SciMedCentral

Annals of Pediatrics & Child Health

Research Article

Prevalence of Enteric Viruses in Children Hospitalized with Acute Gastroenteritis in Northern Jordan

Mamdoh M. Meqdam[1], Malak I. Abusamha[2], Khadija F. Maho[1], Ayat M. Gogazeh[1], Mustafa Ababneh[3], Asem Alkhateeb[2], Samer FM Swedan[1], Muhamad Ali K Shakhatreh[1], and Ziad Bataineh[4]

[1]Faculty of Applied Medical Sciences, Jordan University of Science and Technology, Jordan
[2]Faculty of Science and Arts, Jordan University of Science and Technology, Jordan
[3]Faculty of Veterinary Medicine, Jordan University of Science and Technology, Jordan
[4]Faculty of Medicine, Jordan University of Science and Technology, Jordan

***Corresponding author**

Mamdoh M. Meqdam, Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Jordan University of Science and Technology, P.O. 3030, Irbid 22110, Jordan, Tel: (+962)2-7201000 Ext: 23777; Email: meqdam@just.edu.jo

Submitted: 19 February 2022

Accepted: 12 March 2022

Published: 15 March 2022

ISSN: 2373-9312

Keywords

- Enteric viruses
- Gastroenteritis
- PCR
- RT-PCR
- Jordan

Abstract

Objectives: To detect the prevalence of enteric viruses and to investigate the association between the infection and the clinical data collected from patients.

Methods: Fecal samples collected between September 2019 and August 2020 from 239 Jordanian children aged less than 15 years hospitalized with acute gastroenteritis (AGE), were screened for enteric viruses by polymerase chain reaction (PCR) and reverse transcriptase- polymerase chain reaction (RT-PCR).

Results: A total of 34 out of 239 (14.2%) fecal samples resulted positive for at least one enteric viruses. Viruses detected were rotavirus A (11, 4.6%), astrovirus (9, 3.8%), norovirus (5, 2.1%), adenovirus 40, adenovirus 41, and human bocavirus, each with 3(1.3%) positive samples. Aichivirus, sapovirus, klassevirus, and salivirus A were not detected in any sample. A statistically significant association ($P<0.05$) was observed between female gender and astrovirus (17.6% females vs. 8.8% males). There was no significant association between gender and other viruses. The group having the highest infection rate was 0-1-year-old patients (8.4%) followed by 2-4 years (4.2%). Most viral cases were detected in the winter (38.5%) followed by autumn (35.5%) without recording cases in the spring. Significant associations were seen between the presence of rotavirus and dehydration ($P= 0.00001$) and vomiting ($P = 0.004$), and between norovirus and weakness ($P = 0.047$). The average duration of symptoms was 2-3 days in 25% of the cases. Among the infected patients, 25% were under antibiotic treatment.

Conclusion: Detecting enteric viruses in patients with AGE will aid in the effective care of patients and the development of viral gastroenteritis control measures in the country.

INTRODUCTION

Acute gastroenteritis (AGE), is a leading reason of illness and death around the world. Depending on World Health Organization (WHO) studies, diarrhea is one of the top ten causes of mortality worldwide, most occurring in young children in developing countries [1]. Viruses are responsible for about 70–90% of instances involving all ages of patients [1]. Viral gastroenteritis can range from a self-limited watery diarrheal disease with vomiting, malaise, anorexia, nausea, or fever to acute dehydration that requires hospitalization or even leads to death [1]. Viral AGE is commonly caused by rotaviruses [2], caliciviruses (norovirus and sapovirus) [2], astroviruses [2], and adenovirus 40 (Ad40), adenovirus 41 (Ad41) [2]. New viruses; human bocavirus [3], human coronavirus [3], aichivirus [3], klassevirus [3], salivirus [3], causing AGE were also detected. An estimated five million deaths were caused by diarrhea in children under 5 years old each year, 20% of which were due to rotavirus [4]. In developing countries, an estimated of 870,000 children die from rotavirus diarrhea each year that reflects an urgent need to develop a vaccine [4].

