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Study of Detection of Human Rotavirus in Stool Specimen by ELISA Technique and Association of Enteric Pathogens in Childhood Diarrhoea in a Tertiary Care Hospital, Rajasthan

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Abstract

Background: Rotavirus remains the **single most important cause of severe acute gastroenteritis** in infants and young children worldwide, responsible for an estimated **128,500 deaths annually in children under five years**, with more than 80% occurring in South Asia and sub-Saharan Africa. Despite the introduction of the indigenous oral vaccine **ROTAVAC** into India's Universal Immunization Programme in 2016, region-specific surveillance data from Rajasthan remain scarce. This study was undertaken to detect human rotavirus in stool specimens by ELISA and to evaluate its association with other enteric pathogens in children with acute diarrhoea at a tertiary care hospital in Rajasthan.

Methodology: This **prospective, laboratory-based observational study** was conducted over **12 months (July 2024–June 2025)** in the Department of Microbiology, JNUIMSRC, Jaipur. A total of **174 children aged 2–60 months** presenting to the paediatric OPD with acute diarrhoea (≥3 loose/liquid stools per day) were enrolled by calculated sample size (95% confidence level, 13% expected proportion, ±5% margin of error). Rotavirus antigen was detected in stool by **sandwich ELISA (Rotavirus-antigen-EIA-BEST)**, and stool samples were concurrently cultured on MacConkey and XLD agar with Gram staining, motility testing and a standard biochemical panel to identify co-infecting bacterial and parasitic pathogens.

Results: Of 174 stool samples, **43 (24.71%) were rotavirus-positive** and 131 (75.29%) were negative. Positivity was **highest in the 7–12 month age group (44.2%)** ($\chi^2 = 9.87$, $p = 0.02$). Of 102 male and 72 female children, positivity was comparable between sexes (24.5% vs. 25.0%; $\chi^2 = 0.41$, $p = 0.52$). Rotavirus-positive children showed significantly higher rates of **vomiting (79.1% vs. 52.7%, p = 0.002), severe dehydration (46.5% vs. 24.4%, p = 0.004)** and **≥6 stools/day (72.1% vs. 51.1%, p = 0.01)** compared with rotavirus-negative children. A clear **winter predominance** was observed (51.2% of positive cases; $\chi^2 = 10.9$, $p = 0.004$). Among rotavirus-negative samples, *Escherichia coli* was the most frequently isolated co-pathogen (21.4%), followed by *Shigella spp.* (1.5%), *Giardia lamblia* (0.8%) and *Entamoeba histolytica* (0.8%); 75.6% showed no detectable pathogen on routine culture and microscopy.

Conclusion: Rotavirus continues to be a **dominant and clinically significant cause of childhood diarrhoea** in Rajasthan even in the post-vaccine era, disproportionately affecting infants aged 7–24 months, peaking in winter, and producing more severe clinical disease than non-rotaviral diarrhoea. These findings support **continued ELISA-based surveillance, strengthened vaccine coverage, and integrated WASH interventions** in this region.

Keywords: Rotavirus; ELISA; Childhood diarrhoea; Enteric pathogens; *Escherichia coli*; ROTAVAC; Seasonal variation; Rajasthan; Tertiary care hospital

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Introduction

Diarrhoeal disease remains the **second leading cause of death in children under five years globally**, accounting for approximately **525,000 child deaths annually** according to the World Health Organization, and nearly **13% of all under-five mortality in India**. Among the many infectious causes of acute gastroenteritis, **rotavirus** — a non-enveloped, double-stranded RNA virus of the family Reoviridae — has been consistently identified as the single most important cause of severe disease in infants and young children, irrespective of geography, socioeconomic status, or hygiene standards, owing to its remarkable environmental stability and very low infectious dose.

Globally, rotavirus causes approximately **125 million diarrhoeal episodes and 2 million hospitalizations annually**, with India bearing one of the heaviest burdens — an estimated **78,500 deaths, 872,000 hospitalizations and over 3.2 million outpatient visits per year** among children under five. Following the 2009 WHO recommendation, India introduced the indigenously developed oral vaccine **ROTAVAC** (a monovalent G9P[11] human–bovine reassortant strain) into the Universal Immunization Programme in 2016, administered at 6, 10 and 14 weeks of age. Post-licensure trials demonstrated **moderate efficacy of approximately 55%** against severe rotavirus gastroenteritis in children under two — substantially lower than the >85% efficacy seen in high-income settings — and rotavirus infections have continued to be reported regularly among hospitalized children despite vaccination, reflecting incomplete coverage, reduced vaccine seroconversion in resource-limited settings, malnutrition, environmental enteropathy and high force of infection.

