British Microbiology Research Journal
9(1): 1-11, 2015, Article no.BMRJ.18071
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

Diversity of Rotavirus VP7 and VP4 Genotypes Associated with Severe Childhood Diarrhea in North West Cameroon: Detection of Unusual Strains G1P[6], G2P[6], G2P[8] and G3P[6]

Florence A. Mbuh[1*], Susan Damanka[2], Gorge E. Armah[2], Sunday A. Omilabu[3], Aliyu A. Ahmad[1] and Jarlath U. Umoh[4]

[1]Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Electron Microscopy and Histopathology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana.
[3]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria.
[4]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author FAM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors FAM, SD and GEA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/18071
Editor(s):
(1) Gyanendra Singh, Gene Therapy & Louisiana Vaccine Center, School of Medicine, LSU Health Sciences Center, Louisiana, USA.
Reviewers:
(1) Gouandjika Vasilache Ionela, Virology Unit, Institut Pasteur de Bangui, Avenue Pasteur, Central African Republic.
(2) Mona Zaki Zaghloul, Clinical Pathology Department, Ain Shams University, Cairo, Egypt.
(3) Anonymous, Northwest University, China.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1216&id=8&aid=9677

Original Research Article

Received 1st April 2015
Accepted 29th May 2015
Published 9th June 2015

ABSTRACT

Aims: To determine rotavirus genotypes and distribution among children 0 - 5 years old with severe acute diarrhea in the North West Region (NWR) of Cameroon.
Study Design: Cross sectional.
Place and Duration of Study: Hospitals and health centers in the NWR served as sample

**Corresponding author: E-mail: fa_mbuh@yahoo.com;*

collection centers between January and December 2004.
Methodology: Fifty-six Enzyme Immunoassay (EIA) and Polyacrylamide gel electrophoresis (PAGE) - positive rotavirus stool specimens were analyzed for VP7 and VP4 genotypes by reverse transcriptase polymerase chain reaction (RT-PCR).
Results: A total of 51 (91.1%) samples genotyped as G and P types while the remaining five samples (8.9%) were partially characterized. Four VP7 genotypes (G1, G2, G3 and G9) and two VP4 genotypes, P[6] and P[8] were detected. The predominant G and P types were; G3 (34%) and P[8] (41.1%). Genotypes G1-G3 accounted for 85.8% of isolates while G9 represented 3.6% of isolates. Genotype P[6] represented 30.4% of all VP4 genotypes and was the most widespread strain occurring in all age groups. Twelve single G and P-type combinations were identified. Genotype G1P[8] (19.6%) predominated, followed by G3P[6] (17.9%). Unusual strains detected were G1P[6], G2P[6], G2P[8] and G3P[6] accounting for 32% of cases. Mixed infections were detected from 15 (27%) isolates comprising G1/G3P[6], G2/G9P[6], G2P[6]/P[8] and G1/G3/G8 P[6]/P[8]. Twenty isolates (35.7%) had unusual genotype / electropherotypes combinations. All G9 strains, mixed G genotypes and strains with atypical electrpherotypes occurred in children hospitalized with severe gastroenteritis.
Conclusion: There is high incidence of unusual rotavirus strains circulating in the NWR of Cameroon that could have an impact on rotavirus vaccine performance. Future studies will investigate post vaccine prevalence and characterization of non-typeable strains by other methods.

Keywords: Diarrhea; gastroenteritis; rotavirus; genotypes; Cameroon; Central Africa.

1. INTRODUCTION

Rotavirus-associated diarrhea accounts for about 500,000 deaths yearly in children under years of age worldwide with about 85% of cases occurring in developing countries of Asia and Sub- Saharan Africa [1].

Rotavirus, a genus of the family Reoviridae, has 11 segments of double-stranded RNA as its genome which are enclosed in a triple-layered capsid. These segments encode six structural proteins (VP1-VP4, VP6 and VP7) and six nonstructural proteins (NSP1-NSP6) [2]. Two outer capsid proteins, VP7 and VP4, contain neutralizing epitopes which define serotypes of rotavirus and form the basis for a dual classification of rotaviruses into VP7 (G types) and VP4 (P types) genotypes [3]. Based on diversity of the VP7 and VP4 genes, G and P genotypes have been defined for group A rotaviruses (RVA), respectively, and at least 27 G types and 37 P types have been described [4]. In human rotaviruses, G1, G2, G3, G4, G9, and G12 usually combined with P[4], P[6], and P[8] and are frequently detected throughout the world, with G1P[8] being the most prevalent strain in humans [5].

In order to assess vaccine efficacy rotavirus strain surveillance studies are needed prior to introduction of vaccines in any community [6]. There are very few studies on rotavirus in Cameroon and the Central Africa region. Reports of rotavirus studies in the West and South West Regions of Cameroon showed a wide diversity of strains including unusual genotypes [7]. It was therefore, necessary to determine the circulating strains in the North West Region (NWR) where no such studies have been conducted before the introduction of rotavirus vaccines. Following the World health Organization recommendations to participating nations especially those with high diarrhea related infant mortality Cameroon became the 21st country to introduce rotavirus vaccines in the Expanded Program on Immunization in 2014. This study was supposed to provide baseline data on the diversity and distribution of rotavirus genotypes circulating in the NWR of Cameroon before the introduction of rotavirus vaccines in the country.

