Molecular Epidemiology and Surveillance of Circulating Rotavirus and Adenovirus in Congolese Children With Gastroenteritis

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Infectious Diarrhea caused by rotavirus and adenovirus, is a leading cause of death in children in sub-Sahara Africa but there is limited published data on the diverse rotavirus genotypes and adenovirus serotypes circulating in the Republic of Congo. In this study, we investigated the prevalence of severe diarrhea caused by rotavirus A (RVA) and Adenovirus serotype 40 and 41 in Congolese children hospitalized with severe gastroenteritis. Stool samples were collected from 655 Congolese children less than 60 months of age hospitalized with acute gastroenteritis between June 2012 and June 2013. Rotavirus and adenovirus antigens were tested using commercially available ELISA kits and the RVA G- and P- genotypes were identified by seminested multiplex RT-PCR. Three hundred and four (46.4%) children were tested positive for RVA. Adenovirus infection was found in 5.5% of the 564 tested children. Rotavirus infection was frequently observed in children between 6–12 months (55.9%). The dry season months recorded increased RVA infection while no seasonality of adenovirus infection was demonstrated. The most common RVA genotypes were G1 (57.5%), G2 (6.4%), G1G2 mixture (15.5%), P[8] (58%), P[6] (13.2%), and P[8]P[6] mixture (26%). Additionally, the genotype G12P[6] was significantly associated with increased vomiting. This first study on Congolese children demonstrates a high prevalence and clinical significance of existing rotavirus genotypes. Adenovirus prevalence is similar to that of other Central African countries. This baseline epidemiology and molecular characterization study will contribute significantly to the RVA surveillance after vaccine implementation in the country. ***J. Med. Virol. 9999:XX–XX, 2015.***

KEY WORDS: adenovirus; gastroenteritis; genotypes; Republic of Congo

INTRODUCTION

Rotavirus and adenovirus infections are the leading cause of severe gastroenteritis in children and accounts for at least 4,50,000 deaths per year in children under 5 years of age worldwide [Parashar et al., 2003; Sdiri-Loulizi et al., 2009; Tate et al., 2012]. In the Republic of Congo, Diarrhea causes 7.5% of deaths in children in the same age group [Liu et al., 2015]. The majority of rotavirus-related deaths occur in sub-Saharan Africa, India, and Pakistan [Tate et al., 2012]. Rotavirus A (RVA) is one of the eight groups (Rotavirus A to H), and is also the most common group responsible for endemic human disease [Bass et al., 2007].

RVA strains are classified based on genetic distances and corresponding differences in the antigenic epitopes of VP7 (G types- glycoprotein) and VP4

Grant sponsor: Total E&P Congo

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The authors declare that they have no competing interests.

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Accepted 10 September 2015

DOI 10.1002/jmv.24382
Published online in Wiley Online Library (wileyonlinelibrary.com).

(P types—protease-sensitive), genes. The most commonly occurring G and P genotypes of RVA include G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] [Santos and Hosino, 2005; Matthijnssens et al., 2009; Banyai et al., 2012]. In addition, the less prevalent G and P genotypes including G5, G6, G8, G10, G12, P[6], P[9], P[11], P[14], and P[19] have also been reported [Banyai et al., 2012].

Since 2006, WHO has been supporting the African Rotavirus Surveillance Network to generate information on the burden of RVA diarrhea and the rotavirus genotype profile in children less than 5 years of age. The Republic of Congo was not included in this surveillance network [Mwenda et al., 2014] and limited data on the burden of RVA infection in this country were available when Rotarix® vaccine against RVA infection was introduced in 2014.

Adenoviruses organisms are non-enveloped, double stranded DNA viruses that mostly infect children under the age of two [Wiethoff and Nemerow, 2015]. Serotypes 40 and 41 adenoviruses have been linked to pediatric gastroenteritis [Motamedifar et al., 2013]. In sub-Saharan Africa, gastroenteritis caused by adenovirus is rampant, and the prevalence reported across the continent is between 1 and 8% [Moyo et al., 2014]. To date, there are no licensed vaccines against Adenovirus infection.

In this study conducted in the pediatric ward of the main hospital of Southern Brazzaville, we aim (i) to investigate the prevalence of diarrhea caused by rotavirus and adenovirus, and (ii) to assess the distribution of the respective virus genotypes and their relationship to the severity of the diarrheal disease in Congolese children. Findings from this investigation will provide the first data before the planned introduction of a vaccine against rotavirus infection in the country.

MATERIALS AND METHODS

Sample Collection

From June 2012 to June 2013, stool samples were collected from 655 children (n = 655) less than 60 months of age hospitalized with acute gastroenteritis at Makélékélé Hospital in the Southern part of Brazzaville, Republic of Congo. Adenovirus infection was determined in 564 of the children enrolled because of limited stool material after RVA investigation. The demographic data (such as date of birth, age, gender, body weight, and address), clinical data (such as episodes of diarrhea, vomiting, body temperature), and the information of rehydration therapy were also collected from case report forms and were represented in Table I. The mothers were asked if they breastfed or not. About 80% of mothers with a child less than 15 months provided a positive response. After informed written consent from parents, diarrheal stool was collected in a labeled clean screw-top container within 48 hr after hospital admission. The study was approved by the Institutional ethics committee of Foundation Congolaise pour la Recherche Médicale (FCRM).

Detection of RVA and Adenovirus by ELISA

All stool samples were tested for rotavirus and adenovirus antigens using the *ProSpecT*® Enzyme-linked Immuno-sorbent Assay (ELISA) kit (Oxoid, Cambridge, UK) according to the manufacturer's instructions.

RNA Extraction

The RVA antigen-positive stool specimens were further confirmed and characterized by reverse-transcriptase polymerase chain reaction (RT-PCR) or sequencing. Stool samples were suspended in 1 ml PBS to make approximately 10% solution. This solution was clarified by centrifugation at 13,000*g* for 5 min. The supernatants were transferred to new reaction tubes and the RNA extraction from the supernatants was performed using *QIAamp Viral RNA extraction* kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions.