Since most viruses causing gastroenteritis cannot be isolated in cell culture, direct visualization in stool specimens by electron microscopy is still the mainstay of diagnosis although it is limited to reference laboratories [5]. The introduction of more sensitive techniques for antigen detection in stool-based on immunoassay and molecular biology techniques has improved the diagnosis of newly recognized viruses as norovirus and sapovirus [6,7]. Since the starting of using rotavirus vaccines, hospitalizations and deaths caused by rotavirus significantly decreased [8].

This study aimed to detect the prevalence of rotavirus A, astrovirus, norovirus, ad40, ad41, human bocavirus, aichivirus, klassevirus, and salivirus A as causative agents of AGE among the northern Jordan population and to investigate the association between the infection and the clinical data collected from patients.

MATERIALS AND METHODS

Patients and Specimens

This study was approved by the Institutional Review Board Committee of Jordan University of Science and Technology. Fecal samples were collected from patients with AGE during the period from September 2019 until August 2020 from four hospitals in northern Jordan (Princess Rahma Pediatric Hospital, King Abdullah University Hospital, Abu Obida Hospital and Al-Yarmouk Teaching Hospital). All samples were stored at -20°C until processed. Two hundred and thirty-nine children were included in this study with symptoms like:vomiting, dehydration, abdominal pain, weakness, and fever. Information concerning each patient, including clinical data, age, sex, length of symptoms duration, and use of antibiotics, were collected according to the proposed form.

Fecal suspensions in 1.5 ml sterile Eppendorf tubes (0.5 ml of stool in 450 µl of phosphate buffer saline) were prepared for each stool sample, vortexed, and clarified by centrifugation for 5 min at 3000 rpm. The supernatants were transferred to na ew Eppendorf tube for use and storage at -20° C.

Nucleic Acid Extraction

The viral genome was extracted from the supernatant of fecal suspension using a Quick DNA/RNA Path Miniprep (Zymo USA), according to the manufacturer's instructions. All extraction steps were performed at room temperature (15°C - 25°C) and centrifuged at 11000 rpm for 30 seconds, (unless specified). A volume of 400 µl of the DNA\RNA Shield was added to the fecal suspension, then mixed well. Then 200 µl of the prepared mixture was transferred to a new nuclease-free tube, 2 µl of Proteinase K was added and mixed well. A volume of 200 µl of Pathogen DNA/RNA Buffer was added, mixed well then incubated at R.T for 5 minutes. The mixture was transferred into a Zymo-Spin™ IIC Column in a Collection Tube, centrifuged and the flow-through was discarded. 500 µl of Pathogen DNA/RNA Wash Buffer was added to the column, centrifuged and the flow-through was discarded. This step was repeated. 500 µl of ethanol (100%) was added to the column then centrifuged for 1 minute then re-centrifuged to ensure the removal of any residual ethanol. The collection tube was discarded, and the column was carefully transferred into a new nuclease-free tube. Finally, 50 µl DNase\RNase free water was added directly to the matrix of the column then centrifuged to elute the viral RNA.

Reverse Transcription (RT)

Screening for the presence of dsRNA rotavirus A was determined using Maxime RT PreMix-iNtRON kit with a denaturation step described by Gouvea (9), before the cDNA synthesis, a volume of 5µl RNA, 5 µl from the primer's mix (2.5 µl Beg 9 primer (10 pmol) and 2.5 µl End 9 primer (10 pmol) and 10µl distilled water treated by DEPC. This mixture was denatured at 99o C for five min in a sterile 0.5 ml PCR tube, then it was quenched in ice for five min, then it was added into the Maxime RT PreMix tubes to a total volume of 20 µl then the cDNA synthesis reaction was performed using MultiGene OptiMax Thermal Cycler TC9610-230 (Mayfield Avenue Edison, USA) using the following conditions: 45°C for 60 min to obtain cDNA, followed by 95°C for 5 min to inactivate the RTase.

