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Prevalence, Genotyping, and Seasonality of Rotavirus in Environmental Water Samples and Stool Samples of Gastroenteritis Children in Egypt (2010-2016)

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Research Article

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Abstract

Rotavirus group A (RVA) infection is the most important pathogen of acute diarrheal illness in children and the distribution of this virus in the environment represents threats to Public Health. The aim of the current study was to assess the presence, genotyping, and seasonality of rotavirus in the Egyptian environment and diarrheal specimens of Egyptian children (< five years of age) with acute gastroenteritis to understand the current status of RVA vaccines and future considerations. In the current study, articles contained data on the presence of RVA in clinical and environmental samples published between 2010-2015 were collected. In total, detection of RVA in diarrheal specimens was ranged from 28.3% to 76.9% with a median of 37.8% of analyzed samples. Detection rate of RVA in raw sewage was ranged from 14% to 68.4% with a median of 27.8% while in treated effluent it was ranged from 2.7% to 21% with a median of 11.4%. On the other hand, detection rate of rotavirus in raw Nile water samples was ranged from 13.9% to 52.9% with a median of 31.2% whereas in drinking water it was detected in 23.3%, with prevalence rates ranged from 9.7% and 20.8% of analyzed samples. Furthermore, RVA G1 and P[4] were the most prevalent genotypes in clinical samples. Also, RVA G1was the most common genotype detected in environmental samples. G3P[8] and G1P[4] were the most common genotypes in clinical samples and environmental samples, respectively, whereas G1P[8] was the second most common genotype in both clinical and environmental samples. Moreover, the highest detection rates of RVA in both clinical and environmental samples were found in cooler months (autumn and winter). The current study provides useful data to policy makers to develop potential strategies to enhance vaccine uptake and overall impact.

Keywords: Gastroenteritis; Environment; Rotavirus; Sewage; Vaccine; Water

Introduction

Gastroenteritis is an important illness affecting mainly children < five years of age, worldwide [1]. Enteric viruses are a major cause for gastroenteritis [2] and they usually replicate in the gastrointestinal tract where each gram of stool from infected individual can arise viral particles ranging from 10^5-10^{13} [3,4]. Both asymptomatic and symptomatic shed daily a large number of viruses to the sanitary network. Thus, the wastewater considers as one of the major concentrated sources of human enteric viruses in the environment and pollution of other environmental water matrixes (groundwater, rivers, pond water) may be occurred when sanitary network is broken or when untreated/partially treated wastewater is released directly into the environment [5]. Outbreaks and sporadic cases of gastroenteritis are mostly associated to rotavirus, norovirus, astrovirus, and adenovirus which are also significant cause of water-related diseases [3]. Rotavirus, belonging to family Reoviridae, possess 11 segments of double-stranded (ds) RNA genome surrounded by a non-enveloped triple layered icosahedral capsid. Based on the differences in nucleotide sequence of the two outer layers (VP7 and VP4), rotavirus is classified into several G and P genotypes [6].

Rotavirus as causative agent for severe gastroenteritis in children is responsible for an estimated 24 million outpatient visits, 114 million episodes of gastroenteritis, 2.4 million hospitalizations, and 450,000 deaths annually in children below 5 years of age, most of which are documented in low-income countries [7,8]. Currently, there are two available live oral attenuated vaccines against rotavirus infections: monovalent vaccine (Rotarix, containing human G1P[8] genotype) and pentavalent vaccine (Rotateq, containing G1-G4 and P[8] genotypes), worldwide. Both vaccines are approved by WHO and they have been found to be safe and effective against RVA infections in large-scale clinical trials in Latin America, Asia, Europe, Africa, and the United States [9].

Materials and Methods

For the current systematic review, 13 studies contained data on the occurrence of RVA in clinical and/or environmental samples from Egypt and published between 2010-2016 were collected from google scholar, science direct, and PubMed websites with the topic: monitoring/prevalence/ incidence/epidemiology of rotaviruses in clinical/sewage/water/environmental samples. In addition, our collection for clinical studies was limited to studies that met the following two criteria:1) children < 5 years and 2) the number tested samples > 60 diarrheal samples. The prevalence, genotyping, and seasonality of rotavirus were extracted from all selected studies and compared.

