Prevalence and molecular characterization of rotavirus strains circulating among children with gastroenteritis in Egypt

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Background and objectives
Human rotavirus (RV) is the main cause of diarrheal disease among children younger than 5 years old, worldwide. The aim of the current study was to investigate the prevalence of RV infections and the genotype distribution of RV in Egypt.
Materials and methods
A total of 642 fecal samples were collected from children younger than 5 years of age, suffering from acute diarrhea and attending ten regional public hospitals (n=585) and five private clinics (n=57) located in 6 Egyptian cities between February and June 2021. All samples were screened by immunochromatographic assay to determine RV prevalence. Then RV-positive samples were further subjected for detection of G (VP7) and P (VP4) genotypes by seminested multiplex real-time reverse transcriptase-polymerase chain reaction (RT-PCR).
Results
Out of the 642 children, RV was detected in 268 (41.7%). Inpatients were more likely to be RV-positive (43.2%) than outpatients (26.3%) and most of the positive samples 215/268 (80.2%) were found in children less than 1 year of age. RV infections were more common in males than females (65.3% vs. 34.7%). The VP7 predominant G type was G3 (31.3%), followed by G8 (20.5%), G1 (7%), mixed G infections (6.3%), G2 (1.9%), G9 (1.9%), G4 (1.5%), and G10 (0.4%). The VP4 predominant P type was P[8] (53.7%), followed by P[4] (16%), P[6] (9.3%), P[9] (6.3%), P[11] (4.5%), P[10] (2.6%), and mixed P infections (1.9%). The dominant VP7/VP4 combination was G3P[8] (24.2%), G8P[8] (10%), G8P[4] (5.2%), G1P[8] (4.5%), G8P[6] (3.3%), and G3P[4] (2.2%). Several other combinations were also identified with detection rates less than 2% of positive RV samples. Mixed genotype combinations and partially typed strains were detected in 31.7% and 7.5%, respectively.
Conclusion
This study highlights the necessity for continuous epidemiology and surveillance of RVA infection to improve our control and management of RVA infection. Furthermore, due to the lack of a national anti-RV vaccination program, RV remains the main causative agent for acute gastroenteritis in Egyptian children. Therefore, it is important to introduce RV vaccine into the national immunization program in Egypt free of charge to all infants to reduce the burden of RV gastroenteritis.

Keywords:
children, epidemiology, gstroenteritis, rotavirus, real-time reverse transcriptase-polymerase chain reaction

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Introduction

Acute gastroenteritis (AGE) is a common health problem that leads to morbidity and mortality in children across the world, and mostly in low-income countries. Different infectious agents such as bacteria, viruses, and parasites are well-identified to cause AGE; however, major AGE cases result from viral infections, particularly in children [1]. The most significant viral agents associated with AGE are rotavirus, adenovirus, calicivirus, and astrovirus. Rotavirus (RV) is identified as the primary agent of severe diarrhea among young children and infants, causing 128 515 deaths worldwide each year [2]. The main clinical features

include watery diarrhea along with fever and vomiting, which can result in electrolyte imbalance, dehydration, and mortality in young children less than 5 years [3,4]. RV transmission occurs primarily through the fecal–oral route via person-to-person contact or consumption of fecally contaminated water and food, thus making the waterborne pathway the most important exposure route for RVs [5,6].

RVs within the family Reoviridae are nonenveloped viruses that possess a double-stranded RNA with triple-layered capsid protein. They can be classified into ten serogroups named A–J and K and L based on the sequence of inner viral capsid protein VP6 region [7–9]. Group-A RV is the important cause of dehydration gastroenteritis in children and has been estimated to cause about one million deaths worldwide annually, mostly in developing countries [10]. Based on outer capsid proteins VP4 (P protein) and VP7 (G protein), group-A RV is further divided into 51 P (P [1]–P[51]) and 37 G (G1–G37) genotypes identified in both humans and animal species [11]. Globally, G1, G2, G3, G4, and G9 combined with P[4] or P[8] constitute the most prevalent genotypes in humans [12].

Currently, no specific treatment is available for RV prevention in Egypt where RV vaccines have not yet been introduced into the national immunization program. There are two RV vaccines (RotaTeq and Rotarix) that have been licensed for use in several countries, worldwide. It was reported that their effectiveness is stronger in developed countries than in developing countries [13,14]. The discrepancies in vaccine performance may attribute to the host factors (e.g., malnutrition, histo-blood group antigen, maternal factors, and coadministration with oral polio vaccine), the environmental factors (e.g., reduced vaccine efficacy and effectiveness), and/or the pathogen factors (e.g., co-infection, strain diversity) [15]. Indeed, both vaccines do not provide complete protection against the most prevalent genotypes. RotaTeq contains four G types G1, G2, G3, and G4, and one P type P[8], whereas Rotarix is a monovalent vaccine that includes G1 and P[8] antigens [16]. Several studies showed changes in the predominant circulating RV genotypes after introduction of these vaccines [17–19]. However, it is not clear if these changes are due to natural variation or due to the effect of vaccination [19,20]. Molecular assays (e.g., RT-PCR) have improved our knowledge of the diversity of RV strains because they enable us to track the mutations/changes in the viral genome. So, the current work aimed to evaluate the burden of RV gastroenteritis as well as identifying the most prevalent genotypes of RV among children younger than 5 years and who were hospitalized or visited an outpatient clinic in different regions in the country using seminested multiplex real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

Materials and methods

Study population

Fecal samples were collected from 642 children ≤5 years of age with signs and symptoms of gastroenteritis from February to June 2021. Children were either patients hospitalized (585/642, 91.1%) in a pediatric unit or outpatients consulting (57/642, 8.9%) for gastroenteritis at 10 regional referral hospitals, 2 private clinics, 1 health office, and 2 private labs located in six governorates (Asyut, Faiyum, Cairo, Sharqia, Monifia, and Alexandria) in Egypt, as shown in Fig. 1. Samples were collected in clean containers and transferred on ice to the Virology Lab, Environment and Climate Change Research Institute, National Research Center, within 4 h after collection for analysis.