Because the clinical presentation of rotavirus gastroenteritis — acute watery diarrhoea, vomiting, low-grade fever and dehydration — overlaps substantially with bacterial and parasitic causes, **laboratory confirmation is essential** for accurate diagnosis, case management, surveillance and vaccine-impact evaluation. Among the available diagnostic methods (electron microscopy, latex agglutination, PAGE, ELISA, RT-PCR), **enzyme-linked immunosorbent assay (ELISA)** remains the method of choice for routine clinical and surveillance use owing to its high sensitivity and specificity, batch-processing capability, cost-effectiveness and suitability for tertiary care laboratories. Diarrhoea in children is also frequently associated with co-infecting bacterial and parasitic enteric pathogens — including *Escherichia coli, Shigella spp., Salmonella spp., Giardia lamblia* and *Entamoeba histolytica* — which can influence disease severity, duration and clinical outcome.

Region-specific epidemiological data on rotavirus prevalence and enteric co-infection patterns from **Rajasthan** remain sparse despite the state's considerable rural–urban disparity in sanitation and healthcare access. This study was therefore undertaken to detect human rotavirus in stool specimens by ELISA and to evaluate its association with other enteric pathogens among children under five years presenting with acute diarrhoea at a tertiary care hospital in Rajasthan, generating locally relevant baseline data to inform clinical practice and public health planning.

Aim

To detect human rotavirus in stool specimens by ELISA technique and evaluate the association of enteric pathogens in childhood diarrhoea at a tertiary care hospital in Rajasthan.

Objectives

- **To estimate the prevalence of rotavirus** among children under five years with acute diarrhoea.
- **To estimate the prevalence of other enteric pathogens** (bacterial and parasitic) in the same study population.

Materials and Methods

Study Design and Setting: This was a **prospective, laboratory-based observational study** conducted in the central laboratory of the Department of Microbiology, Jaipur National University Institute for Medical Sciences and Research Centre, Jaipur, Rajasthan, over a period of **12 months (July 2024–June 2025)**.

Study Population and Sample Size: A total of **174 children aged 2–60 months** presenting to the paediatric outpatient department with acute diarrhoea (three or more loose/liquid stools per day, with or without vomiting, fever or abdominal pain) were enrolled. Critically ill children, nosocomial diarrhoea (onset >48 h after hospital admission), and persistent/chronic diarrhoea with mucus and blood were excluded. The sample size was calculated using a **95% confidence level, an expected population proportion of 13%, and a margin of error of ±5%** ($n = z^2p(1-p)/e^2 = 173.79 \approx 174$).

Specimen Collection: Approximately 5–10 g of fresh stool was collected in sterile, wide-mouth, leak-proof containers, transported within 2 hours, or stored at 2–8°C and processed within 24 hours if delayed.

Rotavirus Detection by ELISA

Rotavirus antigen was detected using a **sandwich ELISA (Rotavirus-antigen-EIA-BEST, D-1652)** based on monoclonal and polyclonal antibodies specific to rotavirus antigen. A 10% stool suspension (100 mg stool in 1 mL sample buffer) was centrifuged at 3000 rpm for 20 minutes, and the clear supernatant was tested in duplicate against positive and negative controls, with conjugate and TMB substrate incubation steps performed per kit protocol. Absorbance was read at **450 nm (reference 620–655 nm)**; a difference of ≥0.2 OD units between sample and negative control was interpreted as **positive**.

Detection of Bacterial and Parasitic Enteric Pathogens

Stool samples were examined by **direct microscopy (Gram staining)** and cultured on **MacConkey and XLD agar** at 37°C for 24 hours. Bacterial isolates were identified by colony morphology, Gram staining, motility (hanging-drop method) and a standard **biochemical panel** (indole, methyl red, Voges–Proskauer, citrate utilization, urease, triple sugar iron, oxidase, catalase, mannitol motility and nitrate reduction tests), with bile-esculin testing for suspected *Enterococcus* isolates. Known positive and negative controls (including *E. coli ATCC 25922*) were used for quality control throughout.

Statistical Analysis: Data were entered in MS Excel and analyzed using **SPSS version 21**. Quantitative variables were summarized using measures of central location; normality was assessed by skewness. Categorical variables were expressed as frequencies and proportions and compared using the **chi-square test or Fisher's exact test** as appropriate. A **p value < 0.05** was considered statistically significant. **Institutional Ethics Committee approval** was obtained prior to the start of the study.

Results

Of 174 stool samples collected from children under five years of age presenting with acute diarrhoea, **43 (24.71%) were positive for rotavirus antigen by ELISA**, while 131 (75.29%) were negative — indicating that nearly one-quarter of diarrhoeal cases in this population were attributable to rotavirus infection.

