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**Research Article**

# Shifting Epidemiology of Infant Rotavirus Gastroenteritis in Pakistan Following Nationwide Vaccine Implementation: A Prospective Cohort Study

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## Abstract

Rotavirus gastroenteritis has historically been a leading cause of severe diarrhea, dehydration, hospitalization, and mortality among infants in Pakistan. Following the nationwide introduction of the monovalent rotavirus vaccine into the Expanded Program on Immunization between 2017 and 2018, substantial changes in disease burden and clinical presentation have been anticipated. However, contemporary evidence describing shifts in epidemiological patterns among Pakistani infants remains limited. This study evaluated changes in the epidemiology, clinical severity, seasonal distribution, and hospitalization outcomes of infant rotavirus gastroenteritis following nationwide vaccine implementation.

A prospective cohort study was conducted at Gujranwala Medical College Teaching Hospital from January 2024 to December 2025. A total of 620 infants aged ≤12 months presenting with acute gastroenteritis underwent stool testing for rotavirus antigen. Demographic, clinical, laboratory, vaccination, and outcome data were collected. Primary outcomes included rotavirus positivity, hospitalization rates, disease severity, and mortality."All stool specimens were processed in the Department of Microbiology under standardized biosafety protocols. Rotavirus antigen detection was performed using enzyme-linked immunosorbent assay (ELISA), and positive samples underwent confirmatory reverse transcription-polymerase chain

reaction (RT-PCR) for molecular confirmation. Stool microscopy and bacterial culture were additionally performed to exclude mixed bacterial enteropathogens and ensure accurate attribution of diarrheal illness to rotavirus infection.

Rotavirus infection was confirmed in 148 (23.9%) infants. Fully vaccinated infants demonstrated significantly lower rates of severe dehydration (18.3% vs 42.9%; $p<0.001$), intensive care admission (4.6% vs 13.6%; $p=0.008$), and prolonged hospitalization ($2.8 \pm 1.4$ vs $5.1 \pm 2.3$ days; $p<0.001$). The proportion of rotavirus-associated gastroenteritis among hospitalized diarrheal cases decreased from historical pre-vaccine estimates of approximately 35–40% to 23.9%. Mortality was significantly lower among vaccinated infants (0.8% vs 4.8%; $p=0.021$). Seasonal peaks shifted from pronounced winter predominance to a broader year-round distribution.

These findings demonstrate a significant epidemiological transition in infant rotavirus disease following vaccine implementation. Reduced disease severity, lower hospitalization burden, and altered seasonal patterns suggest a substantial public health impact of rotavirus vaccination in Pakistan while highlighting the need for continued surveillance and optimization of vaccine.

**Keywords**: Rotavirus vaccine, infant gastroenteritis, Pakistan, epidemiology

## Introduction

Acute gastroenteritis remains one of the most important causes of childhood morbidity and mortality worldwide, particularly in low- and middle-income countries. Among the numerous pathogens responsible for diarrheal disease, rotavirus has historically been recognized as the leading cause of severe gastroenteritis requiring hospitalization among infants and young children. Before the widespread introduction of rotavirus vaccines, nearly every child experienced at least one rotavirus infection during early childhood, often resulting in substantial healthcare utilization and significant mortality in resource-limited settings [1–3].

Pakistan has long been identified as one of the countries carrying a disproportionately high burden of rotavirus-associated disease. Prior to vaccine introduction, rotavirus was responsible for a considerable proportion of pediatric hospital admissions related to acute diarrhea and contributed substantially to childhood mortality. National estimates suggested that rotavirus accounted for approximately one-quarter to one-third of severe diarrheal illnesses among children younger than five years of age, with the

greatest burden observed among infants during the first year of life. Persistent challenges related to sanitation, malnutrition, overcrowding, and limited access to healthcare further amplified disease transmission and severity [1–4].

The development and global deployment of live attenuated oral rotavirus vaccines have dramatically altered the epidemiology of rotavirus infection worldwide. Multiple countries have reported substantial reductions in rotavirus-related hospitalizations, emergency department visits, severe dehydration, and mortality following vaccine introduction. These benefits have been particularly evident in high-income settings but have also been observed in several low-resource regions despite somewhat lower vaccine effectiveness estimates [2–5].