Ethical approval

The study was approved by Medical Research Ethics Committee of the National Research Centre, Egypt on the date of 06.07.2023 (Ref No: 129072023). Informed consent was obtained in accordance with the Ethics Committee and approval procedures.

Sample preparation

In order to analyze the specimens, each of the frozen sample was totally thawed, then 1 g was weighed and diluted in 9 ml of phosphate buffer saline (1 : 10). The mixture was vortexed vigorously for 30 s followed by centrifugation at 5000 rpm for 10 min at room temperature. The supernatant was collected for and kept at −20°C until further use.

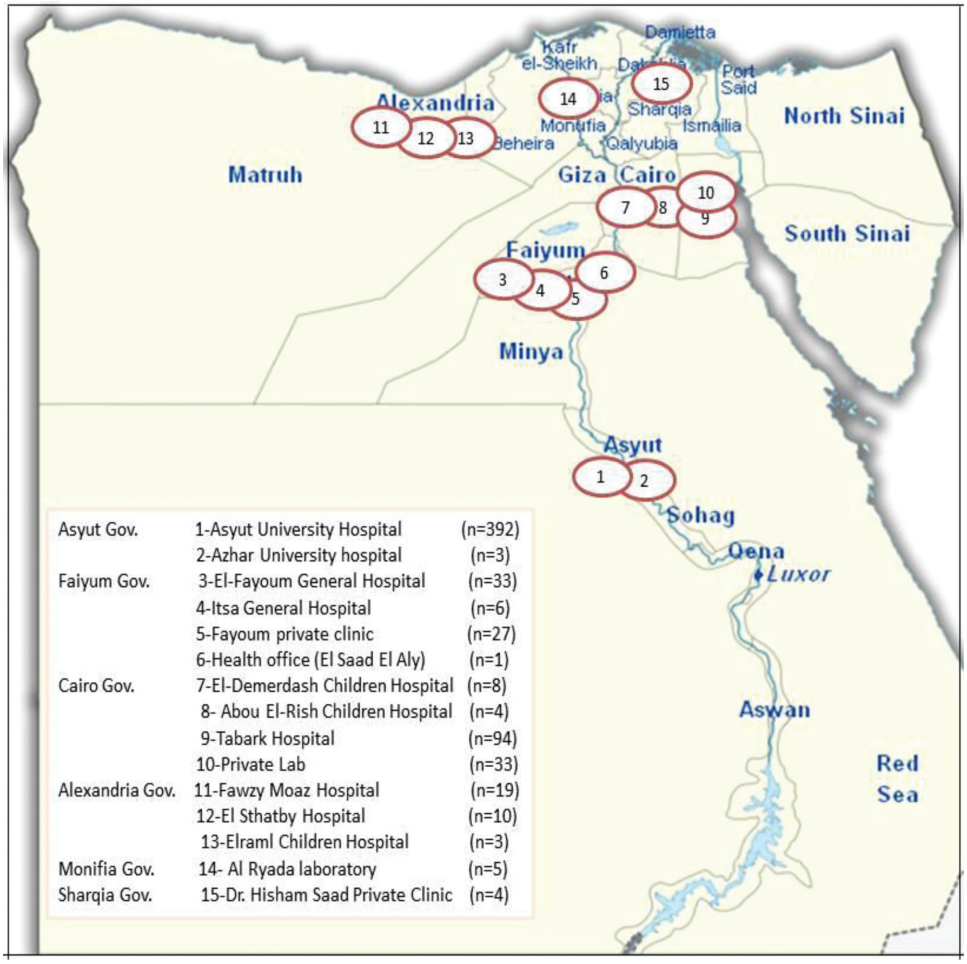
Screening for RV by immunochromatographic method

This method is rapid for the qualitative detection of RV in stool samples. The test was performed according to the manufacturer's manual (Atlas Medical, UK). The sample was added to a dilution buffer supplied by the kit and thoroughly mixed, then four drops were added to the sample well in the test cassette. The result was available after 5–10 min.

RNA extraction and complementary DNA (cDNA) synthesis

Total RNA was extracted from fecal specimens by using the QIAamp Viral RNA Mini spin according to the protocol of the manufacturer (Qiagen,

Figure 1



Specimen collection sites. The samples (*n*=642) were collected from 6 governorates (Asyut, Faiyum, Cairo, Alexandria, Monifia, and Sharqia).

Germany). The cDNA synthesis from the RNA sample was carried out on a 96-well PCR system (Bio-Rad) within two steps according to the manufacturer's instructions using GScript First-Strand Synthesis Kit. In the first step, 4 µl of RNA extract, 1 µl of oligo(dT) as a random primer (50 µM), 1 µl of 10 mM deoxynucleoside triphosphate (dNTP), and 7 µl of nuclease-free water (Qiagen, Germany) to reach a total volume of 13 µl/reaction. The mixture was incubated at 65°C for 5 min and then the tubes were chilled on ice for 5 min. In the second step, 4 µl of 5X reaction buffer, 1 µl of 0.1 M dithiothreitol (DTT), 1 µl of reverse transcriptase, and 1 µl of nuclease-free water were mixed for each sample in the same nanocentrifuge tube to reach a total volume of 20 µl per reaction. The mixture was incubated at 55°C for 1 h, 42°C for 60 min, and RT enzyme was inactivated at 70°C for 15 min. The synthesized cDNA was then stored at −20°C until use.

