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One Year Survey of Human Rotavirus Strains Suggests the Emergence of Genotype G12 in Cameroon

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Abstract

In this study the emergence of rotavirus A genotype G12 in children <5 years of age is reported from Cameroon during 2010/2011. A total of 135 human stool samples were P and G genotyped by reverse transcriptase PCR. Six different rotavirus VP7 genotypes were detected, including G1, G2, G3, G8, G9, and G12 in combinations with P[4], P[6] and P[8] VP4 genotypes. Genotype G12 predominated in combination with P[8] (54.1%) and P[6] (10.4%) genotypes followed by G1P[6] (8.2%), G3P[6] (6.7%), G2P[4] (5.9%), G8P[6] (3.7%), G2P[6] (0.7%), G3P[8] (0.7%), and G9P[8] (0.7%). Genotype P[6] strains in combination with various G-types represented a substantial proportion (N = 44, 32.6%) of the genotyped strains. Partially typed strains included G12P[NT] (2.2%); G3P[NT] (0.7%); G(NT)P[6] (1.5%); and G(NT)P [8] (0.7%). Mixed infections were found in five specimens (3.7%) in several combinations including G1 + G12P[6], G2 + G3P[6] + P[8], G3 + G8P[6], G3 + G12P[6] + P[8], and G12P [6] + P[8]. The approximately 10% relative frequency of G12P[6] strains detected in this study suggests that this strain is emerging in Cameroon and should be monitored carefully as rotavirus vaccine is implemented in this country, as it shares neither G- nor P-type specificity with strains in the RotaTeq and Rotarix vaccines. These findings are consistent with other recent reports of the global spread and increasing epidemiologic importance of G12 and P[6] strains.

Keywords

genotype; surveillance; RT-PCR

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INTRODUCTION

Group A rotaviruses are the most important etiological agents of acute gastroenteritis in infants and young children worldwide [Estes and Kapikian, 2007]. Current mortality estimates demonstrate that about 453,000 children <5 years of age die worldwide each year due to rotavirus [Tate et al., 2012]. Rotaviruses (family *Reoviridae*, genus *Rotavirus*) are icosahedral viruses with genome consisting of 11 segments of dsRNA [Attoui et al., 2012], encoding six structural viral proteins (VP1–VP4, VP6, and VP7) and six non-structural proteins (NSP1–NSP6) [Estes and Kapikian, 2007]. Due to the segmented nature of its genome, novel rotavirus strains can be produced in vivo by exchange of genome segments between two parental strains infecting the same cell, a process called reassortment [Ramig, 1997].

Traditionally, rotaviruses have been classified using a binomial nomenclature GxP[x] based upon serotype and genotype specificities of the outer capsid antigens, VP7 (G-type) and VP4 (P-type) [Estes and Kapikian, 2007]. Thus far, at least 27 G genotypes and 35 P genotypes have been detected from humans and animals [Abe et al., 2009; Esona et al., 2009; Schumann et al., 2009; Solberg et al., 2009; Ursu et al., 2009; Matthijnssens et al., 2011]. Five G genotypes (G1, G2, G3, G4, and G9), and two P genotypes (P[8] and P[4]) predominate worldwide, although genotype P[6] has been recognized as a common cause of diarrhea on several continents and has sometimes been described as a regionally common strain [Steele and Ivanoff, 2003; Gentsch et al., 2005; Santos and Hoshino, 2005; Bányai et al., 2012]. A large number of rare or regionally common strains have been identified during surveillance in anticipation of vaccines introduction including G5, G6, G8, G9, G10, and G12 genotypes and P[1], P[3], P[6], P[9], P[11], P[14], P[19], and P[25] genotypes [Esona et al., 2004, 2009; Gentsch et al., 2005; Rahman et al., 2005; Bányai et al., 2007; Bányai et al., 2012; Castello et al., 2009; Cunliffe et al., 2009; Payne et al., 2009; Martella et al., 2010; Matthijnssens et al., 2010]. Studies from Africa reported high prevalence of genotypes G8 and P[6] in various combinations suggesting that both of these genotypes should be considered common in Africa. [Cunliffe et al., 1999; Armah et al., 2010]. Steele and Ivanoff [2003] concluded that the predominant strains circulating across Africa during 1996–1999 were G1P[6] and G3P[6] strains.

