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Genotyping of Rota Virus Causing Gastroenteritis in Egyptian Children.

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ABSTRACT

Rotavirus (RV) is the major universal early viral infection in childhood gastroenteritis. To identify Rota virus strains causing acute gastroenteritis and its correlation to the disease severity in a sample of Egyptian children in great Cairo. Present study was conducted on 93children presented with acute gastroenteritis during the warm season (March-July).Stool samples were collected from all cases to be tested by PCR for virus detection and typing. Using RT-PCR & PCR test for detection of causative viruses, 53 stool samples were Rota positive. Vomiting, diarrhea and dehydration were found to be significantly higher among Rota virus positive cases ($p<0.005$, <0.005 and <0.05). The most frequent strains were G3P [8] 13.9%, G2P [4] 11.8%, G4P [8] 7.5%. G1P [8]6.45%, G12P [6], G9P [6], G9P [8], G3P [6], G4P [4] and G3P [6] were also detected. The most frequent strains were G3P [8], G2P [4], G4P [8]. Vomiting, diarrhea and dehydration were found to be higher among Rota virus positive cases.

Keywords: Rota virus, Gastroenteritis, children, PCR

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INTRODUCTION

Although the disease burdens of viral Gastroenteritis, little information are known about the causative viruses not only in Egypt but in most of the developing countries [1]. Rota virus is the major cause and the most commonly isolated virus from children with viral gastroenteritis. By the age of 3 years, 90% of children have serum antibodies against one or more types of rotavirus, indicating high levels of exposure [2].

The World Health Organization(WHO)reported rotavirus as the most common cause of severe diarrheal disease hospitalizations in young children in Africa since 2008.Epidemiological study reported 42% annual proportion of Rota virus gastroenteritis among episodes of pediatric gastroenteritis in the Middle East and North Africa region [3- 4].This fact explains the WHO recommendation of using and studying the effect of rotavirus vaccines in developing countries as no data from the current vaccines are available in Africa [5].

Rotavirus is well known to have really wide strain diversity. The inner capsid contains the viral genome of 11segments of double stranded RNA that encode six structural and six nonstructural proteins. Structural proteins which form the viral particle (termed VP viral proteins) have consecutive numbers, VP1, VP2 and so on. Non-structural proteins (NSP) necessary for host cell invasion and replication have also consecutive numbers [6- 7]. The seven major RV groups from A to G are determined by VP6 which is the middle – layer major capsid protein. Two structural proteins constitute the outer capsid of RVs are the VP4 protease sensitive protein and the VP7 glycoprotein. Actually those two proteins are the basics for RV genotype classifications in which the VP4 protein determines the23 P genotype (P represents protease sensitive protein) which have been identified while the VP7 determines the G-types (G represents glycoprotein). These 2 proteins are the principal targets for the neutralizing antibodies and efficacy of RV-vaccines relies on them [8- 9].

The goal of this work is to identify Rota virus strains causing acute gastroenteritis and its correlation to the disease severity in a sample of Egyptian children in great Cairo.

METHODS

This study was conducted on 93children presented with acute gastroenteritis. Seventy-four of them were hospitalized and received intravenous fluids in different hospitals in great Cairo, (Cairo, Giza and Kalyoubia Governorates), Egypt. The other 19 children were presented as outpatient cases at pediatric clinics of the same hospitals. Boys and girls less than 5 years from different social classes having gastroenteritis were recruited in the study. Gastroenteritis was defined if the child experienced at least three watery or loser-than-normal stools and/or forceful vomiting within any 24h period within the previous 3 days [3]. The clinical severity of each child was assessed on the basis of a direct examination and categorized into mild, moderate, and severe using a clinical scoring system [10].Children receiving Rota virus vaccine or any blood products transfusion and cases of nosocomial diarrhea or chronic diarrhea or chronic allergic diseases and children on corticosteroids and immune deficient children, chronic renal disease or cancer were excluded from the study. Samples were collected during the warm season (March to July, 2012).

All cases were subjected to full history taking and thorough clinical examination. Clinical data involving disease manifestations as fever, vomiting, abdominal pain, or bloody diarrhea were collected for all patients. Severity criteria, such as duration of the diarrhea, number of stool motions or bouts of vomiting, range of body temperature, degree of dehydration, were determined for all hospitalized children.