2. MATERIALS AND METHODS

2.1 Study Area and Specimens

In this study we analyze rotavirus strains isolated from an earlier molecular epidemiologic study conducted in the North West Region of Cameroon between January and December 2004 [8]. A total of 543 stool samples were collected from involving children aged 0 – 59 months who sought medical care for acute diarrhea at the Bamenda Regional Hospital; divisional hospitals at Bali, Batibo, Ndop and Santa; Presbyterian Health Center Bafut and the Catholic Health Center Bali in respective health

districts. A case of acute diarrhea was defined as a child with ≥ 3 bowel movement per day with decrease in stool consistency (loose, watery or liquid) and presenting within ≤ 7 days of onset. A child < 60 months of age, male or female suffering from acute diarrhea was considered for inclusion in the study. Any child who met the criteria for inclusion and whose parents or guardian gave informed consent was eligible and enrolled in the study. Consenting parents or guardians completed an interviewer assisted structured questionnaire for socio-demographic and biodata of each child including outcome of the visit whether the child was admitted or treated at the outpatient department.

2.2 Polyacrylamide Gel Electrophoresis

Rotavirus dsRNA genome was extracted from 128/153 stool specimens that previously tested positive for rotaviruses by Enzyme Immunoassay. Briefly, rotavirus antigen detection was performed on 10% stool suspension in phosphate buffered saline using commercial DAKO IDEIA™ Rotavirus kit (DAKO Diagnostics, Sweden) following the manufacturer's instructions. Rotavirus dsRNA was extracted by the Bender method [9] with slight modification for polyacrylamide gel electrophoresis (PAGE) analysis. The extracted dsRNA was applied to separate lanes of a 10% polyacrylamide gel in a discontinuous buffer system overnight for 18-20 hours at 100V. The RNA bands were visualized by the silver nitrate staining technique over an illuminated box [10]. The presence of rotavirus in stool specimens was determined by detection of 11 RNA segments of rotavirus by PAGE as previously described [11].

2.3 Genotyping of Rotavirus Isolates

Rotavirus dsRNA genome was extracted from 56 rotavirus positive stool specimens that were determined to have sufficient genomic RNA by PAGE. The viral genome was extracted from 100 μL of PAGE positive stool specimens in 10% phosphate buffered solution by sodium dodecyl sulphate and Phenol/ Chloroform following standard procedures as previously described [12] and purified with the RNaid® Kit (Bio 101, Carlsbad, USA) as described [11]. Rotavirus G and P types were determined by the standard two-step reverse transcription polymerase chain reaction (RT-PCR) as previously described [13,14].

2.4 Statistical Analysis

Odds ratios with their 95% confidence intervals (CI) were calculated to establish associations between age and rotavirus genotypes using EPI-Info version 3.

3. RESULTS AND DISCUSSION

We present results of rotavirus genotypes and genotype-electropherotype combinations detected in specimens collected in an epidemiologic study conducted in in North West Cameroon in 2004. Results of rotavirus prevalence, electropherotypes, clinical and socio-demographic data were previously reported [8].

3.1 VP7 and VP4 Genotypes

Generally, first and second round products including single and mixed reactivity were obtained in 45 (80%) and 54/56 (96.4%) for VP7 gene and in 52/56 (92.9%) and 53/56 (94.6%) for VP4 gene respectively. A total of 54/56 (96.4%) and 53 (94.6%) isolates were assigned a G and P genotype respectively. Four VP7 genotypes were identified: G1 (30%), G2 (21%), G3 (34%) and G9 (3.6%) all accounting for 91.1% of cases. G8 was detected in a case of mixed infection. With respect to G types mixed infections (G3/G1, G3G2, G3/G1/G8 and G2/G9) were seen in 5/56 (8.9%) cases. Two P genotypes were identified: P[6], 17/56 (30.4%) and P[8] 23/56 (41%) both accounting for 71.4% of isolates. Mixed P types consisting solely of P[6]/P[8] were detected in 13/56 (23.2%) isolates. G3, 15/40 (37.5%) and P[8], 15/40 (37.5%) were the most common genotypes among 40 isolates analyzed from hospitalized children (Table 1). All non-typeable G and P types occurred in hospitalized children.

Although G1 is usually the predominant G type in worldwide studies [5,15] G3 predominated in the NWR of Cameroon. The predominance of this strain in the absence of G4 and P[4] also contrasts with reports in the neighboring West and South West regions where both genotypes were detected at low levels [7]. The prevalence of rotavirus genotypes vary from season to season and between different geographic regions [16] which could account for the differences observed. Four rotavirus genotypes (G1-4 and G9) generally constitute about 80-90% of strains detected in surveillance studies but the increasing reports of unusual strains could impact their global epidemiologic significance. A

typical example is the increasing prevalence of P[6] in clinical infections [5]. High prevalence of G3, P[6] and mixed P types involving P[6]P[8] are common in this region and have been reported at high levels in in Ghana, Nigeria and many parts of Africa [15]. Our study detected a higher rate of P[6] (30.4%) compared to data from other regions of the country [7] where the strain represented only 0.1% of genotypes in circulation. Continuous detection of high levels of P[6] genotypes in clinical specimens may imply strain evolution with increasing pathogenicity or simply due to increasing rotavirus surveillance especially in developing countries. The strain was initially associated with asymptomatic neonatal infections but now causes significant clinical infection in all age groups representing about 33% to 50% of rotavirus disease cases [5,17].

