



Rotavirus Genotypes in Abuja, Nigeria

B. Balarabe-Musa^{1*} and N. T. Dabo²

¹Department of Biological Sciences, University of Abuja, Nigeria.

²Department of Biological Sciences, Bayero University Kano, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Nigeria had planned to introduce the rotavirus vaccine in the National Immunisation Programme in 2014, but this has yet to be done. Nigeria has the continent's highest mortality due to diarrhoeal diseases with little information on specific, prevalent genotypes.

Aim: The study's main objectives were to identify the predominant rotavirus genotypes and examine the effects of existing local vaccination programs on prevailing rotavirus genotypes and on preventing rotavirus diarrhoea.

Methodology: A one-year prospective descriptive study of children under 5 with acute diarrhoea was conducted from June 2018 to May 2019. Children with acute diarrhoea attending Asokoro District Hospital, Abuja. Children without diarrhoea were also recruited as a control group. Rotavirus ELISA and RNA extraction were done with commercially available kits, and positive samples were subjected to RT-PCR and electrophoresis to determine VP7 (G) and VP4 (P) genotypes.

Results: Rotavirus-ELISA was positive among 231 (17.8%) children with diarrhoea and 29 (2.2%) of controls, with November, December. The predominant VP7 genotypes was G1 (n=116, 50.2%) followed by G9 (n=66, 28.5%). Viral Protein, VP4 (P) was mostly P [8] (n=143, 74.8%) followed by P [4] (n=21, 10.9%). The predominant genotype combinations found were G1 P [8] (n=108, 46.7%), G9 P [8] (n=62, 26.8%), and G2 P [4] (n=18, 7.7%). Very few mixed infections were found in the study, 2 (0.8%). Among 94 unvaccinated children with rotavirus isolates that were genotyped, G1 P [8] (n=88, 49.4%) and G9 P [8] (n=43, 24.1%) were predominant. Among 32

vaccinated children, G1 P [8] (n=13, 40.2%) and G9 P [8] (n=9, 28.1%) were predominant.

Conclusion: The emergence of new genotypes such as G 12 P [4] found in this study emphasize the need for continued prospective monitoring of rotavirus at the molecular level to detect new threats to vaccine programs in future.

Keywords: Rotavirus; genotypes; Abuja.

1. INTRODUCTION

Rotavirus is a genus of double-stranded RNA viruses in the family *Riviridae*. There are five species of the virus referred to as A to E. Group A rotavirus is the most common cause of gastroenteritis in humans and causes over ninety percent of infections in humans [1]. Six viral proteins make up the rotavirus protein virion which are called VP1 to VP7. In addition, there are six nonstructural proteins called NSP1 to NSP6. The nonstructural proteins only manifest when a cell is infected with rotavirus. The VP4 and the VP7 determine the serotype of the rotavirus [2]. The VP7 determines the G serotype, and the most common are the G1-G12, while the VP4 determines the P serotypes, and the most common are the P [4], P [6], and P [8] [3]. The G9 genotype has gained global attention in recent years, with the G9P[8] being the most common combination [4].

Rotavirus is the most common cause of severe diarrhoea in children, and almost every child in the world has been infected with rotavirus at some point in their life [5]. The virus is transmitted through the fecal-oral route entering the body to infect and damage the cell lining of the small intestine and cause gastroenteritis, usually in children zero to fifty-nine months [5,6].

The 2013 global estimate for Nigeria showed that 164,010 died due to diarrhoea of which 32, 802 died due to rotavirus diarrhoea [7]. However, reports on rotavirus diarrhea are scarce, and very few have shown the trends, burden, and costs of hospitalisation. According to Jinadu et al. [8], 160 (90.9%) of children positive for rotavirus were under the age of two years, and more males were affected than females [8]. In addition, rotavirus is present in 23% of 260 children with diarrhoea in four northern states [9]. In Zaria, a community-based study reported that rotavirus and astroviruses were significantly associated with acute diarrhoea in children < five years of age [10].

Throughout this study, there was no single publication from Abuja that had been identified.

Hence, this research aims to determine the circulating rotavirus genotypes and their distribution among vaccinated and unvaccinated children.

