Rotavirus Genotypes Associated with Acute Diarrhea in Egyptian Infants

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Background: Before the introduction of rotavirus vaccine in Egypt, information on the burden of disease and the circulating rotavirus genotypes is critical to monitor vaccine effectiveness.

Methods: A cohort of 348 Egyptian children was followed from birth to 2 years of age with twice-weekly home visits to detect diarrheal illness. *VP7* and *VP4* genes were genotyped by reverse-transcription polymerase chain reaction and DNA sequencing.

Results: Forty percentage of children had rotavirus-associated diarrhea at least once by their second birthday. One hundred and twelve children experienced a single rotavirus diarrheal episodes (RDE) at a median age of 9 months; while 27 infants had their second RDE at a median age of 15 months and 1 infant had 3 RDE at the age of 2, 16 and 22 months. Of the 169 RDE, 82% could be assigned a G-type, while 58% had been identified a P-type. The most prevalent genotype was G2 (32%), followed by G1 (24%) and G9 (19%). G2P[4] rotavirus episodes were significantly associated with fever ($P = 0.03$) and vomiting ($P = 0.06$) when compared with other genotypes. G2 strains were the predominant genotype causing 50% of the second RDE while G9 represented 25% of the second RDE.

Conclusions: Genotypes identified are similar to those detected globally except for absence of G4. Our finding that 75% of the second RDE were due to G2 and G9 indicates a possible reduction in natural protection afforded by these types compared with G1, where 90% of G1 cases did not experience a second xposure, indicating greater protection against recurrent symptomatic infection.

Key Words: rotavirus, genotypes, diarrhea, Egypt

(*Pediatr Infect Dis J* 2014;33:S62–S68)

Rotavirus is reported to be the most common cause of severe childhood diarrhea worldwide.[1,2] Each year, in developing nations, rotavirus infections are estimated to result in approximately 500,000 deaths in children under 5 years of age.[2] By the age of 3 years, almost every child worldwide has been infected by rotavirus, although in Africa and Asia, most children have had their first rotavirus infection by 18 months of age.[3,4]

Infections with human rotavirus are common in the first few years of life in both industrialized and developing countries,[5–7] with a spectrum of illness ranging from asymptomatic infection to severe dehydration and death. Mortality is highest in the very young, due to the rapid onset of severe dehydration in this vulnerable population.[1] The incidence rates and severity of disease decline rapidly with increasing age presumably because of acquired immunity, and this has formed the basis for the live-attenuated vaccine approach.[8] However, immunity due to natural rotavirus infection is incomplete, as evidenced by the common finding of a number of repeat infections and illness in infancy and early childhood.[5,6] As rotaviruses are commonly found in children in industrialized countries, and essential control measures such as water, sanitation and hygiene have not reduced infection in these settings, the major control measure likely to have a significant impact on the incidence of severe disease is considered to be vaccination.[1]

Two rotavirus vaccines are available commercially and are being introduced into countries worldwide. Rotarix (GSK Biologicals, Rixensart, Belgium) is a monovalent, attenuated human rotavirus strain that has shown good vaccine efficacy and safety protection in Latin America, Europe and Africa.[9,10] RotaTeq (Merck & Co. Whitehouse, PA) is a pentavalent, bovine human rotavirus reassortant vaccine that has demonstrated good vaccine efficacy and safety in the Unite States, Europe and Africa.[11–13]

Circulating rotaviruses are antigenically and genetically diverse both geographically and temporarily,[14] and concerns exist whether the vaccine may not prevent all types responsible for infection and disease in children. Certainly, the strain diversity observed in Africa and in Asia is high and more variability is seen than in Europe or the United States.[15,16] Thus, knowledge of the genotypic diversity of circulating rotavirus strains in many countries is an important consideration as the rotavirus vaccines are being introduced globally, and it is important to assess how effective these vaccines are against the diversity of strains in circulation in countries such as Egypt.[17] It would be of most benefit to consider the circulating rotavirus strains before, during and after the introduction of the vaccine into populations.

