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# A Clinical and Epidemiological Study of Enterovirus Associated Diarrhea in Hospitalized Children

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## Abstract:

Diarrhea is a leading cause of morbidity and mortality in children less than five years of age. The etiology of diarrhea is unknown in nearly 40% of the cases. Enterovirus as a cause of diarrhea is well known, but there are few studies till date focusing on the epidemiological and clinical variables. To determine the prevalence and seasonal variation and to study the clinical profile and course of enterovirus associated diarrhea in children up to five years of age. A hospital based prospective study was carried out at Vanivilas Childrens' Hospital and Bowring and Lady Curzon Hospital, Bangalore during the period November 2009 to May 2011. Enterovirus in fecal samples of 122 children with acute gastroenteritis was detected by its cytopathic effects in rhabdomyosarcoma and/or HeLa cells, followed by reverse transcription (RT)-PCR and and OPV poliovirus strains. As rotavirus is the most common cause of viral diarrhea in India, the stool samples were also analyzed for rotavirus using polyacrylamide gel electrophoresis. Out of the 122 samples analyzed, 14 (11 sequence analysis of the VP1 gene. All the enterovirus positive samples were tested for wild 5%) were confirmed enterovirus positive. The enterovirus detection was maximal in the months of June to August (monsoon), with a rate of 30.8%. Rotaviral detection rate was 22% and with maximal prevalence in winter months of December to February. Enterovirus detection rate was highest in the age group of less than six months, with a rate of 50%. The primary symptom was watery diarrhea with mean duration of four days and frequency of 7 per day. Fever and vomiting were present in 50% of the cases. Moderate dehydration was observed in 57.1% of the cases. A significant association was observed between enterovirus associated diarrhea and absence of breastfeeding ( $p = 0.016$ ), contact with individuals having diarrhea ( $p=0.009$ ), and advanced protein energy malnutrition ( $p= 0.045$ ). Enterovirus associated diarrhea has a significant prevalence among children less than five years and is common in the monsoon season.

**Keywords:** Diarrhea, enterovirus, gastroenteritis, monsoon, rotavirus

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## Introduction

Diarrhea is one of the leading causes of morbidity and mortality worldwide responsible for 1.8 million deaths every year [1]. There are many causes of diarrhea; but in 40% of the cases, the etiology remains unknown [2].

Viruses known to cause acute gastroenteritis in children are rotavirus, norwalk virus, astrovirus, adenovirus and calicivirus [3]. Though rotavirus is the most common etiological agent of viral diarrhea, enterovirus is also known to cause diarrhea

in children. Although isolated case-studies and small-scale epidemiological studies of children with acute gastroenteritis having enteroviral infections have been reported at various places in the world, a recent five year study in Bangalore revealed a significant association of enterovirus with acute diarrhea [4-

11]. But, the epidemiological and clinical variables associated with enteroviral diarrhea remain largely unexplored, and need to be further investigated. Also, apart from diarrhea, enteroviruses are the most

common cause of aseptic meningitis in children and are associated with other serious disorders such as pleurodynia, pericarditis, myocarditis, encephalitis and the development of type-1 diabetes mellitus in the long term [3].

Enteroviruses belong to the family Picornaviridae. Over 70 serotypes of enterovirus have been isolated from human [12]. The site of replication of enterovirus is the gastrointestinal tract. Occasionally, the virus spreads to other organs causing severe disease, the nature of which depends on the individual enterovirus serotype. Human enteroviruses include polioviruses, coxsackieviruses, enterocytopathic human orphan (ECHO) viruses and enteroviruses 68-71. The genome of the virus consists of a single stranded, positive sense RNA molecule. The viral capsid has 60 copies each of 4 proteins, designated as VP1, VP2, VP3 and VP4, which are arranged with icosahedral symmetry encapsidating the genomic RNA. The virus-neutralizing epitopes reside mainly in VP1 [12].

Epidemiological studies conducted in India and other countries considering the prevalence and seasonal variation of enteroviral diarrhea have been reported in the literature [9, 13-17]. In contrast, there have been very few studies on the clinical profile of enteroviral diarrhea in children [4]. Children below the age of five years are most likely to suffer from the ill effects of acute gastroenteritis, which underlines the need for further research. The present study was undertaken to determine the occurrence and seasonal variation of enterovirus associated diarrhea. An attempt has been made to also study the clinical profile of the illness.

