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Distribution of Serotypes and Genotypes of Rotavirus Strains in Nigerian Children

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Summary

Adah MI, Olaleye OD. Distribution of Serotypes and Genotypes of Rotavirus Strains in Nigerian Children. *Nigerian Journal of Paediatrics* **1998; 25: 20.** Polymerase chain reaction and radioactive dotblot hybridisation techniques were used to analyse 45 rotavirus-positive stool samples for G (serotype) and P (genotype) distribution among Nigerian children. While samples bearing G1 specificities predominated among the females, those bearing serotype G3 were more prevalent among the males ($P<0.05$). VP4 genotypes were evenly distributed among both sexes. Strain type GIP8 was more prevalent among the females than the males ($P<0.05$). The results demonstrate that differences in serotype, genotype and strain distribution among male and female children exist and these differences may be of relevance in formulating the type of vaccine suitable for each gender population.

Introduction

INFECTIOUS gastroenteritis is a major cause of morbidity and mortality among children throughout the world and viruses, particularly rotavirus, constitute the major organisms associated with diarrhoeal diseases. [1 2] World-wide attention has therefore, been focused on ways of reducing the childhood mortality and morbidity due to the virus. The exact parameters of immunity to rotavirus are not cledarly defined; yet it is important to obtain information on epidemiologically important human rotavirus genotypes and serotypes in order to formulate vaccine strategies. In Nigeria, various studies have demonstrated a prevalence rate of rotavirus infection of between 3.6 and 33 percent. [3-9] The molecular characteristics of the local rotavirus strains have been further analysed with a view to providing fundamental data required for vaccine development and application in the country.[9]

Trials of some rotavirus vaccines world-wide have shown varying degrees of success across age and geographical regions. [10-20] Various epidemiological factors such as low infectious dose of the virus, large numbers of infectious particles excreted in the stools of infected individuals, resistance of rotavirus to commonly used disinfectants, ability of the virus to remain viable for long periods of time, malnutrition, race and socio-economic status, have been shown to play important roles in the occurrence, spread, distribution and severity of human rotavirus infection. [12 - 22] Sex differences also occur, with cases of rotavirus infection in males, being up to 20 percent higher than in females as reported by a WHO working group.[22] While one study in Nigeria has shown no sex difference,[5] others have reported a preponderance of males over females. [6 7] The sex distribution of the various rotavirus serotypes and genotypes in Nigeria has not, to our knowledge, been reported in the literature. The aim of the present study therefore, was to

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define the sex distribution of the various serotypes and genotypes of rotavirus among Nigerian children.

Table 1

Sequences, Positions and Sizes of Oligonucleotide Primers used for Amplification

Primers	*Sequence (5'-3')*	*Position*	*Length of Products (base pairs)*	*Strain*
(VP 7)				*(serotype)*
aAT8	GTCACACCATTTGTAAATTCG	178-198	885	69m (8)
aBTI	CCAGTACTCAAATCAATGATGG	314-335	749	Wa (1)
aCT2	CAATGATATTAACACATTTTCTGTG	411-435	652	DS-1 (2)
aDT4	CGTTTCTGGTGAGGAGTTG	480-498	583	ST-3 (4)
aET5	CGTTTGAAGAAGTTGCAACAG	689-709	374	P(3)
aFT9	CTAGATGTAACTACAACTAC	757-776	306	Wi6(19)
(VP4)				*(genotype)*
IT-1	TCTACTTGGATAACGTGC	339-356	356	KU(P8)
2T-1	CTATTGTTAGAGGTTAGAGTC	474-494	494	RV5(P4)
3T-1	TGTTGATTGGATTCCAA	259-278	278	1076(P6)
4T-1	TGAGACATGCAATTGGAC	385-402	402	K8(P9)
5T-1	ATCATAGTTAGTAGTCGG	575-594	594	69M(P10)

Materials and Methods

Three hundred and fourteen stool samples randomly collected from children under the age of five years who presented with diarrhoea in various hospitals in Nigeria were analysed by ELISA radioactive dot-blot hybridisation technique and polymerase chain reaction (PCR) as previously reported.[23] For VP7 serotype determination, the method of Gouvea *et al*,[24] was utilised, while the method of Gentsch *et al*, [25] was used to determine VP4 genotype as previously described.[3] Briefly, dsRNA extracted from stool specimens according to the method of Hering *et al*, [26] and purified with *RNAid PLUS* kit (*Dianova*, Germany) according to the instructions of the manufacturers, was used as template for Reverse-Transcription-PCR (RT/PCR). One hundred picomoles each of Primers, Beg 9 (5' GGTTAAAAG AG AG AATITCCGTCTGG 3') and End9 (5' GGTC AC ATC AT AC AATTCTAATCTAAG 3') were utilised for RT/PCR and mixed with seven percent dimethyl sulphoxide (DMSO) in a total volume of 50pl. The mixture was overlaid by 100µ mineral oil, heated to 95°C for three minutes and then cooled to 37°C in a programmable thermal cycler PTC-100 (*Biozym*, Germany). One volume of two-fold RT-PCR buffer containing dNTPs (5mM each), 100,M Tris-HCL pHL PH 8.0, 3mM $MgC1_2$ and 40mM KCI was mixed with 2U Taq polymerase (GIBCO/BRL) and 100µ M-MLV (murine mouse leukaemia virus) reverse transcriptase. The two mixtures were added and incubated at 37°C for 40 minutes. The PCR programme consisted of 30 cycles at 95°C for one minute, 55°C for one minute and 72°C for two minutes and a final incubation at 72°C for 10 minutes.

