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Descriptive epidemiology of rotavirus infection in a community in North India

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SUMMARY

In India, rotavirus infections cause the death of 98621 children each year. In urban neighbourhoods in Delhi, children were followed up for 1 year to estimate the incidence of rotavirus gastroenteritis and common genotypes. Infants aged $\leqslant 1$ week were enrolled in cohort 1 and infants aged 12 months (up to $+14$ days) in cohort 2. Fourteen percent (30/210) gastroenteritis episodes were positive for rotavirus. Incidence rates of rotavirus gastroenteritis episodes in the first and second year were 0·18 [95% confidence interval (CI) 0·10–0·27] and 0·14 (95% CI 0·07–0·21) episodes/child-year, respectively. The incidence rate of severe rotavirus gastroenteritis in the first year of life was 0·05 (95% CI 0·01–0·10) episodes/child-year. There were no cases in the second year. The common genotypes detected were G1P[8] (27%) and G9P[4] (23%). That severe rotavirus gastroenteritis is common in the first year of life is relevant for planning efficacy trials.

Key words: Diarrhoea, gastroenteritis, rotavirus.

INTRODUCTION

In India, it is estimated that there are 98 621 rotavirus-related diarrhoea deaths in children aged <5 years. Overall 22% of global rotavirus deaths occur in India annually [1]. A safe, effective and affordable rotavirus vaccine is urgently needed in developing countries.

It is important to determine the efficacy of all rotavirus vaccines in countries such as India as the performance of a vaccine may vary in developed and developing countries. To facilitate such evaluations, knowledge regarding the epidemiology of rotavirus infection and its G and P genotypes in the areas where the vaccines are to be tested is essential. Moreover, studies have documented a marked diversity of rotavirus strains as well as the prevalence of unusual strains circulating in India [2–4].

As a part of the Indo-US Vaccine Action Program, a government-sponsored activity to promote new vaccine development, the 116E oral rotavirus vaccine was developed indigenously by Bharat Biotech International Limited (BBIL) in Hyderabad, India (http://dbtindia.nic.in). This virus strain was identified in the late 1980s from neonates born in a tertiary

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hospital, the All India Institute of Medical Sciences, New Delhi [5].

In an attempt to further the clinical development plan of this vaccine, Phase III trials were planned. One of the sites is the setting for the study reported herein. As a preparatory activity for the efficacy trial, we estimated the incidence rates for rotavirus gastroenteritis and the common genotypes in a population. This information will be useful for the planning of rotavirus vaccine trials in India.

METHODS

Subjects and setting

The study was conducted in the urban neighbourhoods of Govindpuri-Tigri-Dakshinpuri and Tughlakabad in South Delhi, India between 31 July 2009 and 4 September 2010. These neighbourhoods have a population of ~150 000 inhabitants residing in around 30 000 households. The median family income is INR6000 (~US$117) per month which is low middle-class income in the Indian context. Residents have easy access to public transportation. Access to telephones is almost universal. In families with young children literacy rates are high; almost all fathers and 80% of mothers have attended school. The currently chosen population reflects the socioeconomic status of about 80% of the Indian population [6].

We conducted an observational study where two cohorts of children were followed for 1 year during which time gastroenteritis episodes were ascertained through weekly household visits. The first cohort (cohort 1) comprised of children enrolled at age $\leqslant$1 week and the second cohort (cohort 2) of those enrolled at age 12 months (up to +14 days).

The protocol was approved by the Ethics Committees of the Department of Biotechnology, the Society for Applied Studies, New Delhi, India and the PATH Human Subjects Protection Committee, USA.

Field workers conducted surveillance in the areas allocated to them and identified newborns and pregnant women (to enrol infants aged $\leqslant$1 week in cohort 1) and infants aged 9–12 months (to enrol infants in cohort 2). Those willing to participate in the study attended the study clinic where informed written consent was obtained from the parents.

Children in the eligible age range were included in the study if the parents agreed to participate, were able to understand study procedures, did not have any illness requiring hospitalization, and the family did not have any confirmed plans to move out of the study area over the next 12 months.

