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## Research Article Prevalence and Genotypes of Rotavirus Infection among Children with Gastroenteritis in Abuja, Nigeria

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### Abstract

**Background and Objectives:** Rotavirus is a major cause of severe diarrhea in children worldwide. The existing genotypes of rotavirus in Nigeria are not widely known. This cross-sectional study investigated the prevalence, risk factors for rotavirus antigens and circulating genotypes amongst children within 5 years of age in Abuja, Nigeria. **Materials and Methods:** Watery and/or semi-solid stool samples were collected from children and preserved in glycerol. A structured questionnaire was used to obtain information on demographic characteristics and clinical details from their parents. Rotavirus antigen was detected in the stool specimens by Rotavirus antigen kit, while genotype characterization for G- and P-type genotypes were by reverse transcription polymerase chain reaction and polyacrylamide gel electrophoresis. Descriptive and chi-square statistics were used to analyze all the data obtained. **Results:** Of 144 stool samples analyzed, 25% (36) had Rotavirus infection, with male dominance than females15.3% (22/144) and 9.7% (14/144), respectively. The age group of less than 10 months had higher rotavirus infection of 13.9% (20/144). The major statistical significant risk factors identified were, use of feeding bottles, p = 0.003 and attendance to day care centers, p = 0.038. The peak of rotavirus infection was April, with a range of January-May. The prevalent genotypes identified were G1, G3, G9, G12, (VP7) and P<sup>8</sup>, P<sup>6</sup> (VP4) with mixed G/P. The G12 genotype was the predominant G-type. The migration pattern of the viral genome showed that the long genomic segments were more than the short ones. **Conclusion:** The G12 genotypes were highly associated with rotavirus-diarrhea and disease amongst the children. There is a need to introduce rotavirus vaccination in the National program on Immunization in Nigeria.

Key words: Rotavirus, G12 genotype, diarrhea, children, vaccination

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

Rotavirus infection is a major cause of non-bacterial gastroenteritis, responsible for severe diarrhea in infants and children Worldwide, causing approximately 453,000 deaths each year, thus having great impact on childhood morbidity and mortality<sup>1</sup>. The burden of rotavirus infection has been estimated to be higher in countries with low income, with Africa having more than half of the deaths<sup>2</sup>. Nigeria was ranked among the countries with developing high mortality due to rotavirus disease, both in children and adults, with 9.5% mortality rate accounting for ~49,974 deaths per annum<sup>3</sup>.

The genus Rotavirus is a member of the family Reoviridae, with 11 segments of double stranded RNA and 18,555 nucleoside base pair. The matured virus particle is non-enveloped and possesses a three layered icosahedra protein capsid, measuring about 75 nm in diameter<sup>4</sup>. The Rotavirus consists of six serological groups (A to F). Groups A to C have been shown to infect humans and Groups D to F have been implicated in Rotavirus diseases of animals<sup>5</sup>.

Group A serotype causes the most severe infections in humans. It has six structural proteins (VP 1, VP2, VP3, VP4, VP6 and VP7) and five Non Structural Proteins (NSP1 to NSP5) Based on antibody neutralization of the outer capsid viral protein antigens (VP7 and VP4), Rotavirus have been grouped into G serotypes (sequel to neutralization by antibodies against VP7 glycoprotein antigen) and P-serotype (with respect to neutralization by antibody against VP4-protease sensitive antigen)<sup>6</sup>.

Rotavirus is transmitted via the feacal-oral route, occurring mostly as a result of poor hygiene<sup>7</sup>. Other major risk factors includes ingestion of contaminated food and water, oral contact with contaminated surfaces of inanimate objects like toys and tables and participation in day care activities especially for children, with the virus likely spreading through faecal-oral contact from other children and day care workers<sup>8</sup>). Transmission may also be via respiratory droplets from sneezes, cough and nose drip<sup>9</sup>. Nosocomial transmission is also frequent in paediatric wards and hospitals with poor sewage treatment and sanitation status.