Polymerase chain reaction (PCR) for RV G and P typing

G- and P-type-specific amplification of VP7 and VP4 fragments was performed in two separate PCR assays using amaR OnePCR kit (Genedirex, Inc), as described previously [21]. For the first-round PCR, VP7-F and VP7-R primers were used for the VP7 gene (881 bp), while Con-2 and Con-3 primers were used for the VP4 gene amplification (876 bp). Briefly, the first PCR round was performed in a final volume 20 µl consisting of 5 µl of cDNA mixed in 10 µl of ready PCR master mix, 1 µl of 10 pmol of each primer, and 3 µl of nuclease-free water. The amplification conditions for VP7 gene amplification were as follows: 94°C for 5 min, followed by 35 cycles at 94°C for 40 s, 52°C for 1 min, and 72°C for 2 min with a final extension step at 72°C for 5 min. The same thermal conditions were used for amplification of VP4 gene, except the annealing step was 50°C for 2 min. The second round for G typing was performed in a final volume 20 µl consisting of 2 µl of the first-round PCR product, 1 µl of 10 pmol of each of specific primers targeted to G1, G2, G3, G4, G8, G9, and G10 plus VP7-R consensus primer (10 pmol) in 10 µl of a PCR master mix. P typing was performed using 2 µl of the first-round PCR product along with 1 µl of

10 pmol of each of specific P[4], P[6], P[8], P[9], P [10], and P[11] primers with a Con-3 consensus primer (10 pmol) in 10 µl of PCR master mix and 1 µl of nuclease-free water. Thermal cycling for G types was performed, including an initial denaturation at 94°C for 5 min followed by 30 cycles at 94°C for 40 s, 42°C for 2 min, and 72°C for 2 min with a final extension step at 72°C for 5 min. The same thermal cycling was applied for P types, except the annealing step was 45°C for 2 min. The amplification products were electrophoresed in 2% agarose gels in Trisborate-EDTA buffer (Thermo Fisher Scientific, USA) along with a 100–1000 bp DNA ladder (Promega, USA) as a standard marker, then the amplicons were analyzed with UV light. Genotypes were determined by the expected sizes of the amplicons (G1: 618 bp, G2: 521 bp, G3: 682 bp, G4: 452 bp, G8: 754 bp, G9: 179 bp, G10: 266 bp, G12: 387 bp, P[4]: 483 bp, P[6]: 267 bp, P [8]: 345 bp, P[9]: 391 bp, P[10]: 583 bp, and P[11]: 312 bp), as indicated previously [21–23]. In each of the 10 genotyping reactions, water instead of RNA extract or the first-round PCR product was applied as a PCR-negative control.

Statistical analysis

Chi-square testing was used to determine significant differences for comparisons of general categorical variables. The results were considered statistically significant at *P* less than 0.05.

Results

Demography data of children with acute gastroenteritis

During the study period from February to June 2021, 642 fecal samples were collected from children with AGE and younger than 5 years of age visiting 10 regional referral hospitals, 2 private clinics, 1 health office, and 2 private labs in Egypt. The age range of patients was from 1 to 59 months and the median age of subjects was 9.5 months. Patients were divided into age groups such as (0–5), (6–12), (13–24), (25–36), (37–48), and (49–60). The age group of 0–12 (n=491) comprised the majority of patients with the rate of 76.5%. There were 112 (17.4%) patients in the age group of 13–24, while age groups of 25–36, 37–48, and 49–60 consisted of 17 (2.6%), 10 (1.5%), and 12 (1.9%) patients, respectively (Table 1).

Detection of RV in children with acute gastroenteritis

RV antigen positivity was detected in 268 (41.7%) samples. Among the samples collected from hospitalized children, 43.2% (253/585) were positive for RV infection, whereas 26.3% (15/57) were positive for RV infection among the outpatient specimens. The highest frequency of RV diarrhea was in infants between 6 and 12 months of age (120/218, 55%), while the lowest number was in those aged 49–60 months (1/12, 8.3%) (Table 2). Furthermore, RV antigen positivity was found to be higher in Feb (55.5%) and March (62%) than in other months

Table 1 Sociodemographic characteristics of children less than or equal to 5 years with acute diarrhea, Feb–June 2021 (n=642)

Variable	Number of GE cases	RV-positive n (%)[#]	RV-negative n (%)[#]	*P* value
Age groups				
1–5	273	95 (34.8)	178 (65.2)	0.002
6–12	218	120 (55)	98 (44.9)	0.00
13–24	112	45 (40.2)	67 (59.8)	0.7*
25–36	17	4 (23.5)	13 (76.5)	0.12*
37–48	10	3 (30)	7 (70)	0.45*
49–50	12	1 (8.3)	11 (91.7)	0.02
Hospitalization				
Inpatient	585	253 (43.2)	332 (56.7)	0.01
Outpatient	57	15(26.3)	42 (73.7)	
Sex				
Male	384	175 (45.6)	209 (54.4)	0.02
Female	258	93 (36)	165 (63.9)	
Nutrition status				
Exclusive breastfeeding	335	127 (37.9)	208 (62)	0.04
Exclusive nonbreast feeding	110	56 (50.9)	54 (49.1)	0.03
Mixed breast-and nonbreast feeding	197	85 (43.1)	112 (56.9)	0.63*
Family size				
≤5	497	204 (41)	293 (58.9)	0.51*
>5	145	64 (44.1)	81 (55.9)	

*P greater than 0.05 was considered statistically not significant. #The percentage was calculated according to the number of rotavirus-positive cases (n=268) or rotavirus-negative cases (n=374).

(Fig. 2). In contrast to RV-negative cases, our finding showed that the rate of RV positivity in males (175/286, 65.3%) was higher than females (93/286, 34.7%). In addition, gastroenteritis due to RV infections was lower in children who were fed exclusively breast milk (Table 1).

Clinical features of acute gastroenteritis in children with or without RV infections

A comparison of clinical differences between children with or without RV gastroenteritis is presented in Table 2. The results demonstrated that all patients suffered from diarrhea. Watery stool appearance was more frequent in those infected with non-RV, while mucoid or bloody stools (P=0.21) were more common in children with RV patients. Additional signs such as vomiting, fever, and/or dehydration were also observed in some RV and non-RV patients. The results showed no statistically significant difference in these clinical features between RV-positive and RV-negative patients (P=0.32).