RVA Genotyping by Seminested Multiplex RT-PCR

RNA extracted from rotavirus positive stool samples was denatured at 95°C for 60 sec and subjected to genotyping by seminested multiplex RT-PCR using primers from the European Regional Rotavirus Laboratory [WHO, 2009]. In the first round RT-PCR, the reverse transcription and amplification of VP7 and VP4 genes was performed in two separate assays with gene specific primers [WHO, 2009] using *Qiagen One Step RT-PCR* kit (QIAGEN GmbH, Hilden, Germany).

Adenovirus DNA Extraction

All adenovirus positive stool samples from ELISA screening were selected for genomic DNA extraction using *QIAamp Fast DNA Stool* Mini kit (*QIAGEN* GmbH, Hilden, Germany), according to the manufacturer's instructions. The extracted DNA was stored at −20°C until used for identification of adenovirus species A and F (serotypes 40 and 41).

Adenovirus Genotyping for Identification of Species A and F (Serotypes 40 and 41)

For detection of adenovirus species A, a singleplex PCR assay was performed by using primers to the fiber gene (Table I) as previously described [Gu et al., 2003]. Briefly, PCR was performed in 50 ml volumes containing 45 ml of reaction mixture and 5 ml of DNA extract. The amplification reaction was carried out in Biometra Professional thermocycler (Vers. 09/07) with (i) a preliminary denaturation for 5 min at 94°C, followed by (ii) 30 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 45 sec, and primer

TABLE I. Sequences of Primers and Probes Used for Identification of Species A and F (Serotypes 40 and 41) of Adenoviruses

Assay	Primer or probe	Sequence	Tm or band size	Reference
PCR	Fw primer (AdVA)	GCTGAAGAAMCWGAAGAAAATGA	1444–1537 bp	Gu et al., 2003
	Rv primer (AdVA)	CRTTTGGTCTAGGGTAAGCAC		
FRET	Fw primer (AdVF40-41)	AACTTTCTCTCTTAATAGACGCC	57°C	Jothikumar et al., 2005
	Rv primer (AdVF40-41)	AGGGGGCTAGAAAACAAAA	62°C	
	Anchor probe[a] (−)	GCGAAGAGTGCCCGTGTCAG	62°C	
	Detection probe[b] (−)	CAAGAGGTGCAgCACTTtGAA	69°C	

[a]The anchor probe was fluorescein labeled at the 5' end with LightCycler (LC) fluorescein and phosphorylated at the 3' end to block extension and has sequence complementarity to both AdV40 and AdV41.
[b]The detection probe was labeled at the 3' end with LC Red 640 and with a mismatch at two positions, 676 (T to C) and 682 (G to T) for AdV40; (−): Negative polarity.

extension at 72°C for 2 min and (iii) a final extension at 72°C for 5 min. Ten microliters of each reaction product was then visualized by SYBR Green staining and UV transillumination following electrophoretic separation on 1% agarose gels.

For detection and identification of adenovirus F species serotypes 40 and 41, real-time PCR assays were carried out in a LightCycler 480 system (Roche) by using fluorescence resonance energy transfer (FRET) probes that target the fiber gene. Primers and probes (Table II) as well as all reaction conditions were as described elsewhere [Jothikumar et al., 2005].

Statistical Analysis

All analyses were performed using the SPSS v17 software and Intercooled STATA v11. The comparison of frequencies between positive and negative rotavirus groups was realized by Chi square or two-sided Fisher's exact test. The *t*-test for equality means was used to compare means between rotavirus versus no infection groups for quantitative variables. The multivariate regression was also employed to determine the possible association of rotavirus genotypes and severity of diarrheal disease and evaluate the effect of potential covariate factors such as clinical and demographic parameters. The significance was set at a *P*-value of less than 0.05.

RESULTS

Demographic and Clinical Characteristics of the Studied Cohorts

The main demographic and clinical characteristics such as age, gender, body weight, body temperature, vomiting, diarrhea, dehydration, and rehydration therapy for all the investigated Congolese children with gastroenteritis are presented in Tables II and III. Out of 304 (46.4%) children stool samples ELISA positive with RVA, the detection was up to 51% in the peak of dry season (August). We found that 55.9% of children aged from 6 to 11 months were positive with RVA followed by children in age group from 1 to 2 years (27.3%). Significantly fewer children with acute gastroenteritis were observed in the age group less than 3 months compared to other age groups. Also, we observed fewer children from 24 to 60 months with RVA gastroenteritis compared to children with non-RVA gastroenteritis in the same age group ($P = 0.003$) indicating that the diarrheal disease in children more than 2 years of age were mainly caused by other factors. We also observed that more children positive with RVA had fever with higher body temperature (>38°C) compared to children negative with rotavirus ($P = 0.0015$). The duration of vomiting and diarrhea in children with RVA gastroenteritis was significantly higher than that in children with non-rotavirus gastroenteritis ($P = 0.001$ and 0.04, respectively) indicating that RVA infection was associated with more severity of diarrheal disease.

Out of the 564 children tested for adenovirus infection, 31 (5.5%) were positive (Table III). Children older than 8 months of age were the most affected (28 out of 31). These older children were more at a risk for adenovirus infection (odds ratio = 7.42, 95% confidence interval, 2.23–24.69, $P = 0.001$). Our results did not show any statistical significance in terms of seasonality of adenovirus infection.

The RVA infection was significantly associated with seasonality as the number of children with rotavirus gastroenteritis was significantly increased during the dry season (from June to September and January to February) compared to the rainy season (from October to December and March to May) ($P < 0.0001$). The peak of RVA infection in the Republic of Congo was in June, July, August, and September whereas few number of rotavirus-associated diarrheal cases was detected in November, December, January, and February (Fig. 1). Of the 564 children in our study, 11 (1.95%) were co-infected with both rotavirus and adenovirus (Table IV).

