Section: Miscellaneous

Original Research Article

ROTAVIRUS DIARRHOEA IN THE POST-VACCINATION PERIOD: CLINICAL PROFILE AND GENOTYPING STUDY

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ABSTRACT

Background: Rotavirus remains a leading cause of acute gastroenteritis in children under five years of age, despite the introduction of rotavirus vaccination into national immunization programs. In the postvaccination period, understanding changes in clinical presentation, disease severity, and circulating rotavirus genotypes is essential for assessing vaccine impact and guiding public health strategies.

Materials and Methods: This hospital-based observational study was conducted among children aged 6 weeks to 59 months presenting with acute diarrhoea at a tertiary care center during the postvaccination period. Demographic details, clinical features, vaccination status, and disease severity were recorded using a structured proforma. Stool samples were tested for Group A rotavirus antigen by enzyme-linked immunosorbent assay (ELISA). Rotavirus-positive samples underwent G and P genotyping using reverse transcription polymerase chain reaction (RT-PCR). Disease severity was assessed using the Vesikari Clinical Severity Score. Statistical analysis was performed to compare clinical and severity parameters between groups.

Results: Out of 163 children with acute diarrhoea, 68 (41.7%) were rotavirus positive. Rotavirus positivity was significantly higher among partially vaccinated and unvaccinated children compared to fully vaccinated children ($p = 0.031$). Rotavirus-positive children had significantly higher rates of vomiting, frequent stools, dehydration, and hospital admission ($p < 0.05$). Severe disease (Vesikari score ≥11) was observed in 36.8% of rotavirus-positive cases compared to 18.9% of rotavirus-negative cases ($p = 0.006$). Among rotavirus-positive children, fully vaccinated children had significantly fewer severe cases and shorter hospital stays compared to partially vaccinated or unvaccinated children ($p = 0.011$ and $p = 0.002$, respectively). Genotype analysis revealed predominance of G9 (25.0%) and G12 (22.1%) genotypes, with P[8] as the most common P type (61.8%). G9P[8] and G12P[6] were the most frequent genotype combinations.

Conclusion: Rotavirus continues to contribute substantially to acute diarrhoeal illness in young children in the postvaccination era. While vaccination does not completely prevent infection, it significantly reduces disease severity and hospitalization. The predominance of emerging genotypes such as G9 and G12 underscores the need for continued clinical and molecular surveillance to monitor vaccine impact and guide future immunization strategies.

Keywords: Rotavirus; Acute gastroenteritis; Postvaccination period; Vesikari score; Childhood diarrhoea.

INTRODUCTION

Acute gastroenteritis remains a leading cause of morbidity and mortality among children under five years of age globally, with rotavirus historically recognized as the most common etiological agent responsible for severe diarrhoeal disease in this age group. Prior to the widespread introduction of rotavirus vaccines, rotavirus accounted for nearly 30–40% of all hospitalizations due to acute diarrhoea and was responsible for approximately 450,000 deaths annually worldwide, predominantly in low- and middle-income countries.[1,2] Despite improvements in sanitation, hygiene, and access to safe drinking water, rotavirus transmission persists due to its high infectivity, environmental stability, and predominant fecal–oral spread.

Rotavirus is a double-stranded RNA virus belonging to the family Reoviridae, characterized by a segmented genome enclosed within a triple-layered capsid. The virus is classified into G (VP7) and P (VP4) genotypes based on outer capsid proteins, both of which play a crucial role in inducing neutralizing antibodies and determining strain diversity.[3] Globally, a limited number of genotype combinations—most commonly G1P[8], G2P[4], G3P[8], G9P[8], and G12P[8]—have been responsible for the majority of human infections; however, significant geographic and temporal variation in circulating strains has been well documented.[4,5]