Infections with strains showing mixed G and P types are not uncommon in Africa. Earlier studies reported significant levels of mixed infections in Guinea Bissau [18] and other parts of the continent [15]. Apart from increasing the risk of reassortment they are also detected more in patients with more severe disease requiring hospitalization. Although it is not clear why mosaic strains are prevalent in some regions it is possible that co-infections with other disease agents and malnutrition that weaken host defense against reinfection may be the mechanism promoting multiple infection and clinical disease manifestation. Common environmental factors such as overcrowding, mass movement and poor living conditions that are prevalent in many developing countries could also facilitate disease transmission leading to mixed infections.

3.2 Age Distribution

A total of 54/56 (96.4%) cases of all G and P types occurred in children less than 24 months of age (Fig. 1). Genotype G1 was not detected from children less than 6 months old while P[6] circulated in all age groups. The odds for any age group having a specific genotype compared with other age groups were: 0 – 6 months, G2 (OR = 2.0; CI: 0.22 - 15.95) and G3 (OR = 1.13; CI: 0.30 - 15.37); 7 – 12 months, G1 (OR = 1.67; CI: 0.36 - 6.72); 13-18 months, mixed infections (OR = 14.33 CI: 1.1 - 405.5); 7 – 12 months, mixed P - types (OR = 1.84; CI: 0.44 - 8.3); 7 – 12 months, mixed P genotypes (OR = 1.84; CI: 0.44 - 8.3). P[8] (OR = 1.79; CI: 0.12 - 53.18) and non-typeable P types (Pnt) (OR = 1.22; CI: 0.37 - 4.1) were more likely to occur in male children who are generally more active than females. Peak rotavirus infection in the NWR was previously reported in ages 7 – 12 months old [8] and this age group also had the highest prevalence of both G and P genotypes. Most cases of rotavirus occur in children younger than 24 months [8,19] although studies in the neighboring Western and South West Regions of the country reported higher prevalence in older children [7]. Mixed genotypes were seen mainly in children less than 24 months of age defining the greater susceptibility of younger children to rotavirus diarrhea but it was not clear why ages 13-18 showed the most prevalence of mixed G types. However, this is usually the transitional age when children begin to temporally move away from supervising parents and could increase the risk of exposure to contaminated fomites and infection. Mixed infections are frequently reported in rotavirus studies worldwide [3,12,16,19] and could be responsible for the high rate of emergence of unusual strains secondary to genetic reassortment.

Table 1. Rotavirus genotypes in children hospitalized with gastroenteritis (2004)

Genotype	P[6]	P[8]	P[6]P[8]	Pnt	Total	Percent
G1	2	5	2	0	9	22.25
G2	0	4	4	0	8	20
G3	8[a]	5	0	2	15	37.5
G9	0	0	2	0	2	5
G1G3	0	0	0	1	1	2.5
G2G3	0	0	1[b]	0	1	2.5
G2G9	1	0	0	0	1	2.5
G1G3G8	0	0	1[c]	0	1	2.5
Gnt	1	1	1	0	2	5
Total	12	15	10	3	40	100

[a]Three cases with short electropherotype; [b]Short electropherotype; [c]Mixed electropherotypes
Gnt = Non-typeable VP7 genotype; Pnt = Non-typeable VP4 genotype

3.3 Sex Distribution

A majority of the genotypes 30/56 (53.8%) were from male patients. Fig. 2 shows the sex distribution of rotavirus genotypes. All G9 strains occurred in female children who were also more likely be infected with G1 (OR = 1.46; CI: 0.40 - 5.32) while male children were more likely to be infected with G2 (OR = 1.28; CI: 0.3 - 5.92) and G3 (OR = 1.81; CI: 0.51- 6.57) strains but the odds were also not significant (P>0.05). Sex analysis of P types showed that male children were more likely to be infected with P[8] (OR = 1.79; CI: 0.12 - 53.18) and non-typeable P types (OR = 1.22; CI: 0.37 - 4.1) and these relationships were also not statistically significant (P>0.05). Reports of higher rate of infection in male children are common [19] but sex did not affect the distribution of rotavirus genotypes causing childhood diarrhea in North West Cameroon.

