Prevalence and Molecular Detection of Rotavirus Gastroenteritis in Children Aged 0-5 Years in Anyigba, Kogi State

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Abstract

Background: Rotavirus is the leading cause of severe acute gastroenteritis in infants and young children, especially in low- and middle-income countries. Malnutrition, poor sanitation, and inadequate access to clean water contribute to its persistence and severity. This study investigated the prevalence and molecular detection of rotavirus infection among children under five years in Anyigba, Kogi State, Nigeria. **Methods:** A total of 150 stool samples were collected from children presenting with diarrhea and screened for rotavirus antigens using a Colloidal Gold Rapid Diagnostic Kit (USA). Positive samples were further confirmed by reverse transcription polymerase chain reaction (RT-PCR). **Results:** Of the 150 children tested, eight (5.3%) were confirmed positive. The prevalence was slightly higher in females (5.8%) than males (5.0%), with no significant association between sex and infection ($p = 0.83$). Children aged 24–36 months recorded the highest prevalence (8.6%), although the association with age was not significant ($p = 0.29$). Higher prevalence was observed among children of caregivers with no formal education (6.8%) and those categorized under "others" occupations (8.3%), with no significant association ($p = 0.64$). Fever ($p = 0.04$) and history of diarrhea ($p = 0.0001$) showed significant associations with infection. **Conclusion:** Rotavirus remains a notable public health concern among children in Anyigba. Strengthened surveillance, increased vaccination coverage, and improved hygiene education are essential to reduce its burden.

Keywords: Rotavirus; Gastroenteritis; Molecular detection; Children under five; Public health surveillance.

Introduction

Rotavirus, a double-stranded RNA virus belonging to the *Reoviridae* family, has been identified as the most common cause of severe acute gastroenteritis in infants and young children, particularly in low- and middle-income countries Crawford et al., 2017; Kotloff et al., 2013. Factors such as malnutrition, poor sanitation, and limited access to potable water have been identified as significant contributors to the persistence and severity of rotavirus infections Omatola et al., 2016 Digwo et al., 2023.

Rotavirus infections are transmitted through the fecal–oral route and are highly infectious, with as few as 100 virions capable of causing disease in a susceptible host. The disease typically manifests with symptoms such as watery diarrhea, vomiting, fever, and dehydration, which can be life-threatening if not properly managed Liu et al., 2016. According to the Global Burden of Disease Study (2019), approximately 185,300 children die each year from rotavirus infection. The recent decline in mortality rates is largely associated with the integration of rotavirus vaccines into national immunization programs beginning in 2013 Global Burden of Disease Collaborators, 2017.

In Nigeria, the prevalence of rotavirus diarrhea has been reported to range between 13.8% and 56% Mado et al., 2022. Although global and regional studies have made substantial contributions to rotavirus epidemiology, local data from sub-national areas in Nigeria, such as Anyigba in Kogi State, remain scarce.

Hence, this study aimed to: Determine the prevalence of rotavirus infection among children aged 0–5 years presenting with gastroenteritis at selected hospitals in Anyigba, Nigeria using Rapid Diagnostic Kits and RT-PCR. And, identify demographic and predisposing risk factors associated with rotavirus infection among children aged 0–5 years presenting with gastroenteritis at selected hospitals in Anyigba.

This research will contribute to national rotavirus surveillance efforts and inform public health policies for effective disease control and prevention.

Materials and Methods

Study Area

This study was conducted in Anyigba, a major town in Dekina Local Government Area of Kogi State, Nigeria, located between latitudes 7°15′ and 7°29′ N and longitudes 7°11′ and 7°32′ E. The town has an estimated population of approximately 130,000 people, comprising diverse ethnic groups. Anyigba lies within the Guinea savanna ecological zone and

experiences a tropical climate characterized by hot, dry conditions, an annual mean temperature of about 25°C, and approximately 1,600 mm of rainfall per year. Agriculture constitutes the primary economic activity of the area Ali et al., 2024.

Study Design and Population

This was a cross-sectional study designed to investigate the prevalence of rotavirus infection among children in Anyigba. Demographic, clinical, and medical history data, along with stool samples, were collected at a single point in time from children under five years of age attending selected hospitals. A total of 150 diarrheic children were enrolled in the study.