The objectives of the present study were to determine the genotypes of circulating rotavirus strains in a cohort of Egyptian children followed from birth to 2 years of age and also to investigate if a specific genotype identified among infants in their first year of life will reduce their risk to subsequent infection of rotavirus at older ages.

MATERIALS AND METHODS

Study Subjects/Sample Collection

The study population consisted of a cohort of children living in a rural district located in the Nile Delta in northern Egypt (Abu Homos), followed from birth to 2 years of age with twice-weekly home visits to detect diarrheal illness. In January 2004, following

Accepted for publication June 19, 2013.

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This work was supported by the Global Emerging Infections and Surveillance System, a Division of the Armed Forces Health Surveillance Center. The study protocol DOD # NAMRU3.2003.0011 (IRB Protocol No. 145), entitled Natural immunity in a cohort of Egyptian children, was reviewed and approved by the US Naval Medical Research Unit No. 3 Institutional Review Board and the Egyptian Ministry of Health in c ompliance with all Federal regulations governing the protection of human subjects. Informed consents were obtained from parents or legal guardians of minors.

The views expressed in this article are those of the author and do not necessarily reflect the official policy or the opinions of the Department of the Navy, Centers for Disease Control and Prevention.

The authors have no other funding or conflicts of interest to disclose.

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ISSN: 0891-3668/14/3301-0S62
DOI: 10.1097/INF.0000000000000052

a house-to-house census by social workers, enrollment of neonates commenced after the mother gave birth and was invited to participate in the study. Before enrollment, written informed consent was obtained from each child's parent or guardian, and the human use guidelines of the US Department of Defense and US Department of Health and Human Services were followed throughout the study.

Active surveillance of the cohort with twice-weekly home visits began in January 2004. If the child had diarrhea, the health social workers obtained a rectal swab, which was placed in Cary-Blair transport medium, and also a stool specimen if available. Upon clinical examination by a study physician, dehydration status was classified according to the World Health Organization criteria as "none," "some" or "severe".[18] When it was deemed necessary by the physician, symptomatic children were referred to a village clinic for further evaluation and rehydration therapy. Deaths and other losses to follow up were recorded at the twice-weekly visits. All individuals in the cohort, irrespective of symptoms, were surveyed once every 2 weeks, at which time a rectal swab placed in Cary-Blair medium and a new stool specimen were collected. At each home visit, information regarding the child's diet and breast-feeding status was obtained.

The study period was January 2004 through April 2007. Crude diarrhea incidence rates and age-specific incidence rates were calculated by dividing the number of episodes by the number of person- years at risk (total person-years of follow up minus the duration of diarrheal episodes after the first day of each episode).

A "diarrheal day" was defined as the occurrence of at least 3 non-formed stools (or at least 1, if bloody) in a 24-hour period. A "diarrheal episode" was defined as beginning on the first diarrheal day after at least 3 consecutive nondiarrheal days and ending on the last diarrheal day to be followed by at least 3 consecutive nondiarrheal days. A "rotavirus diarrheal episode" was defined as a diarrheal episode in which rotavirus solely was detected in a fecal specimen collected during the episode with the absence of other enteric pathogens. All rectal swabs and fecal specimens were transported in ice packs to the Abu Homos field laboratory, where they were refrigerated. Twice weekly, the rectal swabs and fecal specimens were transported in ice packs to the laboratories of US Naval Medical Research Unit 3 in Cairo, where the rectal swabs were evaluated with standard microbiologic methods for bacterial and parasitic pathogens.[19]

For rotavirus, the fecal specimens were kept frozen at −70°C until processed for enzyme immunoassay and polymerase chain reaction (PCR). For enzyme immunoassay, stool suspensions were prepared as 10% (weight/volume) phosphate-buffered saline (pH 7.2) extracts and were tested for rotavirus using a commercial enzyme-linked, immune-sorbent assay kit (Rotaclone; Meridian Diagnostics, Inc., Cincinnati, OH).