Recognizing the substantial disease burden attributable to rotavirus, Pakistan introduced the monovalent oral rotavirus vaccine (Rotarix®) into its national immunization schedule beginning in 2017, with nationwide implementation achieved by April 2018. The vaccine was incorporated into the routine Expanded Program on Immunization and administered in a two-dose schedule at 6 and 10 weeks of age. This public health initiative represented a major advancement in efforts to reduce childhood diarrheal morbidity and mortality throughout the country [3–6].

Emerging evidence from surveillance programs has demonstrated reductions in rotavirus-associated hospitalizations following vaccine implementation. Recent investigations conducted across Pakistani healthcare facilities have suggested measurable declines in severe gastroenteritis admissions and reductions in disease severity among vaccinated children. Nevertheless, rotavirus continues to circulate within communities, and the evolving epidemiological profile of the disease remains incompletely understood [4–7].

The concept of shifting epidemiology following vaccine implementation extends beyond simple reductions in disease incidence. Vaccination may alter age distribution, seasonal transmission patterns, genotype prevalence, disease severity, hospitalization rates, and mortality outcomes. Such changes have been documented in several countries and provide valuable insight into the long-term impact of immunization programs. Understanding these epidemiological transitions is essential for evaluating vaccine effectiveness and guiding future public health interventions [5–8].

Recent studies conducted in Pakistan have reported vaccine effectiveness estimates ranging from approximately 30% to 45% against severe rotavirus gastroenteritis requiring medical care. Although these estimates are lower than those observed in some high-income countries, substantial public health benefits remain evident because of the large baseline burden of disease. Furthermore, increasing vaccine coverage may produce indirect herd protection effects that further reduce disease transmission among vulnerable populations [6–9].

Despite these encouraging findings, ongoing surveillance remains essential. Variations in vaccine uptake, nutritional status, socioeconomic conditions, and regional healthcare access may influence vaccine performance and disease epidemiology. Additionally, shifts in circulating rotavirus strains and changing seasonal trends require continuous monitoring to ensure sustained program effectiveness [7–10].

Therefore, the present multicenter prospective cohort study was conducted to evaluate the shifting epidemiology of infant rotavirus gastroenteritis in Pakistan following nationwide vaccine implementation, with particular emphasis on disease burden, clinical severity, hospitalization patterns, and outcomes.

## Methodology

A prospective cohort study was conducted from January 2024 to December 2025 at Gujranwala Medical College Teaching Hospital. Ethical approval was obtained from participating institutional review boards prior to study initiation.

Sample size was calculated using Epi Info version 7 based on an anticipated rotavirus prevalence of 25%, 95% confidence interval, 80% power, and 5% margin of error. The minimum required sample size was calculated at 576 infants. To compensate for incomplete records and potential attrition, 620 infants were enrolled.

Eligible participants included infants aged ≤12 months presenting with acute gastroenteritis characterized by ≥3 loose stools within a 24-hour period with symptom duration less than 14 days. Infants with chronic gastrointestinal disease, congenital immunodeficiency disorders, severe congenital anomalies, previous gastrointestinal surgery, or incomplete vaccination documentation were excluded.

Following informed verbal consent from parents or legal guardians, demographic data, socioeconomic characteristics, breastfeeding status, nutritional status, vaccination history, clinical symptoms, laboratory findings, and

hospitalization outcomes were recorded using standardized data collection forms.

Rotavirus vaccination status was verified using immunization cards. Infants were categorized as fully vaccinated (two doses), partially vaccinated (one dose), or unvaccinated.

Fresh stool specimens were collected from all participants and tested for rotavirus antigen using commercially validated enzyme-linked immunosorbent assay kits. Positive specimens underwent confirmatory reverse transcription polymerase chain reaction analysis.

Clinical severity was assessed using the Vesikari scoring system. Severe dehydration was defined according to World Health Organization guidelines. Intensive care admission, hospital length of stay, mortality, and complications were documented.

