with acute gastroenteritis in Mansoura, Egypt between January 2019 and February 2020".*
- **Response:** Done.
- *Is the work clearly and accurately presented and does it cite the current literature? Some of the literature cited is not appropriate for the statement/argument being made. Problems with references have been detailed in the report below. Is the study design appropriate and does the work have academic merit? While the work may have merit, the study design is a little confusing as it was not clear when the parasite and bacterial screening was done and when the cases were excluded from the study due to detection of enteric parasites and bacteria. When presented with acute gastroenteritis stool samples were taken and examined for parasite and bacterial pathogens and the remaining sample was preserved if negative the sample was included in the study. It was also not clear if cases were enrolled as outpatients or patients admitted to the Mansoura Hospital. This requires some clarification in the methods section.*
- **Response:** Outpatients and added.

- *Are sufficient details of methods and analysis provided to allow replication by others? No, there were not sufficient details provided for the PCR reactions. In addition, one of the primers was incorrectly labelled. Corrections to these sections have been suggested in the detailed report below. If applicable, is the statistical analysis and its interpretation appropriate? Mostly. When reporting percentage, it is better to provide the numbers used to calculate the prevalence or percentage – make it clearer for the reader to interpret and evaluate the results.*
- **Response:** Added.
- *Are all the source data underlying the results available to ensure full reproducibility? Yes. Are the conclusions drawn adequately supported by the results? Not all of the conclusions are supported. Sapovirus is not an emerging virus, it was always present as a cause of gastroenteritis in children. What has changed is our ability to detect it. The authors should always include person, place and time when discussing sapovirus prevalence from this or other studies.*
- **Response:** Added.

Page 1:

- *Abstract: Background, line 1: Sapovirus has not recently emerged, it was always present but what has changed is our ability to detect it. Rather say that sapovirus has been shown to be an important viral cause of gastroenteritis in children under two years of age. This statement is supported by both the GEMS and MAL-ED studies that used molecular detection methods for sapovirus. Background, line 2: The way that this sentence is written is misleading. There is data to support the circulation of sapovirus in children - see GEMS and MAL-ED which were done in multiple countries in Africa, South East Asia and Latin America. It might be more correct to say that there is limited data on sapovirus in Egypt or in Mansoura, Egypt.*
- **Respond:** Added there is limited data on sapovirus in Egypt or in Mansoura, Egypt.
- *Background, line 4: Please indicate place and time and be more specific for persons. i.e. children <5 years and include "...in Mansoura, Egypt from January 2019 to February 2020."*
- **Response:** Done.
- *Methods, line 7: This section is unclear, consider rewriting as follows: "The cross-sectional study enrolled a 100 children <5 years who presented with acute gastroenteritis at an outpatient clinic in Mansoura, Egypt between January 2019 and February 2020. Clinical data, demographic data and a stool sample was collected from each child. Stools were screened by microscopy for parasites and culture methods for bacteria and excluded from the study if positive for either. Specimens were also screened for rotavirus by enzyme immune assays (EIA) and sapovirus by reverse transcription PCR.*
- **Response:** Done.
- *Results: Please report prevalence as a percentage followed by the numbers used to calculate the percentage i.e. 10% (10/100). This needs to be corrected throughout the paper. Results, line 20: Typically all numbers <10 should be written out. While this is no longer a recommendation by the International Committee of Medical Journal Editors, the*

authors should consider this for the current article under review.

- **Response:** Done.
- *Results, line 21-24: Delete this section from "There was an insignificant..." to the end of the section. Only the main findings should be reported in the abstract so if the results are not significant exclude from the abstract.*
- **Response:** Done.

Page 3:

- *Introduction, paragraph 2, line 5: This is very vague – please define good. Rather state that the sensitivity and specificity of most RT-PCR detection assays for sapovirus are above 90%. Look at article from Yan et al., 2003 J Med Virol and Svraka et al., 2009 J Clin Micro.*
- **Response:** Done.
- *Introduction, paragraph 2, line 6: The primers used in this study amplify the capsid and not the VP1-RdRp junction. Please modify this sentence to reflect this fact.*
- **Response:** Done.
- *Introduction, paragraph 3, line 1: This is not an appropriate reference for this statement. Please insert an appropriate and original reference, try looking at the World Health Organization or similar global bodies that conduct these calculations.*
- **Response:** Done.
- *Introduction, paragraph 3, line 8: Prevention of sapovirus will include access to clean water and uncontaminated food but also on good hygiene habits (WHO's five keys to food safety) and hand washing. Please update sentence to reflect this.*
- **Response:** Added.
- *Methods, paragraph 5, line 5: This is unclear. Were the children admitted to the hospital or were they enrolled from the outpatient clinic? When was a stool specimen taken to exclude parasites and bacteria? Please also indicate if you mean children under five years of age or if you included slightly older children. Methods, paragraph 5, line 4: "...Mansoura, Egypt. The study was conducted from January 2019 to February 2020. All children provided a stool specimen which was screened for parasites by direct microscopy and bacterial aetiology by culture. Children positive for an enteric parasite or bacteria were excluded from the study."*
- **Response:** Done.

