Polish Journal of Microbiology
2016, Vol. 65, No 4, 433–442

ORIGINAL PAPER

Genotyping and Clinicoepidemiological Characterization of Rotavirus Acute Gastroenteritis in Egyptian Children

NIVEEN SAUDY[1]*, WALAA OTHMAN ELSHABRAWY[1], AHMED MEGAHED[2], MONA F. FOAD[1] and ALY F. MOHAMED[3]

[1] Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2] Pediatrics Departments, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[3] VACSERA-Virology Sector, Cairo, Egypt

Submitted 27 November 2015, revised 25 January 2016, accepted 18 April 2016

Abstract

Group A rotavirus (RVA) acute gastroenteritis (AGE) is a common cause of severe childhood diarrhea. The dominant circulating RVA genotypes in a given region may vary between and within the geographic regions and from year to year. Our cross-sectional study was designed to determine the burden of RVA genotypes among children with AGE admitted to referral Children Hospital at Egypt prior to implementation of the vaccine. Stool samples with clinico-epidemiological data were collected from 92 children ≤ 3 years-old with AGE. RVA G and P typing were performed with type-specific primers. RVA was detected in 48.9% of patients. Higher rates of RVA infections, 73.3% were detected in infants < 1 year-old. Breast-fed infants were significantly fewer in RVA positive group ($P = 0.0006$). Non-breast-feeding was a major risk factor for RVA AGE (OR 0.3, $P = 0.02$). RVA diarrhea occurred mostly in autumn and winter months (55.4% and 26.6%) with a significant difference in autumn ($P = 0.0005$) and was associated with vomiting and dehydration (OR; 1.66, $P = 0.021$ & 1.4, $P = 0.03$). RVA genotypes G1P[8] (26.7%), G9P[8] (20%) and G3P[8] (15.6%) were accounting for 62.3% of RVA AGE. G9 was significantly associated with mucus diarrhea, than G1 or G3 which were associated with watery diarrhea ($P = 0.025$). Also, G9 was significantly associated with loose stool for > 5 days ($P = 0.006$) and 54.4% of G9 patients had severe dehydration. The diversity of RVA strains detected in Nile Delta Egypt and emergence of G9 RVA highlight the need to apply vaccines against this genotype in Egypt.

Key words: gastroenteritisin in children, RVA genotypes, clinicoepidemiological characterization in Egypt.

Introduction

The acute gastroenteritis (AGE) caused by Group A rotavirus (RVA) contributes significantly to childhood morbidity and mortality in developing as well as developed countries (Tate *et al*., 2012).

RVA is a double-stranded RNA virus that is classified into 8 serotypes (A-H) based on antigenicity and nucleotide sequence identities of the VP6 intermediate capsid protein and encoding gene (Matthijnssens and Van Ranst, 2012). They exhibit broad genetic and antigenic diversity due to re-assortment among RVA strains and the accumulation of point mutations in the two most external capsid proteins, VP7 and VP4 and have been further categorized in VP4 (P-type) and VP7 (G-type). Currently, there are 35 P-types and 27 G-types with an intratypic variation (Matthijnssens *et al*., 2008; Esona *et al*., 2009; Matthijnssens *et al*., 2011).

Globally, G1, G2, G3, G4, G9 and G12 genotypes are the major RVA circulating genotypes, accounting for over 88% of all strains analyzed worldwide (Santos and Hoshino, 2005). Out of the five, G9 RVAes were the last to emerge and their origin is unclear. Besides humans, G9 RVAes have only been identified from pigs as early as 1980 (Cao *et al*., 2008; Matthijnssens *et al*., 2010). The African RVA Surveillance Network (AfrRSN) reported that G9 RVAes were common after the middle of the 1990s in Kenya, Libya and Tunisia (Mwenda *et al*., 2010).

The most important global strains causing majority of infections are G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]. The examination of the G- and P-type distribution is necessary as the dominant RVA genotypes that circulating in a given region may vary between and within the geographic regions from year to year (Gentsch *et al*., 2005).

Genotyping is based on analysis of the viral RNA by different methods as type-specific PCR (Gentsch *et al*., 1992), restriction fragment length polymorphism (Iturriza-Go'mara *et al*., 2002), sequence analysis (DiStefano *et al*., 2005), oligonucleotide probes (Lovmar *et al*., 2003).

* Corresponding author: N. Saudy, Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt; **e-mail: niveen197@hotmail.com**

RVA infection is highly contagious and not easily controlled by improvements in hygiene and sanitation, as evidenced by similar incidence rates in developed and developing nations (Parashar *et al*., 2006). The only control measure likely to have a significant impact on the incidence of severe disease is vaccination (Matson *et al*., 2010).

WHO recommends surveillance for the burden of RVA disease and circulating RVA strains, before and after inclusion of RVA vaccination in national expanded programs on immunization (WHO, 2009).

The World Health Organization (WHO) has reported that 20–30% of stool samples collected during 2009 from Egyptian children with AGE were positive for RVA (Mansour *et al*., 2013).

In Egypt, there is no well-developed surveillance system for RVA strain identification and the RVA vaccine is not included in the national immunization programs. The private sector only applies RV vaccination (Ortega *et al*., 2009).

Several epidemiological surveys had been done in different Egyptian governorates showing different RVA genotypes with many untypeable strains raising the possibility of spread of additional genotypes (Radwan *et al*., 1997; Naficy *et al*., 1999; Kamel *et al*., 2009). However, there is no data about the RVA strains in Dakhalia Governorate which lies in northern Egypt at the base of the Delta triangle with a population of about 5 million people. The governorate won worldwide fame for hosting specialized medical centers and hospitals that receives patients from all surrounding governorates in Delta.

This pilot study was conducted to evaluate the genotype diversity of RVA strains among children younger than 3 years of age with acute gastroenteritis (admitted to a referral Children Hospital in Dakhalia). We examined the relationship between RVA genotypes and the clinical characteristics of AGE.

Experimental

Materials and Methods

Study population and specimens. The study was conducted at Mansoura University Children's Hospital (one of the main referral hospitals for both urban and rural districts in the Nile Delta) from September 2010 to February 2012 (17 months). The principles outlined in the Declaration of Helsinki were followed and informed consents were obtained from legal guardians after study protocol approval by the local Ethics Committee of Faculty of Medicine, Mansoura University, Egypt. One out of five AGE cases, under the age of 3 years admitted to the Children's Hospital for treatment was randomly selected.