### Material and Methods

One hundred and twenty two children under 5 years of age with acute gastroenteritis admitted to Vanivilas and Bowring & Lady Curzon hospitals were studied in the period from November 2009 to May 2011. Inclusion criteria for the study were children below the age of five years admitted with acute diarrhea, defined as three or more watery stools per day of less than fourteen days duration. Children with chronic diarrhea, persistent diarrhea, transitional diarrhea, dysentery or with antibiotic intake during the course of illness were excluded from the study. Stool samples were accompanied by duly-filled proforma with relevant history and details of clinical examination. Stool samples of symptomatic infants were collected during the acute diarrheal episode and stored in the freezer compartment of refrigerator till

they were transported within the given day. They were analyzed at the Microbiology and Cell Biology Laboratory of the Indian Institute of Science, Bangalore. Supernatants of the stool specimen suspension were centrifuged and stored at -80°C till analysis. The enterovirus was detected in the fecal samples by its cytopathic effects in rhabdomyosarcoma and / or HeLa cells. Only samples that exhibited cytopathic effects in three successive passages were considered positive for enterovirus. This was followed by reverse transcription (RT)-PCR and sequence analysis of the VP1 gene to detect the virus in stool specimens and / or cell culture supernatants.

Extensive polio vaccination is carried out in the age group under study and there is circulation of oral polio vaccine (OPV) polio virus strains in the community. To eliminate the possibility of stool samples positive for poliovirus being counted as enterovirus positive, all the enterovirus positive samples were tested for OPV strains and wild poliovirus strains. Since rotavirus is one of the predominant causes of viral diarrhea in India, the stool samples were also analyzed for rotavirus. Rotavirus was detected by visualization of the viral double-stranded RNA segments by silver staining following polyacrylamide gel electrophoresis.

Admitted cases of gastroenteritis were managed by rehydration with oral rehydration solution or/and intravenous fluids such as Ringer lactate solution as per the severity of dehydration assessed after the clinical examination. Further stool microscopic examination and stool culture to look for bacteria like *Escherichia coli*, parasites such as *Giardia lamblia* and hanging drop preparation to look for *Vibrio cholerae* was carried out only in cases of clinical suspicion or non resolution of diarrhea. Zinc supplementation was done in all the cases.

The study was conducted after obtaining approval from the Institutional Ethical Review Board. Informed consent was obtained from parents or guardians for enrolment of their children in the study.

Statistical analysis: Descriptive statistical analysis was carried out in the present study. The statistical significance was assessed at 5 %. Chi-square/ Fisher Exact test was used to find the significance of the study parameters on categorical scale between multiple groups. One-proportion Z test was performed under the binomial assumption of 0.50 to obtain the frequency distribution of the variables studied.

Season	Number of samples	Number of samples enterovirus positive	Percentage of enterovirus positive	P
Winter (Dec – Feb)	38	1	2.6	0.085
Summer (Mar – May)	36	5	13.9	0.652
Monsoon (Jun – Aug)	26	8	30.8	0.002
Post-monsoon (Sep – Nov)	22	0	0.0	-
<b>Total</b>	122	14	11.5	-

Statistical software: The statistical softwares SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data, and Microsoft word and Excel were used to generate tables.

### Results

Out of the 122 samples, 14 were positive for enterovirus, accounting for detection rate of 11.5%. None of the enterovirus positive samples were

positive for OPV strains or wild polio virus strains, rotavirus or any other diarrheal pathogen. Among the enterovirus negative cases, 2 were positive for *Vibrio cholerae* and 22 were positive for rotavirus. The rotaviral detection rate observed was 18.1%.

The enterovirus detection rate was the highest in the months of June to August (monsoon season), at the rate of 30.8% (Table I). This is associated with a p value of 0.002, indicating a significant association

Seasons	Number of samples	Number of samples with rotavirus positive	Percentage of rotavirus positive	P
Winter (Dec – Feb)	38	14	36.8	0.003**
Summer (Mar – May)	36	4	11.1	0.281
Monsoon (Jun – Aug)	26	0	0.0	-
Autumn (Sep – Nov)	22	4	18.2	0.981
<b>Total</b>	122	22	18.1	-

between monsoon season and enterovirus associated diarrhea. The rotavirus detection rate, on the other hand, was highest in the months of December to February (winter season), at a rate of 36.8% (p value of 0.003) (Table II).