Many of the newly described genotypes are thought to be of animal origin, including G9 that emerged subsequently as globally common G9P[8] strains [Gentsch et al., 2005; Santos and Hoshino, 2005; Bányai et al., 2012]. While G9 strains were reported from the late 1990s in Africa, G12 strains were found spreading only from mid-2000s onward, in Cameroon, Ethiopia, Ghana, Malawi, South Africa, and Zimbabwe [Bányai et al., 2012] at low-to-moderate frequencies (<20%). However, reports from the majority of African countries is scanty and only one third of all countries in the WHO's African region reported any data during 1990–2006 [Todd et al., 2010; Bányai et al., 2012]. Although rotaviruses have been reported as an important etiological agent of diarrhea in Cameroon [Koulla-Shiro et al., 1995; Esona et al., 2003; Ndze et al., 2012], there is a need to obtain comprehensive baseline data on rotavirus genetic diversity for Cameroon in anticipation of the introduction of rotavirus vaccine [Esona et al., 2004, 2009, 2010; Armah et al., 2010].

Two rotavirus vaccines have been developed as effective interventions for severe diarrhea and mortality associated with rotavirus infection. The two live oral vaccines from Merck (RotaTeq, Whitehouse Station, NJ) and GlaxoSmithKline (Rotarix, Rixensart, Belgium) have been licensed in more than 100 countries and have been introduced into routine immunization programs in the United States and some countries in Latin America, Europe, Africa and Asia [Dennehy, 2008]. RotaTeq possesses genes encoding human rotavirus serotypes G1–G4 and P1A[8] on a bovine rotavirus background. This mixture of the five reassortant vaccine strains was designed to stimulate serotype-specific protection to these common rotavirus serotypes. In contrast, a G1P[8] vaccine, Rotarix, was constructed to induce both homotypic responses to G1P[8] strains and heterotypic protective immune responses against different serotypes.

It is considered important for countries considering vaccine introduction into their routine immunization programs to assess the need for the vaccine through disease burden studies. These studies provide baseline data on disease burden and strain prevalence to assess better the impact of the vaccination program on rotavirus disease and circulating strains, and to identify the possible emergence of serotypes that escape vaccine-induced immunity. In this study data on circulating genotypes are presented in the Far Northern (two sites) and North Western (two sites) regions of Cameroon during 2010–2011.

MATERIALS AND METHODS

Study Population and Specimens

In this study, rotavirus strains detected during 2010–2011 in two regions of Northern Cameroon [Ndze et al., 2012] were characterized. In brief, samples were collected from children <5 years of age who presented with acute diarrhea at the Regional Hospital Maroua and at the Domayo Djama integrated health center in the Far North region; and at the Regional hospital Bamenda and at the Esu integrated health centre in the North West region. The population is urban (Maroua and Bamenda) and rural (Esu) and the major occupation in the study area is subsistence agriculture supplemented with fishing and dairy farming in the Far North region.

The Far North is located at latitude 11°00′N and longitude 14°30′E characterized by tropical and Sahelian climates with rainfall ranging from 400 to 900 mm per year. The region is generally dry and hot with rains falling relatively more frequently in the Mandara region. The Far North has two seasons: one dry, and one wet. The climate in Maroua is of the Sahel type. Temperatures reach their highest levels from January to May. The North West region of Cameroon is located between latitude 5°20′ and 7°10′N and longitudes 9°4′ and 11°15′E with altitude range of 300–3,000 m above sea level. Temperatures vary within the year between 10 and 28°C and mean annual rainfall is 2,000 mm, while relative humidity ranges from 49% to 87% [Bayemi et al., 2005].