Virus Detection*:* Stool samples were collected in dry sterile clean plastic cups. Collected samples were diluted and prepared for Rota virus detection by ELISA, PCR, and RT-PCR in the collected samples.

ELISA Test: Rotaviruses was detected in the collected stool samples using RIDASCREEN Kit No. C0901 (r-biopharm, Germany). The tests were done according to the procedures described by manufacturer, samples were considered positive if their extinction in more than 10% above the calculated cut-off and considered negative if its below to the cut-off by 10%.

Reverse transcriptase-polymerase chain reaction (RT-PCR): All collected stool samples were subjected to extraction of both viral RNA and DNA in the samples to facilitate the detection of the RNA Rota virus. Viral genomes were extracted from 10% diluted stool samples and prepared for detection of viral gastroenteritis by both RT-PCR and PCR using sets of oligonucleotide primers specific to strain of Rota virus .cDNA synthesis and PCR amplification: Viral RNA was extracted from 200µl aliquot of 10% stool sample, using Axygen® Kit (Axygen biosciences, Cat. No. AP-MN-BF-VNA-250), according to the instructions of the supplier. Reverse transcription (RT)was carried out using a mixture of 10µl of extracted viral RNA, 5µl random primer (25 pmol/µl), and 20µl DEPC treatedwater. The reaction mixture, 35µl, was heated at 65ºC for 5 min. and chilled on ice for 2 min. 35µl of the reaction mixture was added to 1µl of 200 U/µl M-MLV Reverse Transcriptase (Promega, Cat. # M1701), 10µl RT-buffer, 4µl of 10mM each dNTPs (Promega, Cat. # U1511). The mixture was heated at 42ºC for 60 min and 37ºC for 30 min, for cDNA synthesis, followed by 95ºC for 5 min. Nested RT-PCR the reaction takes place in two rounds; of The first-round PCR was carried out in 50µl reaction mixture containing 10µl of the synthesized cDNA, 5µl 10x PCR Buffer, 4µl of 25mM MgCl2 (Promega, Cat. # A3511), 0.5µl of 5U/µl Go Taq DNA Polymerase (Promega, Cat. # M8305), 1µl (50 pmol/µl) of sense primer, 1µl (50 pmol/µl) of antisense primer, 4µl of 10mM each dNTPs (Promega, Cat. # U1511) and 24.5µl DEPC treated water. For PCR amplification of G type rotaviruses (Table 1), an initial denaturation of 5min at 95ºC was applied and 35 cycles for first round and 30 cycles for 2nd round were used. The PCR profile for first round was 94ºC for 1 min, 52ºC for 1 min, and 72ºC for 1 min, and an additional extension at 72ºC for 7min was applied using a PCR machine (BioMetra, UK). The amplified RT-PCR products were electrophoresed through 1.5% agarose gels in Tris acetate/EDTA buffer against 100bp DNA ladder (Promega, Cat. # G2101) and visualized by staining with ethidium bromide. Bands were visualized under UV light using UV transilluminator. The obtained band size is determined with the reference of the DNA ladder.For the second round; PCR amplification was carried out from the first round PCR reaction in 50µl reaction mixture containing 2µl of 1st round PCR product, 5µl 10x PCR Buffer, 4µl of 25mM MgCl2 (Promega, Cat. # A3511), 0.5µl of 5U/µl Go Taq Flexi DNA Polymerase (Promega Cat. No. M8301), 1µl downstream primer (50 pmol/µl), 1µl of 8 cocktail upstream primers (50 pmol/µl each), 4µl of 10 mM each dNTPs (Promega, Cat. # U1511) and 25.5µl DEPC treated water. The PCR profile for the second round was an initial denaturation of 5min at 95ºC followed by 30 cycle at 94ºC for 1 min, 42ºC for 2 min, and 72ºC for 1 min, and an additional extension at 72ºC for 7min was applied using a PCR machine (BioMetra, UK). The amplified RT-PCR products were electrophoresed through 1.5% agarose gels in Tris acetate/EDTA buffer against 100bp DNA ladder (Promega, Cat. # G2101) and visualized by staining with ethidium bromide. Bands were visualized under UV light using UV transilluminator. The obtained band size is determined with the reference of the DNA ladder.

Table 1: PCR reaction profile for G type Rotaviruses.