Rotavirus infection is neither a priority nor part of routine diagnostic tests in hospital clinical laboratories. The big challenge is that most of the patients are never diagnosed and blind treatment may ensure, especially in scarce resource countries like Africa. Such blind treatments leads to abuse of drugs, predisposing patient to drug resistance and drug reactions and increased mortality due to unknown agent. Thus the goal of this study was to determine the prevalence and risk factors for rotavirus infection in children with Diarrhea and characterize the genotypes of infecting rotaviruses.

#### **MATERIALS AND METHODS**

**Patients:** The study enrolled children attending Out-patient Department of both Government and private Hospitals in Abuja, Nigeria. The locations covered were Kubwa, Gwagwlada, Bwari, Karishi and Nyanya. The children were not hospitalized at the time of study. They were selected based on presenting symptoms of diarrhoea/vomiting and with or without fever. The study period was from October, 2013 to September, 2014.

**Ethical considerations:** The study protocol was reviewed and approved by Ethical Committee for Health Research, Directorate of Medical Laboratory Services, Federal Capital Development Authority, Abuja-Nigeria. Informed written consent was obtained from either of the parents of the children. Information about the children's demographic and risk factors for rotavirus infection was obtained from the parents or caregiver.

**Stool sample collection:** All reagents used for the study were of analytical grade and sterile where necessary. A total of 144 diarrhoeic stool samples were collected from children within the age of 5 years. A diarrhoeic case in this study was defined as a child passing loose, liquid, watery or bloody stool, three or more times in a 24 h period as reported by the mother or caregiver. Information on demographic characteristics, nature of illness and clinical manifestations were obtained from either the parents or caregiver. The stool samples were divided into aliquots and stored in frozen at -95°C, until tested. Two vials of the aliquots were transported to Noguchi Memorial Institute of Medical Research, University of Ghana, Accra, for genotyping analysis.

**Detection of rotavirus antigens:** Ten percent (10%) suspensions of the stool samples were made using phosphate buffered saline (PBS) and subsequently used for the detection of rotavirus antigen using a commercial rotavirus enzyme-linked immunosorbent assay (ELISA) kit (Oxoid, Basingstoke-Hants, United Kingdom). The experiment was carried out according to manufacturer's instructions.

**Reverse transcription and determination of G and P genotypes:** A total of 36 stool samples that were positive for rotavirus antigen were selected for genotyping. Extraction of viral RNA was performed using the Phenol Chloroform method. Briefly, 10% stool suspensions were mixed with 1:10 volume of 1 M sodium acetate containing 1% sodium dodecyl sulphate and incubated for 15 min at 37°C. Viral RNA was extracted with 500 µL volume of a 1:1 phenol-chloroform mixture at 56°C for 15 min and centrifugation at 12,000 rpm for 3 min each recovered supernatant was further extracted thrice by the same procedure described above and the final supernatant placed a sterile tube. The final supernatant was retrieved and placed in a 1.5 mL sterile tube. The 500 µL of 6 M Guanidine Isothiocyanate (GITC) was added to the recovered suspension, vortexed for 30 sec and centrifuged at 12,000 rpm for 5 min. The RNaid<sup>®</sup> kit (Qbiogene, Carlsbad, CA, USA) was used to further extract and purify the RNA according to manufacturer's instructions. The supernatant from the extraction procedure containing the extracted RNA was transferred into sterile tubes and stored at -20°C until needed for RT-PCR reactions.

**VP7 and VP4 RT-PCR:** Reverse transcription PCR of the G and P genes was performed on the purified double stranded RNA (dsRNA). The VP7 gene was reverse transcribed using Beg9 (GGCTTTAAAAGAGAGAATTTCCGTCTGG) and End9 (GGTACACATCATACAATTCTAATCTAAG) primers<sup>10</sup> while the VP4 genes were reverse transcribed using Con3 (ATTTCGGACCATTTATAACC) and Con2 (TGGCTTCGCTCATTTATAGACA) primers<sup>11</sup> (Table 1).

