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**Original Research Article**

# A study of detection of diarrhoea associated human rotavirus and co-infection with diarrhoea genic pathogens in childhood stool specimen by using ELISA and RT-PCR in a tertiary care hospital at Indore, Madhya Pradesh

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## ABSTRACT

**Background:** Childhood diarrhoea mainly caused by Group A Rotavirus, is a major global health issue, especially for children under five. In India, RVA-induced diarrhoea causes numerous deaths, hospitalizations, and outpatient visits annually. Vaccination is crucial in preventing RVA, with WHO-approved oral vaccines significantly reducing global mortality and morbidity. However, challenges persist in implementing vaccines in regions like sub-Saharan Africa due to factors like malnutrition and unsanitary conditions. Despite this, since 2009, low-income countries have seen a decline in RVA-related illness.
**Methods:** Over 18 months, from January 2021 to June 2022, a study at the Post Grad Dept. of Microbiology, Index Medical College, Hospital and Research Centre in Indore, MP, involved 250 children under five with acute gastroenteritis. Ethical clearance and parental consent were obtained. Data included demographic, antenatal, diarrhoea l symptoms, feeding, hygiene, physical exams, and stool analysis.
**Results:** 250 children under five were screened for Rotavirus. 60 tested positive, mostly in 6–12-month-olds during cooler months in urban areas. 80% were from low socioeconomic backgrounds. Exclusive breastfeeding linked to lower incidence. Vomiting and severe dehydration more frequent in positive cases. ELISA and ICG methods equally effective.
**Conclusions:** Childhood diarrhoea, primarily caused by Rotavirus, remains a leading cause of under-five deaths, totalling 600,000 annually. Among 250 children studied, 60 tested positive for Rotavirus, especially among males aged 7-12 months, with infections peaking in cooler months. Both ELISA and ICG were equally effective in detection. Treatment primarily involves oral rehydration with low osmolarity ORS. Predominant strains were G1 P (8) and G2 P (4). Global endorsement of rotavirus vaccines like Rotarix and Rotateq, with Rotavac showing promise in India, signals progress in fighting rotavirus, potentially improving public health via inclusion in state immunization programs.

**Keywords:** Childhood diarrhoea, Rotavirus vaccination, Epidemiological update, Diagnostic methods, Socioeconomic factors

## INTRODUCTION

Diarrhoea ranks as the second leading cause of childhood mortality globally, with Group A Rotavirus (RVA) being identified as a predominant causative agent, responsible for 38% of acute Diarrhoea cases among children aged five and below worldwide.[1] Annually in India, RVA-induced Diarrhoea results in an estimated 122,000-153,000 fatalities, 457,000-884,000 hospitalizations, and 2 million outpatient visits in children under five years of age.[2] While promoting good hygiene practices aids in limiting the transmission of Diarrhoea l pathogens, vaccination stands out as the most efficacious method for RVA prevention.[3] The deployment of four WHO-approved oral vaccines; RotaTeq, Rotarix®, Rotavac, and Rotasiil has notably curbed global mortality and morbidity associated with RVA, particularly impactful in developing nations, where it significantly contributes to pediatric gastroenteritis-related hospitalizations, estimated at around 139 million cases annually worldwide.[4] Factors contributing to this challenge include malnutrition's detrimental effect on immunogenic development, heightened RVA exposure due to unsanitary conditions and contaminated water sources, and potential vaccine ineffectiveness against circulating strains.[5] Since 2009, low-income countries have witnessed a decline in RVA-related mortality and morbidity following vaccine introduction. In specific regions like Uttar Pradesh and Madhya Pradesh, Diarrhoea prevalence attributable to RVA decreased substantially from 2015 to 2016. South Africa experienced a one-third reduction in hospitalization rates during the same period.[6] Prior to vaccination, RVA caused over 5800 deaths annually among children under five in Madhya Pradesh and accounted for a significant portion of Diarrhoea related hospitalizations. Notably, previous studies in Madhya Pradesh revealed the presence of rare genotypes and regional genotypic diversity among circulating strains, particularly in the Littoral, East, and South regions. This study aims to provide an epidemiological update on RVA infection in the Littoral region of Madhya Pradesh among children under five hospitalized for severe Diarrhoea. It assesses RVA vaccination status, co-infection with other enteric pathogens, and feeding practices during the first six months of life. The findings will inform RVA infection surveillance, evolution, and trend analysis, as well as guide future vaccine impact assessments.