Step	Reaction			
	1st round		2nd round	
	Temp (°C)	Time (min)	Temp (°C)	Time (min)
Denaturation	94	1 min.	94	1 min.
Annealing	52	1 min.	42	2 min.
Extension	72	1 min.	72	1 min.
No. of cycles	35		30	
Expected Product	881bp		175-754bp	

Table 2: PCR reaction profile for P type Rotaviruses.

Step	Reaction			
	1st round		2nd round	
	Temp (°C)	Time (min)	Temp (°C)	Time (min)
Denaturation	94	1 min.	94	1 min.
Annealing	50	2 min.	45	2 min.
Extension	72	1 min.	72	1 min.
No. of cycles	35		30	
Expected Product	876bp		276-583bp	

For PCR amplification of P type rotaviruses (Table 2), an initial denaturation of 5min at 95ºC and 35 cycles for first round and 30 cycles for 2nd round. For 1st round PCR cycles profiles was at 94ºC for 1min, 50ºC

for 2min, and 72ºC for 1 min, and an additional extension at 72ºC for 7min. Profile was applied using a PCR machine (BioMetra, UK). The amplified RT-PCR products were electrophoresed through 1.5% agarosegels in Tris acetate/EDTA buffer against 100bp DNA ladder (Promega, Cat. # G2101) and visualized by staining with ethidium bromide. Bands were visualized under UV light using UV transilluminator. The obtained band size is determined with the reference of the DNA ladder.

For the second round; PCR amplification was carried out from the first round PCR reaction in 50µl reaction mixture containing 2µl of 1st round PCR product, 5µl 10x PCR Buffer, 4µl of 25mM $MgCl_2$ (Promega, Cat. # A3511), 0.5µl of 5U/µl Go Taq Flexi DNA Polymerase (Promega Cat. No. M8301), 1µl of a 7 cocktail downstream primers (50 pmol/µl each), 1µl upstream primer (50 pmol/µl), 4µl of 10 mM each dNTPs (Promega, Cat. # U1511) and 26.5µl DEPC treated water. The PCR profile for the second round was an initial denaturation of 5min at 95ºC followed by 30 cycle at 94ºC for 1 min, 45ºC for 2 min, and 72ºC for 1 min, and an additional extension at 72ºC for 7min was applied using a PCR machine (BioMetra, UK). The amplified RT-PCR products were electrophoresed through 1.5% agarosegels in Tris acetate/EDTA buffer against 100bp DNA ladder (Promega, Cat. # G2101) and visualized by staining with ethidium bromide. Bands were visualized under UV light using UV transilluminator. The obtained band size is determined with the reference of the DNA ladder.

Oligonucleotide primers:

Universal viral gastroenteritis oligonucleotide primers sequences were obtained from Bioneer Company, as purified lyophilized primers. Universal primers for G typing rotaviruses was selected for amplification of segment 9 as previously described by Iturriza-Gomara, *et al.*,2004 [11]. These primer pairs are semi nested primers designed to amplify the highly conserved region among all known rotaviruses strains and specific for gene segment 9, as listed and shown in table (3). Universal primers for P typing rotaviruses was selected for amplification of VP7 gene segment as described by Gentsch *et al.*, 1992 [12] and also listed and shown in table (4).

Table 3: The list of primers sets used to detect G genotype of rotaviruses.

Primer	Sequence	Polarity	Location
1st round			
Rota VP7-F10	ATGTATGGTATTGAATATTACCAC	+	51
Rota VP7-R10	AACTTGCCACCATTTTTTCC	-	932
2nd round			
Rota G1-aBT1-10	CAAGTACTCAAATCAATGATGG	+	314
Rota G2-acT2-10	CAATGATATTAACACATTTTCTGTG	+	411
Rota G- 10	ACGAACTCAACACGAGAGG	+	250
Rota G4-2aDT4-10	CGTTTCTGGTGAGGAGTTG	+	480
Rota G8-aAT8-10	GTCACACCATTTGTAAATTCG	+	178
Rota G9-10	CTTGATGGACTAYAAATAC	+	757
Rota G10-10	ATGTCAGACTACARATACTG	+	666
Rota G9-VP7aFT9d-10	CTTGATGTRACTAYAAMTAC	+	757
Rota VP7-R10	AACTTGCCACCATTTTTTCC	-	932

Table 4: The list of primers sets used to detect P genotype of rotaviruses.