Screening for the presence of ssRNA viruses, such as norovirus, astrovirus, sapovirus, aichivirus, klassevirus, and salivirus was conducted firstly by RT reaction, as follows: The extracted RNA was revers transcribed with random primers using Maxime RT PreMix-iNtRON kit, 5µl RNA and 15µl distilled water treated by Diethyl Pyrocarbonate (DEPC), was added into the Maxime RT PreMix tubes to a total volume of 20 µl mixed well and spun down. The mixture was allowed to stand at room temperature for 1-2 min to allow the pellet to dissolve completely. Then, a cDNA synthesis reaction was performed using MultiGene OptiMax Thermal Cycler TC9610-230 (Mayfield Avenue Edison, USA) using the following conditions:45°C for 60 min to obtain cDNA, followed by 95°C for 5 min to inactivate the RTase. β-actin gene was used as an internal expression control.

Polymerase Chain Reaction (PCR)

The presence of the enteric viruses was determined using primers targeting specific genes shown in Table 1. Positive controls (PC) were designed using the GeneBank ® tool, were then synthesized by GeneScript® Biotech Company (New Jersey, USA).

The PCR reactions were performed using 2x PCR master mix solution (i-TaqTM) -iNtRON kit. A volume of 10 µl of 2x PCR master mix solution was added to a sterile PCR tube, 5 µl of the cDNA (or PC) template, 2 µl of 10 pmol primer mix (1 µl from the forward and 1 µl of the reverse) and 8 µl distilled water treated by DEPC (final volume 25 µl). A negative control (mixture without cDNA template) and PC were included in each run. PCR was carried out using MultiGene OptiMax Thermal Cycler TC9610-230 (Mayfield Avenue Edison, USA). Cycling conditions used were adjusted according to the optimization result for the primer as follows:

For rotavirus: PCR activation at 93°C for 3 min, 27 cycles of amplification (1 min at 94°C, 1 min at 45°C, 1 min at 72°C), and a final extension step at 72°C for 7 min. For astrovirus the cycling conditions were PCR at 94°C for 3 min, 35 cycles of amplification (20 s at 94°C, 20 s at 50°C, 1 min at 72°C), and a final extension step at 72°C for 5 min. For norovirus the cycling conditions were PCR at 95°C for 3 min, 40 cycles of amplification (30 s at 95°C, 30 s at 58.8°C, 1 min at 72°C), and a final extension step at 72°C for 5 min. For enteric adenovirus (A – F) primer the cycling conditions were pre-denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 54°C for 45 seconds, and primer extension at 72°C for 2 minutes and a final product extension at 72°C for 5 minutes. For Ad40 primer the cycling conditions were pre-denaturation at 95°C for 5 minutes, followed by 34 cycles of denaturation at 95°C for 30 seconds, annealing at 56.9 °C for 30 seconds, and primer extension at 72°C for 1 minute and a final product extension at 72°C for 5 minutes. For Ad41 primer the cycling conditions were pre-denaturation at 95°C for 3 minutes, followed by 34 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds, and primer extension at 72°C for 1 minute and a final product extension at 72°C for 5 minutes. For human bocavirus was pre-denaturation at 95°C for 2 minutes, followed by 30 cycles of amplification: denaturation temperature 95°C for 30 seconds, annealing temperature 54.6°C for 30 seconds, primer extension at 72°C for 30 seconds, and a final extension temperature of

Table 1: Oligonucleotide primers used for detection of enteric viruses by PCR and RT-PCR assays.