Diarrhea was defined as the occurrence of three or more loose or watery stools in the preceding 24 hours. During this study, 92 acute gastroenteritis patients were included as soon as they were diagnosed by a pediatrician and fresh stool samples were collected from selected patients for RVA detection.

Clinic epidemiologic data including age, sex, source of drinking water, feeding modalities (breast-fed and/or bottle-fed and weaning practice), clinical symptoms such as fever, vomiting and dehydration, and the stool characteristics were recorded for each child.

Detection and typing of RVAes. The stool samples were collected using wide mouthed sterile plastic containers from inpatients within 48 h following their hospitalization (one for microbiological diagnosis and the other for PCR diagnosis).

Microbiological diagnosis (routine stool culture). Cultures for detection of bacteria in stool were performed as follow; about 3–4 loopfuls (1 gm) of stool was added to 20 ml selenite broth (Oxoid) and incubated at 37°C over night then plated out on MacConkey's agar (Oxoid) for 24 hours. The isolated colonies were identified by Gram stain and manual biochemical reaction on Triple Sugar Iron agar (TSI), Christensen's urea agar and Simmon' citrate agar (Oxoid). If no pathogenic bacteria were isolated after 72 hours, the result was deemed negative.

RVA RT-PCR and genotyping. Rotavirus double-stranded RNA (dsRNA) was extracted directly from 10% of fecal suspension of each sample using TRIzol (Life Technologies) and precipitated with isopropanol following the manufacturer's recommendations.

The extracted dsRNA was subjected to G- and P-typing by multiplex reverse transcription-polymerase chain reaction (RT-PCR) with type-specific primers.

Consensus primers Beg9 and End9 were used in the first-round PCR (30 cycles) to amplify the full-length VP7 gene (1,062 bp); cDNA was used in the second-round PCR for G-typing (25 cycles) with primer set aBT1 (G1), aCT2 (G2),aET3 (G3), aDT4 (G4), aFT9 (G9) and primer set FT5(G5), DT6 (G6), HT8 (G8), ET10 (G10), BT11 (G11) (Gouvea *et al*., 1990; 1994).

For P-typing, consensus primers Con2 and Con3 were used in a first-round RT-PCR (30 cycles) to amplify the 876 bp of the VP8 region of the VP4 gene, and the second-round PCR (20 cycles) used primer set 1T-1 (P[8]), 2T-1 (P[4]), 3T-1 (P[6]), 4T-1 (P[9]), 5T-1 (P[10]) (Gentsch *et al*., 1992). All PCR products were analyzed by electrophoresis in 1.2% agarose gels and illumination after staining with ethidium bromide.

Statistical analyses. Statistical analysis was performed using SPSS 19 software for Windows (SPSS Inc, Chicago, Ill). Data were described using mean ±SD for continuous data and frequency (n%) for categorical data. Student's *t*-test was used to compare

means of quantitative data between two groups and one-way ANOVA for multiple groups. For categorical data the Chi^2 or Fisher's exact probability test were applied. *P value* < 0.05 was considered as a level of statistical significance.

Results

A total of 92 children patients with AGE were enrolled during the study period. They were 52 boys, 40 girls; mean age [±SD] was 10.64 ± 7.87 month. Stool specimens were examined for RVA by RT-PCR. RVA was detected in 45 (48.9%) of the cases (RVP group). The other group, RVA negative (RVN), 47 (51.1%), included 34 (37%) patients having bacterial pathogens (*Salmonella* in 20.7%, *Klebsiella* sp. in 4.4%, and *Escherichia coli* in 11.9%), and 13 patients (14.1%) having no detectable pathogen.

Considering the demographic data of studied patients (Table I), the age was comparable between the RVP group and RVN group ($P = 0.92$) and in all age stratifications without statistically significant difference. There were higher rates of RVA infections (33/45; 73.3%) in infants < one year old: [13 (28.9%) in infants aged < 6 months and 20 (44.4%) in infants aged 6–12 months] and the least rates (4/45, 8.9%) were in children > 2 years old. There was no statistical significant association between RVA infection with either sex or residence ($P = 0.2$ and $P = 0.09$).

Among breast fed infants ≤ 12 months of age, the number of RVP cases were significantly lower than RVN cases ($P = 0.0006$). The weaning practice increased significantly the rate of RVP compared with RVN (44.4% *vs.* 23.4 %, $P = 0.047$) (Table I).

RVP diarrhea was found to occur mostly in the autumn and winter months with a significant difference in autumn ($P = 0.0005$) Most RVN cases occurred in summer (65.9 % of cases) with significant difference in their frequency ($P = 0.0001$) than RVP. No RVP cases were detected in spring (Table I).

Regarding the analysis of hygiene factors such as the source of drinking water, 49% of RVP and 31.9% of RVN reported sometimes use of safe water; 40% of RVP and 51.1% of RVN always use it; while 11% of RVP and 17% of RVN did not use this but used tanks for

Table I
Demographic data of studied Egyptian children

Disease characteristics	RVN** N0 (%)	RVP** N0 (%)	Total N0 (%)	*P***
Total	47 (51.1)	45 (48.9)	92 (100)	
Age of child (month) Mean ± SD	10.57 ± 7.7	10.72 ±8.2	10.64 ± 7.8	0.92
< 6	11 (23.4)	13 (28.9)	24 (26.1)	0.67
6–12	17 (36.2)	20 (44.4)	37 (40.2)	0.53
12–24	15 (31.9)	8 (17.8)	23 (25)	0.9
>24	4 (8.5)	4 (8.9)	8 (8.7)	1
Gender				
Male	30 (63.8)	22 (48.9)	52 (56.5)	
Female	17 (36.2)	23 (51.1)	40 (43.5)	0.20
Residence				
Urban	22 (46.8)	13 (28.9)	35 (38)	
Rural	25 (53.2)	32 (71.1)	57 (62)	0.089
Season				
Winter	7 (14.9)	12 (26.6)	19 (20.7)	0. 23
Summer	31 (65.9)	8 (17.8)	39 (42.4)	**0.0001**
Autumn	9 (19.2)	25 (55.6)	34 (36.9)	**0.0005**
Breast feeding (< 12 m)	28	33	61	
Yes	22 (78.6)	11 (33.3)	33 (35.9)	**0.0006**
No	6 (21.4)	22 (66.7)	28 (64.1)	
Weaning	11 (23.4)	20 (44.4)	31 (33.7)	**0.047**
Mothers education: Secondary and above	20 (42.6)	15 (33.3)	35 (38)	0.396
Safe water				
Not always	8 (17)	5 (11)	13 (14.1)	0.55
Yes always	24 (51.1)	18 (40)	42 (45.7)	0.27
Sometimes	15 (31.9)	22 (49)	37 (40.2)	0.136

RVP = RVA positive, RVN = RVA negative

drinking, with no significant difference between both groups (Table I).