Children participating in any other trial, those with signs or symptoms of active sepsis, pneumonia, meningitis or any other disease requiring hospitalization, known cases of immunodeficiency disease or HIV positive, those who had chronic gastrointestinal disease or any other conditions which in the judgement of the investigator warranted exclusion, were not enrolled.

After enrolment, each subject was followed up for 12 months through weekly household visits by field workers. At each visit information on presence of gastroenteritis, signs and symptoms of suspected intussusception and danger signs according to the IMNCI protocol of the Government of India were ascertained [7]. History of gastroenteritis since the last contact was ascertained at each visit. Children with gastroenteritis were visited at home on alternate days until recovery. All variables required for characterization of the Vesikari score (with scores ranging from 0 to 20, where higher scores indicate greater severity) were documented at each visit [8]. A stool specimen was collected for all cases of gastroenteritis as soon as possible after onset of the illness. All children were given oral rehydration solutions (ORS) by the study team for all cases of gastroenteritis. Children with some or severe dehydration were assessed by a study physician and taken to hospital for management.

Rotavirus antigen was detected in stool samples using a commercial enzyme immunoassay (Premier Rotaclone; Meridian Bioscience, USA), according to the manufacturer's instructions. All rotaclone-positive samples were tested for genotypes G1, G2, G3, G4, G8, G9, G12 and P[2], P[4], P[6], P[8], and P[11] by multiplex reverse transcriptase–polymerase chain reaction (RT–PCR) using methods described previously [9, 10]. Specimens that were not typable for the specified G and P types were further tested for presence of VP6 antigen by RT–PCR in order to confirm the presence or absence of rotavirus in these specimens. Specimens testing negative for VP6 were considered to be rotavirus negative.

As a part of routine care, children in cohort 1 were given bacillus Calmette-Guérin (BCG) and '0' dose of oral polio vaccine (OPV), if they had not received this earlier, and three doses of diphtheria, pertussis and tetanus (DPT), OPV and hepatitis B (HepB) at ages 6, 10 and 14 weeks, respectively. Measles vaccine and vitamin A drops were administered at age 9 months. Children in cohort 2 were administered the measles

vaccine (if not received earlier), DPT and OPV booster and two doses of vitamin A drops at 6-monthly intervals.

Case definitions

- *Gastroenteritis*. The passage of $\geqslant 3$ loose or watery stools in a 24-h period with or without vomiting.
- *Recovery from gastroenteritis*. The first day of a gastroenteritis-free period, i.e. <3 loose or watery stools in a 24-h period. Two episodes of gastroenteritis were separated by a 7-day interval between the date of recovery of one event and the date of onset of next event.
- *Rotavirus gastroenteritis*. Presence of gastroenteritis and rotavirus antigen detected in stool using ELISA and RT–PCR for VP6.
- *Severe rotavirus gastroenteritis*. Gastroenteritis with a severity score $\geqslant 11$ using the 20-point Vesikari scale, and rotavirus detected in stools using ELISA and RT–PCR for VP6.
- *Some dehydration*. Presence of any two of the following signs was considered as some dehydration: restlessness or irritability, sunken eyes, drinking eagerly or feeling thirsty, skin pinch goes back slowly [7].
- *Severe dehydration*. This was defined as the presence of any two of the following signs: lethargy or unconsciousness, sunken eyes, not able to drink or drinking poorly, skin pinch goes back very slowly [7].
- *Rehydration*. All children who were administered ORS from other healthcare providers or hospitals or were given intravenous fluids were considered to be rehydrated.
- *Hospitalization*. Inpatient admission for at least 6 h in a hospital or other treatment facility. Two hospitalizations for the same cause were counted twice only if separated by a 2-week interval. However, if the subject was hospitalized for an unrelated cause within 2 weeks, these were counted as separate hospitalizations.

Statistical analysis

Data analysis was performed using Stata, version 10.0 (StataCorp., USA). All enrolled children were included in the analysis until censorship. Disease burden is presented as incidence rates. The incidence rate for gastroenteritis was estimated as the total number of episodes per total child-years of follow-up. The rates for rotavirus gastroenteritis, severe gastroenteritis, and severe rotavirus gastroenteritis were estimated in the same manner.

RESULTS

Of the 323 infants identified through surveillance, a total of 200 infants were enrolled over a 1-month period. A total of 100 infants were enrolled in cohort 1 and another 100 infants in cohort 2. Cohort 1 infants were followed up to age 12 months, and cohort 2 children up to age 24 months.