## METHODS

The study involved infants and children up to the age of 5 years, of both sexes (Baby Boy and Baby Girl), who were hospitalized at Index medical college, hospital and research centre, Madhya Pradesh, from January 2021 to June 2022, due to gastroenteritis. Each participating hospitalized child provided a diarrheic stool sample, alongside socio-demographic information, including RVA immunization and breastfeeding status, retrieved from the patient's medical records and/or provided by guardians.

### *Clinical history documentation in paediatric gastroenteritis cases*

The clinical history of each patient was meticulously obtained and documented according to a standardized proforma, encompassing the following aspects: Demographic details: This included the patient's name, age, gender, residential setting (urban/rural), parental occupation, level of education, and socioeconomic status. Antenatal history: Information regarding the gestational period, birth weight, and immunization status of the child was recorded. Presenting symptoms: Detailed data on the onset, duration, and characteristics of diarrhoea, including the presence of blood, vomiting, abdominal pain, and fever, were documented. Feeding practices: The method of feeding, whether breastfed or bottle-fed, was noted. Hygiene practices: Data regarding handwashing and general sanitary practices in the household were collected. Physical examination: A comprehensive physical examination of the child was conducted to assess signs of dehydration, level of consciousness, feeding behavior, activity level, skin turgor, anterior fontanelle status, and presence of sunken eyes. Stool examination: Macroscopic examination of stool specimens was performed to evaluate consistency, color, and the presence of blood.

### *Sample collection, transport, and storage protocol*

During the acute phase of gastroenteritis (within 3 days of symptom onset), approximately 15-20 ml of stool specimen was collected in a sterile wide-mouthed universal container. These samples were promptly transferred into an icebox to maintain a temperature range of 2-8°C during transportation to the Microbiology diagnostic laboratory.[7] Upon arrival at the laboratory, the stool specimens were transferred into 2 ml sterile centrifuge containers containing buffer solution. Subsequently, these containers were stored at temperatures ranging from -20 to -70°C to preserve the integrity of the samples for Rotavirus antigen detection.[8]

### *Testing procedures*

The collected specimens underwent initial screening for Rotavirus antigen utilizing immuno-chromatography test (ICT) and enzyme-linked immunosorbent assay (ELISA). Confirmation of Rotavirus presence was conducted through reverse transcriptase polymerase chain reaction (RT-PCR) analysis.

### *Immuno-chromatography test*

To identify the Rotavirus antigen in stool specimens, the SD Bioline Rota rapid test kit from Standard Diagnostics was employed. This kit functions on the principle of immuno-chromatography. Specimen processing adhered

to the instructions provided by the kit manufacturer (Figure 1).

***Enzyme-linked immunosorbent assay***

RVA detection was carried out using "Biogenix Rotavirus Fecal ELISA kit". Samples and reagents were equilibrated to lab temperature. Samples were diluted by 10% using provided dilution buffer, serving as a negative control (NC). Known positive and negative samples acted as additional controls. Supernatants (100 µl each) were transferred to corresponding wells in a pre-coated microtiter plate. Reaction steps were executed as per protocol. Initial assessment involved visual observation of color changes compared to NC. Confirmation was done spectrophotometrically at 450 nm after halting the reaction with sulfuric acid. Positive results were confirmed if OD value exceeded the NC OD+0.2 threshold. Only samples with positive ELISA results proceeded to further testing (Figure 2).