Inclusion and Exclusion Criteria

Children under five years presenting with gastroenteritis were eligible for inclusion in the study. The only exclusion criterion was failure to provide informed consent by the parent or caregiver.

Sample Collection and Storage

Approximately 2 mL of stool was collected from each eligible child using sterile, leak-proof containers labeled with unique identification codes. Samples were transported in cool boxes to the laboratory and aliquoted upon arrival. All specimens were stored at $-20°C$ until further analysis.

Rotavirus Detection

Stool specimens were screened for rotavirus antigen using a Colloidal Gold Rapid Diagnostic Test Kit (USA) according to the manufacturer's instructions. Each sample was homogenized in the supplied buffer and applied to the test cassette. Results were interpreted after 15 minutes, with the appearance of a visible test band indicating a positive reaction.

RNA Extraction

Viral RNA was extracted from stool supernatants using the Genezol RNA Extraction Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instructions. Extracted RNA was eluted in RNase-free water and stored at $-20°C$ until use.

cDNA Synthesis

Complementary DNA (cDNA) was synthesized from the extracted viral RNA using reverse transcriptase (Thermo Fisher Scientific, USA) following the manufacturer's protocol. The synthesized cDNA was stored at $-20°C$ until further analysis.

RT-PCR Amplification of VP6 Gene

Molecular detection of rotavirus was performed using reverse transcription-polymerase chain reaction (RT-PCR) targeting the conserved VP6 gene, as described by Iturriza-Gómara et al., 2002. Amplification was performed in a 40 µL reaction mixture containing standard PCR components and VP6-specific primers. PCR products were resolved on a 2% agarose gel stained with ethidium bromide, and the presence of an approximately 379 bp amplicon was considered confirmatory for rotavirus RNA.

Agarose Gel Electrophoresis

PCR products were resolved on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL). Five microlitres of each amplified product were loaded into individual wells alongside a 100 bp DNA ladder. Electrophoresis was performed at 100 V for 45 minutes, and DNA bands were visualized using a gel documentation (Gel Doc) imaging system.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 27. Descriptive statistics were presented in tables to summarize the demographic and clinical characteristics of the participants. The prevalence of rotavirus infection was calculated as the proportion of seropositive cases in the total population and expressed as a percentage. Associations between rotavirus positivity and study variables were assessed using the Chi-square test or Fisher's exact test, where applicable. A p-value of ≤ 0.05 was considered statistically significant at a 95% confidence interval.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committees of Kogi State Teaching Hospital and Maria Goretti Hospital, Kogi State, Nigeria.

Results

Table 2: **Gender and Age Distribution of Rotavirus Infection Among Children**

Variables	Tested	No. (%) +Ve	No. -Ve	p-value
Male	99	5 (5.0)	94	0.83
Female	51	3 (5.8)	48	
Total	150	8 (5.3)	142	
0–12 months	33	2 (6.0)	31	0.29
12–36 months	46	4 (8.6)	42	
36–48 months	31	2 (6.4)	29	
48–60 months	40	0 (0.0)	40	
Total	150	8 (5.3)	142	

This study investigated the prevalence and molecular detection of rotavirus among children and examined its association with socio-demographic characteristics, clinical symptoms, and behavioral risk factors. Of the 150 children tested, eight (5.3%) were confirmed positive. Females (5.8%) and children aged 24–36 months (8.6%) recorded the highest prevalence of rotavirus infection; however, no significant association was observed between sex or age and infection

Table 1: VP6 Primers Used in This Study

Primer Name	Sequence (5′ → 3′)	Description
Beg9	GGCTTTAAAAGAGAGAATTTCCGTCTGG	Forward primer
End9	GGTCACATCATACAATTCTAATCTAAG	Reverse primer

Note: The VP6 gene primers were used for RT-PCR amplification to detect rotavirus RNA.

status ($p > 0.05$) (Table 2).

Children whose caregivers had no formal education showed a higher prevalence (6.8%), although the association was not statistically significant ($p = 0.64$). Similarly, children of caregivers categorized under "others" in terms of occupation had the highest infection rate (8.3%), but this relationship was not significant ($p = 0.64$) (Table 3).