Virus	Primer	Target Region	Sequence (5′ to 3′)	Size (bp)	Ref.
Rotavirus A	Common primer	Beg9	GGCTTTAAAAGAGAG ATTTCCGTCTG G	1068	
		Forward			
		End9	GGTCACATCATACAATTCTAATCTAAG		
		Reverse			
Astro Virus	Mon269	ORF2	CAACTCAGGAAACAG GGT GT	449	[15]
	Mon270		TCAGATGCATTGTCATTGGT		
Norovirus	JV12	ORF1	ATACCACTATGATGCAGATTA	327	[16]
	JV13	(polymerase, RdRp)	TCATCATCACCATAGAAAGAG		
Enteric Adenovius	Ad1	Hexon, Forward	TTCCCCATGGCICAYAACAC	482	[17]
(A-F)	Ad2	Reverse	CCCTGGTAKCCRATRTTGTA		
Ad40	F	Forward	ACCCACGATGTAACCACAGACA	88	[18]
	R	Reverse	ACTTTGTAAGAGTAGGCGGTTTCC		
Ad41	F	Forward	TGGCCACCCCCTCGATGA	381	[19]
	R	Reverse	TTTAGGAGCCAGGGAGTTATA		
Human Bocavirus	Adel-OF	Forward	AGGTAAAACAAATATTGCAAAGGCCATAGTC	732	[20]
	AdelOR	Reverse	TGGGAGTTCTCTCCGTCCGTATC		
Sapovirus	SLV-5317	Capsid, Forward	CTCGCCACCTACRAWGCBTGGTT	434	[21]
	SLV-5749	Reverse	CGGRCYTCAAAVSTACCBCCCCA		
Aichivirus	C94b	3Cregion, Forward	GACTTCCCCGGAGTCGTCGTCT	158	[22]
	AIMP	Reverse	GCRGAGAATCCRCTCGTRCC		
Klassevirus	LG0098	2C, Forward	CGTCAGGGTGTTCGTGATTA	345	[23]
	LG0093	Reverse	AGAGAGAGCTGTGGAGTAATTAGTA		
Salivirus A	VP1	Forward	CCCCRTCAACTTCCAGCAAA	483bp	[24]
	RegionE	Reverse	ACACGAACGATRGAGGTGCT		

72°C for 5 minutes. For sapovirus the cycling conditions were PCR at 94°C for 3 min, 35 cycles of amplification (45 s at 94°C, 45 s at 54.7°C, 1 min at 72°C), and a final extension step at 72°C for 5 min. For aichivirus was pre-denaturation at 95°C for 3 minutes then 34 cycles for denaturation temperature 95°C for 30 seconds, annealing temperature 56.1°C for 30 seconds, extension temperature 72°C for 1 minute, and a final extension at 72°C for 5 minutes. For klassevirus the cycling conditions were PCR at 94°C for 5 min, 40 cycles of amplification (30 s at 94°C, 30 s at 56°C, 1 min at 72°C), and a final extension step at 72°C for 5 min. For salivirus A, the cycling conditions were PCR activation at 94°C for 2 min, 40 cycles of amplification (20 s at 94°C, 10 s at 58°C, 30 s at 72°C), and a final extension step at 72°C for 5 min.

Gel Electrophoresis

After amplification, 5 µl of PCR products for each sample was analyzed using agarose gel electrophoresis 1.5 % agarose in tris-borate ethylenediaminetetraacetic acid (EDTA) buffer (TBE), stained with ethidium bromide (Fisher Scientific, UK); (0.5 µg/ml) to detect the specific amplified product by comparing with 100 base-pairs DNA ladder (iNtron, Sizer ™ -100) DNA Marker Solution. Electrophoresis was carried out at room temperature at 120 volts for 1 hour, then the gels were photographed under a Gel Documentation system.

Statistical Analysis

Statistical analysis of data was done using the Statistical Package for Social Science (SPSS) software (IBM, USA). Comparison between groups was done using Chi-square (X2) test

RESULTS

Table 2 demonstrates the distribution of positive PCR results according to patient gender. A statistically significant association ($P< 0.05$) was observed between gender and astrovirus (8.8 % males vs. 17.6% females). There was no significant association between gender and other viruses.

Figure 1 demonstrates the number of positive results by patient age groups. The positive rate of viral infection was highest in children aged younger than 1-year-old (8.4%, 20 out of 239 specimens), followed by 2-4 years (4.2%, 10 out of 239). Most viral cases were detected in the winter (32.5%), followed by

Table 2: Distribution of positive results by Gender.