Distribution of RVA Genotypes

Out of 304 stool samples collected rotavirus positive children, 219 samples were successfully genotyped by seminested multiplex RT-PCR. The distribution of detected RVA genotypes were presented in Table V.

TABLE II. Demographic and Clinical Characteristics of Congolese Children With RVA

Characteristics	ELISA positive n = 304 (%)	ELISA negative n = 351 (%)	*P*-value
Age (months)			
<3	9 (3.0)	11 (3.1)	NS
3–5	37 (12.2)	34 (9.7)	NS
6–11	170 (55.9)	168 (47.9)	NS
12–23	83 (27.3)	116 (33.0)	NS
24–60	5 (1.6)	22 (6.3)	0.003
Gender			
Male	178 (61.5)	204 (58.1)	
Female	125 (41.1)	146 (42.6)	NS
Missing	1 (0.4)	1 (0.3)	
Body weight (Mean ± sd)	7.6 ± 3.2	7.7 ± 1.9	NS
Body temperature			
<37	14 (4.6)	16 (4.6)	NS
≥37; <38	166 (54.6)	230 (65.5)	<0.0001
≥38; <39	113 (37.2)	90 (25.6)	0.0015
≥39	8 (2.6)	9 (2.6)	NS
Missing	3 (1.0)	6 (1.7)	
Vomiting			
Yes	292 (96.1)	337 (96)	NS
No	12 (3.9)	14 (4)	NS
Duration (Mean ± sd)	1.6 ± 1.4	1.3 ± 1.2	0.001*
Frequency in 24 hr (Mean ± sd)	3.1 ± 1.4	3.0 ± 1.5	NS*
Diarrhea			
Yes	199 (65.5)	265 (75.5))	
No	0	0	
Missing	105 (34.5)	86 (24.5)	
Duration (Mean ± sd)	1.3 ± 0.9	1.1 ± 0.6	0.04*
Frequency in 24 hr (Mean ± sd)	4.7 ± 1.7	4.6 ± 1.7	NS*
Dehydration			
Mild	16 (5.3)	20 (5.7)	
Moderate	267 (87.8)	311 (88.6)	NS
Missing	21 (6.9)	20 (5.7)	
Rehydration therapy			
Oral rehydration	304 (100)	349 (99.4)	NS
Intravenous fluids	277 (91.1)	301 (85.8)	NS
Season			
Rainy season	99 (32.6)	220 (62.7)	
Dry season	205 (67.4)	131 (37.3)	<0.0001

NS: No statistically significant.
*: *t*-test for equality means.

Six G genotypes including G1, G2, G8, G9, G10, and G12 were detected and we observed that the genotypes G1 and G2 were highly predominant (57.5% and 6.4%, respectively). The mixed G genotype accounts for 23.6%, of which the mixture of G1 and G2 accounts for 15.5%. The other less prevalent G genotypes included G9 and G10 (2.3% and 3.2%, respectively) and non-type G was observed to be 2.8%. Related to the distribution of P type, we observed only two P genotypes including P[6] and P[8], of which the types P[8] and P[6] and the mixture of those (P[6]P[8]) were highly predominant (58%,13.2%, and 26%, respectively). In addition, non-genotype P was also less frequent (2.8%). For the combination of G and P genotypes, we observed that the genotype G1P[8] was the most frequent rotavirus genotypes (49.3%) and followed by the genotype G2P[6] (5.5%). Mixed rotavirus genotypes accounted for 31.8%. The mixed genotype G1G2P[6]P[8] was observed with high frequency (12.8%), followed by genotype G1G12P[6][P8] with 4.1% and by genotype G1G2G12P[6][P8] with 0.8%.

Regarding the seasonal distribution of rotavirus genotype, we observed that the genotype G1 was highly circulating in the dry season (from June to September) whereas genotype G2 was only detected predominantly in June. The low frequent genotype G9 was found only in the rainy season (Fig. 2A). The genotypes P[6] and P[8] were also found predominantly in the dry season from June to September (Fig. 2B). In addition, we observed the differential distribution of rotavirus genotypes among different age groups (Fig. 3). However, no difference of rotavirus genotype distribution between males and females was observed (data not shown).

Significance of RVA and Adenovirus Genotypes in Diarrheal Outcomes

We investigated the significance of rotavirus and adenovirus genotype distribution in the clinical outcomes of gastroenteritis. The results showed that the children infected with genotype G12P[6] had significantly higher number of vomiting and diarrhea

TABLE III. Demographics and Clinical Characteristics of Congolese Children With Adenovirus