Overall, the duration of diarrhea or hospital admission to discharge was shorter (1–5 days) among the majority cases either with RV infection or without RV infections. Shorter diarrhea days (1–5 days) were seen in 64.2% of RV-positive cases and 65.2% of RV-negative cases (P=0.98). Diarrhea lasting for 6–10 days was observed among 23.5% of RV-positive cases and 31.3% of RV-negative cases (P=0.03), while the longer duration of diarrhea (>10 days) was seen also among RV-positive cases (12.3%) versus RV-negative cases (3.5%), with a statistically significant difference (P=00001).

Number of GE cases

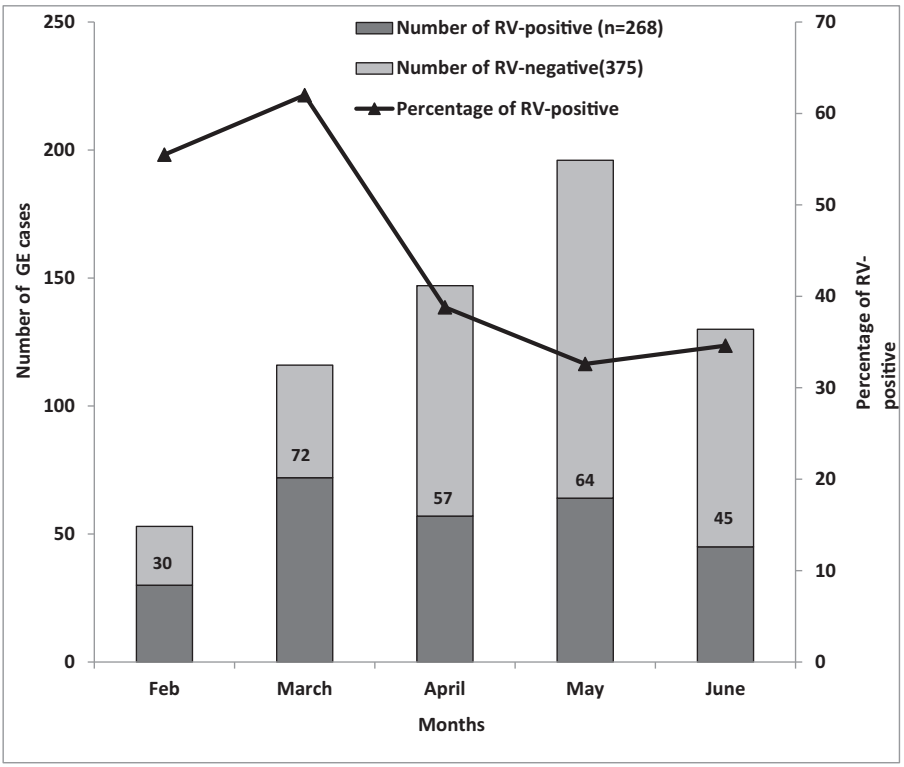
Furthermore, most of gastroenteritis cases had a shorter hospital stay (1–5 days) either in RV-positive

Table 2 Clinical manifestation of children with and without rotavirus gastroenteritis (n=642)

Variable	Number of GE cases	RV-positive *n* (%)[#]	RV-negative *n* (%)[#]	*P* value
Symptoms (diarrhea plus)				
Vomiting only	13	5 (1.9)	8 (2.1)	0.32
Fever only	117	42 (15.7)	75 (20)	
Dehydration only	22	12 (4.5)	10 (2.7)	
Vomiting and fever	47	23 (8.6)	24 (6.4)	
Vomiting and dehydration	48	18 (6.7)	30 (8)	
Fever and dehydration	46	24 (8.9)	22 (5.9)	
Vomiting, fever, and dehydration	314	133 (49.6)	181 (48.8)	
No vomiting, fever, and dehydration	35	11 (4.1)	24 (6.4)	
Appearance of diarrhea				
Watery	345	137 (51.1)	208 (55.6)	0.26
Mucoid	291	127 (47.4)	164 (43.8)	0.37
Bloody	6	4 (1.5)	2 (0.5)	0.21
Duration of diarrhea (day)				
1–5	416	172 (64.2)	244 (65.2)	0.98
6–10	180	63 (23.5)	117 (31.3)	0.03*
> 10	46	33 (12.3)	13 (3.5)	0.00001**
Frequency of diarrhea/24 h				
1–5	344	113 (42.2)	231 (61.8)	0.00*
6–10	191	99 (36.9)	92 (24.6)	0.0007*
> 10	107	56 (20.9)	51 (13.6)	0.01*
Frequency of vomiting/24 h				
1–5	296	127 (47.4)	169 (45.2)	0.58
6–10	99	38 (14.2)	61 (16.3)	0.46
> 10	27	14 (5.2)	13 (3.5)	0.28
Duration of hospitalization (day)				
1–5	565	235 (87.7)	330 (88.2)	0.19
6–10	52	19 (7.1)	33 (8.8)	0.43
> 10	25	14 (5.2)	11 (2.9)	0.14
Outcome at discharge				
Recovered	630	263 (98.1)	367 (98.1)	1.00
Improved	12	5 (1.9)	7 (1.9)	

*P less than 0.05 was statistically significant. #The percentage was calculated according to number of gastroenteritis cases in each variable, separately.

Figure 2



Shows the distribution of rotavirus and non-rotavirus gastroenteries during the period of sample collection (Feb–June). The number inside the bar represents the number of rotavirus gastroenteritis cases in each month.

cases or RV-negative cases (87.7 vs. 88.2%, *P*=0.19). Longer hospital stay of 6–10 days (*P*=0.43) or more than 10 days (*P*=0.14) was observed among RV-positive cases (7.1% vs. 5.2%) and RV-negative cases (8.8% vs. 2.9%).

The lower frequency of diarrhea or vomiting (≤5 times/day) was also the common sign among children with or without RV infections. The frequency of diarrhea (6–10 times/day) was higher in RV-positive cases (36.9%) than those infected with other pathogens (24.6%), with a statistically significant difference (*P*=0.0007). In addition, RV-positive cases showed higher frequency of diarrhea (>10 times/day) than those of RV-negative cases (20.9% vs. 13.6%, *P*=0.01). The higher frequency of vomiting (>10 times/day) was also seen in RV-positive cases (5.2%) than those of RV-negative cases (3.5%) (*P*=0.28), whereas the frequency of vomiting (6–10 times/day) was more frequent among RV-negative cases (16.3%) than those of RV-positive cases (14.2%) (*P* =0.46).