The introduction of live oral rotavirus vaccines has substantially altered the epidemiology of rotavirus diarrhoea. Two vaccines—monovalent (Rotarix®) and pentavalent (RotaTeq®)—have demonstrated significant effectiveness in reducing rotavirus-associated hospitalizations and severe disease globally.[6] In India, the introduction of the indigenous Rotavac® vaccine into the Universal Immunization Programme (UIP) in 2016 marked a critical public health intervention, with phased national scale-up completed over subsequent years.[7] Postvaccination surveillance data indicate a decline in rotavirus-related hospital admissions by approximately 30–50%, along with reductions in disease severity and mortality.[8]

However, the postvaccination period has also raised important epidemiological and virological considerations. While overall disease burden has declined, rotavirus continues to cause breakthrough infections, including cases among vaccinated children, often presenting with milder clinical severity.[9] Additionally, shifts in genotype distribution—commonly referred to as genotype replacement or strain diversification—have been reported following vaccine introduction in several settings.[10] The emergence or increased circulation of non-vaccine or heterotypic strains, such as G9, G12, and unusual G–P combinations, underscores the importance of continued strain surveillance to assess vaccine impact and effectiveness.[11]

Clinical presentation of rotavirus diarrhoea in the postvaccination era may differ from the pre-vaccine period. Classical features such as profuse watery diarrhoea, vomiting, fever, and rapid dehydration remain common; however, studies suggest a trend toward reduced severity, shorter duration of illness, and decreased requirement for intravenous rehydration among vaccinated children.[12] Evaluating the clinical profile of rotavirus diarrhoea in this evolving context is essential for guiding clinical management, resource allocation, and public health policy.

In low-resource settings, where rotavirus disease burden remains disproportionately high, continuous monitoring of circulating serotypes in relation to vaccination status provides critical insights into vaccine performance and viral evolution.[13] Such data are particularly relevant for informing future vaccine formulations, strengthening immunization strategies, and ensuring sustained disease control. Therefore, the present study aimed to evaluate the clinical profile and serotype distribution of rotavirus diarrhoea in children during the postvaccination period, thereby contributing to ongoing surveillance efforts and enhancing understanding of rotavirus epidemiology in the vaccine era.

MATERIALS AND METHODS

Study Design and Setting

This hospital-based observational study was conducted in the Department of Pediatrics in collaboration with the Department of Microbiology at a tertiary care teaching hospital. The study was carried out over a defined postvaccination period, from August 2024 to July 2025, following the introduction and routine implementation of rotavirus vaccination under the national immunization program. The hospital serves as a referral center for urban and surrounding rural populations, providing inpatient and emergency pediatric services.

Study Population

The study population included children aged 6 weeks to 59 months presenting with acute diarrhoea, defined as the passage of three or more loose or watery stools within a 24-hour period, with a duration of less than 14 days. Children presenting to the pediatric outpatient department, emergency department, or requiring hospital admission during the study period were screened for eligibility. Only those with stool samples collected within 48 hours of presentation were considered for inclusion.

Inclusion and Exclusion Criteria

Children meeting the clinical definition of acute diarrhoea and whose caregivers provided informed consent were included in the study. Both vaccinated and unvaccinated children were enrolled to allow evaluation in the postvaccination context. Children with chronic diarrhoea (duration ≥14 days), known gastrointestinal disorders, immunodeficiency states, or those who had received antibiotics within 72 hours prior to presentation were excluded. Children with visibly blood-stained stools suggestive of dysentery were also excluded to minimize inclusion of non-viral etiologies.

Data Collection and Clinical Assessment

After enrollment, detailed clinical information was obtained using a structured case record form. Demographic variables included age, sex, residence, nutritional status, and immunization history, with rotavirus vaccination status verified from immunization cards or caregiver recall. Clinical parameters assessed included duration and frequency of diarrhoea, presence and frequency of vomiting, fever, dehydration status, and need for hospitalization. Dehydration was classified as no dehydration, some dehydration, or severe dehydration based on World Health Organization criteria. Treatment details, including oral rehydration therapy, intravenous fluids, and duration of hospital stay, were recorded. Disease severity was assessed using a standardized scoring system such as the Vesikari Clinical Severity Score, categorizing illness as mild, moderate, or severe.