Characteristics	Patient with ELISA negative. N = 533	Patient with ELISA positive. N = 31	Crude OR (IC 95%)	*P*-value
Demographic				
Age (months) n(%)				
<6	78 (100.0)	0 (0.0)	—	
6–11	264 (93.6)	18 (6.4)	Baseline	
12–23	166 (93.3)	12 (6.7)	1.06 (0.49–2.26)	NS
24–60	25 (96.2)	1 (3.8)	0.59 (0.75–4.58)	NS
Gender n(%)				
Female	221 (94.0)	14 (6.0)	Baseline	
Male	310 (94.8)	17 (5.2)	0.87 (0.42–1.79)	NS
Missing	2	0		
Season n(%)				
Rainy season	306 (95.0)	16 (5.0)	Baseline	
Dry season	227 (93.8)	15 (6.2)	1.26 (0.61–2.61)	NS
Clinical				
Body weight(Mean ± sd)	7.71 ± 2.72	7.73 ± 2.40		NS*
Body temperature n(%)				
<37	25 (100.0)	0 (0.0)	—	
≥ 37; <38	326 (94.5)	19 (5.5)	Baseline	
≥ 38; <39	160 (94.1)	10 (5.9)	1.07 (049–2.35)	NS
≥39	14 (93.3)	1 (6.7)	1.22 (0.15–9.81)	NS
Missing	8	1		
Vomiting n(%)				
No	16 (94.1)	1 (5.9)	Baseline	
Yes	510 (94.4)	30 (5.6)	0.94 (0.12–7.33)	NS
Duration(Mean ± sd)	1.44 ± 1.33	1.67 ± 1.52		NS
Frequency in 24 hours (Mean ± sd)	2.99 ± 1.43	3.33 ± 1.42		NS*
Diarrhea n(%)				
No	0 (0.0)	0 (0.0)		
Yes	533 (94.5)	31 (5.5)		
Duration(Mean ± sd)	1.19 ± 0.79	1.32 ± 1.00		NS
Frequency in 24 hours (Mean ± sd)	4.66 ± 1.76	4.63 ± 1.25		NS*
Dehydration n(%)				
Mild	31 (91.2)	3 (8.8)	Baseline	
Moderate	470 (95.1)	24 (4.9)	0.53 (0.15–1.84)	NS
Missing	32	4		
Rehydration therapy (yes) n(%)				
Oral rehydration	523 (94.4)	31 (5.6)	—	NS
Intravenous fluids	470 (94.2)	29 (5.8)	1.91 (0.44–8.21)	NS

sd: standard deviation; a: Fisher exact test; NS: Not statistically significant.
*: student test.

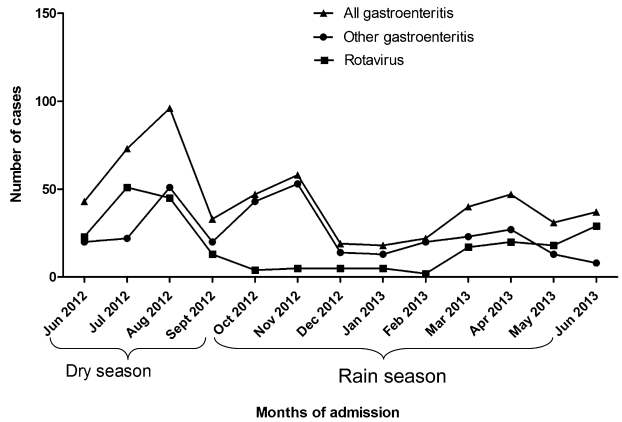

Fig. 1. Monthly distribution of RVA and all gastroenteritis in Congolese children less than 5 years of age.

episodes per 24 hr compared to those infected with major circulating genotype G1P[8] (Fig. 4). This result revealed that genotype G12P[6] was associated with more severity of diarrheal disease in Congolese children. There was no difference of other clinical and demographic parameters (vomiting, vomiting duration, diarrhea, diarrhea duration, dehydration, age, sex, body weight, temperature, and rehydration therapy) in children infected with different rotavirus genotypes (data not shown). Children with more than three vomiting episodes in the 24 hr following hospitalization were more likely to be infected with adenovirus (odds ratio = 2.11, 95% confidence interval, 1.01–4.46, $P = 0.04$).

The analyses were executed for different genotype comparisons with possible associations with severity of vomiting and diarrhea. Only the observation of G12P[6] genotype was significant enough and

TABLE IV. Patients Having Co-Infection

Characteristics	All patient	Patient with RVA	Patient with adenovirus	Patient with co-infection	*P*-value
Demographic					
Age (months) n(%)					
<6	78 (13.8)	34 (15.1)	0 (0.0)	0 (0.0)	
6–11	282 (50.0)	123 (54.4)	11 (55.0)	7 (63.6)	NS
12–23	178 (31.6)	64 (28.3)	8 (40.0)	4 (36.4)	
24–60	26 (4.6)	5 (2.2)	1 (5.0)	0 (0.0)	
Gender n(%)					
Male	235 (41.8)	90 (40.0)	8 (40.0)	6 (54.6)	
Female	327 (58.2)	135 (60.0)	12 (60.0)	5 (45.4)	NS
Missing	2	1			
Season n (%)					
Rainy season	322 (57.1)	160 (70.8)	8 (40.0)	8 (72.7)	
Dry season	242 (42.9)	66 (29.2)	12 (60.0)	3 (27.3)	<0.001
Clinical					
Body weight (Mean ± sd)	7.71 ± 2.71	7.71 ± 1.90	7.9 ± 2.20	7.4 ± 2.87	NS**
Vomiting n (%)					
No	17 (3.1)	6 (2.7)	0 (0.0)	1 (9.1)	
Yes	540 (96.9)	217 (97.3)	20 (100.0)	10 (90.9)	NS
Duration (Mean ± sd)	1.46 ± 1.35	1.60 ± 1.47	1.58 ± 1.47	1.82 ± 1.40	NS**
Frequency in 24 hours (Mean ± sd)	3.01 ± 1.70	3.06 ± 1.43	3.00 ± 1.54	3.91 ± 1.70	NS**
Diarrhea n (%)					
No	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Yes	564 (100.0)	226 (100.0)	20 (100.0)	11 (100.0)	
Duration (Mean ± sd)	1.19 ± 0.80	1.27 ± 0.99	1.05 ± 0.1	2.2 ± 1.79	0.011**
Frequency in 24 hours (Mean ± sd)	4.66 ± 1.74	4.82 ± 1.79	4.35 ± 1.15	5.4 ± 1.34	NS**
Dehydration n(%)					
Mild	34 (6.4)	15 (7.1)	3 (15.8)	0 (0.0)	
Moderate	494 (93.6)	197 (92.9)	16 (84.2)	8 (100.0)	NS
Missing	36	14	1	3	
Rehydration therapy (yes) n(%)					
Oral rehydration	554 (98.2)	224 (99.1)	20 (100.0)	11 (100.0)	NS
Intravenous fluids	499 (88.6)	205 (91.1)	18 (90.0)	11 (100.0)	NS

**: ANOVA test.
NS: No statistically significant.

contributed toward more of episodes of vomiting rather than diarrhea in comparison with G1P[8]. In all cases, multivariate regression was also employed and excluded the effects of potential covariate factors such as clinical and demographic parameters.