Rotavirus RNA Extraction/Genotyping

Viral genomic RNA of stool specimens positive for rotavirus was extracted from a 10% stool suspension (0.1 g or 100 μL stool in 2 mL of a 1:1 Vertrel/Water solution, v/v) using the QIAamp Viral RNA mini kit according to the manufacturer's instruction (QIAgen,Valencia,VA). Following RNA extraction, *VP4* and *VP7* genes were amplified by classical reverse-transcriptase, nested, multiplex procedures (reverse transcriptase PCR) as described elsewhere,[17] using well-established primers sets specific for the different G-type or P-type specific primers.[20,21] Agarose gel electrophoresis and ethidium bromide staining were performed to visualize resulting bands.[17]

Nucleotide Sequencing

For untypeable strains (UTs), the *VP7* and *VP4* genes were amplified by reverse-transcription polymerase chain reaction (reverse transcriptase PCR) and sequencing of each strand of the 2 genes using the same primer as those used for reverse transcriptase PCR. PCR-amplified products of both *VP7* and *VP4* genes were purified with the QIAquick PCR purification kit following the manufacturer's protocol. Direct sequencing of each amplicon was carried out using the dideoxynucleotide chain termination method with the ABI Prism Big Dye Terminator cycle sequencing Reaction kit (Applied Biosystems, Inc., Foster city, CA) on an ABI prism 3100 automated sequencer. Nucleotides sequences were aligned against rotavirus sequences obtained from Genbank using BioEdit software, version 5.1 to ascribe a genotype.

RESULTS

Study Population and Rotavirus Disease

During the study period, 348 newborns enrolled in the cohort experienced 4001 diarrheal episodes with 511 persons-years of follow up. A rectal swab was obtained from every diarrheal episode. Of the 4001 episodes of diarrhea observed in the children during the study, 226 episodes were due to rotavirus; of which 169 episodes, rotavirus was the sole etiologic agent. Additionally, 57 children that were not included for genotyping in this study had rotavirus plus 1 or 2 enteric bacterial pathogen (eg, enterotoxigenic E coli, Shigella spp. or Campylobacter spp.). The overall incidence rate of rotavirus was 0.30 episodes per person-year, while the age-specific incidence rates of rotavirus diarrheal episodes per person-years were 0.22 for infants <6 months, 0.26 for those aged 6–11 months, 0.39 for those aged 12–17 months and 0.32 for children >18 months (Table 1).

Overall, 40% (140/348) of children suffered from a symptomatic rotavirus infection where rotavirus was the sole pathogen in this community setting. A total of 169 symptomatic rotavirus diarrhea episodes (RDE) were identified in 140 children. One hundred and twelve children (80%) experienced a single RDE at a median age of 9 months of age [interquartile range (IQR); 5–15]; while 27 infants (19%) had their second RDE at a median age of 15 months (IQR; 10–20) and 1 infant suffered from 3 RDE at the age of 2, 16 and 22 months. Forty-three episodes were detected in 42 infants < 6 months of age, 56 episodes were from 49 children in the next 6 months of life and the remaining 70 episodes were from 49 children with age >1 year (Table 1). In the 28 cases of sequential rotavirus infections, 71% (20/27) of these episodes occurred in children >1 year, while 25% (7/28) and 4% (1/28) were identified among children in the age group from 6 to 11 months and infants <6 month, respectively. The duration between the first and second episode of rotavirus infection ranged from 1 to 17 months, with a mean of 8 months (data not shown).

Rotavirus Genotyping of the Strains

Of the 164 rotavirus diarrheal episodes evaluated, 135 (82%) could be assigned a G-type, while the remaining 29 (18%) could not be typed (5 samples were not assayed for G-type due to lack of material; Table 1). Only 95 (58%) of the 164 strains could be P-type and the remaining 69 strains (42%) were untypeable for the P genotype. Overall, 17 samples were untypeable for both G- and P-types.

Of the typed strains, G2 was the most frequently identified G genotype (32%), followed by G1 strains (24%) and G9 strains (19%; Table 2). Each of these 3 predominant strains were associated with differing P-types (Table 2). Less common types (G3, G2+G3, G2+G9 and G12) represented 7% of all G genotypes.