Stool Sample Collection and Processing

Fresh stool samples were collected in sterile, wide-mouthed, leak-proof containers within 24–48 hours of presentation. Samples were transported immediately to the microbiology laboratory under cold chain conditions and stored at −20°C until further processing. A portion of each sample was used for initial antigen detection, while the remainder was preserved for molecular analysis and genotyping.

Detection of Rotavirus Antigen

Rotavirus antigen detection was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit specific for Group A rotavirus, following the manufacturer's instructions. Optical density values were read using an ELISA reader, and samples were interpreted as positive or negative based on kit-provided cutoff values. Only ELISA-positive samples were further subjected to molecular characterization and serotyping.

RNA Extraction and Genotyping

Viral RNA was extracted from ELISA-positive stool samples using a standardized commercial viral RNA extraction kit. Reverse transcription polymerase chain reaction (RT-PCR) was performed to amplify the VP7 (G genotype) and VP4 (P genotype) genes using consensus and type-specific primers. Genotyping was conducted through semi-nested multiplex PCR protocols. PCR products were analyzed by agarose gel electrophoresis, and genotype determination was based on expected amplicon sizes. Samples that could not be typed using standard primers were categorized as untypeable.

Vaccination Status Assessment

Vaccination status was classified as fully vaccinated, partially vaccinated, or unvaccinated based on the number of rotavirus vaccine doses received as per the national immunization schedule. Children who had received all age-appropriate doses were considered fully vaccinated. Breakthrough infections were defined as laboratory-confirmed rotavirus diarrhoea occurring in fully vaccinated children.

Statistical Analysis

Data were entered into a spreadsheet and analyzed using statistical software such as SPSS version 20.0. Continuous variables were expressed as mean ± standard deviation or median with interquartile range, depending on data distribution, while categorical variables were expressed as frequencies and percentages. Comparisons between groups were performed using the chi-square test or Fisher's exact test for categorical variables and the Student's t-test or Mann–Whitney U test for continuous variables. A p-value of less than 0.05 was considered statistically significant.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee prior to initiation. Written informed consent was obtained from parents or legal guardians of all participating children. Confidentiality of patient information was strictly maintained, and all laboratory procedures were performed in accordance with biosafety guidelines.

RESULTS

The study included 163 children aged 6 weeks to 59 months presenting with acute diarrhoea. The mean age was 14.8 ± 9.6 months, with the highest proportion of cases observed in the 12–23 months age group (38.0%), followed by 6–11 months (34.4%). Males constituted 58.9% of the study population. A slightly higher proportion of children were from urban areas (56.4%) compared to rural areas (43.6%). Nutritional assessment revealed that 39.9% of children had some degree of undernutrition. Seasonal distribution showed a peak during winter months (43.6%), followed by the monsoon season (33.1%). [Table 1]

Table 1: Baseline Demographic and Epidemiological Characteristics of Children with Acute Diarrhoea (n = 163)

Variable	Frequency (%) / Mean ± SD
Age (months)	14.8 ± 9.6
Age group	
6–11 months	56 (34.4)
12–23 months	62 (38.0)
24–59 months	45 (27.6)
Gender	
Male	96 (58.9)
Female	67 (41.1)

Residence	
Urban	92 (56.4)
Rural	71 (43.6)
Nutritional status	
Normal	98 (60.1)
Underweight	49 (30.1)
Wasted/Severely undernourished	16 (9.8)
Season of presentation	
Winter (Nov–Feb)	71 (43.6)
Summer (Mar–Jun)	38 (23.3)
Monsoon (Jul–Oct)	54 (33.1)

Out of 163 children, 68 (41.7%) tested positive for rotavirus antigen. Rotavirus positivity was significantly higher among partially vaccinated and unvaccinated children compared to fully vaccinated children (p = 0.031). The median age of rotavirus-positive children was significantly lower than rotavirus-negative children [13.2 months (IQR 9–20) vs 16.5 months (IQR 11–28); p = 0.018]. There was no statistically significant difference in rotavirus positivity based on sex (p = 0.762. [Table 2]

