

# Burden of Rotavirus Diarrhoea among Children Less than Five Years of Age Attending a Tertiary Care Institute with Acute Gastroenteritis: A Cross-sectional Study

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## ABSTRACT

**Introduction:** Diarrhoea due to rotavirus continues to cause significant morbidity in children less than five years of age especially in developing countries. Prior to the incorporation of rotavirus vaccine in the national immunisation program the prevalence of rotavirus in stool samples of children in India has been reported to vary from 4.6-33.7%. However, there is not much data regarding the burden of rotavirus diarrhoea after the widespread use of rotavirus vaccine.

**Aim:** To find out the extent to which rotavirus is responsible for causing infection in children under five years of age.

**Materials and Methods:** This was a cross-sectional study carried out in the Department of Microbiology, in collaboration with the Department of Paediatrics, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India, among children who presented with acute gastroenteritis to the biggest tertiary care institute of the region. After seeking written consent from the parents/caretakers stool samples were collected and subjected

to Enzyme Linked Immunosorbent Assay (ELISA). Demographic variables including vaccination status and seasonality were recorded for all the cases. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 23.0 and p-value of <0.05 was taken as significant.

**Results:** Of the 279 stool samples received during the study period, a rotavirus positivity of 17.2% was seen with 31 (63.8%) patients affected being males. A total of 37 (77.1%) children belonged to the age group of 0-12 months and 27 (56.3%) of those affected were from rural areas. Diarrhoea was the most common symptom in 28 (58.3%) patients. Most of the rotavirus positive children, that is, 26 (54.2%) were not vaccinated. Majority of the cases, 34 (70.8%), were seen in winter season.

**Conclusion:** Majority of the children who visited the hospital were not vaccinated and were rotavirus positive despite a robust vaccination program. This puts an emphasis on the need to improve the vaccination rates in order to fight this illness.

**Keywords:** Enzyme-linked immunosorbent assay, Immunisation, Kashmir, Vaccination

## INTRODUCTION

First described by Ruth Bishop and associates in 1973 in children presenting with gastroenteritis, rotaviruses are a leading cause of acute, severe, dehydrating gastroenteritis in children less than five years of age globally, with more than 25 million outpatient visits and around two million hospitalisations attributable to rotavirus infections every year [1,2]. In developing countries, the attack rate is high among children aged 6-12 months whereas in the developed nations children of 12-14 months are predominantly infected [3,4]. The rotaviruses have a Group-Antigen, the protein VP6 present in the middle capsid on the basis of which these are classified into nine groups, namely: Group-A Rotavirus (RVA), Group-B Rotavirus (RVB), Group-C Rotavirus (RVC), Group-D Rotavirus (RVD), Group-E Rotavirus E (RVE), Group-F Rotavirus (RVF), Group-G Rotavirus (RVG), Group-H Rotavirus (RVH) and Group-I Rotavirus (RVI). Among these, RVA, RVB, RVC and RVH are associated to acute gastroenteritis in humans and animals [5]. The virus is shed in very high concentrations and for many days in the stools and vomitus of those infected. Transmission occurs mostly by the faecal-oral route, from one person to another by close contact and via fomites [6]. The most common symptoms are vomiting, diarrhoea and fever that can cause significant dehydration and reduced oral intake often necessitating hospitalisation. In severe cases death can occur if timely intervention is not sought [7]. Despite the availability of a vaccine, clean drinking water supply and good sanitation practices, infections due to rotavirus continue to occur throughout the world.

Rotavirus gastroenteritis is clinically indistinguishable from diarrhoeal diseases caused by other enteric pathogens and laboratory testing is

generally not done; however it is the only way to confirm the diagnosis. In cases with prolonged diarrhoea or complicated cases or immunocompromised patients, when alternative diagnoses are considered, or when epidemiologic or infection control data is desired, it may be valuable to establish rotavirus as the causative agent [8]. The definitive diagnosis of rotavirus gastroenteritis is also paramount in preventing the unnecessary use of antibiotics in such cases.

For laboratory confirmation, antigen testing can be done in stool samples using ELISA or immunochromatography. Other modalities of detection include Reverse Transcription Polymerase Chain Reaction (RT-PCR), assays which is more sensitive and allow genotyping of virus isolates, electron microscopy, polyacrylamide gel electrophoresis, antigen detection assays, and virus isolation [8]. The positivity of rotavirus in stool samples in India has varied from 4.6-33.7% [9]. In Jammu and Kashmir, one study documented the attack rate to be around 20% whereas another study conducted in two major hospitals reported the prevalence of rotaviral diarrhoea to be 45% [10,11]. However, not much data regarding the epidemiology of rotavirus is available from this part of the country especially after the introduction of vaccine against it. The present study was carried out to generate epidemiological data in terms of rotavirus burden in children less than five years of age who attended the paediatric Outpatient Department and/or were admitted at a tertiary care centre in northern India.

