RESEARCH PAPER

Rotavirus and other Diarrheal Disease in a Birth Cohort from Southern Indian Community

R SARKAR, BP GLADSTONE, JP WARIER, SL SHARMA, U RAMAN, J MULIYIL AND G KANG

*From Division of Gastrointestinal Sciences, and *Community Health Department, Christian Medical College, Vellore, Tamil Nadu, India.*

Correspondence to: Dr Gagandeep Kang, Division of Gastrointestinal Sciences, Christian Medical College, Vellore 632 004, Tamil Nadu, India. gkang@cmcvellore.ac.in

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Objective: To describe the incidence, severity and etiology of diarrheal disease in infants and young children residing in an urban slum community in Southern India.

Setting: Three contiguous urban slums in Vellore, Tamil Nadu.

Participants: 452 children participating in a birth cohort study on diarrheal disease; 373 completed three years of follow-up.

Outcome measures: Diarrheal incidence (obtained by twice-weekly home visits) and severity (assessed by the Vesikari scoring system), and etiological agents associated with diarrhea (through examination of stool specimens by bacteriologic culture, rotavirus enzyme immunoassay, PCR for norovirus and microscopy for parasites).

Results: A total of 1856 diarrheal episodes were reported in 373 children. The overall incidence rate of diarrhea was 1.66 episodes per child year for three years, with 2.76 episodes per child year in infancy. The incidence peaked during the months of July and August. Severe diarrhea formed 8% of the total episodes. Rotavirus was the most common pathogen detected, being identified in 18% of episodes. Good hygiene status resulted in 33% protection against moderate-to-severe diarrhea.

Conclusions: This study highlights the burden of diarrheal disease and the important etiological agents of childhood diarrhea in Southern India. Promotion of hygienic behavior through health education may help reduce diarrheal incidence in this and similar communities.

Keywords: *Birth cohort, Diarrhea, Children, Epidemiology, Etiology.*

Acute diarrheal diseases are one of the top five causes of morbidity and mortality worldwide, accounting for 0.52 million deaths annually in children under the age of 5 years [1]. The most common enteropathogens causing diarrhea are rotavirus, *Cryptosporidium* spp., *Salmonella* spp., *Campylobacter*, *Shigella*, diarrheagenic *Escherichia coli*, calicivirus, adenovirus, astrovirus and possibly *Giardia.* Rotavirus, norovirus and diarrheagenic *Escherichia coli.* are responsible for more than half of all diarrheal deaths in under-five children [2].

While mortality is an important measure of disease burden, a true estimate of the global impact includes outcomes from the entire spectrum of mild, moderate and severe forms of disease. Although the diarrhea-related mortality has decreased by 68% between 1990 and 2013 [1], the corresponding decline in disease incidence has been more modest [3]. It has been estimated that approximately 35.2% of all diarrheal episodes are moderate-to-severe in nature [4]; but there are considerable uncertainties surrounding these estimates due to lack of robust country-specific data, especially from regions with the highest disease burden.

Given the importance of diarrheal disease as a cause of both deaths and malnutrition in children [5,6], it is important to have community-based data in order to derive a true estimate of the national disease burden. This study reports incidence, clinical features and etiology of diarrheal disease in a birth cohort in an urban slum community in Southern India.

METHODS

The recruitment and follow-up of the cohort has been previously described [7]. In this cohort, 452 newborn infants were recruited from three contiguous urban slums in Vellore between March 2002 and August 2003. They were followed up with twice weekly home visits by field workers, who enquired about morbidity, until they attained the age of three years. Stool samples were collected whenever a child was found to have an episode of diarrhea. During a diarrheal episode, the child was visited on alternate days until resolution. Detailed clinical data was collected on the onset, duration, frequency, color and consistency of stools, associated vomiting and fever, presence and severity of dehydration, and treatment. The Institutional Review Board of Christian

Medical College, Vellore, approved the study and written informed consent was obtained from parents/guardians of all children prior to enrollment.