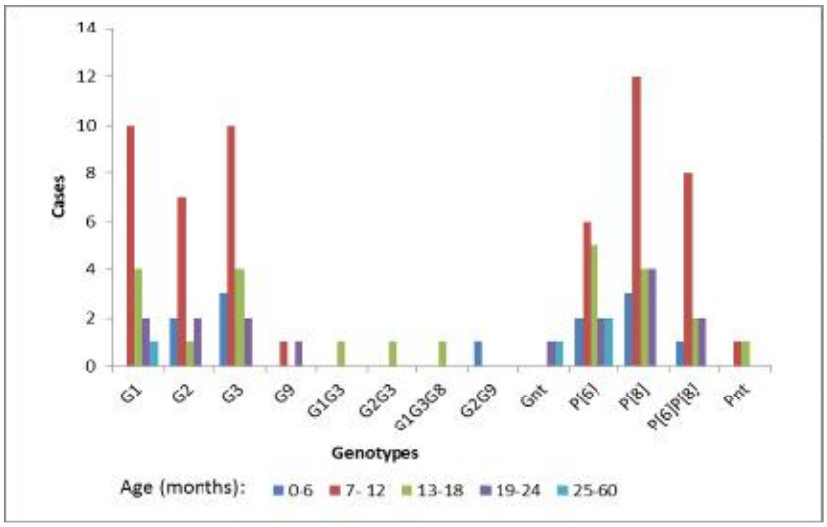

Fig. 1. Age (months) distribution of rotavirus VP7 and VP4 genotypes in North West Cameroon (2004)

Gnt = Non-typeable VP7 genotype; Pnt = Non-typeable VP4 genotype

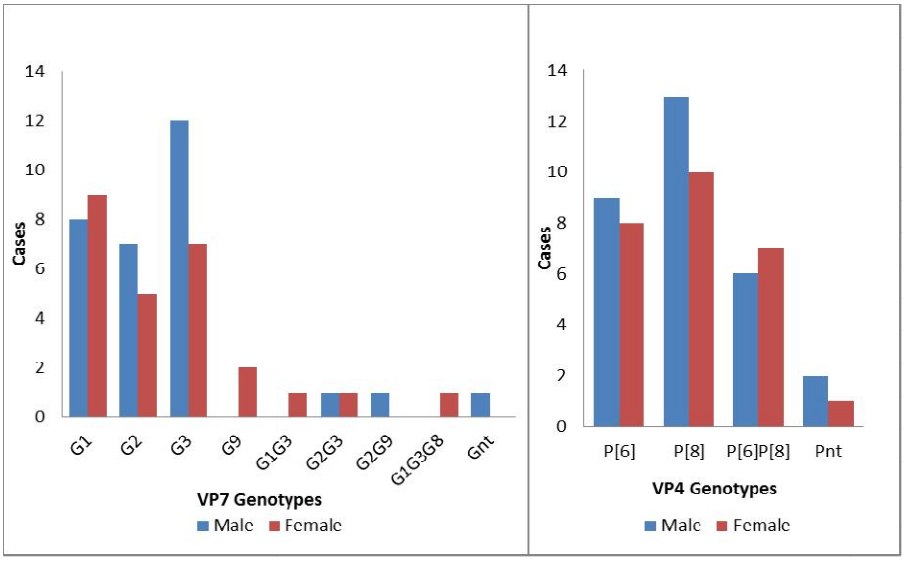

Fig. 2. Sex Distribution of Rotavirus VP7 and VP4 Genotypes in North West Cameroon (2004)

Gnt = Non-typeable VP7 genotype; Pnt = Non- typeable VP4 genotype

3.4 Monthly Distribution of VP7 and VP4 Genotypes

Fig. 3 shows the monthly distribution of genotypes. Most strains circulated between December, 12/56 (21.4%), January 30/56 (53.4%) and February 9/56 (16.1%) which is characteristic of most rotavirus infections [7, 15,17]. A majority of G2 strains 9/11(75%), P[6] 10/18 (55.6%) and mixed G, 3/4 (75%) and P, 8/13 (61.5%) genotypes circulated mostly in January. Many studies have shown wide geographical variation in the prevalence of G and P types across continents as well as local and global temporal changes in the frequency of dominant strains [5,15,19]. Knowledge of the seasonal distribution of rotavirus genotypes is important for strategic planning and effective control measures. However our data was collected only for a period of one year and the results might not reflect the true seasonality of strains but could nevertheless shed light on point prevalence of genotypes.

3.5 G/P Genotype Combination Analysis

A binary characterization was possible with 51/56 (91.1%) isolates comprising 12 G/P constellations with six single and six mixed G/P infections representing 36/56 (64.3%) and 15/56 (26.8%) isolates respectively (Fig. 4). Genotype G1P[8] 11/56 (20%) was the most common strain followed by G3 P[6], 10/56 (18%) and G3P[8] 7/56 (12%). Mosaic strains with either mixed G or P genotypes (G1P[6]P[8], G2P[6]P[8], G3G2P[6], G9G2P[6]) and dual G and P reactivity, G1G3G8P[6]P[8] were seen in 15/56 (27%) cases. Isolates with unusual G/P combinations (G1P[6], G2P[8], G2P[6] and G3P[6]) accounted for 18/56 (32%) cases while 5/56 (8.9%) isolates were partially characterized including three G and two P types with undetermined P and G types respectively.