The number of infants identified, screened, enrolled and reasons for exclusion are shown in Figure 1.

Incidence of gastroenteritis

During the study a total of 210 episodes of gastroenteritis were reported out of which 30 (14·2%) were positive for rotavirus. Five episodes had a Vesikari score $\geqslant 11$ and were positive for rotavirus. The median (range) Vesikari score in the first year of life was 6 (2–17) and 5 (2–16) in the second year of life.

The incidence rate of gastroenteritis was 1·12 episodes/child-year in the first 2 years of life. The incidence rate was higher in the first year; 1·50 episodes/child-year (95% CI 1·48–1·53) compared to second year of life when it was 0·75 episode/child-year (95% CI 0·57–0·93, Table 1).

The incidence rate of rotavirus gastroenteritis was 0·16 episode/child-year (95% CI 0·10–0·22) during the first 2 years of life. The incidence rate of rotavirus gastroenteritis in the first year of life was 0·18 episode/child-year (95% CI 0·10–0·27) and in the second year the corresponding rate was 0·14 (95% CI 0·07–0·21).

The incidence rate of severe rotavirus gastroenteritis (Vesikari score $\geqslant 11$) was 0·05 episode/child-year (95% CI 0·01–0·10) in year 1 while there were no case of severe rotavirus gastroenteritis in the second year (Table 1).

Out of the 30 episodes of gastroenteritis that were associated with rotavirus 17 (57%) occurred in the first year of life and 13 (43%) in the second year of life (Table 2). Of the episodes of rotavirus gastroenteritis five (17%) had a Vesikari score of $\geqslant 11$. All five episodes of severe rotavirus gastroenteritis occurred in the first year of life. Three (10%) episodes of rotavirus gastroenteritis required hospitalization. None of the gastroenteritis episodes were severe (Vesikari score $\geqslant 11$) or required hospitalization in the second year of life.

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Fig. 1. Profile of enrolled infants.* Early sepsis with multiple pustules; † multiple congenital anomalies; ‡ parents HIV positive; extensive rickets with anaemia and splenomegaly; history of seizures; PI, Principal investigator.

Table 1. *Incidence of all gastroenteritis, rotavirus gastroenteritis and severe rotavirus gastroenteritis in the study cohorts*

	Cohort 1 (birth to 12 months) ($n=100$)	Cohort 2 (12–24 months) ($n=100$)	Birth to 24 months
Child-years of follow-up	92·3	94·5	186·8
Gastroenteritis episodes, *n*	139	71	210
Gastroenteritis episodes/child-year, rate (95% CI)	1·50 (1·48–1·53)	0·75 (0·57–0·93)	1·12 (0·97–1·28)
Rotavirus gastroenteritis episodes, *n*	17	13	30
Rotavirus gastroenteritis episodes/child-year, rate (95% CI)	0·18 (0·10–0·27)	0·14 (0·07–0·21)	0·16 (0·10–0·22)
Severe gastroenteritis (Vesikari score ⩾11) episodes, *n*	13	1	14
Severe gastroenteritis episodes/child-year; rate (95% CI)	0·14 (0·04–0·17)	0·01 (−0·01–0·03)	0·07 (0·02–0·09)
Severe rotavirus gastroenteritis (Vesikari score ⩾11) episodes, *n*	5	0	5
Severe rotavirus gastroenteritis episodes/child-year, rate (95% CI)	0·05 (0·01–0·10)	–	0·03 (0·00–0·05)

CI, Confidence interval.

Of the total episodes of gastroenteritis; 108 had scores between 1 and 5 out of which 10% (11/108) were rotavirus positive; 88 had scores between 6 and 10 out of which 16% (14/88) were rotavirus positive; 10 had scores between 11 and 15 out of which 30% (3/10) were rotavirus positive and four had scores between 16 and 20 out of which 50% (2/4) were rotavirus positive.

Episodes with higher Vesikari scores had a significant association with rotavirus positivity (χ^2 for trend $P=0{\cdot}009$).