2. METHODOLOGY

2.1 Study Area

The study was carried out in Abuja, the Federal Capital Territory of Nigeria. Nigeria has a total population of 150 million people according to the 2006 population census, of which about 5 million people live in Abuja [11]. Abuja experiences two weather variations typical to sub-Saharan Africa, the dry season (summer) and the rainy season in between the two seasons. There is a brief interlude of harmattan occasioned by the northeast trade wind, accompanied by dust haze with intensified cold and dry air [12]. The rainy season begins in February and ends in October, daytime temperatures reach 28 °C (82.4 °F) to 30 °C (86.0 °F) and night time is as low as 22 °C (71.6 °F) to 23 °C (73.4 °F). In the dry season, daytime temperatures can soar as high as 40 °C (104.0 °F), and nighttime temperatures are as low as 12 °C (53.6 °F). Even the chilliest nights can be followed by daytime temperatures well above 30 °C (86.0 °F). The high altitudes and undulating terrain of the FCT act as a moderating influence on the weather of the territory [13].

2.2 Study Site

Asokoro (District) General Hospital is a government-owned hospital that provides services in the medical areas such as Paediatrics and child health, Ante-natal, Post-natal, and Obstetrics, Accident and Emergency, Medical laboratory, Radiology, Ophthalmology and so on. The hospital has a patient attendance of over 15,000 monthly, of which 7000 are pediatric inclined. The pediatric ward has 30 beds for hospitalized children.

2.3 Ethical Approval

Ethical approval was obtained from the Federal Capital Development Authority Abuja Research

ethics committee and the Asokoro District hospital research ethics committee. A consent form was administered to the parent/guardian of children before enrolment into the study.

2.4 Inclusion Criteria

All children aged between one month and five years hospitalized or attending the outpatient clinics with a complaint of acute diarrhoea, independent of disease severity. Children were included if the parents provided informed consent and stool sample was collected before leaving the study setting.

2.5 Exclusion Criteria

Parents who left the premises without their child giving a stool sample were excluded from the final analysis as they were unaccompanied minors. A tally was kept to allow the interpretation of data from the children enrolled.

2.6 Laboratory Protocol for Rotavirus

2.6.1 Preparation of faecal supernatants

The faecal supernatant was prepared before ELISA testing. The faecal supernatant was first prepared by adding pea size of solid stool (solid) or 100 μ l (loose) of watery stool to 900 μ l of 0.01M of Phosphate Buffered Saline with pH 7.4 in a 1.5ml Eppendorf tube. The mixture was then vortexed for 30 seconds in a class I biosafety cabinet and centrifuged at 13000 rpm for 10 minutes at 4°C. The supernatants were pipetted off and placed in a clean, sterile safe-lock 1.5 ml Eppendorf tube. The supernatants were frozen immediately at -80°C.

2.6.2 Enzyme-Linked Immunoabsorbent Assay (ELISA) for rotavirus

The enzyme-linked immunoassay (ELISA) is the most recent, sensitive method of detecting rotavirus antigens. The ELISA kits (Rotaclone, Meridian Bioscience, Cincinnati, OH, USA) use monoclonal antibodies sandwich within microtitre wells, which is directed against the VP6 (sixth viral protein). A stool sample suspected to be infected with the rotavirus is added to the well, and an anti-rotavirus monoclonal antibody conjugated to horseradish peroxidase is added. This results to the rotavirus being sandwiched between the solid base and the enzyme-linked

antibodies. The mixture is incubated for one hour at room temperature, and the wells are washed, the color indicator is added, and those that develop blue color are regarded as positive. Thus, the intensity of the blue color determines the concentration of the rotavirus antigen. The ELISA kits came with the following: enzyme conjugate, enzyme-substrate, sample diluents, color developer, positive control, and a stop solution.