Table 3: Prevalence of Rotavirus in Relation to Socio-demographic Characteristics of Parents

Variable	Tested	No. (%) +Ve	No. -Ve	p-value
Parent's Education				
Tertiary	43	2 (4.6)	41	0.64
Secondary	29	1 (3.4)	28	
Primary	34	2 (5.8)	32	
None	44	3 (6.8)	41	
Total	150	8 (5.3)	142	
Parent's Occupation				
Civil Servant	35	1 (2.8)	34	0.64
Farmer	50	3 (6.0)	47	
Business	25	2 (8.0)	23	
Housewife	16	0 (0.0)	16	
Others	24	2 (8.3)	22	
Total	150	8 (5.3)	142	

The clinical symptoms assessed included diarrhea (5.3%), vomiting (6.5%), and fever (7.9%). Fever was the only symptom significantly associated with rotavirus infection ($p = 0.04$), while hospitalization showed a higher infection rate (7.4%) but without statistical significance ($p = 0.27$) (Table 4). Caregiver knowledge appeared to influence exposure, as all positive cases occurred among children whose caregivers lacked prior awareness of rotavirus (5.8%); however, this association was not statistically significant ($p = 0.36$). Attendance at daycare or nursery school (5.7%), as well as playing with toys or books (6.0%), showed no significant association with infection status. A significant relationship was observed among children with a history of diarrhea (17.1%; $p = 0.0001$). Although children whose caregivers did not practice handwashing had a higher prevalence (7.6%), the association was not statistically significant ($p = 0.29$).

Table 4: Prevalence of Rotavirus in Relation to Clinical Symptoms and Risk Factors

Variable	Tested	No. (%) +Ve	No. -Ve	p-value
Diarrhea				
Yes	112	6 (5.3)	106	0.97
No	38	2 (5.2)	36	
Fever				
Yes	88	7 (7.9)	81	0.04
No	62	1 (1.6)	61	
Vomiting				
Yes	92	6 (6.5)	86	0.41
No	58	2 (3.4)	56	
Hospitalization				
Yes	67	5 (7.4)	62	0.27
No	83	3 (3.6)	80	
Knowledge of Infection				
Yes	11	0 (0.0)	11	0.36
No	139	8 (5.8)	131	
Daycare/ Nursery				
Yes	106	6 (5.7)	100	0.75
No	44	2 (4.6)	42	
Plays with Toys/Books				
Yes	116	7 (6.0)	105	0.41
No	34	1 (2.9)	37	
Household Diarrhea				
Yes	115	2 (1.7)	113	0.0001
No	35	6 (17.1)	29	
Handwashing After Toilet				
Yes	98	4 (4.0)	94	0.29
No	52	4 (7.6)	48	
Uncooked Food Consumption				
Yes	114	6 (5.2)	108	0.94
No	36	2 (5.5)	34	

Discussion

Rotavirus remains a leading etiological agent of severe gastroenteritis among children under five years globally, with

significant impact particularly in low- and middle-income countries Alkali et al., 2016. In sub-Saharan Africa, including Nigeria, rotavirus-induced gastroenteritis contributes substantially to childhood morbidity and mortality. This study assessed the prevalence and molecular detection of rotavirus infection among children under five years of age in Anyigba, Kogi State, Nigeria. The observed prevalence (5.3%) is markedly lower than those reported in Nigerian and other African countries: 32.2% in Kaduna Mohammed et al., 2016, 35.0% in Lagos Audu et al., 2002, and 18.5% in Ibadan Omatola et al., 2016. Additionally, it is lower than reports from Ghana (39.8%; Armah et al., 2010, Uganda (45.5%; Nakawesi et al., 2010, and Indonesia (61.0%; Radji et al., 2010. The relatively low prevalence recorded in this study compared to other reports may be attributed to small sample size, geographical and environmental differences, diagnostic sensitivity, timing and season of sample collection, as well as improved vaccination coverage.