With regard to the clinical features of the studied patients (Table II), RVA positive patients showed significant increase in the frequency of reported clinical manifestations than RVN, fever (80% *vs.* 59.6%, $P=0.04$) vomiting (88.9% *vs.* 46.8 %, $P=0.0001$) and dehydration (84.4 % *vs.* 63.8%, $P=0.032$).

The RVP patients had more severe dehydration as compared with RVN group; dehydration was mild in (18.4% *vs.* 76.6%, $P=0.0001$), moderate in (52.6% *vs.* 16.7%, $P=0.0026$), and severe in (29% vs. 6.7%, $P=0.029$) of cases respectively.

Duration of diarrhea with loose stools for ≥ 5 days, (mean ± SD) was shorter in RVN (3 ± 2.5 days) than in RVP (5 ± 3.5 days) ($P=0.58$). The frequency of diarrhea (≥ 5 loose stools/24 hr) was higher in RVP (7 ± 3) than RVN (4 ± 3) ($P=0.36$). As regard vomiting, the duration ≥ 3 days in RVP patients (3 ± 2) was longer than RVN patients (2 ± 1.5) ($P=0.8$). The frequency of vomiting ≥ 5 episodes/24 hr in RVP (5 ±3) was higher than RVN patients (3 ± 2) ($P=0.33$) the difference was statistically insignificant (Table II).

In addition, the results representing delays of medical care were not significantly different between RVP and RVN ones, with a mean period between the onset of the diarrhea and hospitalization of 4.45 and 4.06 days, respectively (data not shown).

Regression analysis (Table III) demonstrated that severity of dehydration is 1.4 times higher in cases of RVA diarrhea (OR 1.4, 95% CI 0.06–2.6, *P* 0.03) than in cases of RVN diarrhea. The presence of vomiting

Table II
Clinical variables of studied Egyptian children

Disease characteristics	RVN** N0 (%)	RVP** N0 (%)	Total N0 (%)	*P***
Total	47 (51.1)	45 (48.9)	92 (100)	
Diarrhea				
Pattern				
Mucus	23 (48.9)	20 (44.4)	43 (46.7)	0.68
Blood	1 (2.1)	0 (0.0)	1 (1.1)	0.55
Watery	40 (85.1)	36 (80)	76 (82.6)	0.59
Frequency (mean± SD)	4 ± 3	7 ± 3	5 ± 3	0.36
Duration (mean± SD)	3 ± 2.5	5 ± 3.5	4 ± 3	0.58
Vomiting				
Yes	22 (46.8)	40 (88.9)	62 (67.4)	**0.0001**
Frequency (mean± SD)	3 ± 2	5 ± 3	4 ± 2	0.33
Duration (mean± SD)	2 ± 1.5	3 ± 2	2.5 ± 1.5	0.80
fever				
Present	28 (59.6)	36 (80)	54 (58.7)	**0.042**
Dehydration	**30 (63.8)**	**38 (84.4)**	**68 (73.9)**	**0.032**
Severe	**2 (6.7)**	**11 (29)**	**13 (19.1)**	**0.0289**
Moderate	**5 (16.7)**	**20 (52.6)**	**25 (36.8)**	**0.0026**
mild	**23 (76.6)**	**7 (18.4)**	**30 (44.1)**	**0.0001**
IVF	40 (97.6)	34 (94.4)	74 (96.1)	**0.048**

RVP = RVA positive; RVN = RVA negative; IVF = Intravenous Fluid

Table III
Relative risk factors associated with RVA diarrhea among studied children.

Factors	*P value*	Odds ratio	(95.0% CI)	
Age (> 10)	0.54	1.08	0.49	2.37
residence (urban)	0.84	1.48	0.48	2.43
Vomiting	**0.021**	**1.66**	**0.744**	**3.7**
Diarrhea duration (> 5days)	0.18	1.95	0.71	5.32
Sex (Male)	0.20	0.602	0.276	1.317
Fever	0.52	1.28	0.59	2.78
Breast feeding	**0.02**	**0.31**	**0.11**	**0.85**
Severity of dehydration	**0.036**	**1.42**	**0.06**	**2.68**
Secondary education	0.52	1.55	0.39	6.09

Table IV
Genotype distribution and the seasonal pattern of studied RVA strains

Genotypes (n)	P						Total	Season					
	P[8]		P[6]		P[4]			Winter		Summer		Autumn	
	No	%	No	%	No	%		No	%	No	%	No	%
G1	12	63.2	1	5.3	6	31.5	**19**	4	21	3	15.8	12	63.2
G9	9	75	3	25	0		**12**	5	41.7	3	25	4	33.3
G3	7	77.8	0		2	22.2	**9**	2	22.2	1	11.1	6	66.7
Mixed G1, G9	1	50	1	50	0		**2**	1	50	0		1	50
G1, G4	2	100	0		0		**2**	0		0		2	100
Untypeable	0		1	100	0		**1**	0		0		1	100
Total	31	68.9	6	13.3	8	17.8	**45**	12	26.6	7	15.6	26	57.8

(odds ratio 1.66, 95% CI; 0.74–3.7 *P* 0.021) was found to be significantly higher in the RVP patients. Children not currently breast fed were at a higher risk of RVA diarrhea (OR 0.3, 95% CI 0.11–0.85, *P* 0.02) than breastfed children.