**VP7 and VP4 genotyping:** The G (VP7) genotyping was performed using an array of primers specific to 8 human rotavirus strains, G1-4, G8, G9, G10, G12) already described by Gouvea *et al.*<sup>10</sup>. Also, P (VP4) genotyping was performed using VP4 specific primers (P<sup>4</sup>, P<sup>6</sup>, P<sup>8</sup>, P<sup>9</sup> and P<sup>10</sup>) described by Gentsch *et al.*<sup>11</sup>. The PCR products from both processes were electrophoresed by adding 8 µL of amplicon to 1.5% agarose gel wells in 0.5 µg mL tris acetic acid EDTA buffer (PH 7.9) at 120 volts for 45 min. (Table 1).

PAGE analysis: Double stranded RNA was extracted using the Bender Buffer method with slight modifications<sup>12</sup>. In brief, 200 µL of Bender buffer was added to 200 µL of 10% stool suspension and vortexed before incubating at 65°C for 30 min. This was followed by the addition of 60 µL of 8 M potassium acetate, mixing and incubation on ice for 45 min. After incubation, the tubes were centrifuged at 10,000 rpm for 20 min and the supernatant transferred into new eppendorf tubes and 950 µL of absolute ethanol were added to the recovered supernatant, incubated on ice for 10 min and centrifuged at 10,000 rpm for 20 min twice. The ethanol was discarded followed by the addition of 200 µL tris EDTA buffer to the pellet and incubation at room temperature for 30 min. Further extraction was performed by the addition of 100 µL of 5M NaCl and 420  $\mu$ L of cold absolute ethanol and incubation at -20°C for 1 h followed by centrifugation at 10,000 rpm for 20 min. The supernatant was discarded and the extracted RNA pellets dried under vacuum.

Electrophoresis was performed on the extracted RNA in a 10% polyacrylamide resolving gel enhanced by 3% stacking gel in a tris-glycine buffer system. The RNA pellets were resuspended in bromophenol blue loading buffer containing glycerol, centrifuged for 10 sec at 10,000 rpm and loaded on to the gel wells. Electrophoresis was conducted at 100 V for 18 h at room temperature. The gels were stained using the Silver nitrate method and the PAGE profiles documented.

**Statistical analysis:** The data were analyzed using descriptive statistics and expressed as means and standard deviations. The variables were compared using chi-square and Fisher's exact test at a 95% confidence interval with probability (P) values less than or equal to 0.05 considered statistically significant. The SPSS package version 25.0 was used for data analysis.

Table 1: Oligonucleotide gene specific primer sequence used for VP7 and VP4 GENOTYPING PCR

Primer	Gene	Sequence (5'-3')	Strain genotype
Gouvea/iturriza-gomara primer	S		
aAT8	VP7	GTCACCATTTGTAAATTCG	G8 (69m)
aBT1	VP7	CAAGTACTCAAATCAATGATGG	G1(Wa)
aCT2	VP7	CAATGATATTAACACATTTTCTGTG	G2(DS-1)
aDT4	VP7	CGTTTCTGGTGAGGAGTTG	G4(ST-3)
aET3	VP7	CGTTTGAAGAAGTTGCAACAG	G3(P)
aFT9	VP7	CTAGATGTAACTACAACTAC	G9(W161)
G10	VP7	ATGTCAGACTACARATACTGG	G10
G12	VP7	CCGATGGACGTAACGTTGTA	G12
Gentsch/iturriza-gomara primei	'S		
1-T1	VP4	TCT ACT TGG ATA ACG TGC	P(8) KU
1-T1VN	VP4	CGT GCA GCT AGG TCA TCT	P(8) Vietnam
1-T1Wa	VP4	CGT GCA ATT GGG TCA TCT	P(8) jrg
2T-1	VP4	CTATTGTTAGAGGTTAGAGTC	P(4) RV5
3T-1	VP4	TGTTGATTAGTTGGATTCAA	P(6) 1076
4T-1	VP4	TGAGACATGCAATTGGAC	P(9) k8
5T-1	VP4	ATCATAGTTAGTAGTCGG	P(10) 69M

#### RESULTS

**Rotavirus antigen detection:** A total of 25% (36/144) of the subjects had human rotavirus antigen detected in their stool sample. The rotavirus infection was distributed among the Abuja districts, with Gwagwalada having the highest prevalence of 8.3% (12/144), followed by Kubwa with 5.6% (8/144). Nyanya, Bwari and Karishi had prevalence rates of 4.9% (7/144), 3.5% (5/144) and 2.8% 4/144), respectively.