## MATERIALS AND METHODS

This cross-sectional study was carried out in the Department of Microbiology, in collaboration with the Department of Paediatrics,

Sher-i-Kashmir Institute of Medical Srinagar, Jammu and Kashmir, India, a tertiary care hospital for a period of 18 months from 15<sup>th</sup> December 2019 to 15<sup>th</sup> June 2021. Ethical clearance was obtained from the Institute's Ethical Clearance Committee bearing the number: RP 54/2020. Written informed consent was sought from the parents/caretakers and a predesigned proforma that included information regarding the age, gender, residence, vaccination status was filled for each participant.

#### Inclusion criteria:

- All children less than five years of age who presented with acute diarrhoea (>3 unformed stools in 24 hours period) to the hospital.
- Children whose parents/caretakers were willing to participate in the study.

#### Exclusion criteria:

- Children with primary diagnosis other than acute gastroenteritis.
- Children  $\geq$  five years of age.

### Study Procedure

**Sample collection and processing:** Stool samples were collected in a sterile container and transported to the laboratory as soon as possible. In case of delay, the samples were stored in a refrigerator at 2-8°C. A 10%-20% suspensions were made using Phosphate Buffered Saline (PBS) for antigen testing. All aliquots of processed samples were stored at -20°C till further testing.

**Enzyme Linked Immunosorbent Assay (ELISA):** ELISA was done for all the samples, using a kit that identifies RVA (Premier Rotaclone Meridian Bioscience Inc. USA). The assay was performed as per the manufacturer's instructions. Briefly, all the reagents were brought to room temperature before use. One mL of sample diluent was added to properly marked tube, using a transfer pipette and the sample resuspended in the sample diluent. Sufficient number of wells were snapped off for samples and the controls and inserted into the microtiter well holder following which two drops (100  $\mu$ L) each of diluted faecal sample, positive control and negative control (sample diluent) and enzyme conjugate were added to the wells. The plate was incubated at room temperature for 60 $\pm$ 5 minutes at the end of which, the liquid was poured out of the wells into a discard vessel. All the wells were filled to the brim with deionised water and the liquid was poured out as in previous step. The washing procedure was repeated four more times (for a total of five washes) after which two drops (100  $\mu$ L) of substrate A solution containing urea peroxide and substrate B solution containing tetramethylbenzidine were added to each well and the plate was incubated for 10 minutes at room temperature. At the end of the incubation period the microtitre plate was examined visually. Spectrophotometric determinations were made by adding two drops (100  $\mu$ L) of stop solution (sulfuric acid) to each well after the 10 minutes incubation at room temperature. The absorbance of each well was read at 450 nm using a >600 nm reference filter (optional) against an air blank within 60 minutes. The positive and the negative controls were validated according to the kit protocol. For visual evaluation, the positive control was deep blue and easily distinguished from the colourless negative control. For spectrophotometric determination, the absorbance of the positive control was  $\geq$  0.3 absorbance.

**Interpretation of results:** Specimens with absorbance units greater than 0.150 on the spectrophotometer were considered positive and those with absorbance equal to or less than 0.150 were considered negative.

### STATISTICAL ANALYSIS

The data were entered into the excel and statistical analysis was done using SPSS software version 23.0. The p-values were calculated using Chi-square test and p-value <0.05 was considered to be statistically significant.