West Africa is known to show great diversity in rotavirus strains and unusual genotypes as well as mixed infections [7,15,20]. Therefore the detection of strains with dual reactivity to either G or P types was not unexpected. However, the detection of a strain with multiple reactivity showing both VP7 and VP4 genotype specificities (G1/G3/G8 P[6]/P[8]) was rather unique in that such combinations have not been previously reported to the best of our knowledge. The mixed reactivity in this case could have resulted from co-infection with more than one mosaic strains with long (possibly G1P[8]) and short electropherotypes (possibly G2P[6], G2P[8], G9P[8], G9P[6], or G2/G9P[6]/P[8]) based on the G and P genotypes detected and high prevalence G2P6 strains in the region with short profiles [17]. Five globally common G/P combinations (G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]) generally represent about 75% of rotavirus strains in circulation [21]. Only two of these (G1P[8] and G3P[8] were detected and accounted for 18/56 (32.1%) of isolates. The significance of this variation will unfold in future surveillance and vaccine efficacy studies. Genotype G1 P[8] is usually the most common strain detected in most epidemiologic studies [6, 7,15,18]. Occurrence of G3P[6], 10/56 (17.9%) as the second most common strain overall could pose a problem in vaccine effectiveness if the current vaccines do not produce sufficient heterotypic antibody protection against globally less common strains such as P[6] if they become prominent in any region. G3P[6] strains have also been reported as the dominant strain in pediatric inpatients in Zimbabwe [19] and protection against the strain in addition to G1P[8] could play a role in reducing rotavirus disease hospitalization in children.

The segmented nature of the rotavirus genome and high rate of mutation [20] suggest that during a mixed infection with different rotavirus strains genetic reassortment (substitution of RNA segments between different RNA strands) could occur giving rise to mosaic strains and unusual genotypes. Mosaic strains occurred mainly in hospitalized children implying that rotavirus could be more severe in West and central African children where such strains are common [7,15,17].

Unlike with many studies in the region that report high levels of untypeable starins [7,15,18], most of our isolates could be characterized by RT-PCR genotyping methods. Failure to type rotavirus strains could result from several factors including presence of rare genotypes, insufficient viral RNA genome, destruction of viral genome during repeated freeze thawing, or due to mutations at specific binding sites in the target genome or primers [18,20,22]. Strains that do not react with RT-PCR genotyping primers could be analyzed by a modification of standard procedures, virus culture and sequencing [18,20, 23] that were beyond the scope of our study.

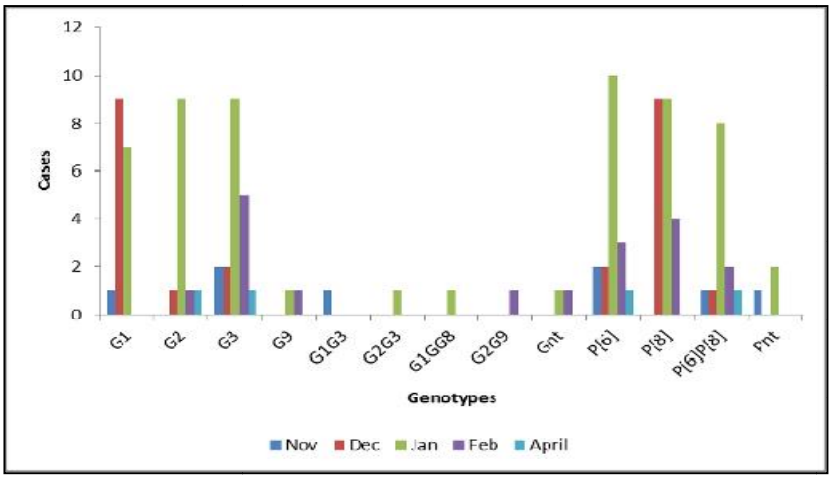

Fig. 3. Monthly distribution of rotavirus VP7 and VP4 genotypes in Cameroon (2004)
Gnt = Non-typeable VP7 genotype; Pnt = Non-typeable VP4 genotype