Diarrhea was defined as the passage of three or more loose watery stools in a 24-hour period, or change in the number or consistency of the stools. An episode of diarrhea was defined as at least one day of diarrhea, preceded and followed by two or more days without diarrhea [7]. A diarrheal episode was said to be associated with a specific pathogen if the pathogen was isolated from stool samples collected at the time of the episode or within a week after the cessation of the episode. In case of rotavirus, it was within a week before or after the actual period of the episode of diarrhea. Acute diarrhea was defined as an episode lasting for less than 14 days and persistent diarrhea as an episode lasting for 14 days or more [8].

Severity of diarrhea was assessed using the Vesikari scale, originally designed for assessing rotavirus disease presenting to hospital [9]. An episode was considered mild for a Vesikari score 5 or less, moderate for 6-10 and severe for 11 or more. Data on baseline socio-demographic characteristics, feeding patterns, and monthly anthropometric (weight and height) measurements were recorded. Height-for age (HAZ), weight-for-age (WAZ) and weight-for-height (WHZ) z-scores were calculated using the 2006 WHO child growth standards as the reference population [10]. Children with HAZ, WHZ and WAZ of <-2 SD were considered to be stunted, wasted and underweight, respectively.

Hygiene status of the household was recorded for all children at the time of recruitment and thereafter at 6-monthly intervals, using a previously validated questionnaire [11], and each household assigned a score ranging from 0 to 21. Households were then classified as good (≥13), poor (10-12) and very poor (≤9) hygiene status based on their score.

Laboratory methods: Microscopy and culture of stool specimens was done on the same day of collection using standard methods to identify various enteropathogens causing diarrhea. Enteropathogenic *E. coli* (EPEC) were identified by serogrouping and other classes of diarrheagenic *E. coli* were not tested [12]. We identified rotavirus in diarrheal stool by ELISA, but also performed RNA extraction and RT-PCR assay even if the screening ELISA was negative [13]. Noroviruses and sapoviruses, which belong to the *Caliciviridae* family, were identified using PCR carried out on a subset of 500 diarrheal samples [14]. *Cryptosporidium* spp. was identified using modified acid-fast staining [15].

Statistical analysis: The overall and pathogen-specific incidence rates of diarrhea episodes, season-specific incidence, and severity and age at infection for specific diarrheal pathogens were calculated using the number of episodes as the numerator and the total child-years of follow-up as the denominator. Factors influencing severity of diarrhea (moderate/severe *vs*. mild) was assessed using logistic regression analysis and odds ratios with 95% confidence intervals (CI) were calculated.

RESULTS

A total of 1856 diarrheal episodes were documented in 373 children, who completed three years of follow-up. Stool samples were collected for 1829 episodes (98.6%). The overall incidence rate of diarrhea was 1.66 episodes per child-year, with the highest incidence of 2.76 episodes per child-year during infancy. During the second and third years of life, diarrheal incidence was 1.28 and 0.94 episodes per child-year, respectively.

Ninety-five percent of the children had at least one episode, and 28% had more than 6 episodes of diarrhea by the time they reached 3 years of age. By 4 months of age, 50% had at least one episode, and at the end of 6 months, 75% had one or more episodes of diarrhea. The highest incidence of diarrhea was observed in children aged between 3 and 8 months.

Median (IQR) duration of a diarrheal episode was 3 (2-4) days. Duration varied with age (P<0.001), with 42% of episodes in first year longer than 3 days, followed by 29% and 22% in second and third years, respectively. A total of 1833 (98.8%) acute and 23 (1.2%) persistent diarrhea episodes were reported in our study.