Identification of rotavirus genotypes in gastrointestinal stool specimens

Fourteen percent (30/210) of gastrointestinal episodes were associated with rotavirus. Of the rotavirus-positive gastroenteritis episodes 27% were G1P8, 23% were G9P4 and 13% each were G2P4 and G0P8 (Table 3). The most common G type was G9 (37%) followed by G1 (33%) while the most common P type was P8 (47%) and P4 (37%). The genotypes that were associated with the five most severe rotavirus

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Table 2. *Non-rotavirus gastroenteritis and rotavirus gastroenteritis in relation to age and severity*

	Number of episodes *n* (%)		Vesikari score ⩾11 *n* (%)		Episodes requiring hospitalization, *n* (%)	
	Birth to 12 months	12–24 months	Birth to 12 months	12–24 months	Birth to 12 months	12–24 months
Non-rotavirus gastroenteritis (*n* = 180)	122 (68·%)	58 (32·%)	8 (6·5·%)	1 (1·7·%)	4 (3·3·%)	1 (1·7·%)
Rotavirus gastroenteritis (*n* = 30)	17 (57·%)	13 (43·%)	5 (29·4·%)	–	3 (17·6·%)	–

Table 3. *Distribution of genotypes in the stool specimens collected during gastroenteritis*

No. of gastroenteritis episodes with stool specimens	210
No. of specimens positive for stool rotavirus	30 (14·%)
Genotypes	*n* (%)
G1P8	8 (27)
G1P0	2
G2P4	4 (13)
G9P4	7 (23)
G9P0	2
G9P8	2
G12P6	1
G0P8	4 (13)

gastroenteritis episodes were G9P0, G1P8, G1P0, G9P8 and G12P6.

Vesikari scores of gastroenteritis episodes

Table 4 displays the individual components of the Vesikari score and the number of episodes fulfilling different criteria. A longer duration of diarrhoea, higher number of maximum diarrhoeal stools and presence of fever were the characteristics that commonly made the total score high.

Age of occurrence and seasonality of gastroenteritis

Of the 210 episodes, 27·% occurred in children aged <5 months, 39·% occurred in children aged 6–11 months and 34·% occurred in children aged 12–24 months.

Of the 30 rotavirus gastroenteritis episodes, 20·% occurred in children aged ⩽5 months, 37·% occurred in children aged 6–11 months and 43·% occurred in children aged 12–24 months.

Of the five severe rotavirus gastroenteritis episodes, none occurred in children aged ⩽5 months, all five episodes occurred in children aged 6–11 months and none in children aged 12–24 months (data not shown in Tables).

In the pooled analysis of the two age cohorts, it was observed that the maximum episodes of rotavirus gastroenteritis occurred in May (20·%), November (17·%) and September (13·%, Fig. 2).

Table 4. *Episodes fulfilling different criteria in Vesikari scoring*

Vesikari score criteria	Total episodes (*N* = 210)
Duration of diarrhoea (days)	
Score 1 (1–4)	139 (66·2)
Score 2 (5)	20 (9·5)
Score 3 (⩾6)	51 (24·3)
Maximum no. of diarrhoeal stools in 24 h	
Score 1 (1–3)	4 (1·9)
Score 2 (4–5)	39 (18·6)
Score 3 (⩾6)	167 (79·5)
Maximum no. of vomiting episodes in 24 h	
Score 0 (0)	149 (71·0)
Score 1 (1)	18 (8·6)
Score 2 (2–4)	33 (15·7)
Score 3 (⩾5)	10 (4·8)
Duration of vomiting (days)	
Score 1 (1)	41 (19·5)
Score 2 (2)	14 (6·7)
Score 3 (⩾3)	6 (2·9)
Fever	
Score 0 (⩽37·0 °C)	138 (65·7)
Score 1 (37·1–38·4 °C)	54 (25·7)
Score 2 (38·5–38·9 °C)	10 (4·8)
Score 3 (⩾39·0 °C)	8 (3·8)
Dehydration	
Score 0 (none)	203 (96·7)
Score 2 (some)	3 (1·4)
Score 3 (severe)	4 (1·9)
Treatment	
Score 0 (none)	189 (90·0)
Score 1 (rehydration)	13 (6·2)
Score 2 (hospitalization)	8 (3·8)

Values given are *n* (%).