In the present study, a total of 44 (97.8%) of the 45 RVP samples were found to be G-typed and only 1 (2.2%) was untypeable. As illustrated in Table IV, the genotype distribution of RVA strains showed that the prevalent G genotypes were G1, G9 and G3 accounted for 19 (42.2%), 12 (26.7%) and 9 (20%) of RVP cases respectively. Mixed G-types reflecting dual infections G1+G9 and G1+G4 were detected in 4 (8.9%) of RVP samples. G2, G6 and G8 were not detected. All RVA strains were P-typeable and three P genotypes P[8], P[6] and P[4] were identified, accounting for 68.9%, 13.3% and 17.8% of cases respectively. The untypeable G strain (2.2%) was P[6] genotype. Of the 45 studied strains, G1P[8] (26.7%), G9P[8] (20%), and G3P[8] (15.6%) were the most prevalent strains and caused 62.3% of RVP cases in Northern Egypt. Other strains were detected in lower frequency as G1P[4] 13.3%, G9P[6] 6.7%, G3P[4] 4.4%, and G1P[6] 2.2%.

Genotype and temporal variations were clarified (Table IV) as following; the RVA genotypes G1 and G3 fluctuated with a characteristic seasonal pattern in autumn. Of the identified 12 G9 strains, 5 cases (41.7%) occurred during the rainy winter season and the remaining 7 cases (58.3%) occurred during the summer and autumn.

Clarification of the clinico-epidemiological features among pure genotypes (n = 40) with exclusion of infection caused by mixed genotypes (n = 4) and untyped strain (Table V), there was a significant difference between genotypes regarding gender ($P = 0.041$), G9 was recorded in higher percentage (66.7%) in male children while G3 was higher (77.8%) in female children.

Pattern of diarrhea significantly differed according to different genotypes ($P = 0.025$). G9 was significantly associated with mucus diarrhea in (66.7%), when compared to either G1 or G3 which were associated with higher frequency of watery diarrhea in (76.5% and 85.7%, respectively). G9 was significantly associated with loose stool for more than 5 days when compared to G3 ($P = 0.006$). Although degree of dehydration did not differ significantly between different genotypes, higher percentage of G9 patients had severe dehydration in (54.5%) than patients of other genotypes (17.6% for G1 and 0% for G3) (Table V).

Discussion

Continuous monitoring of RVA infection surveillance and typing of circulating strains remain valuable all over the world before the introduction of RVA vaccination. In this study we aimed to extend the previous RVA genotype studies conducted in Egypt to provide the baseline information to implement the appropriate vaccines.

In this study, most cases of AGE were infants, 66.3% were infants < one year old which is similar to other Egyptian studies (Naficy *et al*., 1999; Amer *et al*., 2007).

The decline of RVA diarrhea occurred with age confirming the role of the immune system in prevention of the RVA infection; 73.3% were in infants < one year old while the least rates (8.9%) were in children more than 2 years old.

Highlighting the protective effect of breast feeding, the breast fed infants were significantly lower in RVP group than RVN group (33.3%, 78.2%, $P = 0.0006$) and the non-breast feeding was a major risk factor for RVA infection (OR 0.3, 95% CI 0.11–0.85, *P* 0.02). This was because the children were introduced to gradual weaning at age of 6 months with semi solid food and infant ready food mixes thus increasing exposure to water and other environmental sources of contaminations.

Studies in Egypt, Germany, Austria and Malaysia emphasis on the positive relationship between breastfeeding and protection against RVA diarrhea (Naficy

Table V
The clinicoepidemiological features among differ

Total (n = 40)	G1 N = 19	G9 N = 12	G3 N = 9	*P* value*
Age of child (months)				
< 6 (n = 13)	6 (31.5)	4 (33.3)	3 (33.3)	0.334
6-12 (n = 15)	4 (21.1)	7 (58.3)	4 (44.5)	
12-24 (n = 8)	6 (31.5)	1 (8.4)	1 (11.1)	
> 24 (n = 4)	3 (15.9)	0 (0)	1 (11.1)	
Gender (n = 40)				
Male (n = 21)	11 (63.8)	8 (66.7)	2 (22.2)	
Female (n = 19)	8 (36.2)	4 (33.3)	7 (77.8)	**0.041**
Diarrhea (n = 36)				
***Pattern**				
Mucus (n = 13)	4 (23.5)	8 (66.7)	1 (14.3)	
Watery (n = 23)	13 (76.5)	4 (33.3)	6 (85.7)	**0.025**
***Frequency**				
(≥ 5 loose stools/24h)	10 (52.6)	9 (75)	4 (44.4)	0.323
***Duration**				
(loose stools for ≥ 5 days)	16 (84.2)	12 (100)	6 (66.7)	**0.006**
Vomiting (n = 37)				
*Duration (≥ 3 days)	17 (89.4)	11 (91.7)	9 (100)	1.000
*Frequency (≥ 5 episodes/24h)	11 (57.9)	8 (66.7)	6 (66.7)	0.918
Fever (n = 33)	15 (78.9)	10 (83.3)	8 (88.9)	1.000
Dehydration (n = 36)				
Severe (n = 9)	3 (17.6)	6 (54.5)	0 (0)	
Moderate (n = 20)	10 (58.8)	4 (36.4)	6 (75)	
Mild (n = 7)	4 (23.5)	1 (9.1)	2 (25)	0.082

et al., 1999; Plenge-Bönig *et al.*, 2010; Prameela and Vijaya, 2012). On the other hand, many studies were controversy to our results as Prasetyo *et al.* (2015), Wobudeya *et al.* (2011), Misra *et al.* (2007), who concluded that the severe dehydration RVA diarrhea in infants ≤ 6 months old who depend exclusively on breastfeeding was not significantly different from those who did not rely on breastfeeding alone. The human breast milk has shown the presence of secretory IgA antibodies and RVA G9P(5) neutralizing capacity. A strong correlation is seen between the level of anti-RVA antibody and the neutralizing capacity of breast milk samples (Santos *et al.*, 2013).

As, the socioeconomic status of some studied patients was poor, their communities have inadequately treated drinking water and 60% of RVP current cases did not use or sometimes used safe water for drinking but used tanks with communal taps, RVP were significantly higher among the weaned infants (44.4%) than RVN (23.4%) ($P = 0.047$). Mandour *et al.* (2013) performed a study on drinking water samples collected from 14 different locations of Dakahlia Governorate and concluded that in some studied areas, water was polluted and not suitable for drinking purpose. Moreover, rural people keep animals in their dwellings and the animals often use the same water source as the humans (Potgieter *et al.*, 2010).