Vomiting and fever accompanied 297 (16%) and 317 (17%) episodes, respectively. Dehydration, positively associated with age (P<0.001), was seen in 191 (10.6%) episodes with the highest prevalence during 24-30 months of age; majority of them (167, 87.4%) were mild (1-5%) dehydration. Children were taken to an outpatient clinic or a hospital during 1306 (72%) diarrheal episodes and 43 (2.4%) needed hospitalization, of which 11 (0.6%) episodes needed intravenous rehydration. Oral rehydration was given for 1564 (87.4%) episodes. Mucus in stool was observed in 250 (13.5%) episodes whereas bloody diarrhea was reported in 41 (2.2%) episodes. Antibiotics and antimotility drugs were prescribed during 27.5% and 14.6% of the episodes, respectively. Severity of diarrhea was assessed for 1793 (96.6%) episodes wherein 58.4% were mild, 33.4% moderate and 8.2% severe. Antibiotics were prescribed for 19.1% of mild, 35.8% of moderate and 54.2% of severe diarrheal episodes (P<0.001). The proportion of severe diarrhea

episodes was highest in first six months of life (12%) and decreased subsequently.

Of the 1829 episodes of diarrhea for which stool samples were available for analysis, one or more pathogens were isolated from 635 (35.7%) episodes. Isolation of diarrheagenic microorganisms was lower during infancy (28%) than during the later years (45%). Rotavirus was detected in 18% of stool samples, followed by *Giardia* (8%), *Aeromonas* (4%), *Cryptosporidium* (3%), *Shigella* (2%) and *Vibrio cholerae* (1%). The pathogen-specific diarrheal incidence is presented in ***Table* I**. Co-infection with two pathogens was observed in 88 (4.8%) of diarrheal episodes and in 9 (0.5%) episodes, three or more pathogens were detected.

Vibrio cholerae and EPEC infections occurred mostly during infancy, whereas *Giardia* and *Shigella* tended to occur later in life. Among viruses, rotavirus and calicivirus (norovirus and sapovirus) had a mild preponderance during the first year of life. The median (IQR) age at infection and severity scores for different pathogens are presented in ***Table* II**. *Vibrio*-associated episodes were found to be most severe. In general, diarrheal episodes associated with viral pathogens [median (IQR) Vesikari score = 6 (5-9), $P<0.001$] were found to be more severe than those associated with bacterial [median (IQR) Vesikari score = 5 (5-7), $P=0.156$] or parasitic [median (IQR) Vesikari score = 5 (4-7), $P=0.221$] pathogens. Also, antibiotic usage was significantly associated with viral (32.5% *vs.* 26.5%, $P=0.027$), but not bacterial (32.8% vs. 27.3%, $P=0.175$) or parasitic (23.9% *vs.* 28.2%, $P=0.222$) diarrhea.

The highest peak in the incidence of diarrhea was observed during the month of July and August (***Fig.* 1**). When individual pathogens were considered, *Giardia* peaked in August while rotavirus showed two peaks, one in January followed by a smaller peak in June. *Cryptosporidium*, on the other hand, showed a small peak during the cooler months (January-March). Other pathogens did not demonstrate any seasonal variation.

There was no association between diarrhea and breastfeeding or nutritional status, while wasting showed a weak evidence of being a risk factor for moderate/severe diarrhea (OR = 1.26). Household hygiene was positively associated with diarrheal severity, with children from households with poor and very poor hygiene having a 46% and 49% elevated risk of suffering from moderate/severe diarrhea, respectively, as compared to those belonging to families with good hygiene (***Table* III**).

DISCUSSION

The epidemiology of childhood diarrhea in this southern Indian urban slum showed an incidence rate of 1.66 episodes per child-year. The estimate of diarrheal incidence in our study was close to that of 1.69 episodes per child-year among under-five children in a Delhi urban slum [16], but was below the median global estimate of 2.9 episodes [3]. In concordance with what has previously been reported [3], the diarrheal incidence peaked during