Distribution of Adenovirus Genotypes and Serotypes

Among the 31 adenovirus-positive children, the genotyping was successfully determined in 25 cases

TABLE V. Distribution of RVA Genotypes in Congolese Children With Gastroenteritis

	P Type n(%)				
G Type n(%)	P[6]	P[8]	Non type P	Mixed (P[6]P[8])	Total
G1	1 (0.5)	108 (49.3)	4 (1.8)	13 (5.9)	126 (57.5)
G2	12 (5.5)	1 (0.5)	0	1 (0.5)	14 (6.4)
G12	7 (3.2)	0	0	2 (0.9)	9 (4.1)
G9	0	5 (2.3)	0	0	5 (2.3)
G10	0	7 (3.2)	0	0	7 (3.2)
Non type G	0	3 (1,4)	1 (0.5)	2 (0.9)	6 (2.8)
Mixed	9 (4.1)	3 (1.4)	1 (0.5)	39 (17.8)	52 (23.6)
G1G2	4 (1.8)	1 (0.4)	1 (0.4)	28 (12.8)	34 (15.5)
G1G12	2 (0.9)	1 (0.4)	0	9 (4.1)	12 (5.5)
G1G8	1 (0.4)	0	0	0	1 (0.4)
G1G2G12	2 (0.9)	0	0	2 (0.8)	4 (2.8)
G1G8G12	0	1 (0.4)	0	0	1 (0.4)
Total	29 (13.2)	127 (58.0)	6 (2.8)	57 (26.0)	219 (100)

Mixed genotype G: G1G2, G1G6, G1G8, G1G2G6, G1G8G6.
Mixed genotype P: P[6]P[8].

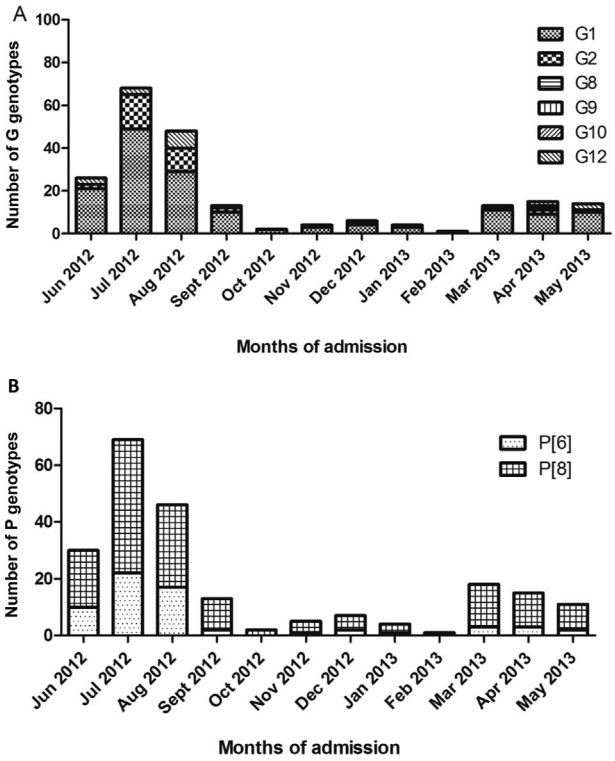

Fig. 2. Monthly distribution of RVA genotypes in Congolese children less than 5 years of age. Distribution of RVA G (**A**) and P genotypes (**B**).

(80.6%). For all 25 cases, only adenoviruses from species F were identified. Of these samples, 11 (36%) carried serotype 40 alone, 4 (16%) specimen contained serotype 41 alone and 10 (32%) contained both serotypes 40 and 41. No adenovirus of species A was identified.

DISCUSSION

In this study, we observed high diverse distribution of RVA genotypes and adenovirus serotypes circulating among Congolese children between 2012 and 2013. The African Rotavirus Surveillance Network was established in 2006 to create the database on the burden of rotavirus diarrhea in children less than 5 years of age in the continent, however, there was no surveillance for the burden of rotavirus gastroenteritis reported from the Republic of Congo [Mwenda et al., 2014]. Therefore our study appears to fill in a regional gap.

The proportion of acute gastroenteritis cases caused by RVA infection detected by ELISA in our study was 46.4% and this result was similar (49.4%) in a study conducted in Ghana from 2006 to 2011 [Enweronu-Laryea et al., 2014]. Other study also reported a higher rate of RVA detection in all gastroenteritis cases to be 56% and up to 95% in the peak of dry season in Nigeria [Tagbo et al., 2014]. From 2006 to 2012, the African Rotavirus Surveillance Network collected totally 38,950 stool samples from children less than 5 years of age hospitalized for acute diarrhea in more than 20 countries and overall 15,313 (40.7%) samples were confirmed positive for RVA by ELISA in 37,585 samples tested [Mwenda et al., 2014]. Our data show that the Republic of Congo is one of the sub-Saharan African countries with higher burden of rotavirus diarrhea compared to data reported by the African Rotavirus Surveillance Network. In other parts of the world, the annual proportions of rotavirus gastroenteritis in all acute gastroenteritis cases were 16–61% in the Middle Eastern and North Africa [Khoury et al., 2011], 22–63.5% in Europe [Ogilvie et al., 2011, 2012], 37.5% in Asia [Kawai et al., 2012], and 33% in Northern Brazil. In addition, our study also showed that the RVA infection was significantly associated with more severity of the diarrheal disease that indicated by more children had higher fever and longer duration of vomiting and diarrhea.