TABLE 1. Incidence and Distribution of Primary and Secondary Rotavirus-associated Diarrhea by Age, Abu Homos, Egypt, 2004–2007

Age Group (Months)	Children With Rotavirus Diarrhea n = 140*	Children With 1 Rotavirus Episode (n = 112)†	Children With 2 Rotavirus Episode (n = 28)‡	Total No of Rotavirus Episodes n = 169	Incidence Rotavirus Episode Person/Yr
<6	42 (30)	41 (37)	1 (4)	43 (25)	0.30
6–11	49 (35)	42 (38)	7 (25)	56 (33)	0.22
12–17	30 (21)	22 (20)	8 (29)	38 (22)	0.26
>18	19 (14)	7 (6)	12 (43)$	32 (13)	0.32

All numbers are expressed as n (%).
*Total number of children and their age at first infection.
†Number of children experienced one rotavirus episode.
‡Number of children and their age at second infection.

Rotavirus Strain and Clinical Features

There was a trend in the age distribution of the common genotypes. For instance, 26% of infants <6 months were exposed to G2 rotavirus genotype infection, while a smaller proportion of cases with G1 (10%) and G9 (14%) type-associated rotavirus were observed in this age group. In the second year of life, 43% of strains among children 12–17 months were G9 (Table 3).

While fever and vomiting characterized most of the episodes regardless of the rotavirus genotype, 76% of the G2P[4] rotavirus episodes were significantly associated with fever (P = 0.03) and 71% were associated with vomiting (P = 0.06) when compared with G1P[8] genotypes (Table 4). No dehydration was reported for any rotavirus cases, except for 2 cases with G2P[4] (2/21 cases) in the age group of 6–11 and 12–17 months.

There was no significant difference in the maximum number of stools per child/day associated with G1-type episode (median=7, IQR: 4–9) and those caused by G2 (median = 5, IQR: 4–7) or G9 episodes (median= 7; IQR: 5–8).

The circulation of rotavirus was year-round, with a higher number of monthly cases of rotavirus diarrhea occurring around the fall season (September to November; Fig. 1). Forty percent (n = 16) of G1 episodes occurred in the month of September, while 31% of G2 and 33% of G9 episodes peaked at the month of November (Fig. 1).

Reinfection by Strain

Rotavirus G2 genotype was the predominant genotype causing 50% (14/28) of the second rotavirus episodes among children with median age 18 months (IQR; 9–20); 43% (6/14) of these episodes were associated with fever and vomiting. No difference in the duration of illness or associated symptoms between the first and second episodes was related to any specific genotype. Twenty-five percentage (7/28) of the second episodes were due to G9 genotype, and together with the G2 genotype, they comprised 75% of sequential RDE, 14% (4/28) were due to G1 and 11% (2/28) were of UT type. Twenty one percent (3/14) of G2, 25% (1/4) of G1

TABLE 2. VP7 and VP4 Genotypes of Rotavirus Isolates, Abu Homos, Egypt, 2004–2007

Genotype	No (%) of Typeabable Specimens VP7 135 (82)	VP4 95(58)	No(%) of Untypeable Specimens VP7 29 (18)	VP4 69 (42)
Common genotypes				
G1P[8]	30 (18)			
G2P[4]	21 (13)			
G9P[8]	19 (12)			
G3[P8]	4 (2)			
Atypical genotypes		83		
G2P[6]	1 (1)			
G12P[6]	2 (1)			
G2[P8]	4 (2)			
G9P[6]	1 (1)			
G9P[4]	1 (1)			
Mixed and Untypeable genotypes				
G2+G3UT	2 (1)			
G2+G9UT	2 (1)			
G9[UT]	9 (6)			52
G2[UT]	26 (16)			
G1[UT]	10 (6)			
G3[UT]	3 (2)			
UTP[8]		12 (7)	12	
UT[UT]			17 (10)	17

Total number of specimens (n = 164) genotyped for the VP7 were 135 and those genotyped for the VP4 were 95. Five specimens were not genotyped due to sample unavailability. G2 was the most frequently (32%) identified G genotype G2[UT] (16%), G2P[4](13%) and others (3%). G1 represented 24% [G1P[8](18%) and G1[UT] (6%)]. G9 represented 20% [G9P[8] (12%), G9[UT](6%) and others (2%)]. Less common types (G3, G2+G3, G2+G9 and G12) represented 7% of all G genotypes.