In the present study, a total of 44 (97.8%) of the 45 RVA positive samples were found to be G typed and only 1 (2.2%) was untypeable. The prevalent G genotypes were G1, G9 and G3, causing 42.2%, 26.7% and 20% of RVP cases respectively.

A series of researches targeting RVA genotyping among children had been conducted in different districts in Egypt (Cairo, Behira, Quliobia, Giza, Alexandria, Fayoum and Sharkia). Genotypes G1, G2 and G4 represented the highest prevalence (Radwan *et al.*, 1997; Naficy *et al.*, 1999; Amer *et al.*, 2007; Kamel *et al.*, 2009; Matson *et al.*, 2010). However, in a nearby governorate (Sharkia), Hashem *et al.* (2012) recorded higher prevalence of G1, G9 and G3 (55%, 14.5% and 22.2%) in accordance with our results in the current study.

Interestingly, an increased prevalence of RVA G3 strains has also been reported elsewhere in the world (Fang *et al.*, 2002). A prolonged low incidence of G3 strains in the community might have induced a lack of G3 specific protective immunity, determining its widespread circulation in Dakhalia in the year studied. Also, G3 strains might have advantages over other G types like a more efficient transmissibility.

Other evidence that G1 and G3 are endemic in our locality is the similarity of RV genotypes in our patients to environmental RV types isolated in same area. A local study was done in 2006–2007 by El-Senousy

and El-Mahdy (2009), who examined RVA strains from water treatment plants in (Meet Khamees) and two compact units (Shoha and Mahalet Damana) from Dakhalia Governorate. Although 15.2% of the positive samples were G untypeable and 9.3% were P untypeable, the distribution for RVA strains was 39.3% P[4]G1, 28.6% P[8]G1, 17.6% P[4]G3, 7.1% P[8]G3, 7.1% P[6]G1. Previously, Kamel *et al.* (2009; 2010) found similar RV isolates were circulating in the environment and in the population. This confirms the value of wastewater screening as a tool for assessing RVs circulating in communities with the benefit of detecting types that cause both clinical and subclinical infections (van Zyl *et al*., 2006).

The current study showed higher prevalence of G9 than before in Egypt that nearly increased two folds than the last study done by Hashem *et al.* (2012) from 14.5% to 26.7% and this may be explained by the high frequency in occurrence of re-assortment, at single or multiple gene segments during mixed infections by strains of human-human or human-animal origin (Chouikha *et al*., 2007) and the escape recognition of the less frequent genotypes as G12 or G9 by the host immune system which recognizes the common G1-G4 genotypes.

Similarly, Than and Kim (2013) identified G9 strain in rural health care centers in South Korea, and reported that this genotype was found to be responsible for 7.4% to 39% of rural infections and much lower, only 1% to 3%, in urban hospitals in other Korean studies.

During the last decade, the G9 genotype has emerged as one of the five most common types worldwide. A high prevalence was detected in Ecuador (72–96%), France (55%) and Italy (84%), whereas a lower prevalence was found in Germany (8%) and the United Kingdom (13%) (Van Damme *et al*., 2007; Endara *et al*., 2007).

In our study, genotype G1P[8] was the most prevalent strains and accounted for (26.7%) of RVA cases. Similarly, the results of the preceding studies (1995–2011) indicated that the G1P[8] strain has been detected at a relatively higher frequency and represented 17.1% and 28.6% of RV strains in studies done by Kamel *et al.* (2009) and El-Senousy and El-Mahdy (2009) in Cairo and Dakhalia Governorates of Egypt, respectively. Moreover, Genotype G9P[8] was the most common combinations of G9 strain and found in 20% of studied RVA cases in corroboration with WHO (2008), which confirmed that G9 is combined more commonly with P[8] than other P genotypes (Agócs *et al*., 2014). Globally, P[8], P[6] and P[4] were the most prevalent P genotypes with the frequent combinations, G1P[8], G3P[8], G4P[8], G2P[4], G9P[8] and G9P[6] (Santos *et al*., 2005).

The pattern of RV mixed infections detected in the present investigation (G1/G4 and G1/G9, 8.9%) differed from other studies (G9/G3 and G9/G1). Mixed infection with different RVA strains might reflect co-infections with 2 different RV serotypes or frequent contamination of water resources with RV strains that could facilitate generation of novel RV strains through re-assortment (Unicomb *et al*., 1999).

In the present study, 2.2 % of the RVP samples could not be G genotyped. However, 38.7% of RVP specimens collected from infants in Cairo by Radwan *et al.* (1997) were untypable. Since RVAes genetically mutate it is to be expected that sometimes today's RT-PCR methodologies are unable to identify all types. The natural variation in the primer binding sites of VP7 genes has been documented from different parts of the world (Espinola *et al*., 2008; Rahman *et al*., 2005).

As observed from these results, the overall change in the distribution of RV genotypes over consecutive RV seasons and across different geographical areas observed in various studies may be partly explained by the coexistence of multiple factors such as anti-RVA immunity in children, the differences in methods and study populations, climate and water supply. Also the genetic mutation and the great diversity within RV strains circulating in humans with change in the geographical spread of genotypes highlights the need for continued surveillance to establish which RV strains are circulating in a community at a given time.

The present study found a clear seasonal pattern of acute RV gastroenteritis that peaked in autumn and winter, with only 17.8% of cases in summer and no RV cases in spring that nearly similar to those observed by Amer *et al.* (2007) and Hashem *et al.* (2012), who reported a marked seasonal peak during the cold months of the year (October-February) with low prevalence in spring and summer.

It is conceivable that when there is a seasonal pattern, children may become infected at later ages, because they have not been continuously exposed to the virus. Indeed, seasonality of RV has been observed in different parts of the world; thus, it would be better for future studies to analyze samples from a "season" rather than a "calendar year".

None of the genotypes exhibited a distinct seasonal pattern. The G1 genotype was the most prevalent genotype in autumn (48%) while in winter G9 was the most prevalent one (41.7%). G3 fluctuated all the year with a seasonal pattern in autumn (24%). To our knowledge no previous reports discussed this issue. Instead of this, it was discussed as year-to-year variations in strain circulation However, the surveillance period of this study is short and long-term observations are required to confirm this pattern.

