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Human G9P[8] rotavirus strains circulating in Cameroon, 1999–2000: Genetic relationships with other G9 strains and detection of a new G9 subtype

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ABSTRACT

Group A rotaviruses (RV-A) are the leading cause of viral gastroenteritis in children worldwide and genotype G9P[8] is one of the five most common genotypes detected in humans. In order to gain insight into the degree of genetic variability of G9P[8] strains circulating in Cameroon, stool samples were collected during the 1999–2000 rotavirus season in two different geographic regions in Cameroon (Southwest and Western Regions). By RT-PCR, 15 G9P[8] strains (15/89 = 16.8%) were identified whose genomic configurations was subsequently determined by complete or partial gene sequencing. In general, all Cameroonian G9 strains clustered into current globally-spread sublineages of the VP7 gene and displayed 86.6–100% nucleotide identity amongst themselves and 81.2–99.5% nucleotide identity with global G9 strains. The full genome classification of all Cameroonian strains was G9-P[8]-I1–R1–C1–M1–A1–N1–T1–E1–H1 but phylogenetic analysis of each gene revealed that the strains were spread across 4 or more distinct lineages. An unusual strain, RVA/Human-wt/CMR/6788/1999/G9P[8], which shared the genomic constellation of other Cameroonian G9P[8] strains, contained a novel G9 subtype which diverged significantly (18.8% nucleotide and 19% amino acid distance) from previously described G9 strains. Nucleotide and amino acid alignments revealed that the 3′ end of this gene is highly divergent from other G9 VP7 genes suggesting that it arose through extensive accumulation of point mutations. The results of this study demonstrate that diverse G9 strains circulated in Cameroon during 1999–2000.

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1. Background

Childhood mortality has been declining worldwide as a result of socioeconomic development and implementation of prevention and survival interventions (Claeson et al., 2000). Group A rotaviruses (RV-A) are the main etiologic agent of acute gastroenteritis in infants and young children worldwide (Estes and Kapikian, 2007) and an estimated 453,000 children aged <5 years die from rotavirus diarrhea each year, with >85% of these deaths occurring in low-income countries of Africa and Asia (Parashar et al., 2009; Tate et al., 2011). Rotaviruses belong to the family *Reoviridae*, and the rotavirus genome consists of 11 double-stranded RNA gene segments that encode six structural (VP) and six non-structural proteins (NSP). Based on the two genes that encode the outer capsid proteins, VP4 (P-type) and VP7 (G-type), a widely used binary classification system was established for RV-A (Estes and Kapikian, 2007). This system has been recently standardized and extended to all 11 genes (Matthijnssens et al., 2008b). To date, at least 27 G, 35

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P, 16 I, 9 R, 9 C, 8 M, 16 A, 9 N, 12 T, 14 E and 11 H genotypes have been identified based on the eleven rotavirus A genes (Esona et al., 2010b; Matthijnssens et al., 2011). In humans, at least five RV-A G types (G1–G4 and G9), and two common P types (P[8] and P[4]) circulate worldwide (Banyai et al., 2012; Gentsch et al., 2005; Santos and Hoshino, 2005). G9 strains emerged in 1990s, and there has been a global description of the appearance and dominance of this genotype (Gentsch et al., 2005; Laird et al., 2003; Matthijnssens et al., 2009; Santos and Hoshino, 2005). Genotype G9 strains with a Wa-like or a DS-1-like genomic configuration or a mixture thereof have been detected sporadically in localized outbreaks (Page et al., 2010). In Cameroon, the first molecular identification of genotype G9 in human samples was reported in a study conducted by Steele and colleagues in 2003 (Steele and Ivanoff, 2003).

At least seven major phylogenetic lineages and eleven minor lineages within G9 VP7 genes have been described (Phan et al., 2007; Wu et al., 2011). A molecular evolutionary analysis study utilizing Bayesian inference supported the idea that one single sub-lineage introduced in the 1980s was responsible for all the worldwide spread of G9 in the 1990s (Matthijnssens et al., 2010).