Primer	Sequence	Polarity	Location
1st round:			
Con3-10	TGGCTTCGCCATTTTATAGA	+	11
Con2-10	ATTTCGGACCATTTATAACC	-	885
2nd round:			
Con3-10	TGGCTTCGCCATTTTATAGA	+	11
Rota P(8)-1T1-10	TCTACTTGGATAACGTGC	-	356
Rota P(4)-2T1-10	CTATTGTTAGAGGTTAGAGTC	-	494
Rota P(6)-3T1-10	TGTTGATTAGTTGGATTCAA	-	278

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Rota P(9)-4T1-10	TGAGACATGCAATTGGAC	-	402
Rota P(10)-5T1-10	ATCATAGTTAGTAGTCGG	-	594
Rota P(11) -10	GTAAACATCCAGAATGTG	-	323
Rota P(8)-1T-1D-10	TCTACTGGRTTRACNTGC	-	356

RESULTS

We examined 93 child aged less than 5 years and diagnosed as cases of acute gastroenteritis during the period from March 2012 to July 2012. They were 52 males and 41 females. Their age ranged from 1.5 m to 60 months. They were mainly from Great Cairo (Cairo, Giza and Kalyoubia governorates).

The clinical data of the studied cases is summarized in table (5). Vomiting was found to be the most frequently reported symptom (in 83cases, 89.2%) in the studied cases, followed by fever (in 81 cases, 87.1%) then diarrhea (in 78 cases, 83.9%).

Comparing Rota virus positive and negative cases, vomiting and diarrhea were found to be significantly higher among Rota virus positive cases ($p<0.005$ in both). Regarding the degree of dehydration, severe dehydration was significantly higher among Rota virus positive group when compared with Rota virus negative group ($p<0.05$) as shown in table (5).

Using RT-PCR & PCR test for detection of viral gastroenteritis, the number of positive stool samples for rotaviruses were 53/93 samples with a prevalence percentage of 56.99%.Four cases of adenovirus were detected by PCR showing a distribution of 4.3% .Only 2 cases of astroviruses by RT-PCR were detected with a 2.15% . Coinfection was found in five cases (5.4%), three cases out of them were of RV–adenovirus coexistence (with a percentage of 3.2%), and two cases (2.2%) were RV– astrovirus co infections.

Table 5: Clinical data of GE cases according to Rotavirus infection

Variable	**Rota positive** mean ± SD		**Rota negative** mean ± SD		**t**	**P#**
Age in years	3.2± 1.6		3.6± 1.9		0.643	0.762
	No	**%**	**No**	**%**	**X^2**	**P***
No. of GE cases	53	56.9	40	43.1	0.108	0.742
Sex: males females	29 24	54.7 45.3	23 17	57.5 42.5	0.177	0.674
Fever	47	88.7	34	85	0.226	0.634
Diarrhea	50	94.3	28	70	7.544	0.005*
Vomiting	52	98.1	31	77.5	7.178	0.006*
Degree of dehydration: Moderate Severe	 25 28	 47.1 52.8	 27 13	 67.5 32.5	2.780	0.035*

by student t test (t)
*by Chi-Square test (X^2)

Table 6: Relative frequencies of individual HRV A Genotypes in stool samples collected from Egyptian children

HRV A Genotypes n=53 %=56.99		
Rotavirus Genotype	No.	%
G3P8	13	13.9
G2P4	11	11.8
G4P8	7	7.5
G1P8	6	6.45
G12P6	4	4.3
G9P6	2	2.15
G9P8	2	2.15

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G3P6	2	2.15
G4P4	1	1.07
NT	5	5.37

As regards genotyping for Human Rotavirus A (HRA); three P genotypes were detected P [8], P [4], P [6] and six G-genotypes were detected where G3, G4 and G9 genotypes were found more than G1, G2, and12.There were ten RV strains detected as combination of G and P genotypes. The frequency of RV strains were G3P [8] and G2P [4], G4P [8], G1P [8], G12P [6], G9P [6], G9P [8], G3P [6], G4P [4] and G3P [6] as shown in table (6).