Clinical features of acute RV gastroenteritis

Clinical features of children with RV infection showed that RV diarrhea is most often accompanied by vomiting, fever, and dehydration (49.6%), followed by fever only (15.7%), fever and dehydration (9%), and vomiting plus fever (8.6%), with a *P* value 0.0009. Watery, mucoid, and bloody stools (*P*=0.003) were observed in 51%, 47.4%, and 1.5% of those with RV infection, respectively (Table 3). The most common length of stay in hospital was greater than 10 days (171/253, 67.6%), followed by ≤5 days (74/253, 29.2%), and only few cases (8/253, 3.2%) were discharged from hospitals to their home within 6–10 days (Table 4). Furthermore, most of children aged 1–5 months (71/93, 76.3%) had shorter hospital stay (≥5 days), but 95% (153/160) of hospitalized children aged 6–60 months had a longer stay (> 10 days) in the hospital, with a statistically significant difference (*P*=0.00) of various age groups.

There was no statistically significant difference in clinical features (diarrhea duration, frequency of diarrhea, and frequency of vomiting) between children with RV infection, where most of RV cases (172/268, 64.2%) recovered from diarrhea within 1–5 days. Longer diarrhea days (6–10 days) and greater than 10 days were observed only in 23.5% (63/268) and 12.3% (33/268) of cases, respectively. On the other hand, the major cases of RV observed fewer frequencies (≤5 times/day) of diarrhea and vomiting (42.2% vs. 47.4%). The frequency of diarrhea and vomiting per day was increased up to 10 times in 36.9% (99/268) and

Table 3 Distribution of signs and symptoms in children less than or equal to 5 years of age with rotavirus gastroenteritis by age groups (*n*=268)

	Sign and symptoms (diarrhea plus)							Appearance of diarrhea		
Age group (month)	Vomiting	Fever	dehydration	Vomiting + fever	Fever + dehydration	Vomiting + dehydration	Vomiting + fever + dehydration	No vomiting+ fever + dehydration	Watery	Mucoid	Bloody
1–5	3	20	4	7	9	4	44	4	46	47	2
6–12	1	19	3	9	9	6	69	4	61	57	2
13–24	0	2	5	7	3	7	18	3	28	17	0
25–36	1	0	0	0	3	0	0	0	2	2	0
37–48	0	1	0	0	0	1	1	0	0	3	0
49–50	0	0	0	0	0	0	1	0	0	1	0
Total *n* (%)	5 (1.9%)	42 (15.7%)	12 (4.5%)	23 (8.6%)	24 (9%)	18 (6.7%)	133 (49.6%)	11 (4%)	137 (51%)	127 (47.4%)	4 (1.5%)
P value					0.0009*					0.003*	

**P* less than 0.05 was statistically significant.

21.2% (38/179) of cases, respectively. The higher frequency of diarrhea and vomiting (>10 times/day) was seen only in 20.9% (56/268) and 7.8% (14/179) of RV cases, respectively (Table 4).

Distribution of G genotypes of group-A RV

G (VP7) and P (VP4) genotypes of RV were carried out on all RV-positive specimens (*n*=268). G types were detected in only 190 (70.9%) by seminested multiplex RT-PCR assay. The most prevalent G type in the population under surveillance was G3, that being identified in 84 out of 268 samples (31.3%), G8 (55/268, 20.5%), then G1 (19/268, 7%), G2 (5/268, 1.9%), G9 (5/268, 1.9%), G4 (4/268, 1.5%), and then G10 (1/268, 0.4%). Mixed infections that include 2 or 3 G strains (G2G3, G3G10, G8G9, G1G2G4, and G8G1G9) were detected in 17 samples (6.3%). The VP7 gene could not be identified in 78 samples (29.1%), as shown in Fig. 3.

Distribution of P genotypes of group-A RV

P-type could be identified in 261 out of 268 cases (97.4%) by seminested multiplex RT-PCR assay. The most common circulating P genotype in the population under surveillance was P[8], that being identified in 144 strains out of 268 samples (53.7%), followed by P [4] (43/268, 16%), P[6] (25/268, 9.3%), P[9] (17/268, 6.3%), P[11] (12/268, 4.5%), and then P[10] (7/268, 2.6%). Thirteen samples showed infections with mixed P genotypes, P[4]P[8] 8(3%) and P[6]P[8] 5(1.9%). The VP4 gene could not be identified in 7 samples (2.6%) (Fig. 4).

Distribution of RV G- and P-genotype combinations

Strains that could be fully G- and P-typed represented 68.3% (183/268) of cases. Strains that could be partially G- or P-typed represented 31.7% (85/268) of the total samples. Among the fully G- and P-typed, single G and P combination were observed in 89% (163/183) of samples, while 10.9% (20/183) had mixed G with or without mixed P RV strains (Table 5). The dominant VP7/VP4 combination was G3 P[8] 65 (24.2%), followed by G8 P[8] 27 (10%), G8 P[4] 14 (5.2%), G1 P[8] 12 (4.5%), G8 P[6] 9 (3.3%), and G3 P[4] 6 (2.2%). Other VP7/VP4 combinations that had detection rates less than 2% were also detected. Mixed genotype combinations and partially typed strains were detected in 31.7% and 7.5%, respectively (Fig. 5).