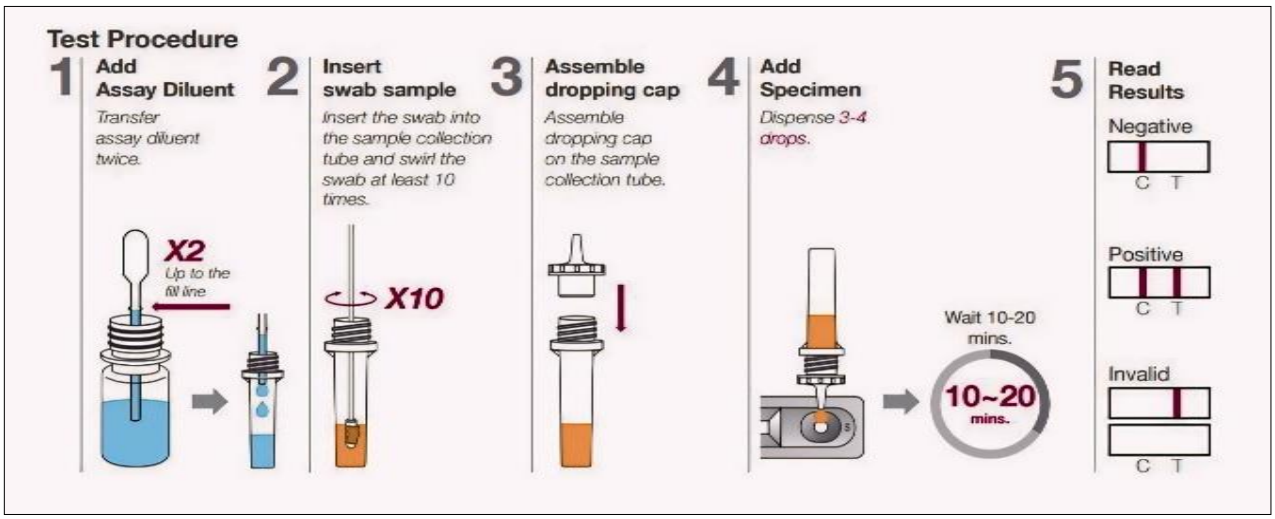


**Figure 1: Immuno-chromatography procedure.**

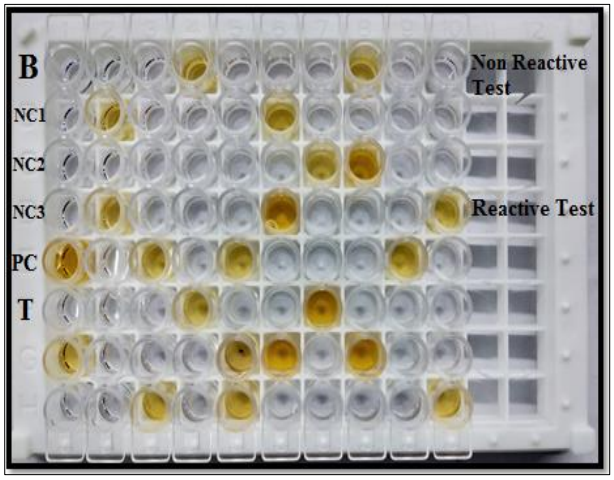


**Figure 2: ELISA result.**

***Reverse transcriptase polymerase chain reaction***

The PureFast R viral nucleic acid mini spin purification kit is equipped with essential components including Proteinase-K, Lysis buffer, Wash buffer-1, Wash buffer-2, Spin columns accompanied by collection tubes, and elution buffer. Designed for the extraction and purification of viral nucleic acids, this kit ensures efficient processing of samples. In conjunction with the purification kit, the HELINI Rotavirus-A Real-time PCR kit, supplied by HELINI Biomolecules, is employed for the real-time PCR analysis of Rotavirus-A. This PCR kit is specifically tailored for accurate and sensitive detection of Rotavirus-A, enhancing the reliability and precision of diagnostic testing procedures.

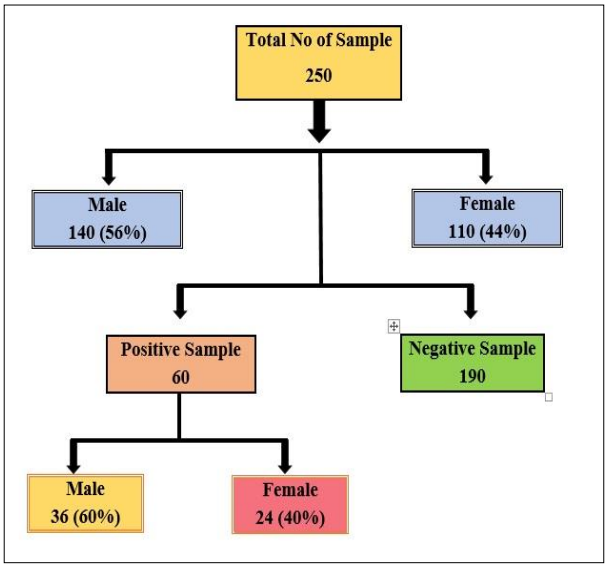


**Figure 3: Demographic data of the patients.**

***Statistical analysis***

Statistical analysis was performed utilizing the GraphPad Prism 5 software. The Chi-square test was employed, and significance was determined at $p<0.05$.[9]

## RESULTS

During the investigation period spanning from January 2021 to June 2022, a cohort comprising 250 children under the age of five, presenting with acute gastroenteritis, underwent screening for Rotavirus antigen. Among these participants, 140 (56%) were male and 110 (44%) were female. Out of the total samples analyzed, 60 tested positive for Rotavirus antigen. Specifically, among the 250 children, 60 exhibited Rotavirus antigen positivity, with 36 (60%) being male and 24 (40%) female (Figure 3).