Results and Discussion

Group A rotaviruses are a major pathogen of acute diarrhea in children below five years of age [10]. They are widely distributed in environment and are released in large mount in the stool of infected persons [11-13]. There are several cases of water-borne outbreaks due to rotavirus infection [14]. This virus has been isolated in groundwater [15], sewage [16], drinking water [17], and river water [18,19]. The current survey aimed to provide better knowledge of rotaviruses epidemiology in the Egyptian environment and children (< 5 years of age) with acute gastroenteritis.

In the current survey, 13 studies [20-32] contained data on the prevalence of RVA in clinical and environmental samples are included. Among them, eight studies [20-22,25-29] contained data on occurrence of RV in clinical samples, three studies [30-32] contained data on the prevalence of RVA in environmental samples, and two studies [23,24] contained data on both clinical and environmental samples Table 1.

Ref.	Data on RV in clinical samples			Data on RV in sewage samples			Data on RV in water samples		
	Prevalence	genotyping	Seasonality	Prevalence	genotyping	Seasonality	Prevalence	genotyping	Seasonality
[20]	✓	-	-	-	-	-	-	-	-
[21]	✓	✓	✓	-	-	-	-	-	-
[22]	✓	✓	✓	-	-	-	-	-	-
[23]	✓	-	-	-	-	-	✓	✓	✓
[24]	✓	-	-	✓	-	-	✓		✓
[25]	✓	-	✓	-	-	-	-	-	-
[26]	✓	-	-	-	-	-	-	-	-
[27]	✓	✓	✓	-	-	-	-	-	-
[28]	✓	✓	-	-	-	-	-	-	-

[29]	✓	-	-	-	-	-	-	-	-
[30]	-	-	-	✓	✓	-	-	-	-
[31]	-	-	-	✓	-	✓	✓	-	✓
[32]	-	-	-	-	-	-	✓	-	✓

Table 1: Data extracted from selected literatures.

Ref.	Study characteristics				Study results		
	Year(s) of Sample collection	Duration of sample collection per month	Age, years	Method	No. with AGE	Prevalence of RV No (%)	Peak Season
[24]	2007-2009	24	< 5	RT-PCR	220	72(32.7)	-
[23]	2008-2009	12	< 5	RT-PCR	120	35(29.2)%	Winter
[21]	2010-2012	17	< 5	RT-PCR	92	45(48.9)	Winter
[22]	2011-2012	12	< 2	EIA	197	77(39.1)	Winter
[20]	2011-2012	12	< 5	RT-PCR	110	35(31.8)	Winter
[25]	2012	4	< 5	ELISA	93	53(57)	-
[26]	2013-2015	18	≤ 24	RT-PCR	100	37(37)	-
[29]	2014 -2015	6	< 5	qr R T-PCR	65	50(76.9)	-
[27]	2015-2016	12	< 5	ELISA	119	37(31)	Spring
[28]	2015-2016	12	< 5	ELISA	198	56 (28.3)	Spring

RT-PCR: reverse transcriptase polymerase chain reaction; qr RT-PCR, quantitative real time RT-PCR; ELISA: enzyme-linked immunosorbent assay; EIA: Enzyme immunoassay.
Table 2: Rotavirus detection among Egyptian children with acute gastroenteritis.

As shown in Table 2, ten studies [20-29] from Egypt contained 1314 specimens from children with acute gastroenteritis were tested for the occurrence of RVA by ELIZA and RT-PCR. Rotavirus detection was ranged from 28.3% to 76.9% with a median of 37.8% of diarrheal samples. Similar results from Nigeria were reported by Japhet, et al. [33] and Uzoma, et al. [34], they found RVA in 34.5% and 37.1% of diarrheal specimens, respectively. However, our analysis is lower than those reported by Lyman, et al. [35] and Pang, et al. [36], they detected RVA infection in 17% and 18% diarrheal samples, respectively.