**Patient characteristics:** Rotavirus antigen was detected more in males than in females. Analysis of the age distribution of children affected by rotavirus showed that the highest

prevalence rate of rotavirus infection was found in children aged between  $\leq 10$  months old. The prevalence seemed to decrease with increasing age (Table 2). The seasonal occurrence of rotavirus indicated a peak period of April, while Infection rates fluctuate between November, March and declined from May, till September, (Fig. 1).

**Assessment of risk factors:** The risk factors for the transmission of rotavirus were also assessed (Table 2). The children that were fed with feeding bottles were more infected, than those that do not use feeding bottles. The use of feeding bottles was a significant risk factor (p = 0.003). Attendance to Day Care centers was also a significant risk

Table 2: Demographic features and risk factors associated with rotavirus infections in infants

Variables	Total: 144	Rotavirus positive (%)	p-value	OR (95% CI)
Sex:			0.560	
Male	82	22 (15.3)		
Female	62	14 (9.7)		
Age group (months):				
<u>&lt;</u> 10	65	20 (13.9)		
	43	13 (9.0)		
21-30	27	2 (1.4)		
31+	9	1 (0.7)		
Exclusive breastfeeding:			0.676	
Yes	44	10 (6.9)		
No	100	26 (18.1)		
Use of feeding bottle:		20 (1011)	0.003	0.30(0.130-0.675)
Yes	73	26 (18.1)	0.005	0.30(0.130 0.075)
No	73	10 (6.9)		
Attendance to day	, ,	10 (0.2)	0.038	0.45(0.208-0.963)
Care Centers:			0.050	0.45(0.200 0.905)
Yes	55	19 (13.2)		
No	89	17 (11.8)		
Contact with pets:			0.689	
Yes	52	14 (9.7)		
No	92	22 (15.3)		
Use of toys:	72	22 (1918)	0.536	
Yes	98	23 (16.0)	01000	
	50	23 (10.0)		
No	46	13 (9.0)		
Fever:	10	13 (2.0)	0.128	
Yes	95	20 (13.9)	0.120	
No	49	16 (11.1)		
Diarrhea/vomiting:	7	10 (11.1)	0.381	
Yes	83	23 (16.0)	0.501	
No	61	13 (9.0)		
Use of ORT (oral rehydration therapy):	01	13 (9.0)	0.919	
	05	24(167)	0.919	
Yes No	95 49	24 (16.7)		
	49	12 (8.3)	0.411	
Previous rotavirus			0.411	
Vaccination:				
Yes	2	0 (0.0)		
No	142	36 (25.0)		

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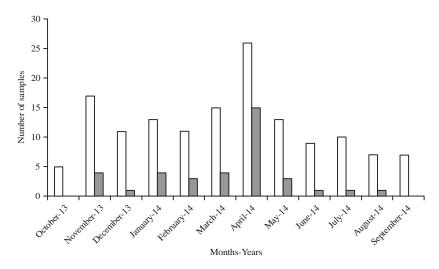


Fig. 1: Seasonal distribution of rotavirus infections during the study period

Genotypes	P Hoshino <i>et al.</i> <sup>6</sup>	P Stupka <i>et al.</i> <sup>8</sup>	PMIX	Total (%)
G1	5	1	3	9 (25)
G3	7	1	2	10 (28)
G9	0	1	0	1 (3)
G12	3	6	2	11 (30)
GMIX	2	1	1	4 (11)
GNT	0	0	1	1 (3)
Total (%)	17 (47.2)	10(27.8)	9 ( 25)	36 (100)

GNT: Genotype non-typable, GMIX: Genotype mix

factor (p = 0.038), infants attending Day Care centers had more rotavirus infections than those who do not attend Day Care Centers. In a multivariate analysis, the use of feeding bottles, OR 0.30 (95% C I: 0.130-0.675) and attendance to day care centre with OR 0.45 (95% C I: 0.208-0.963), were independently associated with the rotavirus infection (Table 2).