Adenovirus infection was detected in 5.5% of cases in our study. These results were similar to the ones of a similar study conducted in the neighboring country of Gabon where a prevalence of 6.3% for adenovirus infection was found [Lekana-Douki et al.,

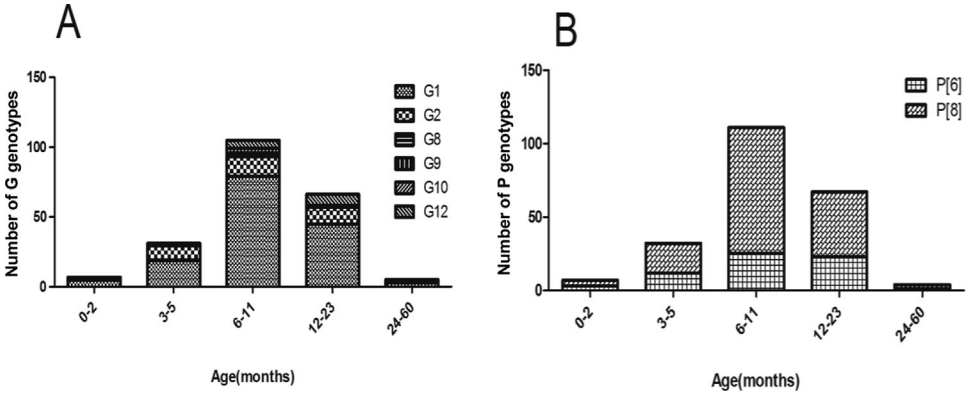

Fig. 3. Rotavirus A genotype distribution among different age groups. (**A**) Distribution of RVA G genotypes; (**B**) distribution of RVA P genotypes.

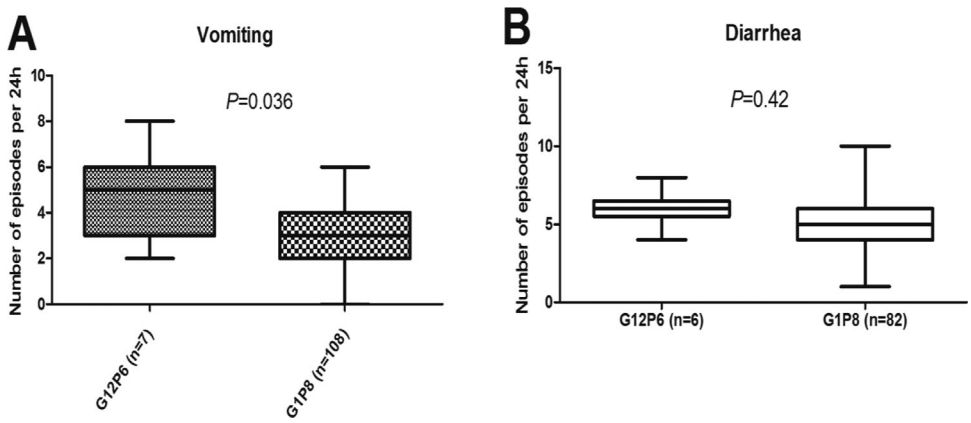

Fig. 4. Association of rotavirus genotypes and severity of diarrheal disease reflected by the number of episodes per 24 h (Frequency in 24 h). (**A**) Comparison of vomiting frequency in Congolese children infected with rotavirus genotype G12P[6] and those infected with rotavirus genotype G1P[8] (major genotype circulating in the Republic of Congo). (**B**) Comparison of diarrhea frequency in Congolese children infected with RVA genotype G12P[6] and those infected with rotavirus genotype G1P[8]. *P* values were calculated by multivariate regression analysis. The other clinical and demographic parameters including vomiting, vomiting duration, diarrhea, diarrhea duration, dehydration, age, sex, body weight, and body temperature were used as covariate factors.

2015]. Our results were slightly higher than those found in other parts of the world such as China where adenovirus positivity rate of 7.1% was also detected in a similar study [Lu et al., 2015]. This slight difference may be due to the fact that the Chinese study primers to detect Adenovirus A–F and retypes 40 and 41, as in our study, but also monitored for 51 serotypes [Lu et al., 2015].

Our study showed that most of RVA gastroenteritis cases were children less than 2 years of age and the main age group was from 6 to 11 months of age. Previous studies reported that RVA gastroenteritis accounted for more than 90% of all the acute gastroenteritis cases in children aged 3–24 months [Enweronu-Laryea et al., 2014; Tagbo et al., 2014; Tsolenyanu et al., 2014]. However, fewer gastroenteritis cases were observed in infants less than 3 months of age and this could be possibly explained that the infants had been receiving the immune protection from breast-feeding against gastrointestinal infections. Different from other sub-Saharan African countries like Nigeria, Togo, and Ghana [Enweronu-Laryea et al., 2014; Tagbo et al., 2014; Tsolenyanu et al., 2014], the peak of RVA infection in the Republic of Congo was from June to September which is the dry season. Similar seasonality was shown in a Gabonese study where rotavirus infection was more common during August and September [Lekana-Douki et al., 2015]. Our study did not show any statistical difference for the seasonal distribution of adenovirus infection, however, the study conducted in Gabon was able to show that adenovirus infection peaked between May and July as well as between February and March.