Table 2: Rotavirus Detection in Relation to Vaccination Status and Demographic Variables (n = 163)

Variable	**Rotavirus Positive (n = 68)**	**Rotavirus Negative (n = 95)**	**p-value**
	Frequency (%) / Median (IQR)		
Vaccination status			
Fully vaccinated	38 (55.9%)	69 (72.6%)	0.031
Partially vaccinated	18 (26.5%)	17 (17.9%)	
Unvaccinated	12 (17.6%)	9 (9.5%)	
Median age (months)	13.2 (9–20)	16.5 (11–28)	0.018
Gender			
Male	41 (60.3%)	55 (57.9%)	0.762
Female	27 (39.7%)	40 (42.1%)	

Vomiting, fever, and high stool frequency were significantly more common among rotavirus-positive children. Vomiting was present in 79.4% of rotavirus-positive cases compared to 51.6% of rotavirus-negative cases (p < 0.001). A significantly higher proportion of rotavirus-positive children had ≥6 stools per day (64.7% vs 41.1%; p = 0.003). Both some dehydration (52.9% vs 33.7%; p = 0.015) and severe dehydration (16.2% vs 6.3%; p = 0.041) were more frequent among rotavirus-positive cases. Hospital admission rates were also significantly higher in rotavirus-positive children (61.8% vs 40.0%; p = 0.007). [Table 3]

Table 3: Comparison of Clinical Profile between Rotavirus-Positive and Rotavirus-Negative Children

Clinical feature	**Rotavirus Positive (n = 68)**	**Rotavirus Negative (n = 95)**	**p-value**
	Frequency (%)		
Vomiting	54 (79.4%)	49 (51.6%)	<0.001
Fever	46 (67.6%)	50 (52.6%)	0.048
≥6 stools/day	44 (64.7%)	39 (41.1%)	0.003
Some dehydration	36 (52.9%)	32 (33.7%)	0.015
Severe dehydration	11 (16.2%)	6 (6.3%)	0.041
Hospital admission	42 (61.8%)	38 (40.0%)	0.007

Rotavirus-positive children had significantly higher disease severity compared to rotavirus-negative children. Severe disease (Vesikari score ≥11) was observed in 36.8% of rotavirus-positive cases compared to 18.9% of rotavirus-negative cases (p = 0.006). The mean Vesikari score was significantly higher among rotavirus-positive children (10.6 ± 2.8) than rotavirus-negative children (8.4 ± 2.5; p < 0.001), indicating greater clinical severity associated with rotavirus infection. [Table 4]

Table 4: Disease Severity Assessment Using Vesikari Clinical Severity Score

Severity category	**Rotavirus Positive (n = 68)**	**Rotavirus Negative (n = 95)**	**p-value**
	Frequency (%) / Mean ± SD		
Mild (<7)	14 (20.6%)	36 (37.9%)	0.018
Moderate (7–10)	29 (42.6%)	41 (43.2%)	-
Severe (≥11)	25 (36.8%)	18 (18.9%)	0.006
Mean Vesikari score	10.6 ± 2.8	8.4 ± 2.5	<0.001

Among the rotavirus-positive samples, G9 (25.0%) and G12 (22.1%) were the most common G genotypes, followed by G1 (20.6%) and G2 (13.2%). P[8] was the predominant P genotype, detected in 61.8% of cases, followed by P[4] (19.1%) and P[6] (8.8%). Mixed or untypeable strains accounted for

10.3% of both G and P genotypes. G9P[8] emerged as the most common genotype combination (23.5%), followed by G12P[6] (16.2%) and G1P[8] (14.7%). Classical vaccine-related strains such as G2P[4] accounted for 11.8% of infections. A relatively high proportion of samples (26.4%) showed mixed or untypeable genotype combinations, indicating considerable strain diversity in the postvaccination period. [Table 5]