TABLE 3. Age-specific Distribution of RDE, Abu Homos, Egypt, 2004–2007

			Common Combinations of VP7 and VP4 Strains n (%)										
			G1 (n = 40)			G2 (n = 47)			G9 (n = 28)		UT (n = 29)		
	Age in Months	No. of RDE	G1P8 (n = 30)	G1UT (n = 10)	Total G1 (n = 40)	G2P4 (n = 21)	G2UT (n = 26)	Total G2 (n = 47)	G9P8 (n = 19)	G9UT (n = 9)	Total G9 (n = 28)	UTP8 (n = 12)	UTUT (n = 17)	Total UT (n = 29)	Other* (n = 25)
<1 Yr	<6	43	3 (10)	1 (10)	4 (10)	3 (14)	9 (35)	12 (26)	3 (16)	1 (11)	4 (14)	7 (58)	7 (41)	14 (48)	9 (36)
	6–11	57	15 (50)	3 (30)	18 (45)	10 (48)	4 (15)	14 (30)	6 (32)	1 (11)	7 (25)	2 (17)	4 (24)	6 (21)	12 (48)
>1 Yr	12–17	38	7 (23)	4 (40)	11 (28)	4 (19)	3 (12)	7 (15)	8 (42)	4 (44)	12 (43)	2 (17)	3 (18)	5(17)	3 (12) (4)
	18+	31	5 (17)	2 (20)	7 (18)	4 (19)	10 (39)	14 (30)	2 (11)	3 (33)	5 (18)	1 (8)	3 (18)	4 (14)	1 (4)

* Others: Mixed and or atypical G/P combinations.

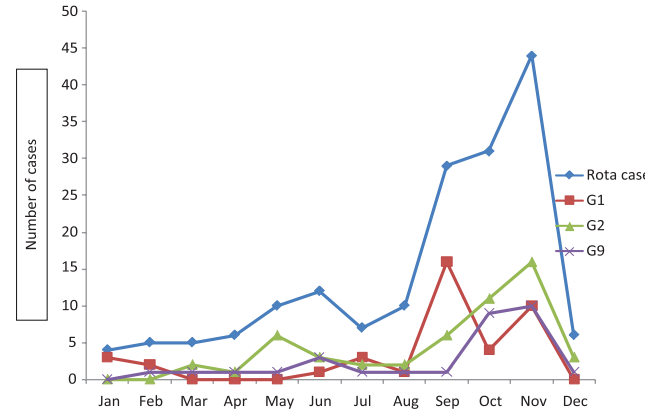

FIGURE 1. Higher number of monthly cases of rotavirus diarrhea occurred during the warm months (September to November). •40% (n = 16) of G1 episodes occurred in the month of September, while G2 and G9 episodes peaked on the month of November.

and 17% (1/7) of G9 cases had the same genotype in their first episode (Table 5). The mean duration between the first and second episodes due to G1 and G9 was 1 and 7 months, respectively, while the duration was 10 months between those caused by G2. Overall, 57% (16/28) of the second episode presented with fever, 39% with vomiting, 36% with both fever and vomiting and 4% with dehydration. There was no difference in the disease symptoms associated with the first and second episodes.

DISCUSSION

This is second largest reported prospective study of rotavirus disease from Egypt and the middle-East region in young children followed from birth, where 20% (28/140) of children presented with 2 rotavirus illnesses as opposed to 3% (12/363) reported in the early study from Egypt.[7] In this study, we report the distribution of rotavirus genotypes isolated from diarrheal episodes in a cohort of 348 Egyptian children followed from birth to 2 years of age during 2004–2007. Forty percentage of the cohort suffered at least 1 rotavirus diarrhea episode with higher frequencies (65%) in children <1 year when compared with those older (35%). This figure is comparable with the 40% reported in another Egyptian study conducted 2 decades ago in infants <1 year of age,[22] but higher than the 23% reported in the Abu Homos clinic-based surveillance conducted during 2000–2002.[23] The high distribution of rotavirus infection in the younger age group in this study, predominantly in infants <12 months of age, is consistent with that reported earlier in Egypt[22] and those reported in other Mediterranean areas and countries, such as Sicily[24] and Italy.[25]