TABLE I SPECIFIC PATHOGENS IDENTIFIED IN 1829 DIARRHEAL EPISODES

Pathogen detected in stool	*No. (%) of diarrheal episodes*	*Incidence rate per child year (95% CI)*
Bacteria		
Aeromonas spp.	69 (3.8)	6.17 (4.72-7.63)
Salmonella spp.	8 (0.4)	0.72 (0.22-1.21)
Shigella spp.	41 (2.2)	3.67 (2.54-4.79)
Vibrio cholerae	18 (1.0)	1.61 (0.87-2.35)
Enteropathogenic E.coli	9 (0.5)	0.81 (0.28-1.33)
Parasite		
Cryptosporidium spp.	57 (3.1)	5.1 (3.77-6.42)
Giardia spp.	148 (8.1)	13.24 (11.11-15.37)
Virus		
Rotavirus	324 (17.7)	28.98 (25.82-32.14)
Norovirus*	35 (7.0)	3.13 (2.09-4.17)
Sapovirus*	18 (3.6)	1.61 (0.87-2.35)

** Number of samples tested = 500*

TABLE II AGE AT INFECTION AND SEVERITY FOR SPECIFIC PATHOGENS IDENTIFIED IN

Pathogen detected in stool	*Age at infection*		*Severity score*	
	n	*Median (IQR)*	*n*	*Median (IQR)*
Aeromonas spp.	69	16 (10-23)	69	5 (4-8)
Salmonella spp.	8	20 (10-24)	5	5 (5-6.5)
Shigella spp.	41	18 (14-27)	41	5(5-6)
Vibrio cholerae	18	11 (5-20)	18	7 (5-13)
Enteropathogenic *Escherichia coli*	9	8 (6-11)	8	5 (5-6)
Cryptosporidium spp.	54	14 (9-24)	53	5 (4-7.5)
Giardia spp.	148	23 (16-28)	146	5 (4-7)
Rotavirus	324	10 (4-17)	209	6 (5-9)
Norovirus*	35	11 (8-11)	35	5 (5-7)
Sapovirus*	18	14 (10-22)	18	5 (4-8)

**Number of samples tested = 500.*

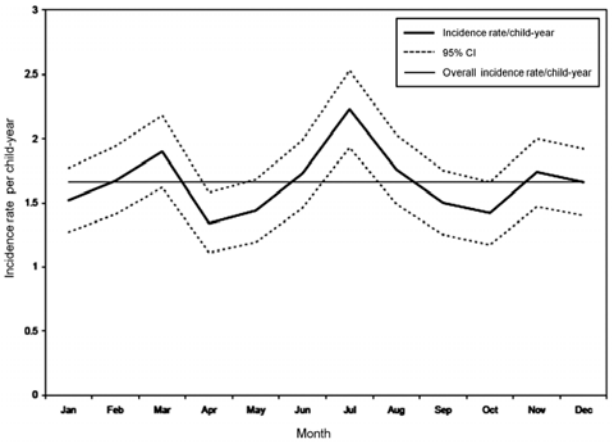

FIG. 1 *Seasonal pattern of diarrheal incidence among children in the birth cohort, followed from March 2002 to August 2006.*

infancy, decreasing steadily thereafter. This peak in incidence during infancy is possibly due to the consumption of foods that are improperly cooked or are prepared under unhygienic conditions [17].

The majority (72%) of the diarrheal episodes in our study resulted in a visit to a doctor, which can be attributed to the free and easy access to health care. The usage of oral rehydration solution (ORS) was also much higher compared to 32% in Tamil Nadu [18] and 39% in a Delhi urban slum [16], which can be attributed to the intensive supervision by the field worker and doctors' advice during each clinic visit. On the other hand, high usage of antimotility and antibiotic drugs was also noticed in this study, which contrasts with the national [19] and international [8] guidelines that recommend against the use medications for childhood diarrhea, except ORS for dehydration, and antibiotics only for certain culture-proven infections. Increased use of antibiotics for the treatment of diarrhea in Indian children has been reported earlier [20].