Discussion

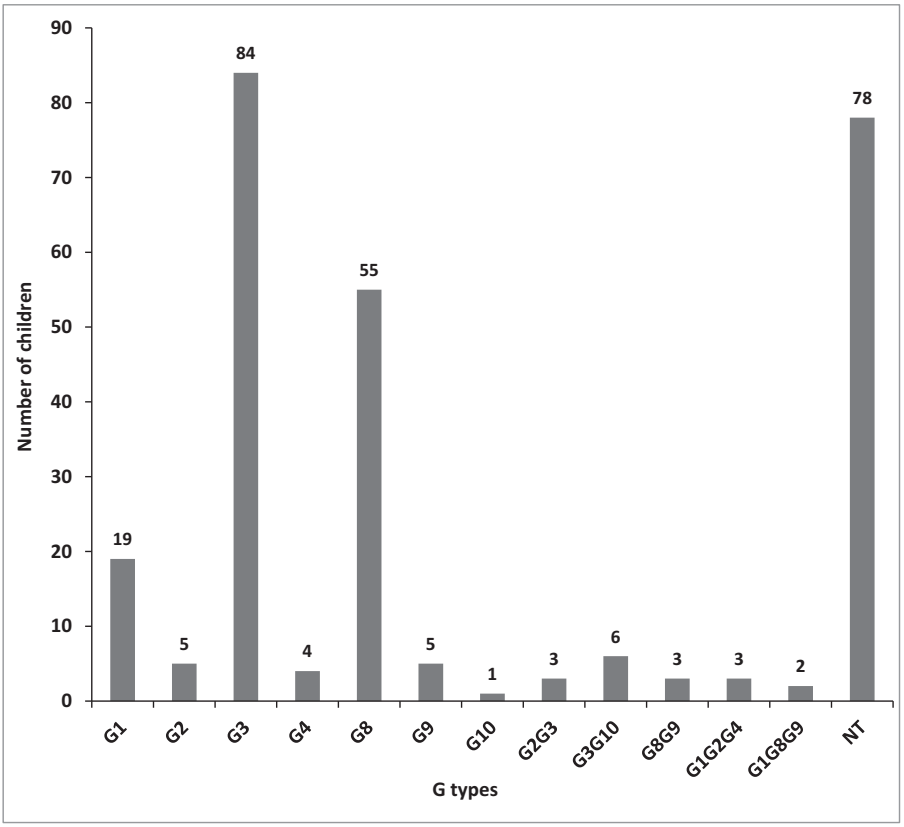
Despite the widespread introduction of RV vaccines, RV A remains one of the most common causes of acute

Table 4 Distribution of duration and frequency of diarrhea and vomiting and days of hospitalization in children less than or equal to 5 years of age with rotavirus gastroenteritis by age groups

Age group (month)	Duration of diarrhea (day)			No. of hospitalized children	Hospitalization			Frequency of diarrhea/24 h			Frequency of vomiting/24 h		
					Days of hospitalization								
	1–5	6–10	>10		0–5	6–10	>10	1–5	6–10	>10	0–5	6–10	>10
1–5	56	26	13	93	71	4	18	42	30	23	42	9	7
6–12	78	29	13	116	2	2	112	44	53	23	62	19	4
13–24	33	5	7	39	1	1	37	21	15	9	20	9	3
25–36	3	1	0	2	0	0	2	2	1	1	1	0	0
37–48	2	1	0	2	0	1	1	3	0	0	1	1	0
49–50	0	1	0	1	0	0	1	1	0	0	1	0	0
Total *n* (%)	172 (64.2)	63 (23.5)	33 (12.3)	253 (94.4)	74 (29.2)	8 (3.2)	171 (67.6)	113 (42.2)	99 (36.9)	56 (20.9)	127 (70.9)	38 (21.2)	14 (7.8)
P value		0.47		-		0.00*			0.42			0.59	

**P* less than 0.05 was statistically significant.

Figure 3

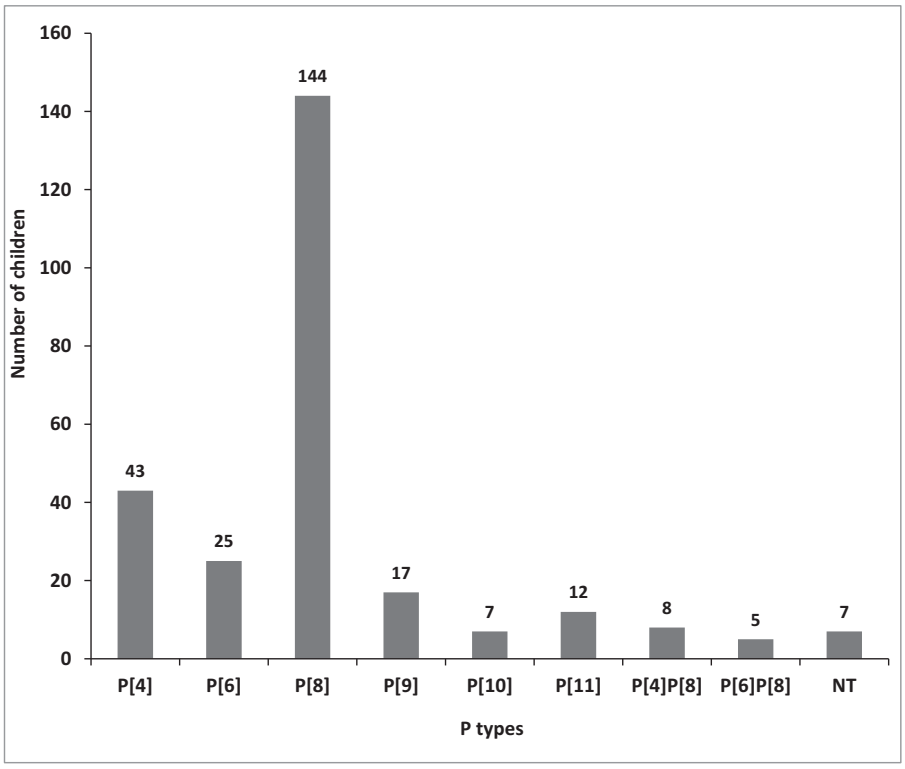


Distribution of rotavirus G types in children with rotavirus infections (*n*=268).

gastroenteritis in most of the world. So, the safety and effectiveness of strain-specific vaccine must be carefully evaluated in post-licensure monitoring programs.