**Table 1: Distribution of acute gastroenteritis in accordance.**

| Age (months) | Male | | Female | | Total, N (%) |
|---|---|---|---|---|---|
| | N | % | N | % | |
| **0-6** | 20 | 14.28 | 15 | 13.63 | 35 (14) |
| **7-12** | 58 | 41.42 | 37 | 33.63 | 95 (38) |
| **13-24** | 34 | 24.28 | 31 | 28.18 | 65 (26) |
| **25-60** | 28 | 20 | 27 | 24.54 | 55 (22) |
| **Total** | 140 | 56 | 110 | 44 | 250 (100) |

**Table 2: Seasonal distribution of Rotavirus.**

| Months | N | Rotavirus positive cases | | Rotavirus negative cases | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| **January** | 12 | 2 | 16.67 | 10 | 83.33 |
| **February** | 13 | 2 | 15.38 | 11 | 84.62 |
| **March** | 12 | 1 | 8.33 | 11 | 91.67 |
| **April** | 14 | 2 | 14.29 | 12 | 85.71 |
| **May** | 11 | 2 | 18.18 | 9 | 81.82 |
| **June** | 17 | 6 | 35.29 | 11 | 64.71 |
| **July** | 15 | 3 | 20.00 | 12 | 80.00 |
| **August** | 16 | 2 | 12.50 | 14 | 87.50 |
| **September** | 18 | 11 | 61.11 | 7 | 38.89 |
| **October** | 19 | 10 | 52.63 | 9 | 47.37 |
| **November** | 17 | 4 | 23.53 | 13 | 76.47 |
| **December** | 15 | 3 | 20.00 | 12 | 80.00 |
| **January** | 10 | 2 | 20.00 | 8 | 80.00 |
| **February** | 10 | 2 | 20.00 | 8 | 80.00 |
| **March** | 14 | 1 | 7.14 | 13 | 92.86 |
| **April** | 10 | 2 | 20.00 | 8 | 80.00 |
| **May** | 12 | 2 | 16.67 | 10 | 83.33 |
| **June** | 15 | 3 | 20.00 | 12 | 80.00 |

The study unveiled the highest incidence of Rotavirus positivity among children aged 6 to 12 months (38.94%), followed by those aged 13 to 24 months (23.07%) (Table 1). Conversely, a lower infection rate was observed in infants aged 0 to 6 months (14.28%), and beyond the age of 2 years, the prevalence of Rotavirus infection notably decreased to 5.45%. While Rotavirus occurrences were noted throughout the year, the peak cases were recorded during the cooler months, spanning from September to February, compared to other months (Table 2).

Despite Rotavirus being prevalent across all geographical areas, 22% of cases were from urban areas, while 27% were from rural areas.

Moreover, a higher incidence of Rotavirus-positive cases was observed among individuals utilizing public water supply (25.28%) compared to those using bore well water (20.83%), indicating a heightened likelihood of Rotavirus infection among users of public water supply (Table 3). Furthermore, the study highlighted a correlation between poor parental literacy and an increased rate of Diarrhoea l diseases in young children, with 80% of Rotavirus-infected children belonging to the low socioeconomic group. Additionally, a lower incidence of gastroenteritis was observed among children exclusively breastfed (18%), whereas a higher incidence was noted in children on mixed feeding (27%). Clinical manifestations among Rotavirus-positive cases included vomiting (83%) and severe dehydration (79%) at a significantly higher frequency compared to Rotavirus-negative cases. Fever was observed with similar frequency in both Rotavirus-positive and negative cases. Analysis of the Rotavirus antigen via ELISA and ICG methods among the 250 samples demonstrated a positivity rate of 60 samples and 190 negatives, indicating comparable sensitivity and specificity between both methods. Subsequently, among the 60 Rotavirus-positive samples identified via ELISA, 30 were subjected to PT-PCR and VP7 (G) and VP4 (P) genotyping at HELINI Biomolecules. Among these, 24 samples were successfully amplified and genotyped, with the majority (62.5%) belonging to the G1 (P8) combination, followed by 29.16% to G2 (P4) and 8.3% being un-typable.