Table 7: Correlations between Rota virus infection and criteria of clinical severity of gastroenteritis in studied cases

		Sex	hospitalization	iv fluids	Dehydration degree	duration of diarrhea	fever	vomiting frequency	vomiting duration
Rota GE	Pearson Correlation	0.035	0.490**	0.163	0.593**	0.308**	0.458**	0.427**	0.283**
	Sig. (2-tailed)	0.740	0.000	0.120	0.000	0.003	0.000	0.000	0.006

** Correlation is significant at the 0.001 level (2-tailed).

Table (7) shows significant positive correlations between RV infection and fever, duration of diarrhea, vomiting frequency as well as its duration. RV infection was also positively correlated to the degree of dehydration and the hospitalization days(r=0.59&0.49 respectively p<0.00 in both).

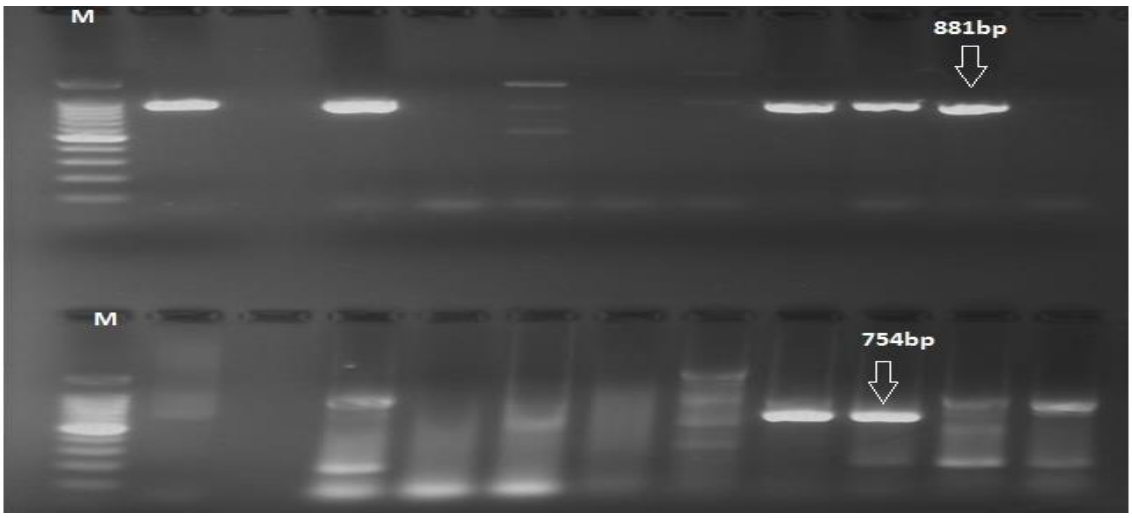

Figure 1: Detection of rotaviruses G genotype by RT-PCR

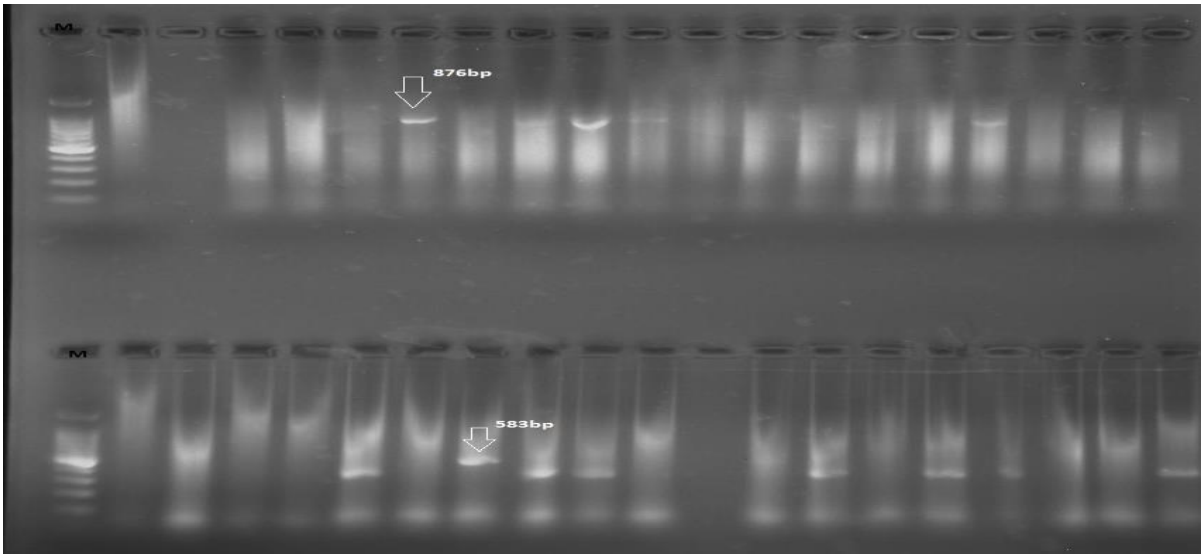

Figure 2: Detection of rotaviruses P genotype by RT-PCR

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DISCUSSION

Viral intestinal infections are the most common cause of acute infectious diarrhea in the pediatric group and accounted for approximately 70% of episodes of acute infectious diarrhea in children [13- 14]. Rotavirus was identified to be responsible for up to 20% of deaths from diarrhea [15]. However, when Middle Eastern and North African countries were compared to each other, episodes of Rotavirus gastroenteritis (RVGE) reported in Saudi Arabia, Tunisia, and Egypt was 16%-23%, a high of61% in Syria was reported while Oman and Kuwait RVGE reached 44%. Based on demographic indicators from UNICEF 2009, the average annual mortality rate in this region was estimated at 39 per 100,000. This rate is considerably higher than in Europe, where rotavirus rarely results in child death (mortality rate below 10 per 100,000) [4].

Rota virus infection affects small bowel entrocytes and causes not only low grade fever and watery diarrhea but vomiting and dehydration as well [13]. The most virulent and commonly isolated strains belong to serotype A as it is an important cause of viral gastroenteritis in children [16-17]. In Asia 37.5% of hospitalized gastroenteritis cases accounted for Rotavirus, it was also associated with approximately145, 000 deaths every year with greatest numbers occurring in India, Pakistan, and Indonesia [18].

In Africa, studies showed higher Rotavirus prevalence among hospitalized children than elsewhere with an estimated range of 29% to52 % of episodes of acute gastroenteritis among children less than 5 years of age. Another finding was the relatively high prevalence of rotavirus infection in young children presenting with diarrheal illness at outpatient clinics in African countries (range 10%-20%) [19-21].