RNA Extraction and Genotyping

This study included a subset of the rotavirus-positive stool specimens analyzed previously by a one-step reverse transcription polymerase chain reaction (RT-PCR) for VP6 protein

[Ndze et al., 2012]. Rotavirus dsRNA was extracted from 100 μl stool suspension with the Promega DNA & RNA Purification kit (Madison, WI) using an automated extractor [Gyuranecz et al., 2011]. The extracted dsRNA of each strain was denatured at 97°C for 5 min and then reverse transcription-PCR (RT-PCR) was carried out using a One-Step RT-PCR kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Previously published consensus primers [Gentsch et al., 1992; Das et al., 1994; Iturriza-Gomara et al., 2001] were used for the amplification of the VP4 and VP7 gene segments. Genotyping of the VP4 and VP7 genes was done by semi-nested RT-PCR assay [Gouvea et al., 1990; Gentsch et al., 1992; Das et al., 1994] using first round PCR products generated by consensus primers. For G- and P-type determination, a series of type-specific primers were utilized (including G1–G4, G8, G9, G10, and G12 primers and P[4], P[6], P[8], P[9], P[10], and P[11] primers [Gouvea et al., 1990; Gentsch et al., 1992; Iturriza-Gomara et al., 2000, 2001, 2004]. For a randomly selected subset of strains representing distinct genotypes sequencing was carried out to confirm G- and P-typing data obtained by the multiplex genotyping PCR assay. Sequencing was also utilized occasionally when samples remained non-typeable.

RESULTS

Prevalence of Individual G Types

A total of 144 samples were subjected to G genotyping by a multiplex RT-PCR method. After the first amplification step, PCR products for the VP7 gene were obtained for 135 (94%) of the specimens, and the remaining nine specimens yielded no visible first-round product. Only the 135 specimens with visible first-round amplification products were subjected to further analysis (Table I).

In all, a total of six different rotavirus VP7 genotypes were observed, including G1, G2, G3, G8, G9, and G12. The predominant genotype was G12 (67.4%), followed by G1 and G3 (8.1% each), G2 (6.6%), G8 (3.7%), and G9 (0.7%). Mixed infections (G1 + G12, G2 + G3, G3 + G8, and G3 + G12) were observed in four specimens (2.9%; Table I).

Prevalence of Individual P-Types

First round PCR products for the VP4 gene were obtained for 135 specimens. A total of three different VP4 genotypes (P[4], P[6], and P[8]) were observed. Genotype P[8] (56.3%) predominated followed by P[6] (32.6%) and P[4] (5.9%). Mixed infections P[6] + P[8] were found in three specimens (2.2%).

G–P Combinations

When G- and P-type data were combined the following genotype prevalence was observed: G12P[8] (54.1%), G12P[6] (10.4%), G1P[6] (8.2%), G3P[6] (6.7%), G2P[4] (5.9%), G8P[6] (3.7%), and G2P[6], G3P[8] plus G9P[8] (0.7% each). The following partial G–P combinations were identified; G12P[NT] (2.2%); G3P[NT] (0.7%); GNTP[6] (1.5%); and GNTP[8] (0.7%). Mixed infections were found in five specimens (3.7%) and presented with these combinations: G1 + G12P[6], G2 + G3P[6] + P[8], G3 + G8P[6], G3 + G12P[6] + P[8], and G12P[6] + P[8].

Regional Distribution of Most Common Rotavirus Strains

The G–P combination analysis by site showed that G12P[8] (54%) dominated in Esu followed by G8P[6] (31%), while in Bamenda G2P[4] (38%) dominated, followed by G12P[6] (33%) and G12P[8] (29%), and in Maroua G12P[8] (59%) dominated, followed by G1P [6] (11%) and G3P[6] (9%; Fig. 1). Mixed infections were detected only in Maroua.

DISCUSSION

The objective of this study was to identify the circulating rotavirus genotypes from infected hospitalized and outpatient children <5 years of age in Cameroon. Previous studies from Cameroon reported the circulation of G1–G5, G8, G9, and G12 VP7 types as well as P[4] and P[6]–P[10] VP4 types during the 1996–2000 and the 2007–2008 surveillance period [Esona et al., 2004, 2009, 2010; Armah et al., 2010; Mwenda et al., 2010], although G12 strains were found only in 11% of samples during 2007–2008 [Mwenda et al., 2010]. Despite the limited length period and the relatively small sample size some novel findings relevant to strain diversity were made in the present study as well.

