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RESEARCH

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Study of Cosavirus, Salivirus, and Bufavirus viruses in children with acute gastroenteritis

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Abstract

Background Acute gastroenteritis (AGE) in children represents a health problem. Besides common enteric viruses such as rotavirus and adenovirus, new viruses such as cosavirus, salivirus, and bufavirus may be associated with AGE. The objective of the study was to detect cosavirus, salivirus, and bufavirus viruses in children below 5 years with acute gastroenteritis by the use of real-time polymerase chain reaction (PCR) besides detection of rotavirus and adenovirus by enzyme-linked immunosorbent assay (ELISA).

Method The study included 150 children ≤ 5 years with community-acquired diarrhea. Stool samples from children were subjected for the detection of rotavirus and adenovirus antigens by ELISA and for detection of buvavirus, salivirus, and cosavirus by real-time PCR.

Results The commonest virus detected in the stool samples of children with AGE was rotavirus 31.3% followed by adenovirus 24%. Among the new viruses studied, salivirus was detected in six samples (4.0%), buvavirus was detected in four samples (2.7%), and cosavirus was detected in two samples (1.3%). The mixed rotavirus detection with studied viruses was 23.4% for adenovirus, 4.3% for calicivirus, and 2.1% for bocavirus, and none of the detected cosavirus was associated with rotavirus. In the studied children, at least one of the new viruses was detected in ten children (6.7%). Buvavirus, salivirus, and cosavirus were detected as a single virus (0.7%) in the children with acute gastroenteritis and buvavirus was detected with cosavirus without other viruses in one sample (0.7%).

Conclusion The study reports the occurrence of buvavirus, cosavirus, and salivirus in the pediatric patients with community-acquired acute gastroenteritis. There was a high prevalence of rotavirus and adenovirus antigens in those patients with low positivity for buvavirus, cosavirus, and salivirus viruses. There is a need for a large cohort study to study the prevalence of buvavirus, cosavirus, and salivirus in pediatrics with acute gastroenteritis and to validate their association with the disease.

Keywords Acute gastroenteritis, Children, Rotavirus, Adenovirus, Buvavirus, Cosavirus, Salivirus

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1 Background

Acute gastroenteritis (AGE) in children remains a major health problem worldwide. It has high morbidity and mortality rates in addition to the financial burden of the disease [1]. The infectious organisms' responsible for AGE include mainly viruses like rotaviruses noroviruses, astroviruses, and human adenoviruses [2]. There is a continuous discovery of new viruses that may be implicated as the etiology of acute gastroenteritis since about 40% of AGE is of unknown etiology [3–5].

Studies have reported the emergence of newly discovered viruses cosavirus (CosV), salivirus (SalV), and bufavirus (BuV) that may be implicated in acute gastroenteritis in children [6]. The CosaV and salivirus are RNA viruses, while bufavirus is a DNA virus.

The CosV is an RNA virus with single RNA strand of around 7.6 Kb organized in a typical picornavirus genome. There are around six species of the virus from A to F; the A species had 24 t genotypes, and D species had five different genotypes [7].

The salivirus is an RNA virus with a single RNA strand genome of around 7.1Kb that is picornavirus genome typically. SalV had one genotype with 2 clusters [8]. Human salivirus has been reported to be associated with AGE from different geographical locations and in sewage from studies in Spain and Hong-Kong [9].

The third virus, bufavirus a single stranded DNA virus, was associated with AGE from children in Burkina Faso, from which it gets its name [10], and from other countries [11–14]. It is a DNA virus that is around 4.9 Kb, that encodes viral structural protein (VP2) and nonstructural protein 1 (NS1). There are three types of the genotypes (BuV1, 2, and 3) [15]; however, due to the diversity of the capsid gene, there may be other undiscovered genotypes [10].

The link of these viruses to AGE as an etiology is controversy and there is a need to detect these viruses in the AGE in children to establish their role. There are various reports about the detection of CosV, SalV, and bufavirus in children with AGE in different geographical regions [7, 10]. However, there are no reports about the detection of these viruses in children from Egypt.

Therefore, the objective of the study is to detect the viruses cosavirus, salivirus, and bufavirus in pediatric patients below 5 years with acute gastroenteritis by using the real-time polymerase chain reaction (real-time PCR) besides rotavirus and adenovirus detection by enzyme-linked immunosorbent assay (ELISA).

2 Methods

This report was a cross-sectional study which included 150 children ≤ 5 years complaining of community-acquired diarrhea. The included children were recruited from outpatient clinics, Egypt, from March 2022 to December 2022. The size of the sample depended upon the time nonprobability sampling consecutive that depended on the time of study in which enrolled pediatric patients with diarrhea with the inclusion criteria will be presented to the clinicians during the time of the study [16].

The inclusion criteria were children ≤ 5 years with diarrhea during the past 72 h. The children with diarrhea due to systemic conditions, drug intake, or associated with hepatic or renal disorders or with isolated bacterial pathogens or parasites were excluded from the study.