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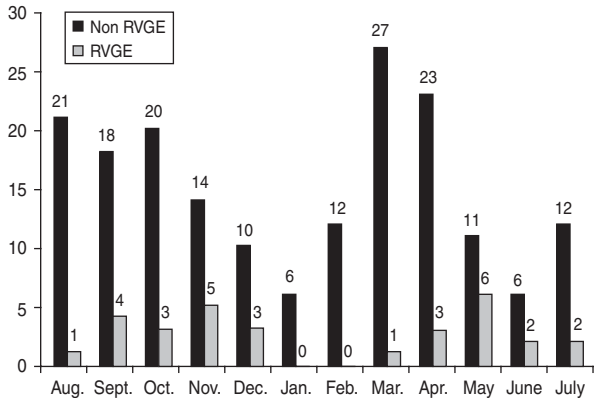

Fig. 2. Seasonality of non-rotavirus gastroenteritis (Non-RVGE) and rotavirus gastroenteritis (RVGE) by calendar month identified during the study.

Intussusception and danger signs

The purpose of ascertaining information on signs and symptoms of suspected intussusception and danger signs was to test out procedures for the Phase III study. During the study period a total of 11 children; seven in the first year of life and four in the second year of life reported presence of a sign and symptom of suspected intussusception. All reported presence of blood in the stool. None reported abdominal distension or lumps, or $\geqslant 3$ episodes of vomiting within an hour. Ultrasonography was performed for all children and none were found to have intussusception.

A total of 66 children; 42 in the first year of life and 24 in the second year of life reported the presence of a danger sign at any visit during the 12-month follow-up period.

DISCUSSION

This study provides an estimate of incidence rate of gastroenteritis, rotavirus gastroenteritis and severe rotavirus gastroenteritis in the first 2 years of life in children in an urban North Indian community.

The notable findings are that the incidence rate of gastroenteritis, rotavirus gastroenteritis, severe gastroenteritis and severe rotavirus gastroenteritis were all higher in the first year than in the second year of life.

The incidence rate of rotavirus seen in this community is lower than that seen in an earlier trial conducted in Delhi [11]. However, it should be noted that in that study the criteria for rotavirus positivity was only stool ELISA while in the present study we used a confirmatory VP6 RT–PCR assay for the ELISA-positive specimens.

The observed rate is also lower to that seen in a community-based study from Vellore in South India in children aged <3 years [12].

Fourteen percent (30/210) of all gastroenteritis cases were associated with rotavirus. Notably around 36% (5/14) of all severe gastroenteritis cases were rotavirus positive, emphasizing the importance of the virus as a cause of severe gastrointestinal illness in children. This is similar to the figure reported in Vellore (34·5%) where the same criteria for severity grading were used [12]. These rates were higher than that observed in Pakistan where the rate was 17% [13]. However, in that trial the severity criteria were different; children who required intravenous hydration were considered as having severe diarrhoea.

There were no distinct peaks of rotavirus gastroenteritis throughout the year although the maximum numbers of episodes were seen in May, November and September. There was no particular association of any genotype by calendar month.

Genotyping results show diversity of rotavirus strains circulating in the community. G1 is the most prevalent strain worldwide, but in this setting G9 was seen to be the most dominant strain with G1 following closely. This is consistent with findings from other parts of India [14]. Out of the five severe rotavirus gastroenteritis episodes, two each had G1 and G2 rotaviruses.

In conclusion, in this study although based on a small sample, rotavirus clearly appears to be the most notable cause of diarrhoeal illnesses in children in the first 2 years of life.

The data generated will be useful for planning efficacy trials of rotavirus vaccines being developed by companies in India. Our data reiterate the importance of developing a cheap and effective vaccine that can be made available to combat rotavirus gastroenteritis in resource-limited settings such as India.

APPENDIX. Members of the Rotavirus Vaccine Development Committee

M. K. Bhan (*Secretary, Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi, India*); John Boslego (*Director, Vaccine Development Global Program, PATH, USA*); Roger Glass (*Director, Fogarty International Center, National Institutes of Health, USA*); Harry Greenberg (*Senior Associate Dean for Research, Stanford University School of Medicine, USA*); George Curlin

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DECLARATION OF INTEREST

None.

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