In the present study, 56.99% of the children presented with gastroenteritis where RV positive. Children presented with diarrhea were significantly higher in Rota positive cases with 94.3%.Interestingly vomiting was found to be the most frequently reported symptom in this study with 89.2% of the studied cases, 98.1% of them were Rota Positive cases. This is in concomitant with what was found in a study done in selected urban African hospitals where vomiting was present in over 78.4% of all rotavirus positive cases [1].

Severe dehydration has been often associated with RV infection and it's the actual cause of death from this infection. Meanwhile, dehydrated children have been found to be two times more likely to have rotavirus diarrhea [22- 23].

In Asia, one study recorded 11.36% severe dehydration in association with Rota virus infection and 88.6% mild form of dehydration. In the Middle East Dehydration was a common health outcome reported by all studies, affecting around 50% of children with RVGE. In 3% to 25% of cases, dehydration was classified as severe [24- 25].

In Africa dehydration and requirement for intravenous fluid were common and contributed to high morbidity as mentioned by many studies [26- 27]

In our study 52.8% of Rota positive cases suffered severe dehydration and 47.1 %suffered moderate degree of dehydration. Regarding fever, 88.7% of Rota virus positive cases had fever. This is in consonance with many studies that report 45% to 84% fever in patients suffering from rotavirus diarrhea [1-28- 29].

Viral typing is necessary for characterizing rotavirus strains, especially focusing on different rotavirus seasons in different locations [22]. Rotavirus diarrhea caused by G1 was scored to be more severe than diarrhea caused by G3.The very wide diversity of RV is mainly due to the great variability in the G and P types where we have at least 27 G- and 35 P- genotypes in humans and animals [30]as well as the new strains generated due to the reassortment which in turn is due to coinfection of a human and an animal RV strain [33].Combined G and P typing such as G2P4, G3P8, G4P8, G9P8, and G1P8 vary from region to region (e.g. G5 types in Brazil, G10 types in India) [32-33].

In the current study, three P genotypes for HRV were detected P [8], P [4], and P [6] while six G-genotypes were detected, G3, G4 and G9 genotypes more than G1, G2, and G12.