2.6.3 RNA extraction from positive samples using the QiagenRNeasy extraction method

Samples that yielded a positive result on screening ELISA were studied further to determine the serotype of rotavirus. RNA was extracted from positive samples from the ELISA tests with QIAGEN kits (QiagenRNeasy, Hilden, Germany). Samples were thawed, and 250 μ l of 10% fecal suspension was placed in a 1.5ml Eppendorf tube. A lysing buffer (RLT buffer) was made in the fume cupboard by adding 100 μ l of beta-mercaptoethanol to 10ml of RLT buffer (from Qiagen kit). 250 μ l RLT buffer was added to all the tubes and left for five minutes and vortexed; RLT buffer is proteolytic. Therefore it denatures the protein and releases the genome. 250 μ l of 70% ethanol was added to the homogenized lysate and mixed. The 2ml Qiagen mini-columns supplied in the kit were labeled, 750 μ l of the sample was added to the columns and was centrifuged at 14600rpm for 15 seconds. The flow-through was discarded into a waste container, 700 μ l of RW1 wash buffer (supplied in the kit) was added to the columns and centrifuged at 14600rpm for 15 seconds. The collection tubes were discarded.

Ethanol was added into the RPE buffer (membrane washing buffer) supplied in the Qiagen kit. Columns were placed in a clean collection tube, and 500 μ l of RPE buffer was added into each tube and centrifuged at 14600rpm for 15 seconds, and the flow-through was discarded, 500 μ l of RPE buffer was added into each column and centrifuged for 2 minutes. Collection tubes were discarded, and columns were placed into clean collection tubes and centrifuged at 14600rpm for 2 minutes. Collection tubes were discarded, and columns were placed in clean 1.5 Eppendorf tubes. The RNA was eluted by adding 50 μ l of HPCL water directly on the silica membrane, and the columns were closed gently and centrifuged at 14600rpm for 1

minute. The columns were discarded, and the RNA was stored at -80°C.

2.6.4 cDNA synthesis

cDNA is used to refer to an mRNA transcript's sequence, expressed as DNA bases in the presence of an enzyme called reverse transcriptase. The enzyme naturally occurs in retroviruses.

The cDNA was prepared with Qiagen kit reagents. 0.5ml tubes were labeled, and 20µl of RNA was placed in each tube. The RNA was placed in the thermal cycler (Labnet International Inc, USA) for 5 minutes to denature at 96°C. The tubes were quickly chilled on ice to avoid RNA strands from delineating. Master mix reagents were defrosted on ice and were prepared with the specifications below. 14.5µl of the master mix was added to each tube of 20µl denatured RNA and were returned to the thermal cycler for 45 minutes with the program of cycles below, cDNA were frozen at -20°C.

2.6.5 G Typing (primary PCR) master mix

Master mix reagents and cDNA's were defrosted on ice. 45µl of the master mix was dispensed into each tube, and 5µl of cDNA was added accordingly. The tubes were arranged in the thermal cycler (Labnet International Inc) with the above program of cycles, and we're set for 3 hours 03 minutes for 35 cycles. The primary PCR products were stored at 4°C for the second amplification.

2.6.6 G typing (secondary PCR) master mix

The secondary PCR products were run on agarose gel immediately and viewed under ultraviolet light; the best images of gels were captured and printed. Bands that were positive for G types 1, 2,3,4,8,9,10, and 12 were recorded according to their molecular sizes. A repeat test was done on samples that showed no bands to confirm that they were true negative.

2.6.7 P typing (primary PCR) master mix

Master mix reagents and cDNA's were defrosted on ice. 22.5µl of the master mix was added to each 0.5ml tube, and 2.5µl of cDNA was accordingly. The tubes were arranged in the thermal cycler (Labnet International Inc) with the above program of cycles, and we're set for 3

hours 05 minutes for 35 cycles. The primary PCR products were stored at 4°C for the second amplification.

2.6.8 P typing (secondary PCR) master mix

Master Mix reagents were defrosted on ice. 45µl of the master mix was added to each 0.5ml tube, and 5µl of the primary PCR products were added respectively. The tubes were arranged in the thermal cycler (Labnet International Inc) with the above program of cycles and were set for 3 hours 04 minutes for 30 cycles. The secondary PCR products were run on agarose gel immediately and viewed under ultraviolet light; the best images of gels were captured and printed. Positive bands for P types 4, 6, 8, and 9 were recorded according to their molecular sizes. A repeat of samples that showed no bands was done to confirm that they were true negativity. In addition, primary PCR products were run on the gel of samples that were PCR negative. This was done to identify higher molecular weight bands, which will inform for another test to confirm negativity or positivity. For quality assurance, samples were selected at random, and all procedures were run to confirm previous results.

