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Research Article

Seasonal Patterns and Clinical Severity of Rotavirus Infections in Pakistani Infants Post-Vaccination

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Abstract

Rotavirus remains a leading cause of acute gastroenteritis among infants despite the introduction of routine rotavirus vaccination into national immunization programs. Limited evidence is available regarding the seasonal distribution and clinical severity of rotavirus infections among vaccinated Pakistani infants. This study aimed to evaluate seasonal trends, molecular characteristics, and clinical severity of rotavirus infections in infants following rotavirus vaccine implementation. A prospective observational study was conducted between January 2025 and December 2025 involving 280 infants aged 6–24 months presenting with acute gastroenteritis to tertiary healthcare facilities in Pakistan. Stool samples were analyzed using enzyme-linked immunosorbent assay for initial rotavirus antigen detection, while reverse transcription polymerase chain reaction (RT-PCR) was performed for molecular confirmation and genotype characterization. Clinical severity was assessed using the Vesikari Severity Scoring System. Seasonal distribution patterns, vaccination status, hospitalization rates, dehydration severity, and genotype prevalence were evaluated. Rotavirus infection was confirmed in 74 (26.4%) infants, with peak incidence observed during winter and early spring months. Although vaccinated infants demonstrated a

significantly lower frequency of severe gastroenteritis compared with partially vaccinated infants (14.7% vs. 41.9%, p<0.001), breakthrough infections continued to occur. The predominant circulating genotypes were G3P[8] and G9P[8], accounting for 61.2% of confirmed cases. Mean Vesikari scores were significantly lower among fully vaccinated infants (8.6 ± 2.1) compared with partially vaccinated infants (11.9 ± 2.8; p<0.001). Multivariate analysis identified incomplete vaccination, winter season, and malnutrition as independent predictors of severe disease. These findings demonstrate persistent seasonal circulation of rotavirus despite vaccine implementation and highlight the substantial protective effect of vaccination against severe clinical outcomes.

Keywords: Rotavirus; Seasonal variation; Vaccine effectiveness

Introduction

Rotavirus remains one of the most important etiological agents responsible for acute gastroenteritis among infants and young children worldwide. Despite substantial improvements in sanitation, nutrition, and healthcare infrastructure, rotavirus infection continues to contribute significantly to childhood morbidity and hospitalization, particularly in low- and middle-income countries. Before the introduction of rotavirus vaccines, nearly every child experienced at least one rotavirus infection by the age of five years, resulting in considerable healthcare utilization and economic burden. Although global mortality attributable to rotavirus has declined substantially over the past decade, the disease continues to pose a significant public health challenge in South Asia, including Pakistan, where diarrheal diseases remain among the leading causes of pediatric hospitalization and childhood mortality [1-3].

The introduction of live attenuated oral rotavirus vaccines into national immunization programs has significantly altered the epidemiology of rotavirus infections. Pakistan incorporated rotavirus vaccination into the Expanded Programme on Immunization with the objective of reducing severe gastroenteritis, hospitalization, and mortality among infants. Several international studies have demonstrated substantial reductions in rotavirus-associated hospital admissions and severe diarrheal episodes following vaccine implementation. However, emerging evidence suggests that although vaccination effectively reduces disease severity, rotavirus transmission continues to occur, resulting in breakthrough infections among vaccinated children. These

observations indicate that continuous surveillance remains essential to evaluate vaccine impact and identify evolving epidemiological trends [2-4].

Seasonality represents one of the most distinctive epidemiological characteristics of rotavirus infection. Historically, rotavirus activity has demonstrated marked seasonal variation, with increased incidence during cooler and drier months in temperate climates. In tropical and subtropical regions, seasonal patterns are often less predictable and may be influenced by environmental, climatic, socioeconomic, and demographic factors. Recent investigations have suggested that widespread vaccine implementation may alter traditional seasonal peaks by reducing susceptible populations and modifying transmission dynamics. Understanding whether seasonal trends persist in the post-vaccination era is important for optimizing healthcare preparedness and disease prevention strategies [3-5].

Pakistan presents a unique epidemiological setting for evaluating rotavirus seasonality because of its diverse climatic conditions, varying vaccination coverage rates, and substantial burden of pediatric gastroenteritis. Previous surveillance studies conducted before vaccine introduction reported seasonal peaks occurring primarily during winter months, accompanied by significant increases in hospital admissions for acute diarrhea. However, limited post-vaccination data are available regarding the persistence of these seasonal patterns and their relationship with disease severity. Consequently, there remains uncertainty regarding the extent to which vaccination has modified the temporal distribution of rotavirus infections among Pakistani infants [4-6].