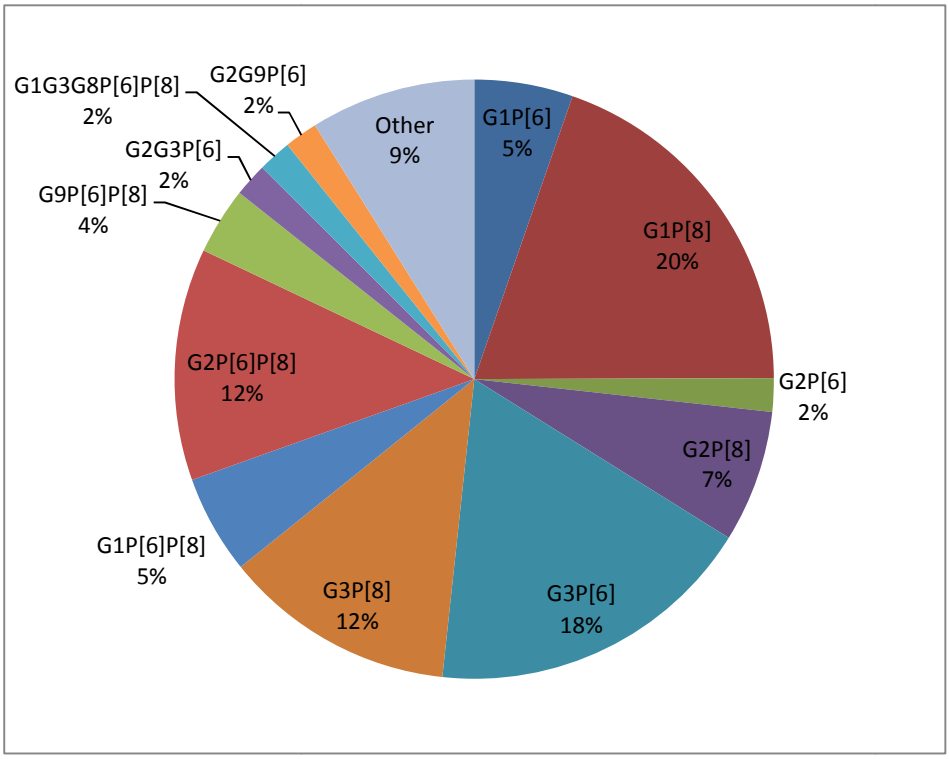

Fig. 4. Frequency (%) distribution of rotavirus G/P combinations in North West Cameroon (2004)
Gnt = Non-typeable VP7 genotype; Pnt = Non-typeable VP4 genotype

3.6 Age (months) Distribution of G/P Combinations

The predominant G/P combinations in different age groups were: 0-3, G2P[6]; 4-6, G3P[8]; 7-9, G1P[8], 10-12: G2P[6]P[8]; 13-18 G3P[6]; 19-24 (G1P[8]; and 25-60, (G1P[6]) with ages 7- 12 showing the highest strain diversity (Table 2). All mixed genotypes occurred in children under 24 months of age while genotype G3P[6], 8/40 (20%) was the most common strain in hospitalized children. All strains with unusual

genotypes occurred in children less than 2 years of age with 13/18 (72.2%) appearing in ages 6 – 18 months and could pose an increased risk in this age group. Scheduled immunization targeting peak age should protect children in this region from severe rotavirus disease.

3.7 Genotype/Electropherotype Combination Analysis

We previously reported results of rotavirus electropherotypes in which strains had predominantly long electropherotypes and a few isolates with short and mixed profiles [8] in which 82/128 (64.6%) specimens were PAGE positive including 76/82 (92%) and 5/82 (6.1%) cases with single long and short electropherotype profiles of which mixed long electropherotype were seen in 10/82 (12.2%) specimens while one sample had both long and short profiles. In this study we further characterized 56/82 PAGE positive rotavirus isolates comprising 40 inpatient and 16 outpatient samples and compared the genotypes with electropherotypes. Several strains showed unusual genotypes/ electropherotype combinations: all G2 and partially characterized strains had long eletropherotypes while G3 strains showed both long and short electropherotype profiles (Fig. 5). Strains with unusual genotype/electopherotype combinations (G2P[6], G2P[8], G3 P[6], G2P[6]P[8], G2G9P[6], G9P[6]P[8], G1G3P[6]) accounted for 20/56 (35.7%) of cases. All strains with short electropheropes had unusual genotypes. Rotavirus genotypes G1 and G3 typically have long electrpherotypes and pair with P[8] while G2, G8 and G9 have short electrpherotype profiles and pair with P[4]. Genotype G1 P[8] is usually the most abundant strain in circulation [5,15]. Abnormal genotype-electropherotype and G/P combinations indicated high diversity of rotavirus strains both at genomic and molecular levels. However, some strains such as G3P[6] that showed short electropherotype profiles might have been strains of G9 misidentified as G3 [20].

G9 strains may also combine with P[6] [5] but strains presented with long RNA profiles and mixed VP4 specificity of P[6]P[8]. Three types of G9 strains have been reported based on electropherotype profile, VP7 and VP4 specificities in which strains either have long or short electropherotypes reacting with either P[4], P[6] or P[8] genotype specific primers [5]. This raises further concerns about the possibility of a complex genetic polymorphism among rotavirus strains that remain to be uncovered and questions about the specificity of genotyping primers for G9 that could react with G3 [20] as well as those for G8 strains that often appear in mixed infections [23]. The detection of all G9 strains, mixed G types and those with atypical G/P - electropherotype combinations in hospitalized children further suggests the possibility of these strains being more virulent although host and environmental factors could also play a role in disease severity. Mixed infections can provide an opportunity for genetic reassortment among rotaviruses [20] that could result in new genotype combinations and unusual strains. Both host and environmental factors could facilitate genetic reassortment among rotavirus strains causing emergence of unusual strain. It is common for humans to cohabit with domestic animals such as goats, sheep, pigs and chicken or cattle in the homes or backyards which could also account for the high rate of unusual strains in the region. Another possibility for unusual strains is the intrinsic nature of the virus as a segmented RNA virus which predisposes to spontaneous mutations during replication.