Enterovirus detected was highest among children of less than six months of age (7 out of 14) (Table III).

Of the fourteen, 9 were male. About 10.7% (3/28) of the diarrheal samples from rural areas and 11.7% (11/94) from the urban areas tested positive for enterovirus with a p value of 0.9. The highest number of enterovirus positive cases out of the cases studied was observed in the lower middle socio-economic segment. Here, 3 out of 17 diarrheal samples tested positive for enterovirus.

**Table III. Comparison of demographic profile of children having enterovirus negative diarrhea with children having enterovirus positive diarrhea.**

Characteristics	Enterovirus negative (n / %)	Enterovirus positive (n / %)
<b>Age</b>		
< 6 months	27 (25%)	7 (50%)
6-12 months	38 (35.2%)	3 (21%)
12-24 months	26 (24.1%)	2 (14%)
24-36 months	13 (12%)	1 (7%)
> 36 months	4 (3.7%)	1 (7%)
<b>Males</b>	66 (61.1%)	9 (64%)
<b>Rural</b>	25 (23.2%)	3 (21.4%)
<b>Urban</b>	83 (76.8%)	11 (78.6%)
<b>Socioeconomic status (Kuppuswamy classification)</b>		
Lower class	58 (53.7%)	6 (42.8%)
Upper lower	14 (12.9%)	5 (35.7%)
Lower middle	36 (33.3%)	3 (21.4%)
<b>Past history of gastroenteritis</b>	52 (48.1%)	8 (57.1%)

Fever was observed in 7 out of the 14 enterovirus positive cases (Table IV). Mean ( $\pm$  standard deviation) duration of fever was 4.2 ( $\pm$ 1.5) days. Mean frequency of loose stools per day was 7 ( $\pm$ 2.0). Out of the 14 children with enterovirus associated diarrhea, 11 had watery consistency of stools. Mean duration of loose stools was noted to be 4.0 ( $\pm$ 2.7) days. Other symptoms observed were vomiting (7/14), mouth ulcers (6/14), cough (5/14), hurried breathing (4/14), decreased urine output (3/14), redness of eyes (1/14), and skin lesions, papulovesicular, on buttocks (1/14).

Enterovirus was detected in the stool samples in 2 out of the 4 children who were never breastfed ( $p = .016$ ) (Table V). Recent contact with individuals having diarrhea was significantly associated with positive enteroviral detection ( $p = 0.009$ ). Grade IV PEM was also significantly associated with enteroviral detection ( $p = 0.045$ ). Out of the 14 children with

positive enteroviral detection, 4 (28.5%) had severe dehydration and 8 of the 14 children (57.1%) had moderate dehydration ( $p > 0.05$ ).

### Discussion

Studies on enteroviral diarrhea in India and other countries across the world have mostly focused on incidence and seasonal prevalence. The novelty of the present study is that it explores the clinical profile of enterovirus associated diarrhea, which has not been considered till date. It demonstrates that enterovirus is responsible for a significant proportion of diarrhea in children, in India. Among the 122 fecal samples of children with acute gastroenteritis analyzed in the present study, enterovirus was detected in 14 samples. The enterovirus detection rate in the present study is similar to the rate observed in other studies [4, 5, 18]. This rate is similar to that observed in the study by Rao et al. for the age group of 0-3 years [9]. In contrast, the rate of excretion of enterovirus in children with diarrhoea in a study in Pakistan was 50-52% [19]; while a detection rate of 3.1% and 2.5% was observed in studies done in Japan and Thailand [16, 17].

The number of children (8/14) with positive enteroviral detection was highest in the monsoon season, in contrast to rotaviral diarrhea, which typically peaks in winter months (similar results observed in the present study). The monthly variation in enteroviral detection in the present study revealed maximum number of cases in the months of May and June, accounting for 35.7% each. It is interesting to note that the months of May and June in the year 2010, in Bangalore urban and rural areas, witnessed good rainfall but were not the months of maximum rainfall for that year (courtesy: Indian Meteorological Department). It is possible that enteroviral prevalence in an area may be linked to various weather changes, rainfall being one among them. Similar results were reported by Patil et al. in the study done in Pune, with the enteroviral isolation rate being the highest in the month of July, accounting for 60% of the enterovirus positive cases [4]. Similar seasonal prevalence was noted by Rao et al. in their study [9]. This is in contrast to the study of infants and children by Phan et al. at Ho Chi Minh city, Vietnam, which reported the highest enterovirus isolation in the month of December [4]. Another study conducted by Maciolek et al. (2002) in the Netherlands on 119 infants younger than 30 days of age found the maximal enterovirus isolation rate in the summer months [7].