2.6.9 Agarose gel electrophoresis

The agarose gel electrophoresis is used to separate PCR products according to their molecular weight by running them on a gel, and their migration is based on the ionic, viscosity, strength, and temperature of the medium they are moving (Nakagomi et al., 1988).

The gel tray was sealed at both ends with tape and an inserting comb. 2.25gms of Gibco electrophoresis agarose (BIOLINE, UK) was added to 150mls of 0.5x TBE in a conical flask and swirled gently until it homogenized. The solution was microwaved for two and half minutes, It was allowed to cool, and 3µl of ethidium bromide (Sigma Chemical Company, Germany) was added and swirled gently until the bromide was homogenized. The agarose was poured into the gel tray and allowed to sit at room temperature.

The 0.5ml Eppendorf tubes were labeled to correspond to the PCR product and one (DNA ladder) ladder. Loading buffer was made up by adding 100µl of concentrated loading buffer to 900µl in a 1.5 Eppendorf tube and kept for subsequent use. The DNA ladder was made by adding 2µl of 1Kb ladder to 10µl of the loading

buffer to make up to 12µl. 5µl of PCR products were added to 10µl of loading buffer to make up to 15µl. The comb and tape were removed from the gel tray, and the tray was immersed in the 0.5x TBE buffer in the horizontal electrophoresis. The prepared PCR products were loaded into the wells and ran at 90 volts for 1 hour. The gel was examined under UV light to identify bands according to their molecular weight.

2.6.10 VP6 Probe for PCR negative samples

A real-time PCR (Taqman) was set up for samples that failed to genotype using RT-PCR to confirm the presence of rotavirus. Briefly, 23µl of the Taqman master mix was added to each well of a 96 well plate. 2 µl of cDNA was added to each well plus controls and were briefly spun in the plate for 5 seconds. The plate was sealed with optical covers and was transferred to an ABI PRISM 7500 SDS Taqman machine (Hilden, Germany) and was cycled using the following temperatures for the following times.

Temperature (°C)	Time (minutes)	Cycles
50	2	x1
95	2	x1
95	15 secs	x35
60	1	x35

Samples that showed negative results were adjusted in the database as negative (previous Elisa results as negative).

3. RESULTS

3.1 Common Genotypes Single and Combined

3.1.1 G typing

A total of 231 rotavirus-positive stools were genotyped (Fig. 1). The VP7 typing showed that G1 is the most predominant genotype typed with 116 (50.2%). This was mostly isolated in unvaccinated participants in the study; G9 was the second most predominant G type found in 66 (28.5%) samples. The third most predominant genotype is the G2 found in 19 samples which constitute 8.2%. G4 was found to be the fourth most predominant G type 8 (3.4%). The G3 and G12 were found in equal proportions in study 6 (2.5%), respectively. Two samples had mixed combinations of G genotype, and in five samples

(2.1%), the G genotype could not be determined.

3.1.2 P typing

Of the 231 samples genotyped, P [8] was found to be the most predominant P-type found in study 143 (74.8%). The P [4] was found to be the second most common 21 (10.9%). P [6] was the third most predominant genotype. The lowest was the P [9] genotype with 5 (2.6%)—there were no mixed types P types found in the study. There was a rather high number of non-typeable P genotypes 14 (7.3%) in the study.

3.1.3 Mixed G and P types

Table 1 and Fig. 3 showed the G and P combinations in the study. A total of 231 samples were genotyped for VP7 (G) and VP4 (P). A total of eight combinations of G and P were identified in the study. G1 P [8] combination was found to be the most predominant, the second-highest combination in the study was G9 P [8] found in 62 samples (26.8%), G2 P [4] is next most predominant with an overall of 18 (7.7%). Other genotypes found in the study include G4 P [8] and G1 P[6]. The combination with the least number in the study is G3 P [4] with 01 (0.4%) and G12 P [4] 2 (0.8%). Two samples were found to be mixed Gs, G1 G9 P[8], which amounts to 2(0.8%) of the overall.