RV antigen was identified, using an immune-chromatographic assay, in 268 (41.7%) of the 642 stool specimens collected from children less than five years of age with AGE. Furthermore, this study demonstrated that the positive rate of RV in pediatric outpatients (26.3%) was less than that in inpatients (43.2%). This finding is in agreement with a report from Shanghai [24,25]. This is probably due to RV being more likely associated with acute gastroenteritis cases that result in hospital admissions for children [26]. In comparison with surveillance studies from Egypt conducted on RV gastroenteritis in children less than 5 years of age between 2010 and 2022, our finding is in accordance with two previous studies where the detection rate of RV was 39% [27,28] lower than some studies' reported

Figure 4



Distribution of rotavirus P types in children with rotavirus infections (*n*=268).

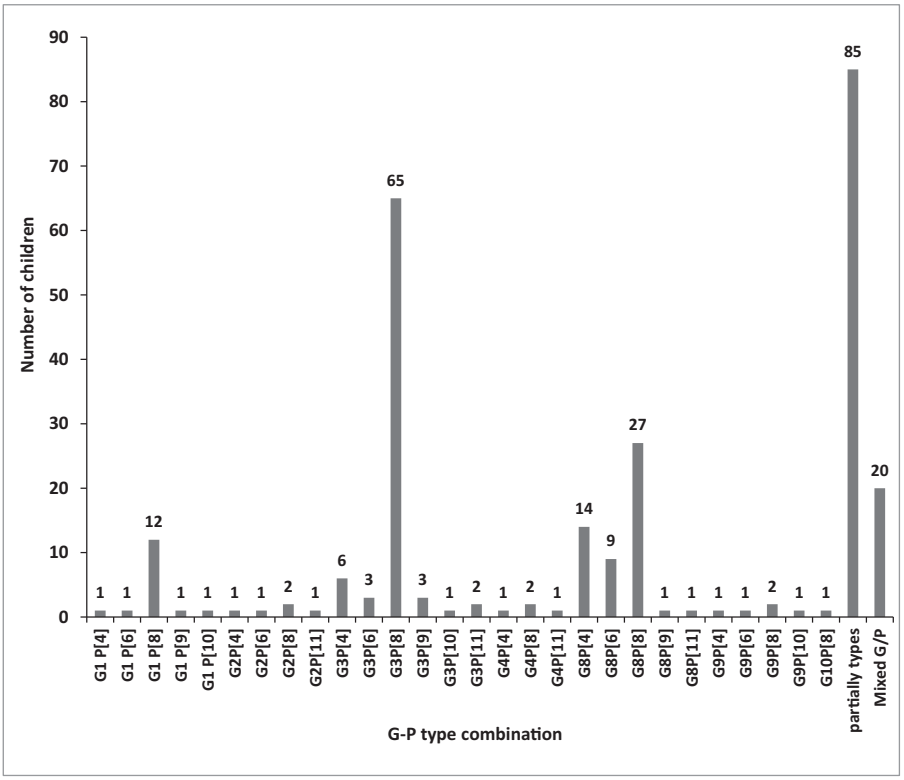
(44–91.4%) prevalence rate [29–32], and higher than those reported in some other studies with detection rates ranging from 10.7% to 37% [33–38]. Globally, the proportion of RV gastroenteritis among hospitalized children younger than 5 years has been estimated to be 30–40% in both low- and high-income countries [25]. These prevalence differences can be attributed to several reasons, including the study population, a different study design, level of socio-economic population, the sampling period (during RV peak season or through the year), type of used diagnostic procedures, and patient age criteria. It is interesting to note that our research involved 10 hospitals and 5 private labs distributed throughout Egypt and thus may well provide the actual epidemiology data of severe RV gastroenteritis

Table 5 Frequency of rotavirus genotypes in Egypt, Feb–June 2021

	No. (%) of strains with P type									
G type	P[4]	P[6]	P[8]	P[9]	P[10]	P[11]	P[8]+P[4]	P[8]+P[6]	NT	Total
G1	1	1	12	1	1	0	1	1	1	19
G2	1	1	2	0	0	1	0	0	0	5
G3	6	3	65	3	1	2	1	0	3	84
G4	1	0	2	0	0	1	0	0	0	4
G8	14	9	27	1	0	1	0	1	2	55
G9	1	1	2	0	1	0	1	0	0	5
G10	0	0	1	0	0	0	0	0	0	1
G8+G9	1	0	2	0	0	0	0	0	0	3
G3+G10	1	0	2	1	0	1	0	0	0	6
G2+G3	1	0	0	1	0	0	0	0	1	3
G1+G2+G4	1	1	0	1	0	0	0	0	0	3
G8+G1+G9	1	0	1	0	0	0	0	0	0	2
NT[a]	14	9	28	9	4	6	5	3	0	78
Total	43	25	144	17	7	12	8	5	7	268

[a]NT, nontypeable.

Figure 5



Distribution of rotavirus G- and P-type combinations among children less than 5 years in Egypt (Feb–June 2021).

among Egyptian children younger than five years of age. However, this study has some limitations as it was performed for a short duration.