Rotavirus-associated diarrhea peaked in late summer (September) to late fall (November), similar to the trend reported in a population-based cohort study conducted in Abu Homos, Egypt, during 1995–1998, where 90% of rotavirus diarrheal episodes occurred between July and November.[26] In a much earlier community-based study conducted in Egypt 2 decades before, rotavirus infection peaked from November to April,[27] which is similar to the pattern seen in other North African countries during the same time period, such as Morocco,[28] Algeria[29] and Egypt.[30] This apparent change in seasonality is interesting and may reflect subtle changes in various factors such as economic conditions, urbanization and population growth or other factors.

In the current cohort from Abu Homos, genotyping of the *VP7* gene showed the presence of diverse rotavirus genotypes with G2 as the most frequently genotype (32%), followed by G1 (24%) and G9 (18%). In this population, G2 rotavirus infection was found

TABLE 4. Distribution of Common Combinations of VP7 and VP4 Strains by Child's age and Clinical Symptoms

Clinical symptoms	Age Groups	Common combinations of VP7 and VP4 strains		
		G2P4 (n = 21)	G1P8 (n = 30)	G9P8 (n = 19)
Fever	0–5	2 (12.5)	1 (5.0)	2 (16.7)
	6–11	8 (50.0)	10 (50.0)	3 (25.0)
	12–17	4 (25.0)	6 (30.0)	6 (50.0)
	18–23	2 (12.5)	3 (15.0)	1 (8.3)
	Total	16 (76.2)*	20 (66.7)	12 (63.2)
Vomiting	0–5	2 (13.3)	1 (5.0)	1 (8.3)
	6–11	9 (60.0)	11 (55.0)	4 (33.3)
	12–17	2 (13.3)	6 (30.0)	5 (41.7)
	18–23	2 (13.3)	2 (10.0)	2 (16.7)
	Total	15 (71.4)†	20 (66.7)	12 (63.2)
Any dehydration	0–5	0 (0.0)	0 (0.0)	0 (0.0)
	6 – 11	1 (50.0)	0 (0.0)	0 (0.0)
	12 – 17	1 (50.0)	0 (0.0)	0 (0.0)
	18 – 23	0 (0.0)	0 (0.0)	0 (0.0)
	Total	2 (9.5)	0 (0.0)	0 (0.0)

*G2P[4] RDE was associated with fever as compared with G1P[8] RDE, $P = 0.03$.
†G2P[4] RDE was associated with vomit as compared with G1P[8] RDE, $P = 0.06$.

to occur in 26% (12/47) in infants during their first 6 months of life, while G1 was prevalent [45% (18/40)] in the next 6 months of life and G9 [43% (12/28)] dominates in the 12–17 months of life. The predominance of G2 genotype in the current study demonstrates the variability of rotavirus strains in Egypt over the course of time when compared with earlier studies in Cairo[22] and Abu Homos.[26] During 1992–1993, Radwan[22] identified G1 (17%), G4 (18%) and mixed genotypes (16%) as the most common genotypes in Cairo. After 2 years, Naficy[26] reported genotypes G1 and G2 with a combined prevalence of 89% in Abu Homos.

This study confirms the earlier report that 17% of 272 children in Egypt experienced >1 symptomatic rotavirus episode.[26] In addition, this study clearly identifies the introduction and emergence of G9 strains as an important type circulating in Egypt, and the absence of G4 strains that circulated previously—both consistent with global trends of strain diversity and distribution.

Human G9 strains have been identified on all human inhabited continents after being first described in 1983 in children with gastroenteritis in Philadelphia.[31] The first report of the G9 strains causing disease in older children and severe disease was documented in the United Kingdom and Nigeria.[32,33] Most strain surveillance, however, is hospital based, with few studies describing the distribution of strains in the community. A prospective and community-based study conducted on children from Latin America indicated the high incidence of G9 strains in the community with a broad spectrum of rotavirus disease severity as measured by wide range of numerical scores from 2 to 20 on the Vesikari clinical score.[34] As a newly emerging human pathogen, better understanding of the association between disease severity and G9 strains should be elucidated from hospital-based studies.