**Clinical manifestations:** The manifestation of symptoms like fever, diarrhea and vomiting, were not significantly associated with Rotavirus infections (Table 2). The number of subjects presenting with fever and who were diagnosed with rotavirus disease were greater than those who did not present with fever. The same pattern of presentation was observed for subjects presenting with diarrhea and vomiting. There was a greater number of subjects who were rotavirus positive among those that employed the use of Oral Rehydration Therapy (ORT) as a control measure for diarrhea, than those that did not use ORT. All 36rotavirus positive stool samples were infants that never received rotavirus vaccine (Table 2).

**Distribution of G and P genotypes:** Thirty-six (36) stool samples that were positive for rotavirus antigen were

genotyped. Table 3 summarizes the distribution of G and P genotypes in the typed samples. A total of 69% (22/36) rotavirus-positive fecal samples had only one rotavirus strain, whereas 11 (31%) had mixed rotavirus strains. The most common G genotypes identified were  $G_1$ , followed by  $G_3$  and  $G_{12}$ . The G9 genotype and the G non-typable (GNT) were the least frequent and seen in one sample each. Among the mixed G-types (G Mix)found in 4 samples, the  $G_{12}$   $G_1$  strain was more common than the  $G_{12}$   $G_3$  strain (Table 3).

Among the P-genotypes, the P<sup>6</sup> genotype were more prevalent in the samples than the P<sup>8</sup> genotype. Among the samples with mixed P genotypes (P mix), the P<sup>8,6</sup> genotype was the most common combination while P<sup>4</sup> was also detected in two samples as a P<sup>8,4</sup> enotype mix. Generally, about one third of the infants typed samples were infected with mixed G/P genotypes. Among the non-mixed rotavirus infections, strains with the single G<sub>3</sub>P<sup>6</sup> genotype predominated (Table 3).

No significant difference was observed in the distribution of rotavirus genotypes between the males and females. The  $G_{12}$  genotype occurred in all ages with its highest frequency seen in children below 10 months old. The genotypes G9 and GNT occurred only in two infants within

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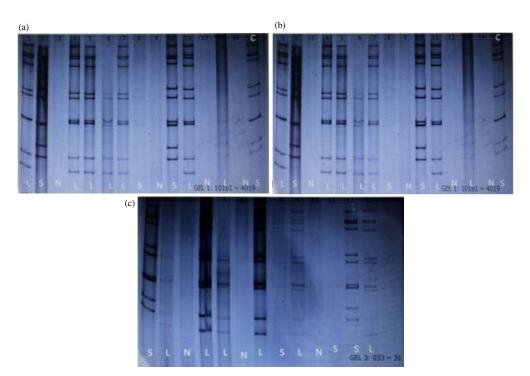


Fig. 2(a-c): PAGE analysis of rotavirus strains in stool samples. Fig. 2a, b and c represents migration patterns for the 36 rotavirus positive samples 1-14, 15-28 and 24-36, respectively. The lanes marked 'C' is the control lane. The lanes marked 'L' indicate gene segment clusters showing long migration patterns while those marked 'S' indicate gene segment clusters showing short migration patterns. Lanes marked 'N' indicates segments showing either mixed long and short RNA migration patterns or faint positive or negative migration patterns

Table 4: Summary of p type distribution by age						
	Age (Months)					
P-Type	0-10	11-20	21-30	31+	Total (%)	
P Hoshino <i>et al.</i> <sup>6</sup>	10	7	0	1	18 (50)	
P Stupka <i>et al</i> . <sup>8</sup>	10	6	1	1	18 (50)	
Total (%)	20 (55.5)	13 (36.1)	1(2.8)	2(5.6)	36 (100)	

the same age group (11-20 months old). Age distribution analysis of the P-genotypes also showed that P<sup>6</sup> and P<sup>8</sup> occurred more in infants aged less than 21 months, with highest frequency seen in children between 6-10 months old (Table 4).

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**Nucleic acid detection:** The nucleic acids of all 36 positive specimens were analyzed by Polyacrylamide Gel Electrophoresis (PAGE) (Fig. 2). The migration pattern of the viral genomic segments showed 47% (17/36) were of long RNA migration pattern while 28% (10/36) showed a short RNA migration pattern and 25% (9/36) showed either mixed long and short RNA migration patterns.