While, RVP showed significant increase in the frequency of reported clinical manifestations, fever, vomiting and dehydration than RVN ($P=0.04$, $P=0.0001$, $P=0.032$ respectively), the multiple regression analysis confirmed that only vomiting and dehydration are major associated presentations of RVA AGE (OR; 1.66, $P=0.021$ and 1.4, $P=0.03$ respectively).

Considering the influence of RVA genotype on the clinical features of AGE, we found that G9 was associated with sever gastroenteritis manifested as mucus diarrhea with loose stool more than 5 days associated with vomiting causing severe dehydration with significant difference than G1 and G3 gastroenteritis that presented mostly with watery diarrhea.

Previous attempts to correlate RVA genotypes and clinical manifestations of diarrhea are limited but no consistent patterns have emerged. An Indian study of hospitalized RVA patients found that G1 caused more severe disease and more severe dehydration than G9 strains (Bahl *et al*., 2005). In the Vizzi *et al.* (2011) study, more than half of the children, who shed G3P[8]/NSP4-E1 and G2P[4]/NSP4-E2 strains showed a severe diarrhea. Linhares *et al*. (2006) reported that circulating RVA belonging to the genotype G9 [8] caused more-severe disease especially under the age of 5 months; the age group targeted for RVA immunization.

Several theories have been put forward to explain these discrepancies. It is known that RV diarrhea can be more severe at younger ages or the newly introduced strains into a community may cause more severe disease due to the lack of pre-existing immunity. Also, there may be year-to-year variations in virulence of particular serotypes or genotypes (Bahl *et al*., 2005; Linhares *et al*., 2006; Schael *et al*., 2009).

Improvements in water supply, hygiene or sanitization are unlikely to decrease the spread of this disease so that vaccines have been identified as the prime means to reduce morbidity and mortality from RVA gastroenteritis (El-Senousy *et al*., 2013). The two available RVA vaccines include the globally common G and P genotypes mimic the protection against severe illness provided by natural infection (Vainio *et al*., 2009).

The main limitation in this study was the low number of samples collected during the surveillance period because not all eligible children were enrolled due to difficulties to obtain consent from all parents. In addition, only one children center hospital participated in the study.

Since the climate varies considerably in different parts of upper and lower Egypt, the differences in temperature and humidity may affect both seasonality and distribution of RVA strains within Egypt. However, limited data are available on the correlation between different genotypes of RVA gastroenteritis, temperature and humidity during various seasons in Egypt.

Conclusion

Our study provides background review to the policy makers before implementation of RVA vaccines in Egypt. It confirms the current burden of RVA AGE in infants and children and shows up the diversity of RVA strains circulating in Dakhalia and neighboring areas in North Egypt. Such variations may be eased by overcrowding population and poor living conditions. The emergence of G9 necessitates the urgent consideration of G9 moiety in RVA vaccines considered for use in Egypt. Year-to-year and geographic variation in the distribution of RVA genotypes underlines the importance of active strain surveillance during several consecutive seasons and supports the need for a vaccine that can provide effective protection against the common RVA types.

Literature

Agócs M.M., F. Serhan, C. Yen, J.M. Mwenda, L.H. de Oliveira, N. Teleb, A. Wasley, P.R. Wijesinghe, K. Fox, J.E. Tate and others. 2014. WHO global RVA surveillance network: a strategic review of the first 5 years, 2008–2012. *MMWR* 25. 63(29): 634–637.

Amer M.A., S.M. Abdel Salam, H.A. Ibrahim and M.A. Farag. 2007. Detection of group A Rota virus and characterization of G type among Egyptian children with diarrhea. *Egyptian J. Med. Microbiol.* 16(1): 123–132.

Bahl R., P. Ray, S. Subodh, P. Shambharkar, M. Saxena, U. Parashar, J. Gentsch, R. Glass, M.K. Bhan and Delhi Rotavirus Study Group. 2005. Incidence of severe rotavirus diarrhea in New Delhi, India, and G and P types of the infecting rotavirus strains. *J. Infect. Dis*. 192(Suppl 1): S114–S119.

Cao D., M. Barro and Y. Hoshino. 2008. Porcine RVA bearing an aberrant gene stemming from an intergenic recombination of the NSP2 and NSP5 genes is defective and interfering. *J. Virol.* 82: 6073–6077.

Chouikha A., I. Fodha, S. Noomen, L. Bouzid, M. Mastouri, I. Peenze, M. De Beer, J. Dewar, A. Geyer, T. Sfar and others. 2007. Group A RVA strains circulating in the eastern center of Tunisia during a ten-year period (1995–2004). *J. Med. Virol.* 79(7): 1002–1008.

DiStefano D.J., N. Kraiouchkine, L. Mallette, M. Maliga, G. Kulnis, P.M. Keller, H.F. Clark and A.R. Shaw. 2005. Novel RVA VP7 typing assay using a one-step reverse transcriptase PCR protocol and product sequencing and utility of the assay for epidemiological studies and strain characterization, including serotype subgroup analysis. *J. Clin. Microbiol*. 43(12): 5876–5880.

El-Senousy W.M. and E.M. El-Mahdy. 2009. Detection and genotyping of RVAes in water treatment plants of El-Dakahlia Governorate. *Egyp. J. Biotechnol*. 31: 25–34.

El-Senousy W.M., Y.E. Shahein, A.B. Barakat, H.E. Ghanem, A.E. El-Hakim and S.M. Ameen. 2013. Molecular cloning and immunogenicity evaluation of RVA structural proteins as candidate vaccine. *Int. J. Biol. Macromol.* 59: 67–71.

Endara P., G. Trueba, O.D. Solberg, S.J. Bates, K. Ponce, W. Cevallos, J. Matthijnssens and J.N. Eisenberg. 2007. Symptomatic and subclinical infection with RVA P[8]G9, rural Ecuador. *Emerg. Infect. Dis*. 13(4): 574–580.

Esona M.D., A. Geyer, N. Page, A. Trabelsi, I. Fodha, M. Aminu, V.A. Agbaya, B. Tsion, T.K. Kerin, G.E. Armah and others. 2009. Genomic characterization of human RVA G8 strains from the Afri-

can RVA network: relationship to animal RVAes. *J. Med. Virol.* 81(5): 937–951.