The children were subjected to medical examination and the condition severity classification was assessed by medical history taking from the parents of each child and recorded by the use of the scale of Vesikari [17]. The Vesikari scale depends upon 20 points calculation regarding the number of diarrhea and its severity with the presence of vomiting, fever, and dehydration. Vesikari score is reported as mild if below 7, moderate from 7 to 10, and severe if ≥ 11[17].

2.1 Stool samples

The obtained stool sample from each child was taken in clean container and transferred within thirty minutes to the laboratory.

3 Stool examination and culture

Stool samples were examined for the presence of r parasites by the method of previously described [18]. The microbiological specific culture was used for exclusion of common bacterial pathogens like *Aeromonas* species, *E. coli O157:H7*, *Pleisiomonas shigelloides*, *Salmonella*, and *Shigella.* Microbiological culture was performed by culture on agar of sheep's blood, plate of MacConkey, plate of sorbitol-MacConkey and Selenite F broth (BBL; Becton Dickinson) was used for Salmonella–Shigella with pre-inoculation. All culture plates were incubated at 35 °C for 24 h [18].

4 Stool examination for rotavirus and human adenovirus antigen

Detection of rotavirus will be performed by commercially available enzyme-linked sorbent assay (ELISA)-RIDASCREEN® kit (R-Biopharm, Germany) and detection of adenovirus was performed by ELISA by RIDASCREEN® Adenovirus (R-Biopharm AG, An der

neuen Bergstraße 17, 64297 Darmstadt, Germany). The kits were used according to the manufacturer instructions.

5 Real-time PCR for CosV, SalV, and bufavirus

5.1 Nucleic acid extraction (DNA–RNA) from stool samples

Nucleic acid was extracted from stool sample, by the use of 400 µL of 12.5% stool suspension in phosphate buffer solution and the RNA, DNA extraction kits (Promega, Madison, WI, USA) were used for the relevant virus according to the manufacturer's instructions. The DNA and RNA were eluted in the final volume of 50 µL and kept frozen at − 20°C till other amplification procedures.

6 Reverse transcription for cosavirus and salivirus

The reverse transcriptase reactions for cosavirus and salivirus were carried on the 5 µL of the extracted RNA by the use of a Promega kit for reverse transcription by the same manufacturer's instructions. The reaction for the reverse transcription was performed at 42°C for 60 min, followed by 70 °C for 15 min.

7 Amplification by real-time PCR

Ready-to-use amplification kit with primers and probe will be used to ready the primers and probes commercial kits: PP-BioMole073 for salivirus, PP-BioMole074 for cosavirus, and PP-BioMole077 for bufavirus (BioMole, Turin, Italy). The amplification was performed by the following sequences: heating for 2 min at 50 °C, 2 min at 95 °C, 45 cycles of 15 s at 95 °C, and 1 min at 60 °C on the 7500 ABI real-time instrument (Life-Technologies).

7.1 Statistical analysis

The SPPS22 was used to analyze the data. The qualitative data were expressed as number and percentages. The comparison between qualitative data was done by the use of Chi-square test and P was significant if it was below 0.05. The numerical data were mentioned as mean and standard deviation (mean ± SD).

Table 1 Demographic and clinical findings of the studied children

	No.%
Sex	
Male	96 64
Female	54 36
Age (mean ± SD)	43.8 ± 13.02
Vomiting	64 42.7
Watery diarrhea	79 52.7
Fever	65 43.3
Vesikari score	
Mild	69 46
Moderate	68 45.3
Severe	13 8.7

8 Results

The study included 150 children with acute gastroenteritis, 96 (64%) males and 54 females (36%), with mean age ± SD 43.8 ± 13.02 months. The presenting symptoms were watery diarrhea 79(52.7%), fever 65 (43.3%), and vomiting 64 (42.7%). The Vesikari score was mainly mild (46%) and moderate (45.3%), Table 1.

The commonest virus detected in the stool samples of children with AGE was rotavirus 31.3% followed by adenovirus 24%. Among the new viruses studied, salivirus was detected in six samples (4.0%), buvavirus was detected in four samples (2.7%), and cosavirus was detected in two samples (1.3%), Table 2. The mixed rotavirus detection with studied viruses was 23.4% for adenovirus, 4.3% for calicivirus, and 2.1% for bocavirus, and none of the detected cosavirus was associated with rotavirus, Table 3.

In the studied children, at least one of the new viruses was detected in ten children (6.7%). Buvavirus, salivirus, and cosavirus were detected as a single virus (0.7%) in the children with acute gastroenteritis and buvavirus was detected with cosavirus without other viruses in one sample (0.7%), data not shown.