In this study, diarrheagenic microorganisms could be isolated from only 35.7% of all reported episodes of diarrhea. Similar findings have also been reported from other population-based studies [21,22]. The proportion of diarrheal episodes from which any pathogen could be isolated was much lower in first year (28%) than the next two years (45%), possibly due to transitional diarrhea of the newborn or weaning diarrhea. Transitional diarrhea can occur as a result of failure of adaptation to enteral feeding and microbial colonisation during the weaning period [23]. Other potential reasons for the large proportion of unaccounted diarrheal episodes are low pathogen yield due to delay in sample collection [24], intermittent pathogen shedding [25], lower sensitivity of conventional diagnostic methods for the detection of stool parasites [26], and the presence of novel diarrheagenic pathogens [27].

An earlier study from Manipal, on hospitalized children with diarrhea showed rotavirus in 5.2%, *Salmonella* in 5.9%, *Shigella* in 5.4%, *Aeromonas* in 4.1% and *Vibrio* in 1.3% of the diarrheal samples [28]. In a multi-centric community-based study on the etiology of acute diarrhea, rotavirus, *Cryptosporidium* spp., enterotoxigenic *Escherichia coli* and *Shigella* were the major causes of moderate-to-severe diarrhea among children in developing countries [29]. In our study too, rotavirus, *Shigella* and *Cryptosporidium* spp. were the commonest pathogens isolated from diarrheal stool samples, thereby highlighting the importance of these pathogens in causing childhood diarrhea.

TABLE III ASSOCIATION OF BREASTFEEDING AND NUTRITIONAL STATUS WITH SEVERITY OF DIARRHEA (*n*=373)

Exposure status	*Diarrheal episodes*		
	Mild, No. (%)	*Moderate/severe, No. (%)*	*Odds ratio (95% CI)**
Exclusive breastfeeding at the time of diarrhea	137 (12)	95 (12)	1.04 (0.76-1.41)
Any breastfeeding at the time of diarrhea	682 (61)	468 (63)	1.06 (0.85-1.32)
Wasted (WHZ<-2 SD)[#]	181 (16)	147 (20)	1.26 (0.94-1.69)
Stunted (HAZ<-2 SD)[#]	493 (44)	317 (42)	0.93 (0.75-1.14)
Underweight (WAZ<-2 SD)[#]	365 (33)	250 (33)	1.03 (0.81-1.31)
Malnourished (wasted/stunted/underweight)[#]	460 (41)	327 (44)	0.91 (0.73-1.12)
Hygiene status[§]			
Good	429 (39)	222 (30)	1[¶]
Poor	290 (26)	222 (30)	1.48 (1.15-1.93)
Very poor	391 (35)	302 (40)	1.49 (1.13-1.97)

**Estimates adjusted for repeated episodes per child; [#]Calculations based on measurement within a month prior to each diarrheal episode; [§]Calculations based on hygiene status assessed at 6 months of age; [¶]Reference category.*

WHAT IS ALREADY KNOWN?

- The etiology of diarrhea in hospitalized children is well documented, but community estimates from longitudinal studies are lacking.

WHAT THIS STUDY ADDS?

- Incidence rate of diarrhea was 1.66 episodes per child year for first 3 years of life; highest incidence (2.76 episodes per child year) is seen in infancy.
- As with hospitalized children, rotavirus was the commonest etiological agent associated with childhood diarrhea in the community.

In our analysis, poor and very poor hygiene status showed a higher risk of getting severe diarrhea. This is in confirmation with studies done in other parts of India which have shown that good hygiene practices within the home, such as washing hands with soap before feeding a child, can reduce the risk of childhood diarrhea [30].

In conclusion, this study provides a better understanding of the etiology of childhood diarrhea in a community setting, besides increasing our awareness about the unnecessary usage of antibiotic and antimotility drugs. Similar to what is observed in hospitalized children, rotavirus was the commonest etiological agent associated with childhood diarrhea in this community. Health education to promote hygiene behavior can be an effective low cost intervention to reduce the incidence of severe diarrhea.

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Contributors: JM and GK: conceived and designed the study, and revised the manuscript for important intellectual content; BPG, JPW, SLS and UR: conducted the study and helped in manuscript writing; RS and BPG: analyzed the data and drafted the manuscript. All authors approved the final version of the manuscript; RS and GK: are guarantors of the paper.

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