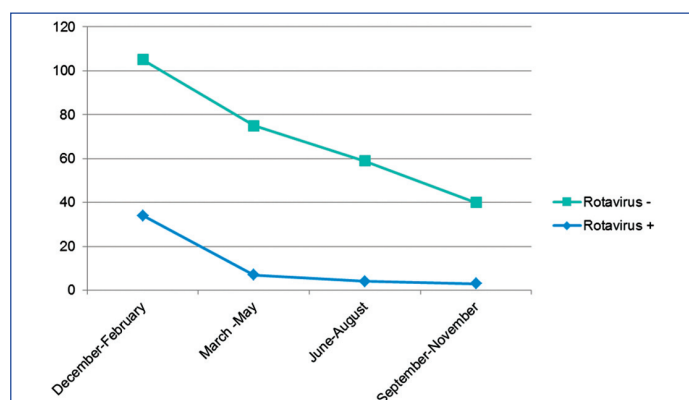
### RESULTS

Of the 279 stool samples received during the study period, 48 (17.2%) were positive and 231(82.8%) negative for rotavirus antigen by ELISA. Statistically significant number of positive samples, 31 (63.8%) belonged to male patients (p-value=0.0012) and majority were in the age group of 0-12 months, 37 (77.1%) (p-value=0.0001). Rural areas represented 27 (56.3%) positive samples. The most common complaint in the group of children whose stool samples were positive for rotavirus was diarrhoea, 28 (58.3%) followed by diarrhoea and fever, 12 (25%). Differences in the demographic parameters between rotavirus positive and negative patients are shown in [Table/Fig-1].

Variables	Rotavirus positive n (%)	Rotavirus negative n (%)	p-value
<b>Gender</b>			
Male	31 (63.8)	94 (40.7)	0.0012
Female	17 (36.2)	137 (59.3)	
<b>Age (months)</b>			
0-12	37 (77.1)	203 (87.9)	0.0001
13-24	11 (22.9)	21 (9.1)	
25-36	0	4 (1.7)	
37-48	0	3 (1.3)	
49-59	0	0	
<b>Locality</b>			
Urban	21 (43.7)	76 (32.9)	0.075
Rural	27 (56.3)	155 (67.1)	
<b>Chief complaint</b>			
Diarrhoea	28 (58.3)	91 (39.4)	0.024
Diarrhoea and vomiting	0	52 (22.5)	
Fever, diarrhoea and vomiting	8 (16.7)	50 (21.7)	
Fever and diarrhoea	12 (25)	25 (10.8)	
Pain abdomen	0	13 (5.6)	
<b>Vaccination status</b>			
Vaccinated	22 (45.8)	127 (54.9)	0.425
Not vaccinated	26 (54.2)	104 (45.1)	

[Table/Fig-1]: Demographic characteristics of patients visiting the hospital with acute gastroenteritis.

Also majority of the children; 26 (54.2%) whose stool sample was positive for rotavirus had not received rotavirus vaccine, whereas 6 (12.5%) had received a full course of vaccine, 11 (22.9%) had received only one dose and 5 (10.4%) two doses of the rotavirus vaccine. Significant number of positive cases were recorded in winter season 34 (70.8%) (p-value=0.0001) [Table/Fig-2]. All the rotavirus positive cases were treated symptomatically (i.v. fluids and oral rehydration therapy as indicated) and discharged from the hospital without any complications after treatment.



[Table/Fig-2]: Distribution of rotavirus positive and negative cases according to the season.

## DISCUSSION

After the introduction of rotavirus vaccine in the national immunisation program in the year 2016, this was the first study that looks into the burden of disease caused by this pathogen from this part of the country. A rotavirus positivity of 17.2% was seen in present study which was less as compared to that reported by Shrestha S et al., where the authors found a positivity of 28% and Binka FN et al., who reported a rotavirus positivity of 39% in their study [12,13]. In a large multi-centre study conducted in hospitals across seven cities of India, between July 2012 and June 2016, stool samples of 35.5% patients were found to be positive for rotavirus by Enzyme Immunoassay (EIA) [9]. However, this was prior to the incorporation of rotavirus vaccine in the national immunisation schedule. Better hygienic practices, awareness of caretakers regarding the disease and its symptoms, vigorous vaccination campaign and the prompt use of oral rehydration solution in case of diarrhoea which circumvents the need for hospitalisation could have been the reason of low rotavirus positivity in present study. Or it could be due to the fact that the study was carried out during the ongoing COVID-19 pandemic which could have resulted in less number of parents seeking medical advice for their children suffering from acute gastroenteritis.

Higher detection of rotavirus in male children (63.8%) than in female children (36.2%) was noted in present study. Results similar to present study have been reported previously by other authors as well [9,12,14,15]. In present study, a significant rotavirus positivity of 77.1% in the stool samples was noted for children in the age group of 0-12 months. Kheyami AM et al., in their study found that 83% of the cases were in age group of 4-23 months [16]. Likewise Agbla JMM et al., in their study found that children aged between 3-24 months were the significantly affected by rotavirus [17], whereas Junaid SA et al., reported that children within the age bracket of 7-12 months had the highest rate of infection [14]. Children less than 6 months of age are protected by maternal antibodies and as weaning starts and the protective effects of breastfeeding decrease they become vulnerable to infections [18,19].