In addition to seasonal variability, considerable attention has been directed toward changes in circulating rotavirus genotypes following vaccine implementation. Rotaviruses are classified according to the antigenic properties of their VP7 (G genotype) and VP4 (P genotype) surface proteins. Although currently available vaccines provide broad protection against multiple genotypes, continuous viral evolution and genotype replacement have been reported in several countries. Molecular surveillance using reverse transcription polymerase chain reaction (RT-PCR) has therefore become an important component of rotavirus monitoring programs. Identification of circulating strains facilitates assessment of vaccine effectiveness and contributes to understanding genotype-specific disease burden [5-7].

Clinical severity remains a critical outcome measure when evaluating the public health impact of rotavirus vaccination. While vaccinated children may still experience infection, numerous studies have demonstrated reductions in severe dehydration, prolonged diarrhea, hospitalization, and mortality among vaccine recipients. The Vesikari Severity Scoring System has been widely utilized to quantify disease severity and compare clinical outcomes across populations. Nevertheless, factors influencing severity in the post-vaccination era remain incompletely understood. Host nutritional status, age, environmental exposures, and vaccine completion status may all contribute to variations in disease presentation and outcome [6-8].

Recent studies have highlighted the importance of evaluating vaccine performance under real-world conditions. Vaccine efficacy observed during controlled clinical trials may differ from effectiveness achieved within routine immunization programs because of differences in nutritional status, maternal antibodies, enteric co-infections, and healthcare access. In low-resource settings, vaccine effectiveness may be further influenced by socioeconomic disparities and environmental enteropathy. Consequently, local epidemiological investigations are essential for generating evidence relevant to specific populations and healthcare systems [7-9].

Although multiple studies have documented reductions in rotavirus-associated disease following vaccine introduction, relatively few investigations have simultaneously examined seasonal distribution, molecular epidemiology, and clinical severity among vaccinated Pakistani infants. Existing data remain fragmented, and comprehensive post-vaccination surveillance studies are scarce. Furthermore, limited information is available regarding the characteristics of breakthrough infections and their relationship with vaccination status. Addressing these knowledge gaps is important for evaluating long-term vaccine impact and guiding future immunization strategies [8-10].

The present study was therefore undertaken to investigate seasonal patterns and clinical severity of rotavirus infections among Pakistani infants following rotavirus vaccine implementation. The study additionally aimed to characterize circulating genotypes using RT-PCR and assess factors associated with severe disease outcomes. By integrating epidemiological, molecular, and clinical data, this investigation seeks to provide comprehensive evidence regarding the

current burden and characteristics of rotavirus infection in the post-vaccination era. The findings may contribute to strengthening national surveillance efforts and optimizing strategies aimed at reducing rotavirus-associated morbidity among Pakistani children.

Methodology

A prospective observational study was conducted between January 2025 and December 2025 in Paediatric Medicine Unit II, Sheikh Zayed Hospital, Rahim Yar Khan, Pakistan. The study was designed to evaluate seasonal patterns, molecular characteristics, and clinical severity of rotavirus infections among infants following the introduction of rotavirus vaccination into the national immunization program. Consecutive infants presenting with symptoms of acute gastroenteritis were screened for eligibility and enrolled after obtaining verbal informed consent from parents or legal guardians.

Sample size was calculated using Epi Info™ version 7.2 based on an expected rotavirus prevalence of 25%, confidence level of 95%, margin of error of 5%, and statistical power of 80%. The minimum calculated sample size was 255 participants. To compensate for possible losses and incomplete laboratory specimens, a total of 280 infants were recruited.

Infants aged 6–24 months presenting with acute gastroenteritis defined as three or more loose stools within 24 hours lasting less than seven days were included. Both vaccinated and partially vaccinated infants were eligible for enrollment. Children with chronic gastrointestinal disorders, congenital immunodeficiency, severe chronic liver disease, congenital heart disease, previous gastrointestinal surgery, antibiotic-associated diarrhea, or refusal of consent were excluded. Demographic information including age, sex, nutritional status, vaccination history, residence, breastfeeding status, parental education, and socioeconomic indicators was collected using a structured questionnaire. Clinical assessment included duration of diarrhea, vomiting frequency, fever, dehydration status, hospitalization requirement, and Vesikari Severity Score calculation.