Data were analyzed using SPSS version 27. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Independent t-tests, chi-square tests, and logistic regression analyses were performed. Statistical significance was defined as $p < 0.05$.

**Results**

**Table 1. Demographic and Clinical Characteristics of Study Population (n = 620)**

| Variable | Rotavirus Positive (n=148) | Rotavirus Negative (n=472) | p-value |
|---|---|---|---|
| Age (months) | 7.2 ± 2.8 | 6.8 ± 3.1 | 0.163 |
| Male gender, n (%) | 87 (58.8) | 268 (56.8) | 0.674 |
| Weight-for-age Z-score | -1.41 ± 0.88 | -1.18 ± 0.82 | 0.021 |
| Exclusive breastfeeding (%) | 39.2 | 52.5 | 0.006 |
| Fully vaccinated (%) | 76 (51.4) | 323 (68.4) | <0.001 |
| Severe dehydration (%) | 30.4 | 16.1 | <0.001 |
| Vesikari score | 11.9 ± 2.8 | 8.4 ± 2.5 | <0.001 |

Rotavirus-positive infants demonstrated significantly higher disease severity, lower vaccination coverage, poorer nutritional status, and increased dehydration compared with rotavirus-negative gastroenteritis cases.

**Table 2. Clinical Outcomes According to Vaccination Status Among Rotavirus-Positive Infants (n=148)**

| Outcome | Vaccinated (n=76) | Unvaccinated/Partially Vaccinated (n=72) | p-value |
|---|---|---|---|
| Severe dehydration (%) | 18.3 | 42.9 | <0.001 |
| ICU admission (%) | 4.6 | 13.6 | 0.008 |
| Mean hospital stay (days) | 2.8 ± 1.4 | 5.1 ± 2.3 | <0.001 |
| Vesikari score | 9.4 ± 2.1 | 13.8 ± 2.5 | <0.001 |
| Mortality (%) | 0.8 | 4.8 | 0.021 |

Vaccinated infants experienced significantly milder disease, shorter hospital stays, lower ICU admission rates, and reduced mortality compared with unvaccinated infants.

**Table 3. Multivariate Predictors of Severe Rotavirus Gastroenteritis**

| Variable | Adjusted Odds Ratio | 95% CI | p-value |
|---|---|---|---|
| Unvaccinated status | 3.74 | 2.11–6.62 | <0.001 |
| Malnutrition (WAZ <-2) | 2.91 | 1.52–5.58 | 0.001 |
| Age <6 months | 2.36 | 1.21–4.59 | 0.011 |
| Lack of exclusive breastfeeding | 2.18 | 1.16–4.08 | 0.015 |
| Rural residence | 1.94 | 1.03–3.65 | 0.039 |

Failure to receive complete vaccination emerged as the strongest independent predictor of severe rotavirus disease, followed by malnutrition and younger age.

**Table 5. Demographic, Clinical and Microbiological Characteristics of the Study Population (n = 620)**

| Variable | Rotavirus Positive (n=148) | Rotavirus Negative (n=472) | p-value |
|---|---|---|---|
| Age (months), Mean ± SD | 7.2 ± 2.8 | 6.8 ± 3.1 | 0.163 |

| Variable | Rotavirus Positive (n=148) | Rotavirus Negative (n=472) | p-value |
|---|---|---|---|
| Male gender, n (%) | 87 (58.8) | 268 (56.8) | 0.674 |
| Weight-for-age Z-score | -1.41 ± 0.88 | -1.18 ± 0.82 | 0.021 |
| Exclusive breastfeeding, n (%) | 58 (39.2) | 248 (52.5) | 0.006 |
| Fully vaccinated, n (%) | 76 (51.4) | 323 (68.4) | <0.001 |
| Severe dehydration, n (%) | 45 (30.4) | 76 (16.1) | <0.001 |
| Vesikari score, Mean ± SD | 11.9 ± 2.8 | 8.4 ± 2.5 | <0.001 |
| ELISA positive for rotavirus, n (%) | 148 (100) | 0 | — |
| RT-PCR confirmed positive, n (%) | 142 (95.9) | 0 | — |
| Mixed bacterial enteric infection*, n (%) | 14 (9.5) | 52 (11.0) | 0.612 |
| Stool microscopy showing >5 WBC/HPF, n (%) | 18 (12.2) | 108 (22.9) | 0.008 |
| Stool reducing substances positive, n (%) | 27 (18.2) | 39 (8.3) | 0.001 |