Table 2. Age distribution of rotavirus genotypes combinations in North West Cameroon (2004)

Genotypes	Age group (months)							
	0-3	4- 6	7-9	10-12	13-18	19-24	25-60	Total
G1P[6]	0	0	0	1	1	0	1	3
G1P[8]	0	0	6	1	2	2	0	11
G2P[6]	1	0	0	0	0	0	0	1
G2 P[8]	0	0	1	1	1	1	0	4
G3P[6]	0	0	4	1	3	2	0	10
G3P[8]	0	2	2	2	1	0	0	7
G1P[6]P[8]	0	0	0	1	2	0	0	3
G2P[6]P[8]	1	0	2	3	0	1	0	7
G9P[6]P[8]	0	0	1	0	0	1	0	2
G2G3P[6]	0	0	0	0	1	0	0	1

Genotypes	Age group (months)							
	0-3	4- 6	7-9	10-12	13-18	19-24	25-60	Total
G2G9[6]	0	1	0	0	0	0	0	1
G1G3G8P[6]P[8]	0	0	0	0	1	0	0	1
G3Pnt	0	0	1	1	0	0	0	2
G1G3Pnt	0	0	0	0	1	0	0	1
Gnt P[6]	0	0	0	0	0	0	1	1
Gnt P[8]	0	0	0	0	0	1	0	1
Total	2	3	17	11	13	8	2	56

Gnt = Non-typeable VP7 genotype; Pnt = Non-typeable VP4 genotype

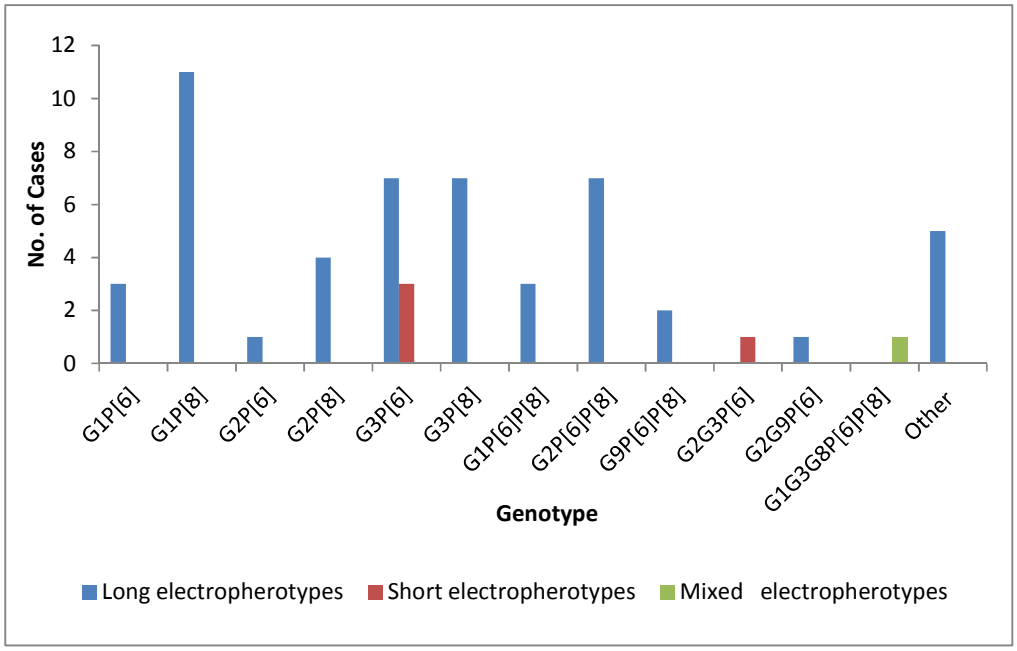

Fig. 5. Rotavirus genotype and electropherotype combinations in North West Cameroon (2004)

4. CONCLUSION

This report presents the diversity of rotavirus G and P types circulating in the NWR of Cameroon in which genotypes G1-3 constituted over 85% of strains with predominance of G1P[8]. G3P [6] emerged as the second most common strain overall and as the predominant strain in hospitalized children with. Unusual strains and mixed infections circulated at high levels including P[6] strains detected in all age groups. These results provide the background for future evaluation of vaccine efficacy and strain evolution in Cameroon and the Central Africa region. Continuous strain surveillance is necessary for a better understanding of rotavirus strain diversity and the epidemiologic significance of G3, G8, G9, P [6] and unusual strains. Follow up studies intend to investigate post vaccination strain prevalence. Further research is also needed to confirm the association of mixed infections and unusual strains with disease severity that may elucidate the high rotavirus disease burden in Sub-Saharan Africa [1] and to clearly identify partially characterized strains and those with atypical genotypes or genotype / electropherotype combinations.

CONSENT

Informed consent was obtained from parents or guardians of all individuals who participated in the study.