The distribution of RVA genotypes is widely diverse in different world regions and keeps changing [Kirkwood, 2010; Kawai et al., 2012; Afrad et al., 2013; Kwambana et al., 2014; Mwenda et al., 2014; Seheri et al., 2014].The genotype profile of RVA shown in the Republic of Congo was also diverse as reported from other countries in Africa, Asia, Europe, and South America [Khoury et al., 2011; Ogilvie et al., 2011, 2012; Kawai et al., 2012; da Silva et al., 2013; Kwambana et al., 2014; Mwenda et al., 2014]. Similar to previous study reported in sub-Saharan Africa [Mwenda et al., 2014], the genotype G1P[8] was the most frequent and the mixed genotypes G and P were also common in the Republic of Congo. In addition, the genotypes G2P[6] and G12P[6] were also found but not common. It appears that, more number of reported cases were during dry seasons, predominated by P6 and P8 genotypes in this season. This is unlikely to predict that, this shall the same P6 and P8 genotypes that may predominate also during rainy season, if large cases are reported. This largely may depend on viral subtypes in circulation, viral replication, and viral transmission ecology. Conversely, the G and P genotypes such as G3, G4, G8, and P[4], which were commonly found in other sub-Saharan African countries [Seheri et al., 2014], were not detected in the Republic of Congo. In addition, the genotype profile in the Republic of Congo also differed from those of a recent study reported a high genetic diversity of rotavirus strains circulating in 16 sub-Saharan African countries (Republic of Congo was not included) from 2007 to 2011 [Seheri et al., 2014]. Of which, the G and P combinations G2P[4], G3P[6], and G8P[6] were observed with high frequency (8.6%,4.3%, and 3.8%, respectively) in 16 studied African countries. Particularly, in Cameroon and the Democratic Republic of Congo (DRC) which are near by the Republic of Congo, the genotype G2P[4] and G8P[6] were also predominant (17% and 5%, respectively) [Seheri et al., 2014]. However, these genotypes (G2P[4], G3P[6], and

G8P[6]) were not detected in the Republic of Congo. Furthermore, we also detected the emerging genotype G9P[8] and rare G and P types such as G9 and G10.

A previous study conducted in Brazil, Mexico, and Venezuela indicated that RVA serotype G9 were significantly associated with more severity score, longer duration of vomiting and diarrhea, higher frequency of diarrhea, increased hospitalization rate, and more-severe dehydration [Linhares et al., 2006]. In addition, the genotype G2P[4] was also associated with more severe gastroenteritis compared to G1P[8] or G4P[8] in Italy [Cascio et al., 2001]. Our results, as in other central African studies [Lekana-Douki et al., 2015], also revealed that the rotavirus genotype G12P[6] contributed to a more severity of diarrheal disease in Congolese children indicating by a higher frequency of vomiting and diarrhea. The genotypes G3P[8] and G2P[4] were not found in the Republic of Congo while the genotype G9 was found with low frequency. Therefore, our finding of more severe gastroenteritis associated genotype (G12P[6]) could generate a vital information on the burden and outbreak of new emerging rotavirus strains.

As in previous studies, our results showed that adenovirus serotype 40 was more common (36%) while serotype 41 was detected in 13% of the cases. Using additional primers, other studies reported higher number of serotypes. For instance, in Gabon Adenovirus serotype 51 was found as it was in a study conducted in China [Lekana-Douki et al., 2015; Lu et al., 2015].

In conclusion, our study reported for the first time the burden of RVA and adenovirus gastroenteritis in Congolese children less than five years of age and provided the important pattern of genotype distribution for these enteric viruses in the Republic of Congo. The adenovirus serotypes found were similar to those found in other places in Central Africa. The rare G and P genotypes such as G9 and G10 were detected in Congolese children and G12P[6] genotype was associated with more severity of gastroenteritis.. The prevalence and/or emergence of unusual or novel RVA strains such as the G12P[6] and non P[8] genotypes are of interest. This finding highlights the need for long-term surveillance of RVA strains in the Republic of Congo, especially after vaccine implementation in the country.

CONTRIBUTORS

FN wrote proposal for funding, overall supervision of the study from study design to finalization of the manuscript. GM collected samples, performed the lab experiments, and data analysis; AS was involved in the study de, development and implementation of the lab techniques, supervision of the hospital study and wrote the manuscript; VM performed the lab experiments and data analysis; JCV was responsible for data entry and data analysis. SK and VT participated in writing manuscript. All the authors approved the final version.

ACKNOWLEDGMENTS

We are grateful to the patients who participated in this study. FN, JCV, GM, TPV are members of CANTAM Network (Central Africa Network on Tuberculosis, HIV/AIDS and Malaria). We thank PD Dr. Philipp Zanger for assistance in study design and implementation of the study, the Robert Koch Institute (Germany) for helpful advices in the development of lab techniques and Dr Tong for assistance in paper writing. This work was funded through a grant provided by the company Total E&P Congo.

REFERENCES

Afrad MH, Hassan Z, Farjana S, Moni S, Barua S, Das SK, Faruque AS, Azim T, Rahman M. 2013. Changing profile of rotavirus genotypes in Bangladesh, 2006–2012. BMC Infect Dis 13:320.

Banyai K, Laszlo B, Duque J, Steele AD, Nelson EA, Gentsch JR, Parashar UD. 2012. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: Insights for understanding the impact of rotavirus vaccination programs. Vaccine 30:A122–A130.

Bass ES, Pappano DA, Humiston SG. 2007. Rotavirus. Pediatr Rev 28:183–191.

Cascio A, Vizzi E, Alaimo C, Arista S. 2001. Rotavirus gastroenteritis in Italian children: Can severity of symptoms be related to the infecting virus? Clin Infect Dis 32:1126–1132.

da Silva SL, de Fátima Dos Santos Guerra, do Socorro Lima de Oliveira A, da Silva Dos Santos F, de Fátima Costa de Menezes EM, Mascarenhas JD, Linhares AC. 2013. Diversity of rotavirus strains circulating in Northern Brazil after introduction of a rotavirus vaccine: High prevalence of G3P[6] genotype. J Med Virol 86:1065–1072.

Enweronu-Laryea CC, Sagoe KW, Mwenda JM, Armah GE. 2014. Severe acute rotavirus gastroenteritis in children less than 5 years in southern Ghana: 2006–2011. Pediatr Infect Dis J 33: S9–S13.

Gu Z, Belzer SW, Gibson CS et al. 2003 Multiplex, realtime PCR for quantitative detection of human adenovirus. Journal of Clinical Microbiology 41:4636–4641.

Jothikumar N, Cromeans TL, Hill VR, Lu X, Sobsey MD, Erdman DD. 2005. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 an 41. Appl Environ Microbiol 71:3131–3136.