Gender	Rotavirus +ve	Astrovirus +ve	Norovirus +ve	Ad40 +ve	Ad41 +ve	Human Bocavirus +ve
Male	6 (17.6%)	3 (8.8%)	4(11.8%)	1(2.9%)	1(2.9%)	2(5.9%)
Female	5 (14.7%)	6(17.6%)#	1(2.9%)	2(5.9%)	2(5.9%)	1(2.9%)
Total	11	9	5	3	3	3

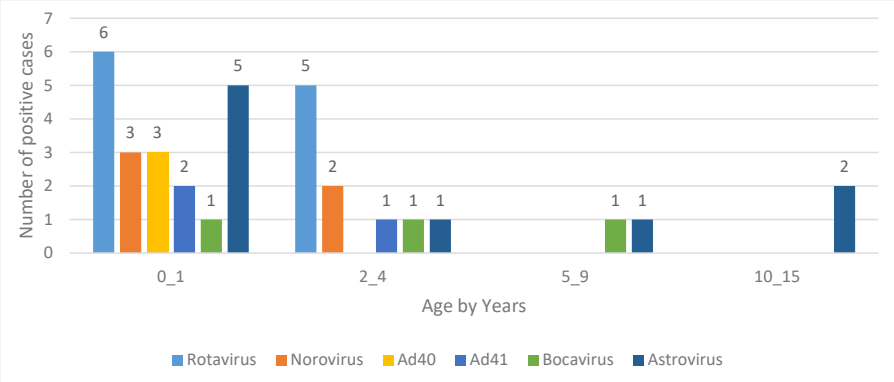

Figure 1 Distribution of positive results by patient age groups.

autumn (30%), without recording any cases in the spring (Table 3). Significant associations were seen between the presence of rotavirus and dehydration (P = 0.00001), and vomiting (P = 0.004), and between norovirus and weakness (P = 0.047) (Table 4). The average duration of symptoms was 2-3 days in 25% of the cases. Among the infected patients, 25% were under antibiotic treatment

DISCUSSION

Two decades ago, AGE was considered as the leading cause of mortality in children globally with a high rate of deaths, 10% in hospitalized children [19]. Diarrhea illness causes more than 1.3 million deaths and was reported as the fourth leading cause after recording 146000 deaths among children <5 years old in 2010 [19].

It is mandatory to understand the possible viral etiological agents associated with diarrhea among the Jordanian population. This study focused on the prevalence of enteric viruses infections in children with AGE in northern Jordan over a one-year period. In our findings, rotavirus is recognized as the major viral agent that causes AGE associated with infantile diarrhea followed by astrovirus, norovirus, adenovirus, and bocavirus respectively. Generally, the positive rate of each virus vary by the region, in Tunisia, viruses causing AGE were rotavirus (22.5%), norovirus (17%), astrovirus (4 %), aichivirus (3.5 %), enteric adenovirus (3%), and sapovirus (1%) [20]. Another Egyptian study between 2006 and 2007 recorded that rotavirus and norovirus were the main causative agents of viral gastroenteritis in Cairo, followed by adenoviruses and astrovirus [21]. In Saudi Arabian study, rotavirus has been found in the majority of the patients, followed by adenovirus, norovirus, and astrovirus [22].

The infection rate with rotavirus before the introduction of the RotaTeq vaccine into the National Vaccination Program in Jordan in 2015 ranges between 35-50 % [23-25]. In a 2020 study [14] that was carried out after the introduction of the RotaTeq vaccine, rotavirus infection recorded a 5.8%, showing a significant decrease in the prevalence of rotavirus infection among the studied individuals. In this study, a similar rate of rotavirus infections was reported (4.6%). The reason behinds these reductions are most probably due to the introduction of the rotavirus vaccine. One study in Jordan showed that the infection rate with norovirus was 11.4% compared to a low of 2.1% in

Table 3: The association between viral infection and seasons.

Season (Months)	Rotavirus +ve	Astrovirus +ve	Norovirus +ve	Ad40 +ve	Ad41 +ve	Human Bocavirus +ve
Autumn (1.Sep-30.Nov)	3(8.8%)	3(8.8%)	0(0.0)	2(5.9%)	2(5.9%)	2(5.9%)
Winter (1.Dec-28.Feb)	8(23.5%)	2(5.9%)	2(5.9%)	1(2.9%)	0(0.0)	0(0.0)
Spring (1.Mar-31.May)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Summer (1.Jun-31.Aug)	0(0.0)	4(10%)	3(8.8%)	0(0.0)	1(2.9%)	1(2.9%)

Table 4: The association between viral infections and clinical observations.