Espinola E., A. Amarilla, J. Arbiza and G.I Parra. 2008. Sequence and phylogenetic analysis of the VP4 gene of human RVAes isolated in Paraguay. *Arch. Virol.* 153(6): 1067–1073.

Fang Z.Y., H. Yang, J. Qi, J. Zhang, L.W. Sun, J.Y. Tang, L. Ma, Z.Q. Du, A.H. He, J.P. Xie and others. 2002. Diversity of RVA strains among children with acute diarrhea in China: 1998–2000 surveillance study. *J. Clin. Microbiol.* 40: 1875–1878.

Gentsch J.R., R.I. Glass, P. Woods, V. Gouvea, M. Gorziglia, J. Flores, B.K. Das and M.K. Bhan. 1992. Identification of group A RVA gene 4 types by polymerase chain reaction. *J. Clin Microbiol.* 30(6): 1365–1373.

Gentsch J.R., A.R. Laird, B. Bielfelt, D.D. Griffin, K. Banyai, M. Ramachandran, V. Jain, N.A. Cunliffe, O. Nakagomi, C.D. Kirkwood and others. 2005. Serotype diversity and reassortment between human and animal RVA strains: implications for RVA vaccine programs. *J. Infect. Dis.* 192(Suppl 1): S146–159.

Gouvea V., R.I. Glass, P. Woods, K. Taniguchi, H.F. Clark, B. Forrester and Z.Y. Fang. 1990. Polymerase chain reaction amplification and typing of RVA nucleic acid from stool specimens. *J. Clin. Microbiol.* 28(2): 276–282.

Gouvea V., L. de Castro, M.C. Timenetsky, H. Greenberg, and N. Santos. 1994. RVA serotype G5 associated with diarrhea in Brazilian children. *J. Clin. Microbiol.* 32(5): 1408–1409.

Hashem S.E., A. Sahar, S.A. Shoman, S.A. Zaki and A.F. Elsayed. 2012. Isolation and molecular genotyping of group A RVA strains circulating among Egyptian infants and children. *Austr. J. Basic App. Sci.* 6(6): 361–367.

Iturriza-Go'mara M., C. Wong, S. Blome, U. Desselberger and J. Gray. 2002. RVA subgroup characterization by restriction endonuclease digestion of a cDNA fragment of the VP6 gene. *J. Virol. Meth.* 105: 99–103.

Kamel A.H., M.A. Ali, H.G. El Nady, A. de Rougemont, P. Pothier and G. Belliot. 2009. Predominance and circulation of enteric viruses in the region of Greater Cairo, Egypt. *J. Clin. Microbiol.* 47(4): 1037–1045.

Kamel A.H., M.A. Ali, H.G. El-Nady, S. Aho and P. Pothier. 2010. Evidence of the co-circulation of enteric viruses in sewage and in the population of Greater Cairo. *J. Appl. Microbiol.* 108: 1620–1629.

Linhares A.C., T. Verstraeten, J. Wolleswinkel-van den Bosch, R. Clemens and T. Breuer. 2006. RVA serotype G9 is associated with more-severe disease in Latin America. *Clin. Infect. Dis*. 43(3): 312–314.

Lovmar L., C. Fock, F. Espinoza, F. Bucardo, A.C. Syvänen and K. Bondeson. 2003. Microarrays for genotyping human group a RVA by multiplex capture and type-specific primer extension. *J. Clin. Microbiol*. 41(11): 5153–5158.

Mandour R.A., A.A. Ghanem and S.M. El-Azab. 2013. Correlation between lead levels in drinking water and mothers' breast milk: Dakahlia, Egypt. *Environ. Geochem. Health* 35(2): 251–256.

Mansour A.M., M. El Koutby, M.M. El Barbary, W. Mohamed, S. Shehata, H. El Mohammady, M. Mostafa, M.S. Riddle, P.J. Sebeny, S.Y. Young and others. 2013. Enteric viral infections as potential risk factors for intussusception. *J. Infect. Dev. Ctries*. 15; 7(1): 28–35.

Matson D.O., I.A. Abdel-Messih, C.D. Schlett, K. Bok, T. Wienkopff, T.F. Wierzba, J.W. Sanders and R.W.Jr. Frenck. 2010. RVA Genotypes among hospitalized children in Egypt, 2000–2002. *J. Infect. Dis.* 202(S1): S263–265.

Matthijnssens, J.1., M. Ciarlet, E. Heiman, I. Arijs, T. Delbeke, S.M. McDonald, E.A. Palombo, M. Iturriza-Gómara, P. Maes, J.T. Patton and others. 2008. Full genome-based classification of RVAes reveals a common origin between human Wa-Like and porcine RVA strains and human DS-1-like and bovine RVA strains. *J. Virology* 82: 3204–3219.

Matthijnssens J., E. Heylen, M. Zeller, M. Rahman, P. Lemey and M. Van Ranst. 2010. Phylodynamic analyses of RVA genotypes G9 and G12 underscore their potential for swift global spread. *Mol. Biol. Evol.* 27(10): 2431–2436.

Matthijnssens J., S. De Grazia, J. Piessens, E. Heylen, M. Zeller, G.M. Giammanco, K. Bányai, C. Buonavoglia, M. Ciarlet, V. Martella and others. 2011. Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A RVA strains. *Infect. Genet. Evol.* 11(6): 1396–1406.

Matthijnssens J. and M. Van Ranst. 2012. Genotype constellation and evolution of group A RVAes infecting humans. *Curr. Opin. Virol.* 2(4): 426–433.

Misra S., T.K. Sabui, S. Basu and N. Pal. 2007. A prospective study of RVA diarrhea in children under 1 year of age. *Clinical Pediatrics* 46(8): 683–688.

Mwenda J.M., K.M. Ntoto, A. Abebe, C. Enweronu-Laryea, I. Amina, J. Mchomvu, A. Kisakye, E.M. Mpabalwani, I. Pazvakavambwa, G.E. Armah and others. 2010. Burden and epidemiology of RVA diarrhea in selected African countries: preliminary results from the African RVA Surveillance Network. *J. Infect. Dis.* 202(Suppl): S5–S11.