Category	Number (n)	Percentage (%)
Total samples	174	100
Rotavirus positive	43	24.71
Rotavirus negative	131	75.29

Table 1. Overall Detection of Rotavirus by ELISA (n = 174)

Among the 43 rotavirus-positive children, the highest proportion occurred in the **7–12 month age group (19 cases; 44.2%)**, followed by 13–24 months (12; 27.9%), 25–60 months (7; 16.3%), and 2–6 months (5; 11.5%). This age distribution was statistically significant ($\chi^2 = 9.87$, $p = 0.02$).

Age Group	Positive (n = 43)	% within Group
2–6 months	5	11.5%
7–12 months	19	44.2%
13–24 months	12	27.9%
25–60 months	7	16.3%

Table 2. Association between Rotavirus Infection and Age Group (n = 174)

Of the 174 children, 102 (58.6%) were male and 72 (41.4%) were female. Rotavirus positivity was observed in 25 males (24.5%) and 18 females (25.0%); this difference was **not statistically significant ($\chi^2 = 0.41$, $p = 0.52$)**.

Gender	Positive	Negative	Total
Male	25	77	102
Female	18	54	72

Table 3. Sex-wise Rotavirus Positivity (n = 174)

Rotavirus-positive children showed significantly higher rates of **vomiting (79.1% vs. 52.7%, p = 0.002)**, **severe dehydration (46.5% vs. 24.4%, p = 0.004)**, and **≥6 stools/day (72.1% vs. 51.1%, p = 0.01)** compared with rotavirus-negative children. Fever was more frequent among rotavirus-positive children (65.1% vs. 56.5%) but did not reach statistical significance (p = 0.32).

Clinical Symptom	Rotavirus + (n = 43)	Rotavirus − (n = 131)	p value
Vomiting	34 (79.1%)	69 (52.7%)	0.002
Fever	28 (65.1%)	74 (56.5%)	0.32
Severe dehydration	20 (46.5%)	32 (24.4%)	0.004
≥ 6 stools/day	31 (72.1%)	67 (51.1%)	0.01

Table 4. Clinical Symptoms in Rotavirus-Positive vs. Rotavirus-Negative Cases

A distinct **winter predominance** was observed, with 22 cases (51.2%) occurring between November and February, followed by summer (13 cases; 30.2%) and monsoon (8 cases; 18.6%). This seasonal variation was statistically significant ($\chi^2 = 10.9$, $p = 0.004$).

Season	Positive (n = 43)	Percentage (%)
Winter (Nov–Feb)	22	51.2%
Summer (Mar–Jun)	13	30.2%
Monsoon (Jul–Oct)	8	18.6%

Table 5. Seasonal Distribution of Rotavirus Cases (n = 43)

Among the 131 rotavirus-negative cases, *Escherichia coli* was the most frequently isolated pathogen (28 cases; 21.4%), followed by *Shigella spp.* (2; 1.5%), *Giardia lamblia* (1; 0.8%) and *Entamoeba histolytica* (1; 0.8%). No pathogen was detected on routine culture and microscopy in **99 cases (75.6%)**.

Category	Pathogen / Status	n	%
Rotavirus-positive (n = 43)	Rotavirus	43	24.71
Rotavirus-negative (n = 131)	*Escherichia coli*	28	21.4*
	Shigella spp.	2	1.5*
	Giardia lamblia	1	0.8*
	Entamoeba histolytica	1	0.8*
	No pathogen detected	99	75.6*

*Table 6. Enteric Pathogens Identified by Stool Culture and Microscopy (*percentage of rotavirus-negative cases, n = 131)*

Discussion

Diarrhoeal disease remains a leading cause of childhood morbidity and mortality worldwide, and **rotavirus** continues to be recognized as the most virulent viral agent responsible for severe paediatric gastroenteritis. Global Burden of Disease estimates attribute approximately **128,500 under-five deaths annually** to rotavirus, with more than 80% occurring in South Asia and sub-Saharan Africa, and recent modelling suggests rotavirus still contributes to nearly 20% of diarrhoeal deaths globally despite widespread vaccination. India bears a disproportionate share of this burden, and although **ROTAVAC** has reduced rotavirus-related hospitalizations and deaths since its 2016 introduction into the Universal Immunization Programme, complete elimination has not been achieved — a pattern this study's findings from Rajasthan, a region with scarce prior surveillance data, closely mirror.

The reliability of **ELISA** as the diagnostic method in this study is well supported: prior work has demonstrated 97% concordance between ELISA and PAGE, sensitivity of 95.24% and specificity of 97.47% against immunochromatography, and detection of viral loads as low as 381.5 particles/mL — considerably more sensitive than lateral-flow assays. The WHO similarly recommends ELISA for rotavirus surveillance and vaccine-impact assessment, supporting its use in this study for comparability with national and international data.