ETHICAL APPROVAL

This study was approved by the North West Regional Delegation of Public Health, Cameroon.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, et al. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis. 2009; 200(Suppl 1):9 -15
2. Estes MK. Rotaviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE. Fields Virology, 5th ed. Kluwerhealth/Lippincott, Williams and Wilkins; 2007.
3. Cunliffe NA, Gondwe JS, Broadhead RI, Molyneux ME, Woods PA, Bresse JS, Glass RI, Gentsch JR, Hart CA. Rotavirus G and P Types in children with acute diarrhea in Blantyre, Malawi from 1997 to 1998: Predominance of novel P[6]G8 strains. J Medical Virol. 1999;57:308-12
4. Trojnar E, Sachsenröder J, Twardziok S, Reetz J, Otto PH, et al. Identification of an avian group A rotavirus containing a novel VP4 Gene with a close relationship to those of mammalian rotaviruses. J Gen Virol. 2013;94:136-42.
5. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol. 2005;15:29-56.
6. Mwenda, JM, Tate JE, Steele D, Parashar UD. Preparing for the scale-up of rotavirus vaccine introduction in Africa: Establishing surveillance platforms to monitor disease burden and vaccine impact. Pediatr Infect Dis J. 2014;33:1–5.
7. Esona MD, Armah GE, Steele AD. Rotavirus VP4 and VP7 Genotypes Circulating in Cameroon: Identification of unusual types. J Infect Dis. 2010; 202(Suppl):205-11.
8. Mbuh FA, Armah GE, Omilabu SA, Ahmad AA, Umoh JU. Molecular epidemiology of group A human rotaviruses in North West Region, Cameroon. Pan Afr Med J. 2012; 12:108. Epub 2012 Aug 15.
9. Flook PK, Wilson MD, Post RJ. The use of repetitive DNA probes in the analysis of natural populations of insects and parasites. In: Berry RJ, Crawford TJ and Hewitt GM, ED. Genes in Ecology. British Ecological Society/Blackwell Scientific Publication, Oxford; 1992
10. Herring AJ, Inglis NF, Ojeh CK, Sondgrass DR. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J Clin Microbiol. 1982;16(3):473-7.
11. Kobayashi N, Lintag IC, Urasawa T, Taniguchi K, Saniel MC, Urasawa S. Unusual human rotavirus strains having subgroup I specificity and “long” RNA electropherotype. Arch Virol. 1989;109:11-23
12. Steele AD, Alexander JJ. Molecular epidemiology of rotavirus in black infants in South Africa. J Clin Microbiol. 1987;25; 2384-87.
13. Gouvea V, Glass IR, Woods P, Taniguchi K, Clark HF, Forrester B, Fang ZY. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol. 1990;28: 276-82
14. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, et al. Identification of group a rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol. 1992;30:1365-73.
15. Steele AD, Ivanoff B, The African Rotavirus Network. Rotavirus strains circulating in Africa during 1996 – 1999: Emergence of G9 strains and P[6] strains. Vaccine. 2003;21:361-67.
16. Banyai K, Lazlo B, Duque J, Steele D, Nelson AS, Gentsch JR, Parashar UD. Systemic 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. 2012; 30(Suppl 1):A122-30.
17. Asmah RH, Green J, Armah GE, Gallimore CI, Gray JJ, Iturriza-Gomara M, Anto F, Oduro A, Binka FN, Brown DWG, Cutts F. Rotavirus G and P genotypes in Ghana. J Clin Microbiol. 2001;39:1981-1984
18. Fischer TK, Page NA, Griffin DD, Eugen-Olsen J, Pedersenag, Valentiner-Branth P, et al. Characterization of incompletely typed rotavirus strains from Guinea Bissau: Identification of G8 and G9 types and a high frequency of mixed infections. Virology. 2003;311:125-33.
19. Mukaratirwa A, Berejena C, Nziramasanga P, Shonhai A, Mamvura TS, Chibukira P, Mucheuki I, Mangwanya D, Kamupota M, Manangazira P,

Tapfumaneyi C, Gerede R, Munyoro M, Mwenda JM, Mphahlele JM, Seheri ML, Peenze I, Gonah AN, Maruta A, Tengende MB. 2014. Epidemiologic and genotypic characteristics of rotavirus strains detected in children less than 5 years of age with gastroenteritis treated at 3 Pediatric Hospitals in Zimbabwe During 2008–2011. Pediatr Infect Dis J. 2014;33:45–48.

20. Mitui MT, Chandrasena TNGA, Chan PKS, Rajindrajith S, Nelson EA, et al. Inaccurate identification of rotavirus genotype G9 as genotype G3 strains due to primer mismatch. Virol J. 2012;9:144. Doi: 10.1186/1743-422x-9-144.
21. Gentsch JR, Laird AR, Bielfelt B, Griffin DD, Banyai K, Ramachandran M, Jain V, Cunliffe NA, Nakagomi O, Kirkwood CD, Fischer TK, Parashar UD, Bresee JS, Jiang B, Glass RI. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. J Infect DIS. 2005; 192(Suppl. 1):146-159.
22. Fischer TK, Gentsch JR. Rotavirus typing methods and algorithms. Rev Med Virol. 2004;14:71-82.
23. Enweronu-Laryea CC, Sagoe KW, Damanka S, Lartey B, Armah GE. Rotavirus genotypes associated with childhood severe acute diarrhoea in Southern Ghana: A cross-sectional study. Virology Journal. 2013;10:287. Doi:10.1186/1743-422x-10-287.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1216&id=8&aid=9677