Naficy A.B., R. Abu-Elyazeed, J.L. Holmes, M.R. Rao, S.J. Savarino, Y. Kim, T.F. Wierzba, L. Peruski, Y.J. Lee, J.R. Gentsch and others. 1999. Epidemiology of RVA diarrhea in Egyptian children and implications for disease control. *Am. J. Epidemiol.* 150(7): 770–777.

Ortega O., N. El-Sayed, J.W. Sanders, Z. Abd-Rabou, L. Antil, J. Bresee, A. Mansour, I. Adib, I. Nahkla and M.S. Riddle. 2009. Cost-benefit analysis of a RVA immunization program in the Arab Republic of Egypt. *J. Infect. Dis.* 200: S92–98.

Parashar U.D., C.J. Gibson, J.S. Bresse and R.I. Glass. 2006. RVA and severe childhood diarrhea. *Emerg. Infect. Dis.* 12: 304–306.

Plenge-Bönig A., N. Soto-Ramírez, W. Karmaus, G. Petersen, S. Davis and J. Forster. 2010. Breastfeeding protects against acute gastroenteritis due to RVA in infants. *Euro. J. Ped.* 169(12): 1471–1476.

Potgieter N., M.C. de Beer, M.B. Taylor and A.D. Steele. 2010. Prevalence and diversity of RVA strains in children with acute diarrhea from rural communities in the Limpopo Province, South Africa, from 1998 to 2000. *J. Infect. Dis*. 202(Suppl): S148–55.

Prameela K.K. and L.R. Vijaya. 2012. The importance of breastfeeding in rotaviral diarrhoeas. *Mal. J. Nutrition*. 18(1): 103–111.

Prasetyo D., I.M. Sabaroedin, Y.S. Ermaya and Y. Soenarto. 2015. Association between severe dehydration in RVA diarrhea and exclusive breastfeeding among infants at dr. Hasan Sadikin General Hospital, Bandung, Indonesia. *J. Trop. Med.* ID 862578: 4.

Radwan S.F., M.K. Gabr, S. El-Maraghi and A.F. El-Saifi. 1997. Serotyping of group A RVAes in Egyptian neonates and infants less than 1 year old with acute diarrhea. *J. Clin. Microbiol.* 35(11): 2996–2998.

Rahman M., R. Sultana, G. Podder, A.S.G. Faruque, J. Matthijnssens, K. Zaman, R.F. Breiman, D.A. Sack, M. Van Ranst and T. Azim. 2005. Typing of human RVA serotypes: Nucleotide mismatches between the VP7 gene and primer are associated with genotyping failure. *Virol. J.* 2: 24–29.

Santos N. and Y. Hoshino. 2005. Global distribution of RVA serotypes/genotypes and its implication for the development and implementation of an effective RVA vaccine. *Rev. Med. Virol.* 15: 29–56

Santos N., E.M. Volotao, C.C. Soares, G.S. Campos, S.I. Sardi and Y. Hoshino. 2005. Predominance of RVA genotype G9 during the 1999, 2000, and 2002 seasons among hospitalized children in the city of Salvador, Bahia, Brazil: Implications for future vaccine strategies. *J. Clin. Microbiol*. 43: 4064–4069.

Santos S.M.R., T.L. Ferreira, V.S. Quintal, S.B. Carbonare and M. Tino-De-Franco. 2013. Milk from Brazilian women presents

secretory IgA antibodies and neutralizes RVA G9P[5]. *J. Ped.* 89(5): 510–513.

Schael I.P., R. González and B. Salinas. 2009. Severity and age of RVA diarrhea, but not socioeconomic conditions, are associated with RVA seasonality in Venezuela. *J. Med. Virol*. 81(3): 562–567.

Tate J.E., A.H. Burton, C. Boschi-Pinto, A.D. Steele, J. Duque and U.D. Parashar. 2012. WHO-coordinated Global RVA Surveillance Network. 2008 estimate of worldwide RVA-associated mortality in children younger than 5 years before the introduction of universal RVA vaccination programmes: a systematic review and meta-analysis. *Lancet Infect. Dis*. 12(2): 136–41.

Than V.T. and W. Kim. 2013. Prevalence of rotavirus genotypes in South Korea in 1989–2009: implications for a nationwide rotavirus vaccine program. *Korean J. Pediatr.* 56(11): 465–473.

Unicomb L.E., G. Podder, J.R. Gentsch, P.A. Woods, K.Z. Hasan, A.S. Faruque, M.J. Albert and R.I. Glass. 1999. Evidence of high--frequency genomic reassortment of group A RVA strains in Bangladesh: emergence of type G9 in 1995. *J. Clin. Microbiol.* 37(6): 1885–1891.

Vainio K., S.A. Nordbø, G. Njølstad, G. Størvold. H. Døllner, C. Midgaard, F.J. Bosse, A.G. Rognlien, A. Rojahn, K.O. Wathne and others. 2009. Detection and characterization of group A RVAes in children hospitalized with acute gastroenteritis in Norway, 2006–2008. *J. Med. Virol*. 81(10): 1839–1844.

Van Damme P., C. Giaquinto, M. Maxwell, P. Todd, M. Van der Wielen on behalf of the REVEAL Study Group. 2007. Distribution of RVA genotypes in Europe 2004–2005 the: REVEAL Study. *J. Infect. Dis*. 195(Suppl 1): S17–25.

van Zyl W.B., N.A. Page, W.O. Grabow, A.D. Steele and M.B. Taylor. 2006. Molecular epidemiology of group A RVAes in water sources and selected raw vegetables in southern Africa. *Appl. Environ. Microbiol*. 72(7): 4554–4560.

Vizzi E., O. Piñeros, G.G. González, J.L. Zambrano, J.E. Ludert and F. Liprandi. 2011. Genotyping of human RVAes circulating among children with diarrhea in Valencia, Venezuela. *J. Med. Virol.* 83(12): 2225–2232.

Wobudeya E., H. Bachou, C.K. Karamagi, J.N. Kalyango, E. Mutebi, H. Wamani. 2011. Breastfeeding and the risk of RVA diarrhea in hospitalized infants in Uganda: a matched case control study. BMC Pediatrics. 11(17): 1–7

World Health Organization (WHO). 2009. RVA vaccines: an update. World Health Organization. *Weekly Epidemiological Record*. 84: 533–540.