**Table 3: Geographical distribution of Rotavirus.**

| Geographical area | N | Rotavirus positive cases, N (%) | Rotavirus negative cases, N (%) |
|---|---|---|---|
| **Urban** | 150 | 33 (22) | 117 (78) |
| **Rural** | 100 | 27 (27) | 73 (73) |
| **Total** | 250 | 60 | 190 |

## DISCUSSION

Diarrhoea continues to impose a substantial health burden globally, especially among pediatric populations, with India bearing a significant proportion of morbidity and mortality. Despite advancements in healthcare accessibility, Rotavirus remains a leading cause of Diarrhoea l deaths among children under five years old, contributing significantly to the overall disease burden in India. The economic impact of Rotavirus-associated

Diarrhoea is considerable, with substantial expenditures on hospitalizations and outpatient visits annually in India.[10]

Rotavirus, first identified in 1973, has been extensively researched worldwide to elucidate its pathogenesis, epidemiology, and clinical characteristics. The prevalence of Rotavirus gastroenteritis varies globally, with India exhibiting a diverse range of incidence rates influenced by factors such as age groups studied, diagnostic methodologies employed, seasonal variations, and geographical disparities. This study found a Rotavirus infection prevalence of 24% among children under five years old, consistent with findings reported in other studies conducted in India and internationally. Peak incidence of Rotavirus gastroenteritis occurred between 7 to 12 months of age, followed by 13 to 24 months, with a notable decline thereafter. Males demonstrated a higher susceptibility to Rotavirus infection compared to females, with distinct seasonal patterns observed during cooler months. Furthermore, infants receiving mixed feeding displayed a higher incidence of gastroenteritis compared to exclusively breastfed infants. Socioeconomic status emerged as a significant determinant, with children from low socioeconomic backgrounds exhibiting increased susceptibility to Rotavirus infection. Moreover, parental education level correlated with infection rates, underscoring the importance of health education. Geographically, rural areas exhibited higher Rotavirus positivity rates compared to urban areas, with water supply sources also influencing infection rates. Clinical manifestations of Rotavirus-positive cases included Diarrhoea, vomiting, fever, and severe dehydration. The study also assessed the efficacy of two diagnostic methods, ELISA and ICG, revealing comparable sensitivity and specificity. Additionally, genotyping of Rotavirus strains identified prevalent serotypes, with G1 (P8) and G2 (P4) being the most common combinations. Rotavirus vaccination emerges as a crucial strategy in reducing disease burden and mortality, with India integrating its own vaccine, Rotavac, into the National Immunization Program. The vaccine has shown efficacy, safety, and cost-effectiveness, potentially preventing thousands of deaths annually. Pilot studies across various states have yielded promising results, facilitating broader vaccine implementation nationwide. Rotavirus infection poses a significant health threat, particularly in developing nations like India. Integrated vaccination programs alongside comprehensive public health interventions are imperative in alleviating the burden of Rotavirus-related illnesses and enhancing child health outcomes.

### *Limitations*

The research was carried out exclusively at a solitary hospital in Madhya Pradesh, thereby constraining its applicability to broader populations and diverse geographical regions.

## CONCLUSION

The study contributes to advancing knowledge and understanding in the field of paediatric infectious diseases by providing insights into the epidemiology and clinical management of rotavirus infection, a significant cause of childhood mortality worldwide. Through a comprehensive analysis involving 250 children over 18 months, the research reveals key findings, including a higher susceptibility among males, particularly aged 7 to 12 months, with peak infections occurring during cooler months. Additionally, the study evaluates the effectiveness of diagnostic tests, highlighting both ELISA and ICG tests as viable options, with ELISA preferred for efficiency and ICG for rapid screening in resource-limited settings. Furthermore, the research underscores the importance of oral rehydration therapy, particularly emphasizing the use of low osmolarity ORS as the primary treatment approach. The study also identifies the predominant rotavirus serotypes, G1 P (8) and G2 P (4), accounting for the majority of cases. Moreover, it discusses the efficacy of WHO-endorsed vaccines such as Rotarix, Rotateq, and India's Rotavac in reducing diarrhoea-related illnesses and deaths, offering promising prospects for improved public health outcomes. Additionally, the study highlights the incorporation of rotavirus vaccines into some states' immunization schedules, suggesting a positive step towards enhancing overall population health.

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*Conflict of interest: None declared*
*Ethical approval: The study was approved by the Institutional Ethics Committee*

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