#### DISCUSSION

The present study was undertaken to assess the prevalence and genotype of rotavirus infections among infants aged 5 years and below in Abuja, Nigeria. Rotavirus infection was found in 36 out of the 144 samples taken with children aged 10 months or less being affected.

Rotavirus has been found to be a major cause of childhood diarrhea that leads to hospitalization of children less than 5 years of age with associated high morbidity and mortality rates<sup>1</sup>. A prevalence rate of 25% observed in this study was similar to previous study by Mohammed *et al.*<sup>7</sup> in Kaduna, Nigeria where a prevalence rate of 32.2 and 7.6% from diarrheic stools was reported. A prevalence rate of

25.5 and 28.9% was reported from Ghana and Abidjan<sup>13,14</sup>, respectively, while in Uganda, Bwogi *et al.*<sup>15</sup> reported 37% higher occurrence of rotavirus infection. The detection of rotavirus as aetiologic agent of gastroenteritis indicates the wide spread of the viral infection within the areas surveyed and this constituted a major health problem.

The study revealed a predominance of rotavirus infection in male subjects compared to the females. This report is similar to that of Tagbo *et al.*<sup>16</sup> and Bonkoungou *et al.*<sup>17</sup>. The reason for this predominance remains unclear though a possible explanation might be the suggestion that boys are 2 times more likely to be hospitalized than girls hence explaining the slight predominance by males.

The high prevalence of rotavirus in children less than 10 months old. This age pattern of rotavirus infection can be attributed to the absence of well-developed immune system to offer protection against rotavirus, especially as majority of the children analyzed were not vaccinated against rotavirus. It has been suggested that as children grow and have repeated exposure to the virus, they eventually become immune to and/or possibly become carriers of the virus<sup>18</sup>. This finding is in contrast to the report of Dennehy<sup>19</sup> that reported that rotavirus infection is more in children aged 9-15 months. The reduction of rotavirus infection as age increases is similar to a previous study by Grace and Jerald<sup>18</sup>.

In a multivariate analysis, the independent risk factors for the transmission of rotavirus were the use of feeding bottles and attendance to day care centres. These are part of environmental factors that may be related to poor hygiene and lack of care<sup>20</sup>. Viral transmissions and infections possibly occur via fecal contamination of caregivers hands during changing of diapers of other infected children, contaminated hands of staff and children touching contaminated surfaces (like tables, toilet seats and toys) that are used by most of the children. Another possible source of infection is the respiratory route, since rotavirus, like influenza, can spread by large droplets that travel short distances suspended in the air (typically less than 3 ft.). These droplets are usually produced through sneezing and coughing as children do not reliably cover their mouth and nose properly when sneezing and coughing. The significant association seen in the use of feeding bottle could be attributed to use of improperly washed feeding bottles, touching of feeding bottle cap by children with contaminated hands during feeding and use of contaminated water in preparing foods put in the feeding bottle.

Analysis of the seasonal distribution of rotavirus reflected an increase in infection rate during the dry cooler period of the year with its peak within the months of November and April. This is similar to seasonality trends in other parts of Nigeria<sup>21</sup> and Africa<sup>22</sup>. Seasonal changes observed occasionally in temporal distribution of rotavirus cases can be explained by the variability of mean temperature, relative humidity and rainfall.

The rotavirus genotypes identified in the study shows that G/P antigens combinations of G3P<sup>6</sup> (19.4%), G12P<sup>8</sup> (16.6%) and G1P<sup>6</sup> (13.8%) were the three most prevalent genotypes. The genotype, G9P<sup>8</sup> was also detected in an 11-month child, which contrast previous study in Lagos Nigeria that found G9 infections to exist in older children greater than 24 months<sup>23</sup>. The G9P<sup>8</sup> genotypes was more prevalent among children five years in Uganda<sup>15</sup>. The findings supported the premise that G9 strains remained a major rotavirus genotypes. The different G genotypes play a role in the severity of the diarrhea associated rotavirus infections. On global basis, most severe infections are caused by 5 G-types (G1-G4 and G9) although considerable differences exist between countries<sup>1</sup>. The finding of G1, G3, G4 and G9 in this study, is in line with the globally identified diarrheic serotypes and to an extent correlate with the study in Surabaya, Indonesia<sup>23</sup> in which the authors observed a significant association diarrheal severity with the different genotypes. A frequency of diarrhea greater than 10 times daily was reported in children infected with G3, G4 and G9 rotavirus strains while an insignificant correlation was reported between the different P-types and severity of diarrhea. In this study the presence of G3, G4 and G9 may present a severe rotavirus disease.