Ref.	Study characteristics				Study results Prevalence of rotavirus n (%)		
	Year(s) of Sample collection	Duration of sample collection per month	Place	Number of raw Nile and drinking water samples	Raw Nile water	Drinking water	Seasonality
[23]	2006- 2008	24	Meet Khames, Shoha and Mahalet Damana WTPs	72 Raw Nile water 72 Drinking water	20(27.7)	12(16.6)	autumn and winter
[24]	2007-2009	24	Meet Khames, Shoha and Mahalet Damana WTPs	72 Raw Nile water 72 Drinking water	38(52.7)	15(20.8)	autumn and winter
[31]	2009-2011	24	El-Giza WTP	24 Raw Nile water 24 Drinking water	7(29.2)	3(12.5)	autumn and winter
[32]	2010-2012	24	Shark El Mansoura, Depo Awam, El Dnabik WTPs	72 Raw Nile water 72 Drinking water	10(13.9)	7(9.7)	autumn and winter
Total				240 Raw Nile water 240 Drinking water	75 (31.2%)	37 (15.4 %)	

Table 3: Prevalence of rotavirus in water samples collected from Water treatment plants.

As shown in Table 3, four studies [23,24,31,32] contained data on the presence of RVA genome in 480 water samples (240 raw Nile water and 240 drink water samples) by RT-PCR to evaluate of the role environmental water as possible vehicle for transmission of rotavirus. Overall, RVA genome was detected in 23.3% of all analyzed samples. In raw Nile water, detection rate of RVA was ranged from 13.9% to 52.9% with average of 31.2% of tested samples. In drinking water, detection rates for rotavirus ranged from 9.7% to 20.8% with a median of 40% of analyzed samples. In previous studies conducted in Pakistan and China, RVA was detected in in 9.4% and 31% of water samples, respectively [37,38].

Three studies were found during the period 2010-2015 to contain data on the detection of RVA genomes in 158 wastewater samples (79 raw sewage and 79 treated effluent) by RT-PCR to evaluate of the role inadequately wastewater treatment as source of rotavirus in aquatic environments [24,30,31] Table 4. Overall, RVA genome was detected in 19.6% of wastewater samples. This finding is lower than those reported by Ruggeri, et al. [39] and Sdiri-Loulizi, et al. [40], which found RVA in 60.4%, 32%, of sewage samples, respectively. However, our result was higher than those reported by Motayo, et al. [41], who detected RVA in 10.3% of sewage samples.

Ref.	Study characteristics			Study results			
	Year(s) of Sample collection	Duration of sample collection per month	Place	Number of raw and treated sewage samples	Prevalence of rotavirus n (%)		Seasonality
					Raw sewage	Treated sewage	
[30]	2006-2007	11	Zenin, and El Berka WWTPs	36 raw sewage 36 treated sewage	5(14)	1(2.7)	-
[24]	2007-2009	24	Meet Khames WWTP	19 raw sewage 19 treated sewage	13(68.4)	4(21)	-
[31]	2009-2011	24	Zenin WWTP	24 raw sewage 24 treated sewage	4(16.7)	4(16.7)	autumn and winter
Total				79 raw sewage 79 treated sewage	22 (27.8)	9 (11.4)	-

Table 4: Prevalence of rotavirus in wastewater samples collected from wastewater treatment plants.

In the current study, RVA detection rate in raw sewage was ranged from 14% to 68.4% with a median of 27.8% whereas RVA detection rate was ranged from 2.7% to 21% with a median of 11.4% of treated effluent samples (Table 4). This result is lower than those detected by Dubois, et al. [42], who detected RVA genomes in 42% and 67% of raw sewage and treated effluent samples, respectively. In Iran, RVA genomes were also detected in 73.33% and 26.67% of raw sewage and treated effluent samples [43]. Also, He XQ, et al. [44] found RVA in 100% of raw sewage samples and 90% of treated sewage samples. Differences in prevalence rates of the current survey as compared to other studies may be occurred due to the differences in the methods used for concentration and detection of RVA. In addition to the differences in the number of tested samples with geographical area and the level of economic status.