Table 2 The prevalence of the studied viruses in the children

	No.%
Rotavirus	4731.3
Adenovirus	3624
Cosavirus	21.3
Bufavirus	42.7
Salivirus	64.0

Table 3 The association of viruses with rotavirus

	Rotavirus positive (n = 47) No.%	Rotavirus negative (n = 103) No.%	*p*
Adenovirus	11 23.4	25 24.3	1.00
Bufavirus	1 2.1	3 2.9	1.00
Cosavirus	0 0	2 1.9	1.00
Salivirus	2 4.3	4 3.9	1.00

Chi-square test

9 Discussion

The common signs and symptoms of AGE in children are vomiting, fever, appetite loss, and abdominal cramps. The severe AGE may lead to dehydration and hospitalization [19–21].

In the present study, the severe Vesikari score was defined in 8.7% of the children. Previous study reported similar severity of AGE in children [22]. The presenting symptoms were watery diarrhea 79(52.7%), fever 65 (43.3%), and vomiting 64 (42.7%). A previous study of the AGE in children below 5 years and vomiting in. reported fever and vomiting as a common symptom in children with AGE [22]. The vomiting is a leading cause for the severity of AGE and failure of oral rehydration therapy.

The common etiology of AGE in children in less developed countries is viral pathogens [23]. The present study shows high prevalence of rotavirus (31.3%) followed by adenovirus (24%). The mixed rotavirus detection with studied viruses was 23.4% for adenovirus. The prevalence of adenovirus as an etiology for AGE ranged from 1% up to 23% in different geographical locations [24–29]. The range of rotavirus detection in AGE was from 16.7% up to 73.7% [30–33].

In a previous study from Egypt, a higher prevalence rate was reported for rotavirus (58%) and a lower rate for adenovirus (6.7%) and mixed rotavirus and adenovirus was 8% in children admitted to hospital [34]. The difference in the presence of rotavirus and adenovirus may be attributed to the age of the included children and the type of acute gastroenteritis, as the included children in the present study were children 5 years with community-acquired diarrhea. However, there is a need to introduce the rotavirus vaccine in the national program the vaccination of children in Egypt.

In the studied children, at least one of the new viruses was detected in ten children (6.7%). Among the new viruses studied, salivirus was detected in six samples (4.0%), buvavirus was detected in four samples (2.7%), and cosavirus was detected in two samples (1.3%). A previous study reported detection rates of these new viruses in 11 patients (5.4%) with CosV 2 (1.0%), SalV 7 (3.5%), and 2 BuV (1.0%) [6].

The ranges of detection rates of cosavirus in diarrheal children in previous studies ranged from 1% up to 3.6% in children with diarrhea (1%) [35, 36]. The detection rates of BuV 0% to 4.0% in patients of all ages [[10, 12, 37–39]. SalV had a prevalence ranges from 0.1 up to 8.8% in patients with gastroenteritis [4, 14, 40–48]. The discrepancy of the detection rates of these virus may be attributed to the difference in the patients age in various studies, whether children or adults.

In the present report, mixed presence of buvavirus was detected with cosavirus without other viruses in one sample (0.7%) and mixed with rotavirus was present in 4.3% of the stool samples for salivirus, and in the stool samples 2.1% for bufavirus. The mixture of enteric rotavirus with the bufavirus and salivirus may indicate environmental contamination of soiled water as a possible source of infection [48]. There were various studies that indicated the wide distribution of salivirus in different environmental samples [29–32]. This finding may indicate a fear-oral transmission of the virus. There is a need for larger children studies from multiple geographical regions in Egypt to assess the association of these viruses with AGE in children.

10 Conclusion

The study shows the presence of buvavirus, cosavirus, and salivirus in the pediatric patients with community-acquired acute gastroenteritis. There was a high presence of rotavirus and adenovirus antigens in those patients with low positivity for buvavirus, cosavirus, and salivirus viruses. There is a need for a large cohort study to study the prevalence buvavirus, cosavirus, and salivirus in children with acute gastroenteritis and to validate their association with the disease.

Abbreviations

AGE	Acute gastroenteritis
BuV	Bufavirus
CosV	Cosavirus
ELISA	Enzyme-linked immunosorbent assay
NS1	Nonstructural protein 1
PCR	Polymerase chain reaction
SalV	Salivirus
VP2	Viral structural protein

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Author contributions

OAMS designed the study, collected clinical data from the studied children, and wrote the article. MEZ shared the laboratory study, the draft preparation of the article and data analysis of the study. AGE shared the laboratory study and draft preparation of the article. AZMH collected clinical data from the studied children and wrote the article. EHM shared the laboratory study and draft preparation of the article. All authors read and approved the final manuscript.

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Availability of data and materials

https://Mendeley.com/datasets/24zn3vxmwb.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Mansoura Faculty of Medicine, Egypt (R.22.12.1987). The study was performed according to the Declaration of Helsinki. Informed written consent was obtained from each child's parent.

Consent for publication
Not applicable.

Competing interests
There are no competing interests for any of the authors.

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