A total of six different rotavirus VP7 genotypes were identified, including G1, G2, G3, G8, G9, and G12. The predominant genotype was G12 (67.4%; 91/135) in combination with P[8] (54.1%) and P[6] (10.4%) genotypes. Although G12 strains were in circulation in 2007/2008 in sub-Saharan regions [Mwenda et al., 2010], to the best of our knowledge, no study in Africa has demonstrated such a high prevalence of G12 strains, suggesting a major out-break of this strain in the study area during 2010/2011. In neighboring and other sub-Saharan countries different strains were more prevalent in this period as documented in the annual report of the WHO's Global Rotavirus Surveillance Network information bulletin [WHO, 2012]. For example, G3P[6] and G12P[8] strains were prevalent in Togo, G2P[4] and G2P[6] predominated in Guinea Bissau and in Democratic Republic of the Congo, G3P[6] was common in Nigeria, and G9P[8] was abundant in Cote d'Ivoir. Genotype G12 strains (mainly G12P[8]) were found to be in circulation at relatively low detection rates in Nigeria, Ghana, and Democratic Republic of the Congo, but were also detected in East African countries, including Uganda and Tanzania, and were very prevalent in Ethiopia (with >30% relative frequency). Data on strain prevalence from this period have been not specified for Cameroon in the WHO's Global Rotavirus Information and Surveillance Bulletin.

Data on mixed infections and non-typeable strains in this study showed lower frequency than reported usually from developing countries [Bányai et al., 2012]. The utilization of an improved genotyping protocol implemented by the European rotavirus strain monitoring network (EuroRotaNet [Iturriza-Gomara et al., 2009, 2011]) combined with nucleotide sequencing could be one reason that the proportion of non-typeable strains could be minimized. In fact, Esona et al. [2010] demonstrated that the majority of non-typeable strains collected across Africa could be genotyped and most of them belonged to one of the major genotypes when improved typing reagents (i.e., modified typing primers) or methods (i.e., sequencing) were utilized. Nonetheless, in this study a small portion (~6%) of strains that did not produce a first round product were omitted from routine genotyping and it is

possible that some of the rare human rotavirus genotypes were present among these few strains.

Some studies have shown that both vaccines currently in use worldwide are protective against a variety of circulating strains. Examples include G1 to G4, G8, and G9. Vaccine effectiveness has been demonstrated against G12 strains in the United States with an estimated 85–100% protection [Payne et al., 2011], which was somewhat reduced in parts of Africa (20–60% effectiveness) [Madhi et al., 2010; Cunliffe et al., 2012; Steele et al., 2012]. Current theories suggest that vaccine failure may not be a direct consequence of the circulation of uncommon rotavirus strains in African countries [Cunliffe et al., 2012]. Nonetheless it will be important to monitor the spatiotemporal dynamics of emerging strains, which might become predominant after rotavirus vaccines are used in massive immunization programs. Those strains that share no G- and P-type specificities with strains in RotaTeq and Rotarix may be challenging to existing rotavirus vaccines. This hypothesis was raised during mass vaccination conducted with the Rotarix vaccine in parts of Latin America, Australia, or Europe, where G2P[4] strains dominated other strains over several consecutive rotavirus seasons. However, rotavirus experts agree that the possibility that this re-emergence of G2P[4] strains was the result of natural fluctuation of rotavirus strains could not be excluded either [Matthijnssens et al., 2009].

The findings from this study document the rotavirus genotypes circulating in Cameroon and the emergence of genotype G12. The predominance of genotype G12 strengthens the evidence indicating that G12 is emergent worldwide. Any vaccine used in Cameroon will need to be effective against unusual rotavirus strains, such as G8, G12 and P[6]. The detection of emerging new strains re-enforces the need for enhanced rotavirus surveillance in humans and animals. Complete genome studies are needed to understand better the full picture of the circulating rotavirus strains in this geographic region.