Page 4:

- *Methods, PCR for sapovirus: Primer name incorrect - SLV5749 forward, please correct. Please also provide the concentration that the primers were used at or the reference of the method used in the lab with enough information to be replicated by the person reading it. These primers were also originally designed by Yan, H., F. Yagyu, S. Okitsu, O. Nishio, and H. Ushijima. 2003. Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. J. Virol. Methods 114:37-44. Please use the original reference and not Svraka. Results - Please report prevalence as a percentage followed by the numbers used to calculate the percentage i.e. 10% (10/100).*

This needs to be corrected throughout the paper. Discussion, line 1: Data from the GEMS and MAL-ED studies support this statement. Have a look at these studies.

- **Response:** Added.
- *Discussion, line 2: Using the word developing, underdeveloped or developed is not appropriate to describe a country as it is not well defined i.e. all countries could technically be described as developing. Rather use the word low-income or high-income as this has been defined by the World Bank.*
- **Response:** Done.
- *Discussion, paragraph 2: This paragraph is a repetition of the results and not a discussion. Please rewrite this section.*
- **Response:** Done.
- *Consider "The present study including children <5 with diarrhoea in an outpatient/hospital (please indicate the correct setting as it is unclear from the article presented) setting showed that the majority of cases experienced fever, vomiting and abdominal pain; common symptoms of viral gastroenteritis. These patients usually present to outpatient clinics and do not require hospital admission except in rare instance of dehydration. The findings support the results noted in this study."*
- **Response:** Done.
- *Discussion, paragraph 3, line 4: "A previous study in Nigeria in 2014-2015 revealed..." Please also indicate if children were under 5 years and if by general population you mean everyone over five years or all ages.*
- **Response:** Below five, added.
- *Discussion, paragraph 3, line 8: Please provide the time period and be more specific about the place and age of the cases - person, place and time.*
- **Response:** Done.
- *Discussion, paragraph 4, line 6: "A previous systematic review...of sapovirus in African countries..."*
- **Response:** Done.
- *Discussion, paragraph 4, line 9: Please provide the persons, place and time for the stated sapovirus prevalence?*
- **Response:** Done.

Page 5:

- *Discussion, paragraph 2, line 7: "...role of improvement of water and sanitation..."*
- *Discussion, paragraph 2 line 10: "...improving food safety and access to clean water and improved sanitation services..."*
- **Response:** Done.
- *Discussion, paragraph 3, line 3: Change mandatory.*

- **Response:** Done.
- *Discussion, paragraph 4, line 7: "...circulation of enteric viruses including sapovirus..."*
- **Response:** Done.
- *Conclusions – Please remove the word emergence and frequent as these are not appropriate given the data presented. Also include the place i.e. Mansoura, Egypt.*
- **Response:** Done.

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 12 May 2021

<https://doi.org/10.5256/f1000research.33041.r83889>

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Marta Diez Valcarce 

Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

The article titled 'Molecular study of sapovirus in acute gastroenteritis in children: a cross-sectional study' by El Sayed Zaki *et al.* includes some very interesting data on the prevalence of sapovirus in Egypt.

Overall, the article is well presented but there are a few minor comments that I would like to make, and also some edits that I consider necessary for clarity and accuracy.

- In the abstract section, results paragraph, I think it could be informative which months are considered winter season in Egypt, as this varies in different parts of the world.
- In the introduction I would suggest to write the family *Caliciviridae* in italics. Also, and that is a major edit that need to be done, the sapovirus genus contains or can be divided into 15 genogroups, not genotypes.
- I am not sure what the authors mean when they say that the primers used depend upon the use of a segment from VP1 encoding gene compared to the RdRp region. I am not aware of any comparison made between the capsid and the polymerase for classification of sapovirus. In some cases, both regions are sequenced to give a more accurate classification, but not compared. Maybe the authors could rewrite this sentences so they are clearer.

- The third paragraph of the introduction mentioned a reference but I think the authors misinterpreted the findings of that work. What the article said is that globally, diarrhea is the second cause of mortality and the most common cause of illness in children younger than 5, and that it causes approximately 2 million deaths per year. But that is diarrhea, from any cause, not only sapovirus. Please correct.
- In the methods section, under the stool samples paragraph the acronym for the molecular detection method used is incorrect, it should read RT-PCR and not RT-PVR.
- Also in the methods section, when the mentioned the Ridascreen method to detect rotavirus, I think they should refer to the method as a semiquantitative one, since as they mentioned, the color intensity is proportional to the concentration of rotavirus present compared with the control.
- I also suggest to rewrite the part in which they explain how the kit uses monoclonal antibodies that specifically react with the VP6 protein (not the protein of the six viral genes, please correct).
- In the statistical analysis paragraph, if results were expressed as numbers and percentages, those are quantitative values, not qualitative. Please correct.
- In the discussion section, the fifth paragraph, I suggest to replace the word 'management' for 'treatment'. Also, I would rewrite the sentence and instead of using the word 'argument', I would say something like: There are conflicting/contradictory opinions about the role of water sanitation improvement in the prevention of sapovirus ...
- I suggest to replace the expression 'an insignificant difference' for 'no significant difference', throughout the manuscript. Similarly, I also suggest to just mention acute gastroenteritis with all the letters once, with the acronym AGE between brackets, and from then on just use AGE throughout the article.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular epidemiology of caliciviruses

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 May 2021

Maysaa El Zaki, Mansoura Faculty of Medicine, Mansoura, Egypt

Dear Dr. Diez Valcarce,

Thanks for your valuable comments. I have made all the required corrections.

Best Regards.

Competing Interests: No competing interests were disclosed.

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