According to gender findings, the detection rate of RV gastroenteritis was higher in males than in females (65.3% vs. 34.7%), which is also in accordance with previous work from Egypt and other countries [32,39,40], suggesting that males are more susceptible to infection with RV as they may have a high rate of RV infection in their stool excretion [32,37,38,41]. Furthermore, it is well-known that exclusive breastfeeding offers protection of children against RV gastrointestinal both in developing and developed countries [42–45] and this is similar to our finding. This protective effect may be due to the presence of abundant immunoglobulin A (IgA), lactoferrin, mucin, and lactadherin in breast milk, which inhibits replication of RV [46]. Several studies showed a significant increase in the age distribution of RV [47–49]. This study suggests that the younger children are more susceptible to RV infection than the older group where the peak RV infection was detected in children aged 6–12 months. Similar findings have been reported in previous studies from different countries, including Egypt [32,50,51]. This observation could possibly be due to mothers starting supplementary feeding or weaning their infants, which may raise the risk of gastrointestinal disease because of immature immune systems in children [51]. Subsequently, when preparing supplemental feeds, poor hygiene practices may also be responsible for diarrhea in children [44,52]. It may also be due to lowbreastmilk RV-IgA levels in children during the first year of life. It was reported that RV-specific IgA is sometimes not detected in stools of children below 1 year of age due to their low concentrations, while the maximum concentrations of RV fluorescent antibody were detected in children at age 1–3 years [53]. The low rates of RV infections in children over one year could be explained by the development of natural immunity due to their exposure to RV infections during the first 2 years of life [54]. Valazquez *et al.* [55] reported that by age 24 months, 96% of children experienced a primary RV infection, while 70% of those children during the same period experienced a second infection. Overall, based on these data, it seems important to focus the RVGE surveillance research on younger Egyptian children for RV immunization programs.

As indicated by our findings, RV-associated diarrhea is characterized by diarrhea, vomiting, and fever that lead to severe dehydration and increased hospitalization

rates [56]. In this study, we did not find significant differences between RV-diarrhea cases and those infected with other enteropathogens when compared according to clinical features such as vomiting, fever, or clinical dehydration in patients at admission [47]. Furthermore, although we did not assess the level of acute diarrhea severity in this research, this symptom appeared less severe in RV acute gastroenteritis children, who had lower durations of diarrhea and hospitalization than that found in non-RV acute gastroenteritis controls. Previous studies reported inconsistent results about the link between RV and severe diarrhea. In a hospital-based study, it was demonstrated that children with RV infection had severe dehydration less frequently than those with other enteropathogen infections [57]. In some case–control studies, RV was also observed not to be linked to severe dehydration [58–60]. Conversely, several studies showed that diarrhea due to RV infection was particularly severe compared with other enteropathogen diarrhea [61–64]. Therefore, it is difficult to distinguish the RVA infection based on clinical characteristics. This is because clinical features of RV gastroenteritis do not differ significantly from those induced by other pathogens [65].

Globally, the G-genotypes G1, G2, G3, G4, G9, and G12 while human P-genotypes P[4], P[6], and P[8] are the most common RV genotypes that affect humans [13,66]. All these G-genotypes, except G12, and P-genotypes were detected in this study. Interestingly, the current study detected G10, P[10], and P[11] strains for the first time in clinical samples from Egypt. Worldwide, six genotype combinations of G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] genotypes were found to be the predominant cause of human disease [67]. However, rare, unusual, or novel strains can be generated occasionally by various mechanisms that may include coinfection with animal and human strains or as a result of reassortment [68–70]. Among G/P combinations, the current result observed that G3 genotype combined with P[8] was the most common combination detected among RV patients. This observation is in agreement with other studies from Egypt, Qatar, Taiwan, Colombia, Indonesia, South Korea, and the United States [27,29,47,71–74]. A recent review on rotaviral diarrhea worldwide has found the G3P[8] strain to be one of the six most prevalent genotypes globally, causing about 90% of the RV associated with severe diarrhea requiring hospitalization [75].

G8P[8] was detected for the first time and as the second most prevalent genotype after G3[P8] in our study. A similar genotype distribution was documented in studies from South Korea and ASEAN countries [76–78]. Moreover, the G8P[8] genotype was reported as the most predominant strain in Japan and in the postvaccine period in Korea [79,80]. In this study, G8P[4] was the third most prevalent genotype (5.2%) followed by G1P[8] (4.5%). In Egypt, G1P[8] was found to be the most common genotype in samples collected between 2000–2002 (56%) and 2015–2016 (29.7%) [33,81]. Additionally, unusual G–P combinations (e.g., G1P[9], G1P[10], G2P[11], G3P[9], G3P[10], G3P[11], G4P[4], G4P[11], G8P[4], G8P[6], G8P[9], G8P[11], G9P[10], and G10P[8]) were detected in this study for the first time in Egypt. However, these genotypes have been identified in multiple countries [47,73,76,77]. These unusual genotypes could be raised due to mixed infections, resulting in intragenotype reassortment and evolution of new genotypes. In fact, these genotypes are not included in the formulations of the current available vaccines (Rotarix and RotaTeq), so it is unknown if they offer a protective immunity against these uncommon strains. However, all the strains covered by these vaccines were detected in this study, highlighting the need for these vaccines to reduce RV diseases in our country. Of note, it was reported that RV vaccination provided higher protection against RV gastroenteritis in developed countries (85–100%) than those in developing countries (46–77%) [82].

Moreover, RV genotype mixed infection prevalence has risen worldwide from 7.9 to 11.7% from 1997 to 2007 [83,84]. The rate of RV mixed infection in this study is 7.5% that is similar to previous studies [85–87]. This percentage is lower compared with other studies from Italy (12%), Austria (12%), and Spain (18%) [88]. This finding indicates that the children most likely acquire infections from different sources, and could be sources of novel global strains. A total of 29.1% and 2.6% of the study specimens were G-nontypeable and P-nontypeable even with various primer sets, respectively. The primer mismatch resulting from the higher RV diversity could be the reason of genotyping failure.

Conclusion

In conclusion, this study shows that RVs are the main causative pathogen of gastroenteritis among Egyptian children and are associated with severe symptoms in infants. Also, this study has described the genetic diversity of group-A RV strains circulating in Egypt. We identified seven different RV G serotypes (G1, G2,

G3, G4, G8, G9, and G10), six different P serotypes (P [4], P[6], P[8], P[9], P[10], and P[11]), and twenty-seven different single G–P combinations. G3 genotype combined with P[8] was found to be the predominant combination followed by G8P[8], G8P[4], G1P[8], G8P[6], and G3P[4]. It is important to introduce RV vaccine into the national immunization program in Egypt free of charge to all infants to reduce the burden of RV gastroenteritis.

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Conflicts of interest

There are no conflicts of interests.

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