Worldwide, G1P8, G2P4, G3P8, G4P8 and G9P8 were reported to be the most commonly known genotypes from 1994 to 2003 and were responsible for nearly 90% of childhood RV diseases [3]. An interesting

research done in china from January 2005 till December 2006,researchers found that among 8 RV infected hospitalized infants, 7 were of the G3P8 RV strain and only one was G9P836. Also Bangladesh to be considered because it is a developing country like Egypt and it is an Asian country, a study was conducted from 2006-2012 and there was no mention of the G3P8 strain whatsoever where G1P8 was 22.4% and G_9P_8 was 20.8%.

In 2006-2008, G4P [8] became the most prevalent genotype combination in Iran with a decline in G1P [8] proportion [35], meanwhile in Turkey, the G1P [8] was the predominant genotype combination while G4P [8] declined [36]. On the other hand, a Turkish study done in 2013 on 1297 child,G9P(8) was the most frequent genotype with a rate of 34.4%, followed by G2P (4) (23.0%) and G1P (8) (19.7%) [37].

In Saudi Arabia, there was a decrease in both G1P[8] and G9P[8] (16% to11%), with an increase in G2P[4] during the period from2002-2003 to 2004-2005 [38- 39]. In the same time frame but in Tunisia, G1P[8] and G1P[6] were replaced by a variety of genotype combinations including G1P[4], G9P[8], and G3P[8],with the emergence of G9 serotypes during 2003-2005 [40-42]. Shifting to Morocco by the period between 2006-2009, a study reported G1P8 to represent 55% of RV strains, while the G3P8 strain was only 0.4 % [43- 44]

Africa has been reported to unusual serotypes at an incidence of 27%, practicallyG_3P_8 is not really an unusual serotype but comparing its incidence to G_1P_8 worldwide shows that G_1P_8 is responsible for only <30% of infections in Africa, Asia and South America [45] .One study found G1P8 serotype the most prevalent among infants aged 3.1–9 months and G3P6 frequently identified among neonates less than 3 months [46].

In order to provide a wide scope of vision concerning the different RV strains found in Egypt throughout the past years; Long ago in 1995-1996, one study showed that the G_2P_4 strain represented 67% of RV-GE cases in a rural region in Egypt [47]. Later on in 2003, an Egyptian research work reported G1P8 and G9P8 to be the most prevalent strains [48]. However, by that time, G_1P_8 was the most common strain worldwide.

By the 2006-2007 the G_2P_4 strain represented 40.8% while the G_1P_8 strain was only 17% and the G12 was the third most common one in a study done at Giza governorate and Cairo. Definitely, those results were unusual compared to other parts of the world where G_1P_8 was the most predominant. During the same period 2006-2007,but in Zenin and El-Berka regions in Greater Cairo, G_1P_8 and G_9P_8 strains were the prevalent strains while G12 was a rare one [49].

Back to the current study,G3P [8] and G2P [4], G4P [8], G1P [8], G12P [6], G9P [6], G9P [8], G3P [6], G4P [4] and G3P [6] were the most prevalent combination. In our study, 13.9% of children who suffered Rotavirus gastroenteritis were belonging to the G_3P_8 strain followed by G2P4, G4P8 with 11.8% and7.5% respectively. These results goes with many researchers who reported that G_3P_8 was one of the 5 strains which are associated with 80% - 90% of the childhood RV burden disease all over the world [50-52]. In Palestine in spite of its presence in Asia it is geographically very close to Egypt. Accordingly it is important to report that an Israelian research work emphasized the presence of the relatively uncommon G_3P_8 serotype at a high percentage together with other serotypes like (G_1P_4, G_2P_8, G_9P_4 and $G_{12}P_8$) in 2009. They all represented 26.9% which is a considerable percentage and were considered in vivo reassortants [25]. However, in 1991-1994 another Israelian study reported that G_2P_4 and G_3P_8 serotypes were present merely in a minor group of patients. Nevertheless, G_1P_8 was 60.2% and G_4P_4 was 32.3% [53]. It is very much worth noting that Asia has also been reported to be of a specific nature and challenges where it embraces the unusual serotypes of RV at a 14% incidence rate.

Here we would like to emphasize to what we mentioned before about particular nature and the diversity of RV strains reported in Africa (and to some extent Asia) when compared to other parts of the world which provides the toughest challenges to vaccination.

In a conclusion, the most frequent Rota strains encountered during the warm season in great Cairo were G3P[8], G2P[4], G4P[8]. Vomiting, diarrhea and dehydration were found to be higher among Rota virus positive cases.

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