Observation	Rotavirus +ve	Astrovirus +ve	Norovirus +ve	Ad40 +ve	Ad41 +ve	Human Bocavirus +ve
Clinical diagnosis						
Dehydration	4(11.8%)#	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Weakness	3(8.8%)	4(11.8%)	4(11.8%)#	0(0.0)	2(5.9%)	2(5.9%)
Vomiting	7(20.6%)#	2(5.9%)	0(0.0)	1(2.9%)	1(2.9)	1(2.9%)
Fever	3(8.8%)	2(5.9%)	3(7.5%)	1(2.9%)	2(5.9%)	0(0.0)
Abdominal pain	0 (0.0)	4(11.8%)	1(2.5%)	1(2.9%)	0(0.0)	0(0.0)
Duration of symptoms (days)						
1	2(5.9%)	3(8.8%)	1(2.9%)	0(0.0)	1(2.9%)	1(2.9%)
2_3	7(20.6%)	5(14.7%)	3(8.8%)	3(8.8%)	2(5.9%)	1(2.9%)
> 4	2(8.8)	1(2.9%)	1(2.9%)	0(0.0)	0(0.0)	1(2.9%)
Antibiotics received						
Yes	2(5.9%)	1(2.9%)	3(8.8%)	0(0.0)	0(0.0)	3(8.8%)
No	9(26.5%)	8(23,5%)	2(5.9%)	3(8.8%)	3(8.8%)	0(0.0)

#: significant association

this study which may be explained by low sample collection due to coronavirus pandemic. The infection rate for astrovirus and human bocavirus was 3.8% and 1.3% respectively. Up to our knowledge, no studies were carried on astrovirus and human bocavirus causing AGE in Jordan. Adenovirus 40 and 41 infection rates each with 1.3% was comparable with a previous study in Jordan (1.1%) [26].

A statistically significant association (P< 0.05) was observed between gender and astrovirus (8.8 % males vs. 17.6% females), with no significant association between gender and other viruses. Similar findings were reported by Nasab et al., 2020 where the ratio of males: females was 1:2 [27]. The majority age group affected by enteric viruses was 0–1-year-old patients (58.8%) followed by 2-4 years old (29.4%). This age pattern is consistent with other studies from different countries confirming that the main affected age group was below five years old [10,28,29].

In this study, the most viral cases were detected in the winter (38.5%), followed by autumn (35.5%), without recording any cases in spring. Our findings were nearly in line with a study from north Africa [30]. These differences may be due to environmental factors humidity and temperature [31]. In this study, the detected enteric viruses, in general, were highly associated with weakness (44%), followed by vomiting (35.3%), and fever (32.4%). In this study, rotavirus records a significant association with vomiting (20.6%), and dehydration (11.8%). In a previous study in Bangladeshi, it was reported that rotaviruses were significantly associated with dehydration and vomiting (P<0.05) [32]. In another study in Saudi Arabia, data showed that the rotavirus infection was mostly accompanied by vomiting (72.3%) and dehydration (81.9%) [22]. Norovirus reported a significant association with weakness (11.8%). Previous studies showed that norovirus infections were mostly accompanied by abdominal pain at 21.2% [33], and fever at 30% [34]. Differences may reflect variations in the treatment approaches between patients among age groups, symptoms identified in older patients typically included a larger and more diverse range of physical symptoms [35].

In the present study, the average duration of symptoms was 2-3 days which was in line with previous studies [24]. In our study, 25% of the enteric viruses patient were under antibiotic treatment, which necessitates introducing a sensitive viral testing kit to confirm the detection of viruses in the fecal samples of AGE patients to reduce the unnecessary antibiotics treatment

ACKNOWLEDGEMENTS

This work was funded by Jordan University of Science and Technology (grant No. 332/2019)

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Ethical clearance of the study & permission number-IRB 13/124/2019 dated- 21May 2019.