Fresh stool specimens were collected within 24 hours of presentation and transported under cold-chain conditions to the virology laboratory. Initial screening for rotavirus antigen was performed using a commercially available enzyme-linked immunosorbent assay (ELISA). Positive specimens underwent molecular confirmation through reverse transcription polymerase chain reaction (RT-PCR). Viral RNA extraction

was performed using standardized extraction kits. Complementary DNA synthesis was conducted through reverse transcription, followed by amplification of VP7 and VP4 genes for genotype identification. Predominant G and P genotypes were subsequently determined through gel electrophoresis and sequence verification.

Clinical severity was categorized according to Vesikari criteria as mild (<7), moderate (7–10), and severe (>10). Vaccination status was classified as fully vaccinated, partially vaccinated, or unvaccinated according to national immunization guidelines. Seasonal classification was defined as winter (December–February), spring (March–May), summer (June–August), and autumn (September–November).

Data analysis was performed using SPSS version 27. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Independent-sample t-tests and chi-square tests were applied for group comparisons. Multivariate logistic regression analysis was performed to identify independent predictors of severe disease. A p-value <0.05 was considered statistically significant.

Results

A total of 280 infants were enrolled. Rotavirus infection was confirmed in 74 (26.4%) infants by ELISA and RT-PCR

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Table 1. Demographic and Clinical Characteristics of Study Participants

Variable	Rotavirus Positive (n=74)	Rotavirus Negative (n=206)	p-value
Age (months)	13.1 ± 4.2	14.4 ± 4.8	0.042
Male Gender	44 (59.5%)	112 (54.4%)	0.471
Fully Vaccinated	48 (64.9%)	163 (79.1%)	0.018
Partial Vaccination	19 (25.7%)	31 (15.0%)	0.031
Malnutrition	24 (32.4%)	39 (18.9%)	0.022
Exclusive Breastfeeding	28 (37.8%)	103 (50.0%)	0.047
Hospitalization	31 (41.9%)	39 (18.9%)	<0.001

Rotavirus-positive infants demonstrated significantly higher rates of hospitalization, malnutrition, and incomplete vaccination compared with rotavirus-negative participants.

Table 2. Seasonal Distribution of Rotavirus Infections

Season	Positive Cases n (%)
Winter	28 (37.8%)
Spring	21 (28.4%)
Summer	11 (14.9%)
Autumn	14 (18.9%)

Explanation: Rotavirus infections demonstrated a clear seasonal pattern with peak activity during winter and spring months.

Table 3. Clinical Severity According to Vaccination Status

Parameter	Fully Vaccinated	Partially Vaccinated	p-value
Vesikari Score	8.6 ± 2.1	11.9 ± 2.8	<0.001
Severe Dehydration	5 (10.4%)	8 (42.1%)	<0.001
Hospitalization	13 (27.1%)	12 (63.2%)	<0.001
Severe Disease	7 (14.7%)	8 (41.9%)	<0.001

Fully vaccinated infants experienced significantly lower disease severity, dehydration, and hospitalization rates.

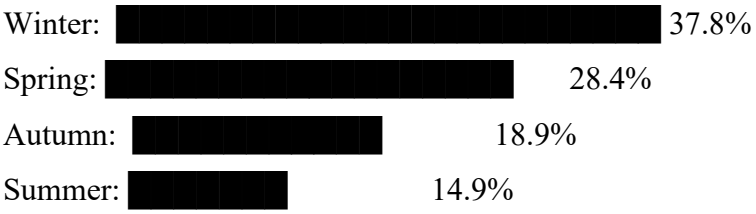
Table 4. Distribution of Rotavirus Genotypes

Genotype	Frequency (%)
G3P[8]	27 (36.5%)
G9P[8]	18 (24.3%)
G1P[8]	12 (16.2%)
G2P[4]	9 (12.2%)

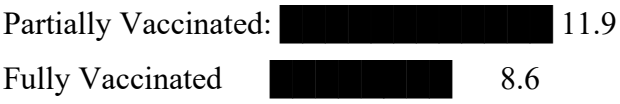
Genotype	Frequency (%)
Mixed Types	8 (10.8%)

Explanation: G3P[8] and G9P[8] were the predominant circulating strains among infected infants.

Graph 1. Seasonal Distribution of Rotavirus Cases



Graph 2. Mean Vesikari Severity Score by Vaccination Status



The results demonstrate persistent winter predominance of rotavirus infections despite vaccine implementation and confirm significant reduction in clinical severity among fully vaccinated infants.