Although the current survey was limited to studies published between 2010-2016, however clinical sample collections were started from 2007-2016 whereas environmental sample collections were started from 2006-2012. Based on the periods of sample collections we classified the current survey into different periods using single or combined studies as follows: the prevalence of RVA gastroenteritis were shown during 2007-2009 [23,24], 2010-2012 [20-22,25], 2013-2015 [26,29], 2015-2016 [27,28] whereas RVA genome in sewage was shown during 2006-2007 [30], 2007-2009 [24], and 2009-2011 [20] and RVA genome in water was shown during 2006-2008 [23], 2007-2009 [24], 2009-2011 [31], and 2010-2012 [32]. Our results demonstrated that the higher prevalence of rotavirus infections (55.8%) among children was found in the diarrheal specimens collected during the period 2013-2015 whereas the higher detection rates of RVA in both wastewater (44.7%) and water (36.8%) samples were found in samples collected during the period 20017-2009, which *could* be a result of differences in the time of sample collection.

For rotavirus genotyping, two studies [23,28] contained data on RVA G types in 85 environmental and clinical samples. In total, G1, G3, G4, G9, and G10 were the circulated G serotypes in both environmental and clinical samples, with G1 being the most prevalent (24%), followed by G3 (18%), mixed G types (13%), then G9 and G10 (3.5%), and G4 (1.2%) of all analyzed samples (data not shown). On the other hand, one study [23] contained

data on the presence of P serotypes in 30 environmental samples where P[4], P[6], and P[8] the circulated P types among children with acute diarrhea, with P[4] being the most common P types (56.7%) followed by P[8] (33.3%), and P[6] (10%) (data not shown). In agreement with the current observation, G1 was the most common circulating genotype in both environmental and clinical samples [36,39,43,45]. Also, P[4] was the predominant rotavirus genotype in urban Karen environmental samples, Kenya [46].

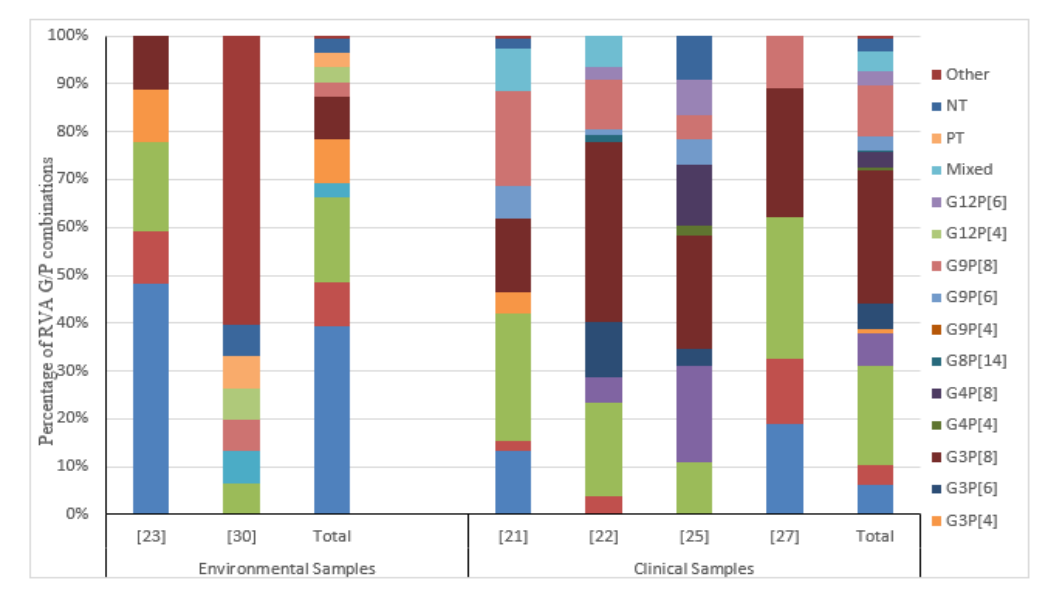

Figure 1: Distribution of RVA G-P combination genotypes in environmental and clinical samples by study; total number of environmental samples = 33 and total number of clinical samples = 212.