To our knowledge, G9 was not detected in a recent hospital-based study examining enteric viruses conducted in Cairo,[35] nor in an earlier cohort of children assembled from 2 villages near Abu Homos during 1995–1996.[30] The first description of G9 strains in Egypt in 2002 indicate that this strain has also emerged in Egypt as in other locations.[17] The emergence of new rotavirus strains exhibits the dynamic evolution of human rotaviruses and may provide challenges for the efficacy and design of new rotavirus vaccines worldwide.

In this study, a few apparently "reassortant" viruses were identified, which carried a G1P[4] background. Most human rotaviruses show a strong association of the G1 serotype with a P[8] VP4 genotype, and the P[4] genotype is normally determined in viruses

TABLE 5. Distribution of G Types for the First and Second Episodes of Rotavirus-Associated Diarrhea Among 28 Children, Abu Homos, 2004–2007

Genotype First Episode	Second Rotavirus Episode						
	Rotavirus Genotypes n (%)						
	G1	G2	G3	G9	G12	UT	Total*
G1	1 (25)	3 (75)	0	0	0	0	**4**
G2	0	3(30)	0	5 (50)	0	2 (20)	10
G3	0	2 (100)	0	0	0	0	2
G9	1 (20)	3 (60)	0	1 (20)	0	0	5
G12	0	1 (100)	0	0	0	0	1
UT	1 (25)	1 (25)	0	1(25)	0	1(25)	4
ND (n = 2)	1 (50)	1 (50)	0	0	0	0	2

G2 was the predominant type causing 50% (14/28) of the second rotavirus episode in 64% of children >18 month of age; 43% (6/14) of these episodes were associated with fever and vomiting.

The mean duration between the first and second episodes due to G2 type was 10 months, while the mean duration between the first and second episodes due to G1 and G9 was 1 and 7 months, respectively.

*Total number of genotype associated with the first rotavirus diarrheal episode.

with a G2 VP7 serotype.[36] Reassortments between naturally cocirculating strains that coinfect 1 host, while apparently rare, have been recorded to occur and drive the molecular evolution of "new" strains.[37] The high rate of mixed rotavirus infection identified in the current study is considered a prerequisite for the occurrence of rotavirus reassortment events, that may lead to the appearance of new strains and new variants. The increased diversity in rotavirus strains and the high rate of mixed infections have defined a profile characteristic of developing countries, as previously described in studies conducted in Tunisia, Brazil and Bangladesh.[38–40]

It was possible to determine age-specific genotype variation in the second episode of rotavirus diarrhea, where 50% of those attacking children > 1 year. Age-specific variation in the pathogenesis has been reported.[41] The observation that the same genotype was only present in few numbers of the second episodes differs from the first study conducted from Egypt[7] and indicates a possible role of genotype-specific natural immunity.

Some limitations of this study include the lack of serum antibody data to help indicate genotype-specific immunity developed from the first rotavirus episodes. In addition, rotavirus cases were detected based on testing of stool specimens by enzyme immunoassay, which although widely used, may have missed low levels of rotavirus excretion. The fact that we only used passive recruitment of diarrhea cases may mean that we missed occurrences of rotavirus excretion as was observed in a recent study in India.[42] Finally, the current study did not include and genotype the RDE that were coinfected with other enteric copathogen (in the first and second RDE) to avoid confusion with causality when another pathogen was involved. We had a large number of P untypeable strains (42%). However, this was not surprising since the big bulk of these strains (n = 52) have identified G type and most probably we expect that these strains may possess variants that were not picked due to primer mismatch with the majority belonging to G2 and G9.

In view of the common occurrence of multiple infections caused by G2 and G9 types in sequential rotavirus infection, G1 infections may be associated with the development of a greater degree of immunity to subsequent other rotavirus strains. The high rate of occurrence of these globally spread genotype infections early in life supports the high value of introducing current vaccines that are composed of these genotypes into the national immunization schedule.

ACKNOWLEDGMENTS

The authors are grateful to CAPT Jesse Monestersky for his review and critique of this article.

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