In the second amplification and serotyping reaction, 5 µ of the RT/PCR amplification product was used as template and RT omitted. The template was mixed with 100 pmol each of the serotype specific primers (Table 1) and the common primer End9, 3mM $MgCl_2$, 5mM each of dNTPs, and IU of Taq polymerase in a 10 X PCR buffer in a final volume of 100µ1. The mixture was overlaid with 100µl of meneral oil and the same PCR programme was repeated.

For VP4 genotyping, reaction parameters remained the same except that the primers utilised included Con2 (5' ATTTCGG ACC ATTTATAACC 3') and Con3 (5' TGGCTTCGCCATITTATAGACA

3') for RT/PCR while the VP4 genotype-specific primers are shown in Table 1. Con 3 was used as the common primer in the second amplification and typing reaction. The PCR programme consisted of 95°C for five minutes, 50°C for 30 seconds, 72°C for one minute, 95°C for 30 seconds and the cycle was repeated 30 times followed by a final five minutes incubation at 72°C. Further analysis for VP7 serotype determination utilised the radioactive dot-blot hybridisation method described by Zheng *et al.* [27] The comparison of prevalence values between the sexes was done using the student's t test.

Table II

Serotype and Genotype Distribution of Rotaviruses according to Sex

	Male	*Female*
VP7 G Sterotypes		
G1	5	8
G3	8	6
GIG3	4	5
GIG8	1	-
GIG9	1	-
G3G8	-	2
ND	3	2
Total	22	23
VP4P Genotypes		
P8	6	7
P6	8	8
P4P6	1	1
P8P6	4	3
P8P4P10	1	-
ND	2	4
Total	22	23

ND = Not determined

Results

Details of the distribution of rotavirus strains, serotypes and genotypes according to the sexes in the present study are presented in Tables II and III. VP7 G serotype distribution showed that while samples bearing GI were more predominant among females, those bearing G3 serotypes were significantly more prevalent among males (P<0.05). Samples bearing G serotypes GIG8 and GIG9 were detected only in the males, but samples with G3G8 specificities were detected only among the females. VP4 genotypes were evenly distributed among both sexes, except one sample bearing P8P4P10 specificity that was detected in a male child.

Strain types G3P6, GIG3P6P8, G3P6P8, P6P8 were equally distributed among both sexes, but strain type G1P8 was more prevalent among female children (P<0.05). Strain types G1G3P6, GrG8P4P6 and G3G8P8 were only detected among female children, while strain types G1P6, G1G8P6, G1G9P6, G3P8, G1G3P6P4 and G1G3P4P8P10 were detected only among male children.

Table III

Distribution of Rotavirus Strains and mixed Infections of Strains according to Sex

Rotavirus Strains	Male	Female
GIP8	4	6
G3P6	4	4
G3G8P4P6	-	1
GIG9P6	1	-
G3P8	1	-
GIP6	2	-
GIG3P6	-	4
GIG3P8	1	-
GIG3P6P8	1	1
G3P8P6	1	1
*P6P8	1	1
GIG3P6P4	1	-
GIG3P4P8P10	1	-
G3G8P8	-	1
GIG8P6	1	-
ND	3	4
Total	22	23

* VP7 Serotype not determined
ND = Not determined.

Discussion

It has been reported that rotavirus infection is more common in males than in females, but the 4:3 ratio of the disease is consistent with that observed in other childhood diseases.[28] Previous studies from northern Nigeria [67] have shown higher preponderance of male children infected with rotavirus as compared to females, a difference that was how-

ever, not significant. In any case, another study from Nigeria had shown no difference between the sexes.[5] The distribution of rotavirus serotypes and genotypes according to the sexes, has hitherto, not been evaluated in Nigeria, neither have the exact parameters of immunity to the virus been clearly defined. Yet, it is important to obtain information on epidemiologically important human G and P types in order to formulate a vaccine strategy.[3] The current study has indicated that G1 serotypes are currently more prevalent among female children in Ibadan whereas, male children have a higher preponderance of G3 serotypes; conversely, VP4 genotypes were evenly distributed among both sexes.

Rotavirus strain G1P8 was more prevalent among the female children in the present study, while strain G3P6 was evenly distributed among both sexes. To our knowledge, no information exist in the literature regarding the distribution of rotavirus G and P types according to the sexes anywhere in the world. Whether the findings in this study are due to a greater susceptibility of either sex to these G and P types and strains is not clear. However, it is noteworthy that strain G1P8 which has world-side distribution, was more prevalent among the female children studied. These results show that differences in serotype, genotype and strain distribution among male and female children exist and these differences may be relevant in formulating the type of vaccine suitable for each gender population. Notwithstanding this, vaccine application utilising tetravalent vaccine may be able to protect Nigerian children against severe rotavirus diarrhoea irrespective of the gender. In view of the small sample size analysed in this study, it is suggested that further and more extensive studies be undertaken to determine a comprehensive distribution of rotavirus strains among children in the country.

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