Age-specific analysis showed the highest rotavirus positivity (8.6%) among children aged 12–36 months. Similar findings were reported in Kaduna Mohammed et al., 2016 and Paraguay Coluchi et al., 2002. In contrast, studies conducted in Ibadan, Jos, and Zaria reported higher infection rates among children less than 24 months old Aminu et al., 2010; Junaid et al., 2011; Omatola et al., 2016. The peak rotavirus infection among children aged 24–36 months may be linked to behavioral factors.

At this developmental stage, children are more mobile, interact frequently with peers, and engage in hand-to-mouth activities, including contact with contaminated toys or surfaces, which heighten exposure to the virus transmitted via the fecal–oral route Omatola et al., 2016. The absence of infection in the 48–60 month age group may indicate acquired immunity through vaccination Mado et al., 2022. With respect to gender, no statistically significant association was observed between sex and rotavirus infection, though a slightly higher prevalence was recorded among females (5.8%). This contrasts with earlier reports from Enugu and Abuja, which documented higher rates among male children Tagbo et al., 2014; Theophilus et al., 2018.

Analysis based on parental education revealed that children whose parents had no formal education exhibited the highest prevalence of infection (6.8%), consistent with earlier findings in Ibadan Omatola et al., 2016. The absence of a statistically significant relationship suggests that rotavirus infection can occur across all educational backgrounds, potentially due to the virus's persistence on surfaces and objects, making it difficult to eliminate solely through hygiene and awareness measures. Interestingly, Mohammed et al., 2016 observed higher prevalence among children whose parents had attained primary and secondary education, indicating that additional socio-environmental factors may influence transmission dynamics.

Regarding parental occupation, the highest prevalence (8.3%) was recorded among children whose parents fell into the "Others" occupational category. This may reflect the influence of broader socio-economic and environmental determinants, including overcrowding, poor access to potable water, inadequate sanitation, low health literacy, and limited healthcare access. Unemployment and unstable income sources, common within this group, may also limit access to vaccination and proper nutrition—both crucial in reducing rotavirus infection risk.

Clinical manifestations among infected children included diarrhea (5.3%), fever (7.9%), and vomiting (6.5%), with fever showing a significant association with infection. These symptoms align with classical rotavirus gastroenteritis presentation and previous reports from Ibadan Omatola et al., 2016 and India Chavan et al., 2013. Furthermore, rotavirus infection was more prevalent among hospitalized children, consistent with findings by Patić et al., 2022, who also observed increased infection rates among hospitalized pediatric patients.

Caregiver awareness assessment revealed that all rotavirus-positive cases occurred among children whose caregivers had no prior knowledge of the virus. Although not statistically significant ($p = 0.36$), this finding underscores the need for improved public health education. Limited awareness may contribute to poor hygiene and delayed healthcare-seeking behavior, increasing exposure risk. A higher prevalence was observed among children attending daycare or nursery school, as well as those with household diarrhea or shared-item contact (e.g., toys, books). While no significant association was found between toy use and infection ($p = 0.41$), a significant association existed between household diarrhea and rotavirus positivity ($p = 0.0001$). These observations align with previous findings Omatola et al., 2016, emphasizing the role of fomites in viral transmission, particularly through contaminated shared objects handled by asymptomatic children.

Limitations

This study has certain limitations. Firstly, the findings may not be fully generalizable to the entire pediatric population of Anyigba, as data were obtained solely from children who sought medical care in selected hospitals. Secondly, the RT-PCR analysis targeted only specific rotavirus genes (VP6); hence, the study did not determine circulating genotypes or strains of rotavirus within the study area. Future studies incorporating molecular characterization and wider community-based sampling are recommended to provide a more comprehensive understanding of rotavirus diversity and epidemiology in the region.

Conclusion

The findings of this study indicate that rotavirus infection continues to pose a public health concern among children in Anyigba, Kogi State, although the observed prevalence is lower than previously reported rates. This underscores

the importance of sustaining and strengthening routine rotavirus immunization programs. It is also recommended that rotavirus screening be included in the diagnostic evaluation of diarrheal cases that test negative for bacterial and parasitic pathogens. Broader, multicenter studies incorporating both rural and urban populations would provide a more comprehensive understanding of the true burden and spatial distribution of rotavirus infection in the region.

Consent for Publication

All authors approved the final draft of the manuscript and provided consent for publication.

Availability of Data and Materials

All data used in the study are included within this manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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