Table 5: Distribution of G, P Genotypes and Predominant G–P Genotype Combinations among Rotavirus-Positive Cases (n = 68)

Genotype	Frequency (%)
G types	
G1	14 (20.6)
G2	9 (13.2)
G3	6 (8.8)
G9	17 (25.0)
G12	15 (22.1)
Mixed/Untypeable	7 (10.3)
P types	
P[8]	42 (61.8)
P[4]	13 (19.1)
P[6]	6 (8.8)
Mixed/Untypeable	7 (10.3)
Genotype combination	
G9P[8]	16 (23.5)
G12P[6]	11 (16.2)
G1P[8]	10 (14.7)
G2P[4]	8 (11.8)
G3P[8]	5 (7.4)
Mixed/Untypeable	18 (26.4)

Among rotavirus-positive children, severe disease was significantly less common in fully vaccinated children compared to partially vaccinated or unvaccinated children (23.7% vs 53.3%; p = 0.011). The mean duration of hospital stay was significantly shorter among fully vaccinated children (3.1 ± 1.2 days) compared to partially vaccinated or unvaccinated children (4.4 ± 1.6 days; p = 0.002). Although severe dehydration was less frequent in fully vaccinated children, the difference did not reach statistical significance (p = 0.148). [Table 6]

Table 6: Clinical Severity and Hospital Outcomes by Vaccination Status among Rotavirus-Positive Children (n = 68)

Parameter	Fully vaccinated (n = 38)	Partially/Unvaccinated (n = 30)	p-value
	Frequency (%) / Mean ± SD		
Severe Vesikari score (≥11)	9 (23.7%)	16 (53.3%)	0.011
Severe dehydration	4 (10.5%)	7 (23.3%)	0.148
Mean hospital stay (days)	3.1 ± 1.2	4.4 ± 1.6	0.002

DISCUSSION

This study provides an updated evaluation of the clinical profile and molecular epidemiology of rotavirus diarrhoea in children during the postvaccination period, highlighting the continuing burden of disease, evolving genotype distribution, and the modifying effect of vaccination on clinical severity. Despite the introduction of rotavirus vaccines under the national immunization program, rotavirus remained a significant contributor to acute diarrhoeal illness, with an overall positivity rate of 41.7%. This finding is consistent with recent Indian hospital-based surveillance reports by Girish Kumar et al., Chaudhary et al., and Dalal et al., that have documented rotavirus detection rates ranging from 30% to 45% in the post-vaccine era, indicating sustained virus circulation despite declining severity and mortality.[14-16]

The age distribution observed in this study, with the highest burden in children aged 6–23 months, aligns with the known epidemiology of rotavirus infection. This age group represents a period of waning maternal antibodies, increasing exposure to environmental pathogens, and incomplete vaccine-induced immunity in partially vaccinated children. Similar age clustering has been reported in multicentric Indian surveillance study by Dahake et al., and global post-vaccination analyses by Hasso-Agopsowicz et al.[17,18] The male predominance noted in the present study has been consistently described in earlier literature and is likely attributable to differential healthcare-seeking behavior rather than biological susceptibility.[19]

A key finding of this study is the significantly higher rotavirus positivity among partially vaccinated and unvaccinated children compared to fully vaccinated children. Although breakthrough infections were documented among fully vaccinated children, vaccination was associated with a clear reduction in disease severity. This supports accumulating evidence that rotavirus vaccines, while not fully preventing infection, substantially mitigate severe disease outcomes.[20,21] The lower median age among rotavirus-positive children further underscores the vulnerability of younger infants and the importance of timely vaccine completion.[20]