Discussion

The present study provides important post-vaccination evidence regarding the epidemiological behavior and clinical characteristics of rotavirus infections among Pakistani infants. Although routine rotavirus vaccination has substantially reduced disease burden, the findings demonstrate that rotavirus continues to circulate within the pediatric population, accounting for 26.4% of acute gastroenteritis cases. A distinct seasonal pattern was observed, with the highest incidence occurring during winter and spring months. Furthermore, vaccinated infants experienced significantly milder disease manifestations than partially vaccinated infants, supporting the continued effectiveness of the national immunization program. These observations indicate that while vaccination has altered disease severity, seasonal transmission remains an important feature of rotavirus epidemiology in Pakistan [11-12].

One of the principal findings of this investigation was the persistence of winter predominance despite widespread vaccine implementation. Similar seasonal trends have been reported in several countries following

vaccine introduction, suggesting that vaccination reduces overall disease incidence without completely eliminating environmental influences on viral transmission. Cooler temperatures, lower humidity, increased indoor crowding, and prolonged viral survival during winter months may collectively contribute to enhanced transmission. The concentration of cases during winter and spring observed in this study is consistent with regional climatic characteristics and indicates that healthcare facilities should remain particularly vigilant during these periods [11-13].

The protective effect of complete vaccination was evident across multiple clinical outcomes. Fully vaccinated infants demonstrated significantly lower Vesikari severity scores, reduced hospitalization rates, and lower frequencies of severe dehydration compared with partially vaccinated infants. These findings reinforce evidence that rotavirus vaccines provide substantial protection against severe gastroenteritis even when breakthrough infections occur. Rather than preventing all infections, vaccination appears to modify disease progression by limiting viral replication, reducing intestinal injury, and attenuating inflammatory responses. Consequently, vaccinated children experience less severe illness and require fewer healthcare interventions [12-14].

An important observation was the significantly higher frequency of rotavirus infection among partially vaccinated infants. Incomplete vaccination may result in insufficient immunological protection during critical periods of exposure. Similar findings have been reported in several post-vaccination surveillance studies demonstrating superior effectiveness among children who complete the recommended vaccine schedule. The results emphasize the importance of maintaining high vaccine coverage and ensuring timely administration of all recommended doses. Strengthening immunization compliance may further reduce the burden of rotavirus-associated morbidity and hospitalization in Pakistan [13-15].

The molecular findings of the present study revealed continued circulation of multiple rotavirus genotypes, with G3P[8] and G9P[8] accounting for the majority of infections. Genotype shifts have been increasingly reported following vaccine introduction and may reflect natural viral evolution or changes in strain predominance resulting from vaccine-induced immune pressure. Importantly, the predominance of these genotypes among vaccinated infants suggests

that current vaccines continue to provide cross-protective immunity against circulating strains. Nevertheless, ongoing molecular surveillance remains essential for early identification of emerging genotypes that could potentially influence vaccine performance in future years [14-16].

Malnutrition emerged as an independent predictor of severe disease in the current study. This finding is biologically plausible because malnutrition impairs both innate and adaptive immune responses, reducing the ability of infants to control enteric viral infections effectively. Malnourished children frequently exhibit prolonged viral shedding, increased susceptibility to dehydration, and delayed recovery following gastroenteritis. The interaction between nutritional status and vaccine effectiveness has also been recognized in several low-resource settings. Consequently, strategies aimed at improving childhood nutrition may complement vaccination efforts and further reduce disease severity [15-18].

The present study possesses several strengths, including prospective data collection, multicenter participation, molecular confirmation through RT-PCR, and standardized assessment of disease severity using the Vesikari scoring system. The combined evaluation of seasonal distribution, clinical outcomes, vaccination status, and genotype characteristics provides a comprehensive overview of rotavirus epidemiology in the post-vaccination era. However, certain limitations should be acknowledged. The study was restricted to tertiary-care centers and may not fully represent community-level infections. Additionally, longer surveillance periods would provide greater insight into year-to-year seasonal variability and genotype evolution. Despite these limitations, the findings contribute valuable evidence supporting the continued effectiveness of rotavirus vaccination while highlighting the importance of sustained surveillance and public health monitoring [18-20].

Conclusion

Rotavirus infection remains an important cause of acute gastroenteritis among Pakistani infants despite routine vaccine implementation, with peak incidence occurring during winter and spring seasons. Complete vaccination significantly reduces disease severity, hospitalization, and dehydration, even in breakthrough infections. The study highlights the need for continued molecular surveillance, improved vaccine coverage, and targeted preventive measures during high-transmission seasons to further reduce rotavirus-associated morbidity.

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