For frequencies of G and P combinations in this study, two studies [23,30] contained data on G/P combinations in 33 environmental samples whereas four studies [21,22,25,27] contained data on G/P combinations in 212 clinical samples. In Environmental samples, G1P[4] was the most prevalent genotype (39.4%), followed by G1P[8] (18%). G1P[6], G3P[4], and G3P[8] genotypes were detected in similar percentage (9%) and also G2P[8], G9P[8], G12P[4] genotypes, partially typed, and non-typeal samples were equally distributed in 3% of tested samples (Figure 1). In agreement with previous study from Nigeria, G1P[4] was the most circulated genotype in environmental samples [41]. In clinical samples of current survey, G3P[8] was the most circulated genotype among children with acute diarrhea (27.8%), followed by G1P[8] (20.7%), G9P[8] (10.8%), G2P[4] (7%), G1P[4] (6%), and G3P[6] (5%). Uncommon genotypes with detection rates lower than 5% were G1P[6] (4%), mixed genotypes (4%), G9P[6] (2.8%), G12P[6] (2.8%), G4P[8] (3.3%), no-typeal (2.8%), G3P[4] (0.9%), G4P[4] (0.5%), and G6P[14] (0.5%) among children with acute gastroenteritis (Figure 1). Similar finding was reported by Shoeib, et al. [22] and Li, et al. [47], which documented that G3P[8] was the most circulated genotype among children with acute diarrhea.

Collectively, G3P[8] and G1P[4] were the most common genotypes in clinical samples and environmental samples, respectively, this difference may be attributed to the differences in the time and area of collection of clinical and environmental samples. However, G1P[8] was the second most common genotypes in both clinical and environmental samples which agree with other previous studies [47]. In comparison with study conducted in Saudi Arabia, G1P[8] (44%) was the most prevalent genotype among children with acute diarrhea, followed by G2P[4], G9P[8], G12P[8], and G3P[8] [48]. In study from Nigeria, G3P[6] (24.5%) was the most common genotype, followed by G1P[6] (12.2%) and G12P[8] (10.2%) of the diarrheal samples, respectively [34]. Kittigul, et al. [49] reported that the most common genotype was G1P[8] (27%), followed by G2P[4], G3P[8], and G9P[8] of diarrheal specimens collected from Thailand.

Based on the current data, the both vaccines (Rotarix and Rotateq) seem to be effective against RVA infections in Egypt. However, updating vaccine formulation are required to contain other circulating genotypes (such as G9 and G10, G1P[4], G2P[4], and G3P[8], and G9P[8]) in our community. Furthermore, most of the included studies [21-24,27,28,31,32] demonstrated that the peak of incidence of rotavirus in both clinical and environmental samples was occurred in cooler months (autumn and winter), which agree with other studies [43,45]. It has been suggested that survival of infectious RV is increased in cooler months with low relative humidity, where this relative drop in humidity and the relatively dry soil moisture combined with rainfall can lead to increase the aerial transport of contaminated fecal and dried materials [50,51].

Conclusion

The current study provides important data on the circulated rotavirus serotypes in the Egyptian environment and responsible for sever gastroenteritis in our community and therefore the data of the current research can help us in evaluation the efficacy of current RVA vaccines in order to protect our community against all currently distributed rotavirus strains. Future studies including both clinical and environmental samples collected from different arears and for long periods are required for molecular characterization and sequencing of rotavirus strains in the environment and population to evaluate the efficacy of the current vaccines.

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