3.2 G Combination with Non-Typable P

This G9 P [NT], G12 P [NT] combination was found in 4 out of 231 samples (1.7%). G1 G9 P [NT] was the second-highest at 3 (1.2%), Others include G1 P [NT] and G2 P[NT] with 1 (0.4%).

3.3 P Combination with Non-Typable G

Only two specimens in the study were found with this combination [NT] P6 1(0.4%) and [NT] P8 4(1.7%). There were no specimens in the overall study that was G and P untypeable.

3.4e Double Combination of Genotypes (G and P)

This was found in three specimens, and they are G1 G9 P[NT] with an overall total of 3(0.8%).

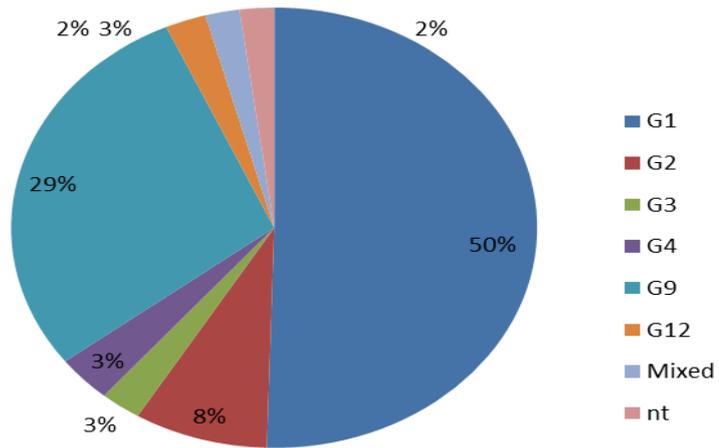


Fig. 1. Common rotavirus G types in Abuja (N=231)

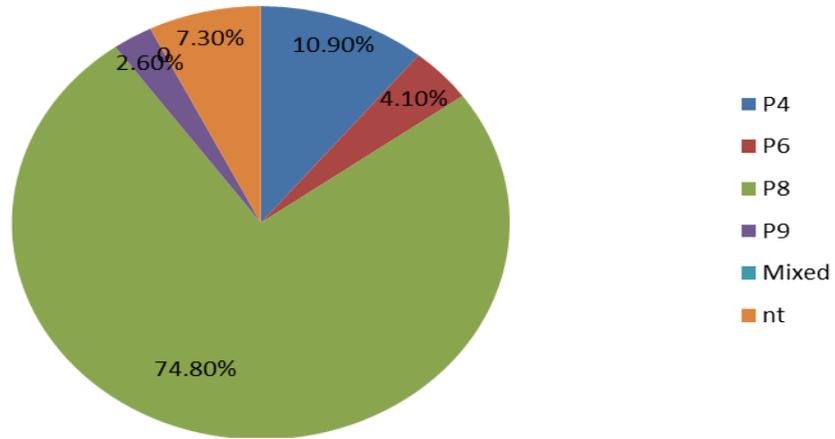


Fig. 2. Common rotavirus P types in Abuja (N=231)