In order to gain insight into the degree of genetic variability of G9P[8] strains circulating in Cameroon, Central Africa, sequence determination and phylogenetic analysis of all eleven genome segments from G9P[8] RV-A strains detected in two different geographic regions of Cameroon (Southwest and Western Regions) was performed in order to infer the genetic relationship of Cameroonian strains with G9P[8] worldwide. The results of these studies revealed a new G9 genetic variant circulating in Cameroon during the 1999–2000 rotavirus seasons.

2. Material and methods

2.1. Fecal samples, strains and nomenclature

Fifteen diarrheic stool specimens collected from children <5 years of age, genotyped as G9P[8] (Esona et al., 2010a), were obtained during the 1999–2000 rotavirus season in two different geographic regions in Cameroon (Southwest and Western Regions). The strains and nomenclature are shown in Table 1.

2.2. Viral RNA extraction, amplification, and sequencing

Viral RNA from each of the 15 specimens was extracted from a 10% stool suspension made from 0.1 g or 100 μl stool in 2 ml of a 1:1 Vertrel/Water solution using either a commercial RNA extraction kit (NucliSens automated extractor, BIOMERIEUX, Durham, NC) according to the protocol specified by the manufacturer or a silica binding method described previously (Boom et al., 1990).

Previously published forward and reverse primers (Das et al., 1994; Gentsch et al., 1992; Iturriza-Gomara et al., 2001, 2002; Kerin et al., 2007; Matthijnssens et al., 2006; Mijatovic-Rustempasic et al., 2011) were used for the amplification of the different gene segments. The extracted dsRNA of each strain was denatured at 97 °C for 5 min and RT-PCR was carried out using a one step RT-PCR kit (Qiagen, Inc., Valencia, CA) according to manufacturer's instructions. Reverse transcription (RT) of each gene from each sample was carried out for 30 min at 42 °C, followed by 15 min at 95 °C to inactivate the reverse transcriptase and activate the Taq polymerase. The cDNA was then subjected to 35 cycles of PCR in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Inc., Foster City, CA) using the following conditions: 30 s at 94 °C; 30 s at 42 °C; 45–90 s at 72 °C (depending upon the expected size of the amplified gene fragment), followed by a 7 min final extension at 72 °C. Amplicons were analyzed by gel electrophoresis in 1% SeaKem agarose gels (Thermo Fisher Scientific, Inc., Waltham, MA) then excised and purified with the QIAquick Gel Extraction kit (Qiagen, Inc., Valencia, CA) following the manufacturer's instructions.

DNA cycle sequencing of each amplicon was performed with the same consensus primers used for RT-PCR, using a Big Dye Terminator cycle sequencing Ready kit v1.1 (Applied Biosystems, Inc., Foster City, CA). Previously published primers homologous to internal regions of each gene segment were also used (Mijatovic-Rustempasic et al., 2011). Cycle sequencing products were purified using Centri-sep spin columns (Princeton Separations, Inc., Adelphia, NJ), dried in a DNA speed Vac^{R} (Savant Instruments, Inc., Holbrook, NY) and reconstituted in 15 ml Hi-Di formamide. Automated separation and base-calling of cycle sequencing products was performed using an ABI 3130xl sequencer (Applied Biosystems, Foster City, CA). Overlapping sequence fragments were assembled and edited using Sequencher 4.8 (Gene Codes Corporation, Inc., Ann Arbor, MI).

Table 1
Characteristics of the Cameroon G9P[8] strains.

	Genotypes												
	VP7	Lineage	VP4	Lineage	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Length of gene sequenced (nucleotide)[a]	840		834		1191	1917	1367	1187	1043	948	930	525	591
Presence of ORF[b]	P		P		C	P	P	P	P	C	C	C	C
Strain name													
RVA/Human-wt/CMR/6735/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6778/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6779/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6788/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6791/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6796/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6807/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6806/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6795/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6777/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6790/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6792/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6793/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6805/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/CMR/6794/1999/G9P[8]	G9	III	P[8]	P[8]-3	I1	R1	C1	M1	A1	N1	T1	E1	H1

[a] Length of gene sequenced in nucleotides.
[b] P and C denotes partial or complete ORF, respectively.