The **24.71% rotavirus positivity** observed here is consistent with multicentric Indian surveillance: 35.5% (Giri et al., pre-vaccine, seven-site study), 37.1% (nationwide surveillance, 2005–2016), 35% in Pune, 41.1% in Chennai, and 24–30% in Indore, as well as comparable rates internationally (32.4% in Burkina Faso; 29.6% in Kenya). This concordance strengthens confidence in the present findings and confirms that Rajasthan's rotavirus burden is comparable to other high-burden regions, reflecting shared drivers such as moderate vaccine efficacy in resource-limited settings, incomplete coverage, malnutrition, poor sanitation and circulating strain diversity.

The **peak positivity in the 7–12 month age group (44.2%)** aligns with established epidemiology: maternally derived antibodies wane after six months just as complementary feeding and increased environmental contact heighten faecal–oral exposure, while gut mucosal immunity remains immature. The **absence of significant gender difference (p = 0.52)** in this study, despite the male predominance reported in several other Indian series, suggests that apparent sex differences in hospital-based rotavirus data more often reflect **healthcare-seeking behaviour** than true biological susceptibility, since no sex-specific tropism of rotavirus has been demonstrated experimentally.

The clear **winter predominance (51.2% of cases; p = 0.004)** replicates a globally reproducible feature of rotavirus epidemiology, driven by enhanced viral environmental stability at lower temperatures and reduced UV inactivation, compounded by crowded indoor contact during cooler months. The **significantly greater clinical severity** of rotavirus-positive cases — higher vomiting, dehydration and stool frequency — is explained mechanistically by rotavirus-induced villous atrophy and malabsorption together with **NSP4-mediated secretory diarrhoea**, underscoring the importance of early recognition and prompt rehydration in this group.

Among rotavirus-negative children, **28.3% (37/131) had an identifiable bacterial or parasitic pathogen**, most commonly *Escherichia coli* (21.4%), while 71.7% showed no detectable organism — a lower co-pathogen detection rate than the 40–55% reported from Kenya and Cameroon, potentially reflecting differences in diagnostic methodology (routine culture and microscopy versus molecular assays), prior antimicrobial exposure, or regional epidemiological variation.

Strengths and Limitations

Strengths

- **One of the few hospital-based studies from Rajasthan** to systematically evaluate rotavirus prevalence, age/seasonal distribution, and clinical severity in the post-vaccine era, filling a significant regional data gap.
- **Use of a validated, internationally standardized ELISA method** ensures comparability with national and global surveillance data.
- **Detailed clinical correlation** between rotavirus status and symptom severity strengthens the study's public health relevance.

Limitations

- **Single-center design** limits generalizability, and hospital-based sampling may overestimate prevalence relative to community settings.
- **No molecular genotyping** of rotavirus strains was performed, precluding assessment of circulating genotypes or vaccine-escape variants.
- **Cross-sectional design** precludes definitive causal inference between rotavirus infection and clinical outcomes.

Conclusion

Rotavirus remains a **major cause of severe acute gastroenteritis** among children under five years in Rajasthan, despite ROTAVAC's introduction into the Universal Immunization Programme, with **24.71% ELISA positivity** in this cohort. Infection was significantly associated with **younger age (7–12 months), winter seasonality, severe dehydration, frequent vomiting, and higher stool output**, confirming that rotavirus-associated diarrhoea is clinically more severe than diarrhoea from other causes. The persistence of infection despite vaccination reflects moderate vaccine efficacy, incomplete coverage, malnutrition, environmental exposure and genotype diversity, and underscores the continued value of **ELISA-based laboratory confirmation** for case management, surveillance and vaccine-impact evaluation. This study provides valuable baseline regional epidemiological data supporting integrated prevention strategies combining vaccination, sanitation, early diagnosis and effective clinical care.

Recommendations

- **Strengthen rotavirus surveillance** through routine ELISA testing in district and tertiary hospitals, integrated with existing diarrhoeal disease monitoring.
- **Improve vaccine coverage**, particularly among rural, migrant and socioeconomically disadvantaged populations.
- **Introduce molecular surveillance** (genotyping) to track emerging strains and possible vaccine-escape variants.
- **Promote early diagnosis and rational management**, avoiding unnecessary antibiotic use and ensuring prompt rehydration therapy.
- **Strengthen WASH (Water, Sanitation and Hygiene) interventions** and community nutrition programs.

- **Ensure health-system preparedness** (ORS, IV fluids, zinc, trained personnel) with seasonal planning for winter case surges.

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