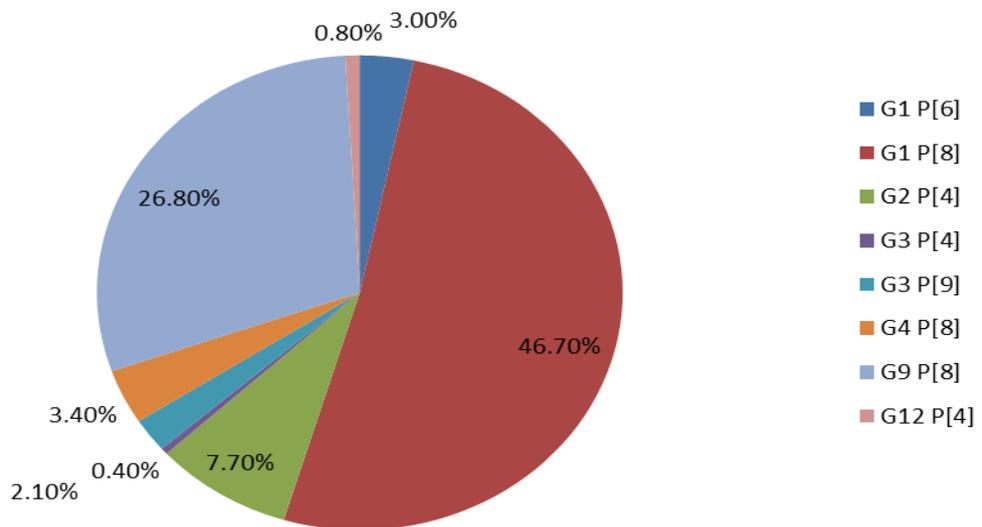


Fig. 3. Predominant G and P combinations in Abuja (N=231)

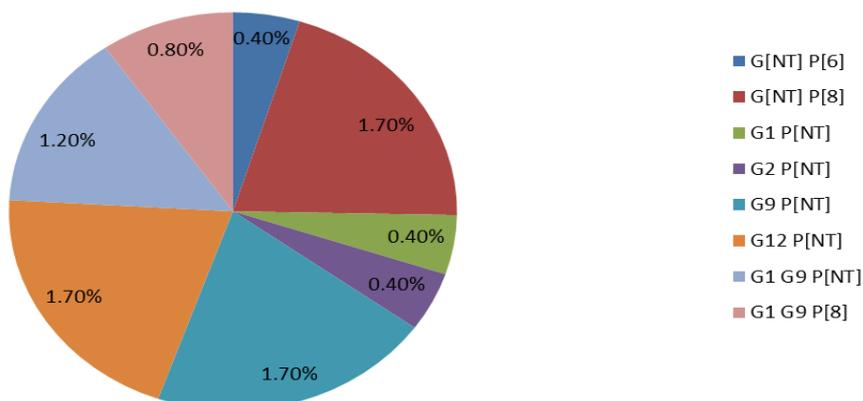


Fig. 4. Combination of G and P non-typeable

Table 1. Common Genotypes single and combined

Genotypes	Percentages % (n=231)	
G TYPES	Frequency	
G1	116	50.2
G2	19	8.2
G3	6	2.5
G4	8	3.4
G9	66	28.5
G12	6	2.5
Mixed	5	2.1
nt	5	2.1
TOTAL G's	231	100
P TYPES		
P4	21	10.9
P6	8	4.1
P8	143	74.8
P9	5	2.6
Mixed	-	-
nt	14	7.3
TOTAL P's	191	
G1 P[6]	7	3.0
G1 P[8]	108	46.7
G2 P[4]	18	7.7
G3 P[4]	1	0.4
G3 P[9]	5	2.1
G4 P[8]	8	3.4
G9 P[8]	62	26.8
G12 P[4]	2	0.8
Non-typeable		
G[NT] P[6]	1	0.4
G[NT] P[8]	4	1.7
G1 P[NT]	1	0.4
G2 P[NT]	1	0.4
G9 P[NT]	4	1.7
G12 P[NT]	4	1.7
G1 G9 P[NT]	3	1.2
Mixed Infection		
G1 G9 P[8]	2	0.8
TOTAL	231	231

Table 2. Showing frequency of genotypes among vaccinated and unvaccinated children (N=231)

Genotypes	Vaccinated patients	Unvaccinated patients	Unknown	Total
G1 P[6]	-	7	-	7
G1 P[8]	13	88	7	108
G2 P[4]	2	13	3	18
G3 P[4]	-	1	-	1
G3 P[9]	1	4	-	5
G4 P[8]	3	5	-	8
G9 P[8]	9	43	10	62
G12 P[4]	1	1	-	2
G1 P[NT]	-	1	-	1
G2 P[NT]	-	1	-	1
G9 P[NT]	1	3	-	4
G12 P[NT]	-	4	-	4
G[NT] P[6]	-	1	-	1
G[NT] P[8]	2	1	1	4
G1 G9 P[8]	-	2	-	2
G1 G9 P[NT]	-	3	-	3
Total	32(13.8%)	178 (77.0%)	21(9.0%)	231(100%)