REFERENCES

1. Bányai K, Estes MK, Martella V, Parashar UD. Viral Gastroenteritis. 2018; 392: 175-186.
2. Desselberger U. Viral Gastroenteritis. Medicine (United Kingdom). Elsevier; 2017; 45: 690-694.
3. Chaimongkol N, Khamrin P, Suantai B, Saikhreang W, Thongprachum A, Malasao R, et al. A Wide Variety of Diarrhea Viruses Circulating in Pediatric Patients in Thailand. Clin Lab. 2012; 58: 117-123.
4. De Zoysa' I, Feachem2 RG. Interventions for the Control of Diarrhoeal Diseases among Young Children: Rotavirus and Cholera Immunization. Bull World Health Organ. 1985; 63.
5. Donelli YG, Ruggeri FM, Tinari A, Marziano ML, Menichella D, Caione D, et al. A Three-Year Diagnostic and Epidemiological Study on Viral Infantile Diarrhoea in Rome. Epidem Infect. 1988; 100: 311-320.
6. Lopman B, Vennema H, Kohli E, Pothier P, Sanchez A, Negredo A, et al. Increase in Viral Gastroenteritis Outbreaks in Europe and Epidemic Spread of New Norovirus Variant. Lancet. 2004; 363: 682-688.
7. Gallimore CI, Pipkin C, Shrimpton H, Green AD, Pickford Y, Mccartney C, et al. Detection of Multiple Enteric Virus Strains Within a

Foodborne Outbreak of Gastroenteritis: An Indication of the Source of Contamination. Epidemiol Infect. 2005; 133: 41-47.

8. Alrwashdeh M, Almaayteh M, Kaabneh A, Haddadin W, Ababneh M. Prevalence of Rotavirus Infection in Pediatric Patients after Introduction of the Rotateq Vaccine in Jordan. 2020; 4860: 4853-4860.

9. Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, et al. Polymerase Chain Reaction Amplification and Typing of Rotavirus Nucleic acid from Stool Specimens. J Clin Microbiol. 1990; 28: 276-282.

10. Lee JI, Lee GC, Oh YH, Lee YK, Kim MY, Lee CH. Molecular Characterization of Partial-Open Reading Frames 1a and 2 of the Human Astroviruses in South Korea. Virol J. 2010; 7: 3-7.

11. Kim JS, Kim HS, Hyun J, Kim HS, Song W. Molecular Epidemiology of Human Norovirus in Korea in 2013. Biomed Res Int. 2015; 2015.

12. Xu W, Mcdonough MC, Erdman DD. Species-Specific Identification of Human Adenoviruses by a Multiplex PCR Assay. J Clin Microbiol. 2000; 38: 4114-4120.

13. Jiang S, Dezfulian H, Chu W. Real-Time Quantitative PCR for Enteric Adenovirus Serotype 40 in Environmental Waters. Can J Microbiol. 2005; 51: 393-398.

14. Samarbaf-Zadeh A, Pirmoradi R, Shamsizadeh A, Makvandi M. Prevalence of Adenoviruses 40 And 41 in Children Less than Five Years Suffering from Acute Gastroenteritis Hospitalized in Ahvaz Abuzar Hospital. Jundishapur J Microbiol. 2010; 3: 48-52.

15. Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. A Novel Bocavirus Associated with Acute Gastroenteritis in Australian Children. Plos Pathog. 2009; 5.

16. Khamrin P, Okame M, Thongprachum A, Nantachit N, Nishimura S, Okitsu S, et al. A Single-Tube Multiplex PCR for Rapid Detection in Feces of 10 Viruses Causing Diarrhea. J Virol Methods. 2011; 173: 390-393.

17. Alcalá AC, Pérez K, Blanco R, González R, Ludert JE, Liprandi F, et al. Molecular Detection of Human Enteric Viruses Circulating Among Children with Acute Gastroenteritis in Valencia, Venezuela, Before Rotavirus Vaccine Implementation. Gut Pathog. 2018; 10.

18. Yip CCY, Lo KL, Que TL, Lee RA, Chan KH, Yuen KY, et al. Epidemiology of Human Parechovirus, Aichi Virus and Salivirus in Fecal Samples from Hospitalized Children with Gastroenteritis in Hong Kong. Virol J. 2014; 11: 1-10.