Clinically, rotavirus-positive children exhibited a more severe disease profile compared to rotavirus-negative cases, characterized by higher rates of vomiting, frequent stools, dehydration, and hospital admission. These findings reinforce the established pathophysiological basis of rotavirus infection, wherein viral enterotoxin NSP4 induces secretory diarrhoea, disrupts intestinal epithelial integrity, and activates the enteric nervous system, leading to profuse diarrhoea and vomiting.[22] The significantly higher Vesikari scores among rotavirus-positive children corroborate observations from both pre- and post-vaccine study by Rahman et al., although the proportion of severe cases appears reduced compared to the pre-vaccination era.[23]

Importantly, vaccination status significantly influenced clinical severity among rotavirus-positive children. Fully vaccinated children experienced fewer severe cases and shorter hospital stays compared to partially vaccinated or unvaccinated children. This finding is consistent with Indian study by Dhalaria et al., and global study by Aliabad et al., demonstrating a 40–60% reduction in severe rotavirus diarrhoea and hospitalization following vaccine introduction.[24,25] The absence of a statistically significant difference in severe dehydration between vaccinated and unvaccinated groups may reflect early healthcare presentation, improved case management, or limited sample size within subgroup analyses.

The molecular characterization revealed a predominance of G9 and G12 genotypes, with P[8] as the most common P genotype. This shift toward non-G1 genotypes has been increasingly reported from India by Devi et al., and other low- and middle-income countries by Manouana et al., and Gikonyo et al., following vaccine introduction.[26-28] While G1P[8] remains an important strain, the emergence and sustained circulation of G9P[8] and G12P[6] highlight ongoing viral evolution and strain replacement dynamics. These genotypes are not unexpected, as rotavirus vaccines exert selective immune pressure, potentially favoring heterotypic strains with partial immune escape capabilities.[29]

The high proportion of mixed and untypeable strains observed in this study further underscores the genetic diversity of circulating rotavirus strains in the postvaccination period. Similar findings have been reported in Indian surveillance networks and raise important considerations for continued genomic monitoring.[24] Nevertheless, current evidence suggests that available rotavirus vaccines provide substantial cross-protection against severe disease caused by diverse genotypes, supporting their continued public health value.[29]

Seasonal variation, with a peak during winter months, remains unchanged in the post-vaccine era and is consistent with established rotavirus transmission patterns in tropical and subtropical regions.[30] This persistence suggests that vaccination modifies disease severity rather than eliminating seasonal transmission.

Limitations

This study has certain limitations that should be considered while interpreting the findings. Being a single-center, hospital-based study, the results may not be fully generalizable to the broader community, particularly to children with mild diarrhoeal illness who do not seek hospital care. The observational design limits causal inference regarding vaccine effectiveness, and the sample size, although adequate for descriptive analysis, may have limited the power of subgroup comparisons, especially for dehydration severity and less common genotypes. Vaccination status in a subset of children relied on caregiver recall, which may have introduced recall bias. Additionally, molecular characterization was restricted to G and P genotyping; whole-genome sequencing was not performed, which could have provided deeper insights into reassortment events and emerging strains. Despite these limitations, the study provides valuable real-world data on the clinical and molecular epidemiology of rotavirus in the postvaccination period.

CONCLUSION

Rotavirus continues to be a significant cause of acute diarrhoeal illness among young children in the postvaccination era, particularly affecting infants and toddlers. While breakthrough infections were observed, rotavirus vaccination was clearly associated with reduced disease severity, fewer severe cases, and shorter hospital stays, reinforcing the substantial protective benefit of vaccination against severe outcomes rather than complete infection prevention. The predominance of G9 and G12 genotypes and the presence of diverse and mixed strains highlight the dynamic nature of rotavirus epidemiology and the need for sustained molecular surveillance. From a public health perspective, these findings underscore the importance of achieving high and timely rotavirus vaccine coverage to maximize protection during the most vulnerable age period. Strengthening routine immunization services, minimizing missed or delayed doses, and improving caregiver awareness remain critical strategies for reducing severe rotavirus disease burden. Continued integration of rotavirus strain surveillance with national diarrhoeal disease monitoring programs is essential to track genotype shifts, assess long-term vaccine impact, and inform future vaccine policy and formulation.

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