These differences in the seasonal prevalence could be

**Table IV. Comparison of clinical profile of children having enterovirus positive diarrhea with children having enterovirus negative diarrhea and rotavirus positive diarrhea.**

Characteristics	Enterovirus positive cases	Enterovirus negative cases (including rotavirus positive cases)	Rotavirus positive cases
<b>Fever</b>	7/14 (50%)	57/108 (52.7%)	15/22 (68.1%)
<b>Mean duration of fever in days (<math>\pm</math> SD)</b>	4.2( $\pm$ 1.5)	2.9 ( $\pm$ 1.58)	2.7 ( $\pm$ 1.4)
<b>Watery consistency of stools</b>	11/14 (78.5%)	87/108 (80.5%)	18/22 (81.8%)
<b>Mean frequency of loose stools / day</b>	7 ( $\pm$ 2.0)	8 ( $\pm$ 1.8)	9 ( $\pm$ 3.0)
<b>Mean duration of loose stools in days</b>	4 ( $\pm$ 2.7)	3.3 ( $\pm$ 2.0)	3 ( $\pm$ 1.6)
<b>Vomiting</b>	7/14 (50%)	71/108 (65.7%)	19/22 (86.3%)
<b>Decreased urine output</b>	3/14 (21.4%)	31/108 (28.7%)	9/22 (40.9%)
<b>Severity of dehydration</b>			
Mild	2/14 (14.2%)	23/108 (21.3%)	2/22 (9%)
Moderate	8/14 (57.1%)	48/108 (44.4%)	14/22 (63.6%)
Severe	4/14 (28.5%)	37/108 (34.2%)	6/22 (27.3%)

due to the climatic, rainfall and geographical differences between the various regions where the studies were conducted. However, interestingly, no significant difference in the detection rate of the enterovirus was observed between rural and urban areas in the present study, indicating that the prevalence of enteroviral infection may not vary much within a given geographical location.

The age group with the highest isolation was less than six months of age (50%). The gender-wise distribution of enterovirus positive cases was 64.2% male and 35.8% female. The highest percentage of enterovirus positive cases out of the cases studied was seen in the lower middle socio-economic segment (17.6%).

The clinical profile of children who tested positive for enterovirus was the following: The predominant symptom was watery diarrhea with mean duration of four days and frequency of 7 per day. Other features were fever and vomiting. Moderate dehydration was

observed in most of the cases. Study conducted by Patil et al reported severe disease in 53% of enterovirus positive cases followed by moderate disease in 46%. However, as all the samples were collected from hospitalized cases, there may be greater number of moderate and severe cases of dehydration reported in the present study and the study by Patil et al. There was no significant association of the other features of enteroviral infection such as conjunctivitis, meningoencephalitis, upper respiratory tract infection, lower respiratory tract infection, pleurodynia, and hand, foot and mouth disease with enterovirus isolation in the stool. Past history of gastroenteritis did not correlate significantly with enterovirus associated diarrhea. Recent contact with diarrhea and Grade IV PEM were significantly associated with positive enteroviral culture with a p value of 0.009 and 0.045, respectively. Lack of breastfeeding was significantly associated with enterovirus associated diarrhea (p of 0.016). The protective effect of breastfeeding due to

passage of maternal antibodies in breastmilk has been demonstrated [20]. Infants breastfed for more than two weeks had fewer enterovirus infections by the age of 1 year compared with those exclusively breastfed for less than 2 weeks. As the incidence of enterovirus associated diarrhea is highest in the age group of less than six months of age, the importance of breastfeeding in the prevention of repeated diarrheal episodes is further emphasized.

A limitation of the present study was the population sampling, which was predominantly from an urban area, and the small sample size. A large scale community based study is recommended to assess the burden of enteroviral diarrhea.

### Conclusions

Enterovirus associated diarrhea is prevalent in the rainy season and occurs in epidemic outbreaks. Acute watery diarrhea with fever and vomiting leading to moderate dehydration is the clinical profile of children having enteroviral diarrhea. Enterovirus is an etiological agent for a significant proportion of childhood diarrhea. Further systematic research on enterovirus is required to assess the importance of developing a vaccine for enteroviral diarrhea.

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