3.5 Frequency of Genotypes among Vaccinated and Unvaccinated Children

Overall, in the study, 178 samples (77.0%) were found in unvaccinated children, 32 (13.8%) were from the vaccinated children, and 21 (9.0%) were detected in children with unknown vaccination status. The most predominant combination is the G1 P[8] with 88 (49.4%), found most among unvaccinated children. The second most predominant genotype among the unvaccinated children is the G9 P [8] with 43(24.1%) and G2 P [4] with 13 (7.3%). The next most common combination in the overall study is the G9 P [8] with (26.8%) and also G2 P [4], which accounts for 18 specimens (7.7%). The least common combinations in the study include G3 P[4], G2 P [NT], and G[NT] P[6] with 1 (0.4%) each. Most of these combinations were found in unvaccinated children, with the least among vaccinated children.

4. DISCUSSIONS

Two hundred and thirty-one (231) specimens were genotyped in this study. Five G types were found in the study. Each of the global predominant genotypes (G1, G2, G3, G4, G9, and G12) was found in the study. This finding is similar to a study conducted in Nigeria, in Jos Plateau in 1998, where all global epidemiological predominant VP7 genotypes were found among the children in the study [14].

In the current study, G1 was the most predominant 116 (50.2%) among all G-types. This is similar to several other studies published from Nigeria since the current study was commenced. A further study reported a high G1 proportion of 33% in four locations surveyed in Niger State [15]. A similar study from Kano State reported a similar finding, with G1 having a 35.0% [16]. Another study in Jos Plateau state of Nigeria; reported a low incidence of the G1 in a one-year study [17]. A study similar to current research findings, which had G2 as the most predominant G1 genotype, was conducted in Lagos, Nigeria [42-46]. The study reported that G1 was predominant with 62.5% [18]. Previous studies, as evidenced above, showed high G1 in Nigeria even though the rotavirus vaccine is yet to be introduced in the national immunization program [32-41].

G3 and G9 were found second and third most predominant in the study, with 6 (2.5%) and 66 (28.5%), respectively. This is similar to a recent study in Ghana done by Armah et al which revealed a high prevalence of G9 strains [19]. G9 strains were reported for the first time in Ghana since 2003 and had since been on the increase [20]. In Nigeria, a one-year study in Jos Plateau state (1998-1999), G9 was found in the majority of the positive rotavirus cases in the study 17.3% [17]. A study from Kano in 2010 reported a low percentage of G9 genotype among the study participants [16].

G12 was found in a high proportion, while G10 was not found in the study. G10 was reported in

low proportion in a study in Ghana, which suggests that there could have evolved through reassortment between bovine and human strains [19].

Many studies show the predominant G types globally to include G1, G2, G3, G4, and G9 (Santos and Hoshino, 2005, [19,21,22]. This study conforms to this trend worldwide and especially the serotypes common in West Africa, except that there were a high number of G12 strains 21 (16.8%), found to be the fourth most predominant G type. The reason for this is not yet known, but G12 has been reported in other studies in Ghana and Guinea Bissau [21-23]. Therefore, the high moderate numbers of G12 strains found in this study are a cause for concern.

The VP4 was also characterized for the 231 positive rotavirus cases in the study. P [8] was found to be most predominant in 143 participants, who accounted for 74.8%, followed by P [4] found in 21 participants who accounted for 10.9% and P [6] in 5 participants with 2.6%. A previous study from Nigeria reported that P [6] was the most predominant genotype found, especially among children from southern Nigeria compared to those in northern Nigeria. The authors added that P [6] was mostly found among neonates that are babies less than one month [24].

This finding agrees with previous studies on the predominant rotavirus VP4 circulating in the world and West Africa (Santos and Hoshino, 2005) [19]. A similar report was published from rural Ghana with P [4], P [6], and P [8] dominating all VP4 [25,23]. A study conducted in four locations of Niger State of Nigeria also reported a high rate of P [6] (22%) [15].

Very few mixed rotavirus infections were found in the study, as defined by more than one G and/or P-type, with only 2 (0.86%). Mixed infections have been reported in higher proportions in other studies, such as that by Horwood et al. [23], in which a mixed infection rate of 11.9% was reported. A previous study reported that children from northern Nigeria had very high rates of mixed infection of rotavirus [24], also seen in a recent study conducted in four states (Zamfara, Kebbi, Sokoto, and Kaduna) in north-western Nigeria [26]. In another study, Lagos state in the South West also reported high rates of mixed infection at 26.7% [27].