Kawai K, O'Brien MA, Goveia MG, Mast TC, El Khoury AC. 2012. Burden of rotavirus gastroenteritis and distribution of rotavirus strains in Asia: A systematic review. Vaccine 30:1244–1254.

Khoury H, Ogilvie I, El Khoury AC, Duan Y, Goetghebeur MM. 2011. Burden of rotavirus gastroenteritis in the Middle Eastern and North African pediatric population. BMC Infect Dis 11:9.

Kirkwood CD. 2010. Genetic and antigenic diversity of human rotaviruses: Potential impact on vaccination programs. J Infect Dis 202:S43–S48.

Kwambana BA, Ikumapayi UN, Sallah N, Dione M, Jarju S, Panchalingham S, Jafali J, Lamin M, Betts M, Adeyemi M, Akinsola A, Bittave O, Jasseh M, Kotloff KL, Levine MM, Nataro JP, Corrah T, Hossain MJ, Saha D, Antonio M. 2014. High genotypic diversity among rotavirus strains infecting gambian children. Pediatr Infect Dis J 33:S69–S75.

Lekana-Douki SE, Kombila-Koumavor C, Nkoghe D, Drosten C, Drexler JF, Leroy EM. 2015. Molecular epidemiology of enteric viruses and genotyping of rotavirus A, adenovirus, and astrovirus among children under 5 years old in Gabon. Int J Infect Dis 34:90–95.

Linhares AC, Verstraeten T, Wolleswinkel-van den Bosch J, Clemens R, Breuer T. 2006. Rotavirus serotype G9 is associated with more-severe disease in Latin America. Clin Infect Dis 43: 312–314.

Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE. 2015. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: An updated systematic analysis. Lancet 385:430–440.

Lu L, Jia R, Zhong H, Xu M, Su L, Cao L, Dong Z, Dong N, Xu J. 2015. Molecular characterization and multiple infections of rotavirus, norovirus, sapovirus, astrovirus, and adenovirus in outpatients with sporadic gastroenteritis in Shanghai, China, 2010–2011. Arch Virol 160:1229–1238.

Matthijnssens J, Bilcke J, Ciarlet M, Martella V, Bányai K, Rahman M, Zeller M, Beutels P, Van Damme P, Van Ranst M. 2009. Rotavirus disease and vaccination: Impact on genotype diversity. Future Microbiol 4:1303–1316.

Motamedifar M, Amini E, Talezadeh SP. 2013. Frequency of rotavirus and adenovirus gastroenteritis among children in Shiraz, Iran. Iran Red Crescent Med J 15:729–733.

Moyo SJ, Hanevik K, Blomberg B, Kommedal O, Nordbø SA, Maselle S, Langeland N. 2014. Prevalence and molecular characterisation of human adenovirus in diarrhoeic children in Tanzania: A case control study. BMC Infect Dis 14:666.

Mwenda JM, Tate JE, Parashar UD, Mihigo R, Agócs M, Serhan F, Nshimirimana D. 2014. African rotavirus surveillance network: A brief overview. Pediatr Infect Dis J 33:S6–S8.

Ogilvie I, Khoury H, El Khoury AC, Goetghebeur MM. 2011. Burden of rotavirus gastroenteritis in the pediatric population in Central and Eastern Europe: Serotype distribution and burden of illness. Hum Vaccin 7:523–533.

Ogilvie I, Khoury H, Goetghebeur MM, El Khoury AC, Giaquinto C. 2012. Burden of community-acquired and nosocomial rotavirus gastroenteritis in the pediatric population of Western Europe: A scoping review. BMC Infect Dis 12:62.

Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. 2003. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 9:565–572.

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

Sdiri-Loulizi K, Gharbi-Khelifi H, de Rougemont A, Hassine M, Chouchane S, Sakly N, Pothier P, Guédiche MN, Aouni M, Ambert-Balay K. 2009. Molecular epidemiology of human astrovirus and adenovirus serotypes 40/41 strains related to acute diarrhea in Tunisian children. J Med Virol 81:1895–1902.

Seheri M, Nemarude L, Peenze I, Netshifhefhe L, Nvaga MM, Ngobeni HG, Maphalala G, Maake LL, Steele AD, Mwenda JM, Mphahlele JM. 2014. Update of rotavirus strains circulating in Africa from 2007 through 2011. Pediatr Infect Dis J 33:S76–S84.

Tagbo BN, Mwenda JM, Armah G, Obidike EO, Okafor UH, Oguonu T, Ozumba UC, Eke CB, Chukwubuike C, Edelu BO, Ezeonwu BU, Amadi O, Okeke IB, Nnani OR, Ani OS, Ugwuezeonu I, Benjamin-Pujah C, Umezinne N, Ude N, Nwodo C, Ezeonyebuchi MC, Umesie E, Okafor V, Ogude N, Osarogborum VO, Ezebilo SK, Goitom WG, Abanida EA, Elemuwa C, Nwagbo DF. 2014. Epidemiology of rotavirus diarrhea among children younger than 5 years in Enugu, South East, Nigeria. Pediatr Infect Dis J 33:S19–S22.

Tsolenyanu E, Seheri M, Dagnra A, Djadou E, Tigossou S, Nvaga M, Adjeoda E, Armah G, Mwenda JM, Atakouma Y. 2014. Surveillance for rotavirus gastroenteritis in children less than 5 years of age in Togo. Pediatr Infect Dis J 33:S14–S18.

Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2012. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: A systematic review and meta-analysis. Lancet Infect Dis 12: 136–141.

WHO. 2009. Manual of rotavirus detection and characterization methods. Available online at: http://whqlibdoc.who.int/hq/2008/who_ivb_08.17_eng.

Wiethoff CM, Nemerow GR. 2015. Adenovirus membrane penetration: Tickling the tail of a sleeping dragon. Virology 479-480: 591–599.