The presence of the unusual rotavirus genotype, G12, being an emerging strain is a source of concern. The G12 strain had been reported previously in Nigeria by Ayolabi et al.24. The identification of this strain in the present study is consistent with recent reports of the possible global presence and spread of this serotype highlighting its increasing epidemiological importance and confirming it as an emerging strain although its origin is yet to be established. It has been speculated that the occurrence of this new G12 strain may be due to genetic reassortment of nucleotide sequences of other strains. This strain has been regarded as a "traveler's rotavirus strain" as it has been reported to have entered Saudi Arabia (for instance) through visitors coming in from Nepal and India<sup>25</sup>. Hence this G12 strain should be seen as an emerging strain exhibiting high capacity to spread among children just like other human rotavirus strains of the G types and requires further studies and investigations. G12 was identified in combination with P<sup>8</sup> in 16.6% of the strains and with P<sup>6</sup> in 8.3% of the strains representing a ratio of 2:1.

The infections by rotavirus of mixed strains observed in this study seem to be a common finding in Nigeria, as it has consistently been reported in other studies<sup>26,27</sup>. This could also be attributed to naturally occurring reassortment among

Rotaviruses<sup>28</sup>. It could also be due to varying concentrations of rotavirus strains that resulted in an uneven degree of PCR amplification, making interpretation of PCR gel band patterns difficult to read<sup>27</sup> or even incorrect primer binding due to genetic drift on rotavirus genome. The presence of one G non-typeable strain (GNT) is also a common feature of rotavirus molecular characterization studies in most developing countries. As observed in this study, the GNT strain existing as mixed infection with P<sup>8,6</sup> strain may represent either a strain with accumulated point mutations in the used primer binding sites, novel strains yet to be identified or even too few virus particles with intact RNA in the stool sample.

Global reports have shown PAGE to be a potential tool in understanding and studying the molecular epidemiology of rotaviruses infections<sup>22,29</sup>. In this study, 27 different electropherotypes (17 long and 10 short electropherotypes) were found. The predominance of long electropherotypes over the short electropherotypes is not unusual as it's in accordance with previous findings and now seems to be a routine<sup>14,30</sup>. Twenty-five percent of the stool samples identified as positive by ELISA did not show clear or any RNA pattern on PAGE. This could be attributed to insufficient intact RNA in the specimens detectable in PAGE, given that the sensitivity limit of PAGE stained by silver nitrate has been shown to be 3.4 ng of viral genomic RNA<sup>31</sup>. There is also the possibility that RNA in the samples might have degenerated due to poor storage resulting from power failure. However, it is important to also note that development of extensive genomic diversity among rotaviruses can also occur due to genetic reassortment as genetic rearrangement occurs either naturally or through drug or immune pressure mounted by the host system could also be a factor.

#### CONCLUSION

In this study, it was observed that rotavirus is responsible for some of the cases of severe gastroenteritis in children under the age of 5 years in Abuja, Nigeria. Children aged 10 months and below were the most affected with G1 and P<sup>6</sup> genotypes being the most prevalent. The use of feeding bottles and attendance to Day Care facilities were found to be the most significant risk factors for rotavirus infection.

#### SIGNIFICANCE STATEMENTS

The present study puts forth vital epidemiological data on the prevalence, genotypic pattern, seasonal distribution and risk factors of rotavirus infection among children aged less than 5 years in Abuja, Nigeria. Such information is required in designing and implementing necessary preventive and curative measures aimed at stemming the menace of the viral gastroenteritis such as vaccination programs, that is non existence in Nigeria. This study will also provide preliminary bases on which further studies of molecular sequencing analysis and constant monitoring of genetic drift and shift especially in G12 and the non type able strains would be based.

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