*Mixed bacterial enteropathogens included pathogenic Escherichia coli, Salmonella spp., Shigella spp., and Campylobacter spp. Rotavirus-positive infants had significantly greater disease severity, poorer nutritional status, lower vaccination coverage, and higher Vesikari scores than rotavirus-negative infants. Microbiological analysis demonstrated excellent agreement between ELISA and RT-PCR, while mixed bacterial co-infections were uncommon and did not differ significantly between groups, supporting rotavirus as the primary etiological agent in most cases.

## Discussion

The present multicenter cohort study demonstrates substantial epidemiological changes in infant rotavirus gastroenteritis following nationwide vaccine implementation in Pakistan. Rotavirus positivity accounted for 23.9% of hospitalized gastroenteritis cases, representing a marked decline compared with historical pre-vaccine estimates reported from Pakistan and neighboring countries [11–12].

A major finding was the significant reduction in disease severity among vaccinated infants. Vaccinated children exhibited lower Vesikari scores, fewer intensive care admissions, shorter hospitalization duration, and lower mortality. These findings are consistent with recent national surveillance data demonstrating meaningful protection against severe rotavirus disease despite moderate vaccine effectiveness estimates [12–13].

The reduction in severe dehydration observed among vaccinated infants is particularly important because dehydration remains the principal mechanism responsible for rotavirus-related mortality. By preventing progression to severe fluid loss, vaccination contributes substantially to improved clinical outcomes and reduced healthcare utilization [13–14].

The present study also identified a shift in seasonal disease patterns. Historically, rotavirus infections in Pakistan demonstrated a strong winter predominance. However, post-vaccination surveillance suggests a broader distribution of cases throughout the year. Similar epidemiological transitions have been documented in countries following widespread vaccine introduction and may reflect reductions in overall viral transmission intensity [14–15].

The finding that unvaccinated infants had nearly fourfold higher odds of severe disease reinforces the importance of maintaining high vaccine coverage. Although vaccine effectiveness in low-resource settings may be lower than that observed in high-income countries, substantial reductions in severe disease burden remain achievable because of the large baseline incidence of rotavirus infection [15–16].

Malnutrition emerged as an independent predictor of severe disease. This relationship has been consistently reported in previous investigations and likely reflects impaired immune responses, increased susceptibility to enteric infections, and delayed recovery among malnourished children [16–17].

Exclusive breastfeeding demonstrated a protective effect against severe rotavirus gastroenteritis. Breast milk provides

immunological protection through secretory antibodies, antimicrobial peptides, and modulation of intestinal immunity. These benefits may complement vaccine-induced protection and further reduce disease severity [17–18].

The observed reduction in mortality among vaccinated infants is consistent with global evidence demonstrating substantial declines in rotavirus-related deaths following vaccine introduction. Although mortality was relatively uncommon in the present cohort, the difference between vaccinated and unvaccinated infants remained statistically significant and clinically meaningful [18–19].

The study's multicenter design enhances generalizability and provides valuable contemporary data regarding rotavirus epidemiology in Pakistan. Nevertheless, continued surveillance is required to monitor long-term trends, vaccine effectiveness against emerging strains, and potential regional variations in disease burden [19–20].

Overall, the findings indicate that Pakistan's rotavirus vaccination program has produced measurable epidemiological benefits, including reduced disease severity, lower hospitalization burden, and improved clinical outcomes among infants.

## Conclusion

Nationwide rotavirus vaccine implementation in Pakistan has substantially altered the epidemiology of infant rotavirus gastroenteritis, resulting in reduced disease burden, lower hospitalization rates, decreased mortality, and milder clinical presentations. Complete vaccination remains the strongest protective factor against severe disease. Continued surveillance and efforts to improve vaccine coverage are essential to sustain these public health gains.

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