Twenty samples were untypeable (non-typeable for G and/or P-type). A high number of untypeables were also reported in a similar study [26] and in another study from Nigeria, there was a much greater proportion of untypeable isolates at 45% [28]. This suggests that more detailed investigation needs to be done to ascertain links to previous genotypes, the discovery of new or unusual VP7 of rotavirus.

4.1 Common Genotypes among Unvaccinated Children and Children with Unknown Vaccination History

Unvaccinated children and children with unknown history of vaccination were more common, with most rotavirus genotypes found among these groups [47-53]. The genotypes that predominate in this group are G1 P [8], G9 P [8], G2 P [4], G12 P [4] and G3 P [9]. Children with no vaccination history had G9 P [8] followed by G1 P [8] and G2 P [4]. G2 P [4] was found mostly in this group. In Brazil, after introducing the rotavirus vaccine in 2006, the most frequent genotype isolated from 2006-2011 was G2 P [4] and after 2011 it was reduced [29]. Another study found G2 P [4] most predominant among vaccinated and unvaccinated children [30]. The same study reported that G9 P [8] was also found in both groups (vaccinated and unvaccinated) but in moderate to high proportions. It may be that this occurrence may be due to natural variation or the effect of the vaccination. Another study in Portugal again reported a high proportion of G2 P [4] strains among unvaccinated children, followed by G9 P [8] [31], which suggests that since these children were not previously vaccinated, the existence of the G2 P [4] and G9 P [8] may be the natural fluctuation of rotavirus genotypes. Therefore, more surveillance should be in place before the introduction of the vaccine and after.

5. CONCLUSION

Few of the parents had good knowledge about the availability of rotavirus vaccines, and only a quarter could remember if/how often their child might have been vaccinated. Vaccination cards were often not available for verification for the study.

A greater proportion of children with diarrhoea with less severe disease (judged by activity scores) had been vaccinated than the children with worse activity scores.

Using the indirect estimate method of Poole, a vaccine protective efficacy of 74.3% was estimated. Looking at the rotavirus genotypes determined in 231 subjects, in the group of vaccinated children previously given R1V vaccine, there were 13(5.6%) G1 genotypes compared to 7 in the group of children of uncertain vaccination status. However, G1 was found in 88 (38.0%) of isolates and was found most in unvaccinated children. There were no other major differences in genotypes and combinations between vaccinated and unvaccinated groups except the novel genotype G12 was found in 4 (1.7%) of unvaccinated children. This genotype has recently emerged in several continents and maybe more pathogenic. This requires further monitoring.

6. RECOMMENDATIONS

The surveillance of diarrhoeal disease in Nigeria, the largest country in Africa, is inadequate for meaningful analysis of disease trends. Oral rehydration is managed according to WHO recommendations, but there is marked overprescribing of antibiotics and this needs to be addressed.

The lack of availability of virological investigations in most clinics in Nigeria means that specific access would be needed to support and monitor the effectiveness of vaccination campaigns. Nevertheless, results of this work suggest that Abuja closely resembles most other states in Nigeria and nearby countries in the incidence and prevalence of rotavirus diarrhoea in children under the age of 1 and slightly older and indirectly support the effectiveness of the monovalent vaccine.

The findings support the planned (but delayed) introduction of the rotavirus vaccine in Nigeria but emphasize the need for adequate investment in quality assured surveillance and virological surveillance to monitor this. In addition, the emergence of new rotavirus genotypic combinations may pose a threat to vaccine efficacy in the future. Henceforth, the use of a pentavalent vaccine may be considered in the future.

CONSENT

All authors declare that 'written informed consent was obtained from the patient to publish this case report and accompanying images. A copy of the written consent is available if requested.

ETHICAL APPROVAL

The ethics committee of the Asokoro District Hospital, Abuja, approved this research.

DISCLAIMER

The products used for this research are commonly and predominantly used in our research area and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather, it was funded by the personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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