19. Wang H, Naghavi M, Allen C, Barber RM, Carter A, Casey DC, et al. Global, Regional, and National Life Expectancy, All-Cause Mortality, and Cause-Specific Mortality for 249 Causes of Death, 1980–2015: A Systematic Analysis for the Global Burden of Disease Study 2015. Lancet. 2016; 388: 1459-1544.

20. K S-L, H G-K, A De R, S C, N S, K A-B, et al. Acute Infantile Gastroenteritis Associated with Human Enteric Viruses in Tunisia. J Clin Microbiol. 2008; 46: 1349-1355.

21. Kamel AH, Ali MA, El-Nady HG, De Rougemont A, Pothier P, Belliot G. Predominance and Circulation of Enteric Viruses in the Region of Greater Cairo, Egypt. J Clin Microbiol. 2009; 47: 1037-1045.

22. Meqdam MM, Thwiny IR. Prevalence of Group A Rotavirus, Enteric Adenovirus, Norovirus and Astrovirus Infections among Children with Acute Gastroenteritis in Al-Qassim, Saudi Arabia. Pakistan J Med Sci. 2007; 23: 551-555.

23. Khitam Salem K, Bdour S, Zeller M, Van Ranst M, Matthijnssens J. Genotypes of rotavirus strains circulating in Amman, Jordan in 2006/07 and their significance for the potential effectiveness of future rotavirus vaccination. Arch Virol. 2011; 156: 1543-1550.

24. Nafi O. Rotavirus gastroenteritis among children aged under 5 years in Al Karak, Jordan. EMHJ. 2010; 16: 1064-1069.

25. Kaplan NM, Kirby A, Abd-Eldayem SA, Dove K, et al. Detection and molecular characterization of rotavirus and norovirus infections in Jordanian children with acute gastroenteritis. Arch Virol. 2011; 156: 1477-1480.

26. Meqdam MMM, Youssef MT, Nimri LF, Shurman AA, Rawashdeh MO, Al-Khdour MS. Viral Gastroenteritis among Young Children in Northern Jordan. J Trop Pediatr. 1997; 43: 349-352.

27. Nasab SDM, Zali F, Kaghazian H, Aghasadeghi MR, Mardani R, Gachkar L, et al. Prevalence of Astrovirus, Adenovirus, and Sapovirus Infections among Iranian Children with Acute Gastroenteritis. Gastroenterol Hepatol from Bed to Bench. 2020; 13: S122-127.

28. Nafi O. Rotavirus Gastroenteritis among Children Aged Under 5 Years in Al Karak, Jordan. East Mediterr Heal J. 2010; 16: 1064-1069.

29. Kreidieh K, Charide R, Dbaibo G, Melhem NM. The Epidemiology of Norovirus in the Middle East and North Africa (MENA) Region: A Systematic Review. Virol J. 2017; 14: 1-10.

30. D'Souza RM, Hall G, Becker NG. Climatic Factors associated with Hospitalizations for Rotavirus Diarrhoea in Children Under 5 Years of Age. Epidemiol Infect. 2008; 136: 56-64.

31. Bern C, Unicomb L, Gentsch JR, Banul N, Yunus M, Sack RB, et al. Rotavirus Diarrhea in Bangladeshi Children: Correlation of Disease Severity with Serotypes. J Clin Microbiol. 1992; 30: 3234-3238.

32. Roodsari SR, Bitajian F, Gachkar L, Jadali F, Adabian S, Sajadi Nia RS, et al. Detection of Noroviruses Isolated from Children with Acute Gastroenteritis by RT-PCR in Iran. Arch Pediatr Infect Dis. 2013; 1: 57-60.

33. Gruber JF, Bowman NM, Becker-Dreps S, Reyes Y, Belson C, Michaels KC, et al. Risk Factors for Norovirus Gastroenteritis among Nicaraguan Children. Am J Trop Med Hyg. 2017; 97: 937-943.

34. Yates P, Miaskowski C, Cataldo JK, Paul SM, Cooper BA, Alexander K, et al. Differences in Composition of Symptom Clusters between Older and Younger Oncology Patients. J Pain Symptom Manage. 2015; 49: 1025-1034.