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Glossary

NT	non-typeable

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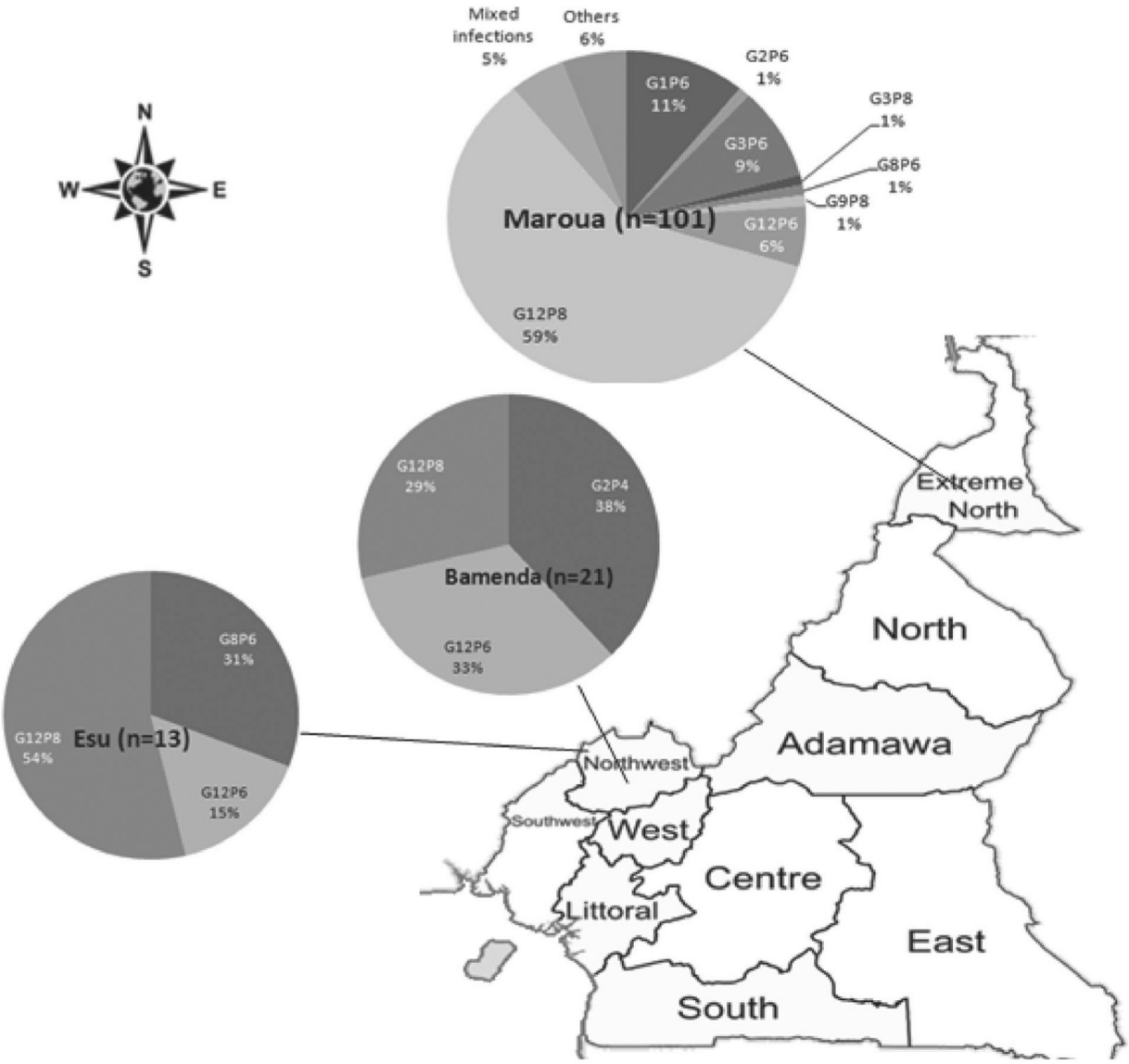

Fig. 1.

Rotavirus G and P genotypes combinations circulating in Northern Cameroon (North West: Esu and Bamenda; Far North: Maroua).

TABLE I.

Rotavirus G- and P-Types Circulating in the North West and Far North Regions of Cameroon (2010–2011)

	No. of strains of the following G-type											
P-type	**G1**	**G2**	**G3**	**G8**	**G9**	**G12**	**G1G12**	**G2G3**	**G3G8**	**G3G12**	**GNT**	**Total**
P[4]	—	8	—	—	—	—	—	—	—	—	—	8
P[6]	11	1	9	5	—	14	1	—	1	—	2	44
P[8]	—	—	1	—	1	73	—	—	—	—	1	76
P[6] + P[8]	—	—	—	—	—	1	—	1	—	1	—	3
P[NT]	—	—	1	—	—	3	—	—	—	—	—	4
Total	11	9	11	5	1	91	1	1	1	1	3	135

NT, non-typeable.