Most of the positive samples in this study were received from children residing in rural areas, 27 (56.3%). Improper sanitation and inaccessibility to proper drinking water in rural areas and low vaccination rates as compared to urban areas could be one of the factors contributing to such an observation. In a study by Tian Y et al., the authors reported higher positivity in cases from urban areas (23.9%) [20], which was in contrast to what was observed in present study. Since rotavirus infection is primarily acquired by feco-oral route it is quite natural that the most common symptoms are those related to the gastrointestinal tract. Diarrhoea was also the most common symptom in the group of children whose stool samples were positive for rotavirus, 28 (58.3%) followed by diarrhoea and fever, 12 (25%) and diarrhoea fever and vomiting 8 (16.7%). Present study results are in accordance to what has been reported by Martinez-Gutierrez M et al., where the authors found that among the rotavirus A-infected children, diarrhoea, fever and vomiting were the most common symptoms [18]. Many other study have also reported diarrhoea fever and vomiting to be the most common symptoms associated with rotaviral gastroenteritis [12,15,21].

An increased incidence of rotavirus infections during winter or cooler months of the year have been well documented from several parts of the globe [3,22,23]. Because the survival of rotavirus is better in cooler conditions with low relative humidity, it has been hypothesised that a relative drop in humidity and rainfall combined with drying of soils might increase the aerial transport of dried, contaminated faecal material containing rotavirus [3,23]. Findings from previous Indian studies have also corroborated this. The same was seen in present study where the highest number of cases of acute gastroenteritis sought medical attention during winter season (70.8%); particularly in the months of October and November.

Majority of the children (54.2%) positive for rotavirus in present study had not received rotavirus vaccine, whereas 12.5% had received a full course of vaccine, 22.9% had received only one dose and 10.4% two doses of the rotavirus vaccine. Martinez-Gutierrez M et al., in their study found that most of the rotavirus-positive children had received two doses of rotavirus vaccine; 86.2%, whereas 6.9% had received atleast one dose and 6.9% did not have a vaccination card to confirm rotavirus vaccination [18]. [Table/Fig-3] summarises the published literature providing an insight into the impact of rotavirus vaccine on the prevalence of rotaviral diarrhoea in different parts of India [9,15,24-33].

Author name, reference	Publication year	Prevalence (%)	Rotavirus vaccine introduced in National Immunisation Schedule
Giri S et al., [9]	2019	35.5	No
Girish Kumar CP et al., [15]	2020	36.3	No
Mathew A et al., [24]	2014	35.9	No
Goel AK et al., [25]	2021	11.02	Yes
Dalal P et al., [26]	2021	17.1	Yes
Sharma J et al., [27]	2021	23.3	Yes
Goru KB et al., [28]	2021	7.4	Yes
Chaudhary P et al., [29]	2021	18.8	Yes
Dass SM et al., [30]	2018	18.3	Yes
Kumar A et al et al., [31]	2022	14.58	Yes
Ghoshal V et al., [32]	2020	29	Yes
Gupta V et al., [33]	2021	11.33	Yes
Present study	2023	17.2	yes

**[Table/Fig-3]:** Compilation of studies published in the literature related to the prevalence of rotaviral diarrhoea in children less than five years of age [9,15,24-33].

A decrease in the rate of hospitalisations due to rotavirus infection after the introduction of rotavirus vaccine has been reported worldwide. In present study the increased positivity of rotavirus antigen in stool samples of unvaccinated children substantiates the fact that the vaccine is effective in reducing the overall burden of rotavirus illness.

### Limitation(s)

Since the study was carried out in a hospital setting a true estimate of the burden of rotaviral diarrhoea in our community can be determined by community based surveillance only. Also, genotyping of the positive samples was not done, which would have given information about the circulating strains.

### CONCLUSION(S)

Rotavirus infection continues to occur in children under the age of five years. Because of similar symptoms most of the diarrhoeal illnesses are treated as bacterial or parasitic in origin. Rotavirus assay should be performed in cases in which bacterial and parasitic assays all show negative results. There is a possibility of overestimation or underestimation of the rate at which the cases of acute gastroenteritis visit the hospitals because of potential referral of these patients to other hospitals especially in the peripheral regions. Continued surveillance of rotaviral illness carried out across different socio-economic strata of our society can in the long run provide meaningful insights so as to guide the vaccination campaign against this disease in future.

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**Authors' contributions:** AAM: Collected the samples and data from the patients, performed ELISA and wrote the first draft of the study. BAF: Gave the concept for the study, supervised the study and reviewed the literature. NB: Wrote the final draft of the study, did the literature research and performed analysis of the study. MB: Helped in sample collection and provided the clinical details of the patients.

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