PREVALENCE OF ROTAVIRUS DIARRHEA AMONG OUT-PATIENTS AND HOSPITALIZED PATIENTS: A COMPARISON

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Abstract. The prevalence of rotavirus diarrhea was compared in two settings, among children attending outpatient clinics and those hospitalized (inpatients) at Pune, India. A total of 489 and 628 fecal specimens were collected during October 1993 to September 1996 from outpatients and inpatients respectively. Overall occurrence of rotavirus diarrhea was more among hospitalized children. Using the stratification on the variable age, it is shown that age is indeed a confounding variable. The important finding of the study was, in ≤6 months age group, it was observed that the occurrence of rotavirus diarrhea was more in the outpatients (30.26%) than among the inpatients (10.11%). Children of this age group are likely to be partially protected by maternal antibodies. The effect of seasonality and sex distribution did not differ in the two settings. It was found that G2 serotype was the major cause of diarrhea among the outpatients.

INTRODUCTION

Rotaviruses have been shown to be the single most important worldwide cause of severe diarrhea in young children (Kapikian, 1993; Kapikian *et al*, 1989; LeBaron *et al*, 1990). It is a major cause of death in young children in developing countries (Guerrant *et al*, 1990). Rotaviruses are also a common cause of mild diarrhea in children. Such mild diarrhea cases seek consultation at outpatient clinics.

Systematic surveillance for rotavirus among children hospitalized for diarrhea and among children having mild disease is needed to estimate the overall prevalence of rotavirus diarrhea. This in turn will provide an estimate of burden of disease on our health system. Also such a study will be useful to improve our understanding of the epidemiology of rotavirus in different settings.

Information of rotavirus diarrhea in children attending outpatient clinics is very limited (Koopman *et al*, 1984; Kotloff *et al*, 1988; Pitson *et al*, 1986). Most of the studies on rotavirus diarrhea have not compared severe and mild diarrhea in children in the same community. For comparative studies on outpatients and inpatients, fecal specimens were collected from patients attending outpatient clinics for diarrhea between October 1993 and September 1996.

Analysis of different aspects of epidemiology *viz* age, sex and season has been published for hospitalized cases for the duration, July 1992 to June 1996 (Kelkar, 1999). Furthermore, the time series analysis using ARIMA modeling has also been published (Purohit *et al*, 1998). We have carried out comparative analysis of these factors in both the settings over the common study period, *viz* October 1993 to September 1996 in the present manuscript.

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MATERIALS AND METHODS

Collection of specimens

Specimens from children ≤5 years age

group, having diarrhea were collected from outpatient clinics and a hospital.

1) Children attending outpatient clinics: Fecal specimens were collected from patients attending private clinics for diarrhea. These patients did not require hospitalization hence were treated at home. For this, five pediatricians from one locality named Deccan Gymkhana, in Pune city, were requested to send the patients having acute diarrhea to a pathologist in the same area. Approximately 80% of the specimens were obtained from only one clinic. A majority of the patients attending this busy clinic were from the lower socio-economic group because charges were minimum. The specimens were stored at -20ºC at the pathology laboratory. Subsequently, the specimens were brought on wet ice every week by National Institute of Virology, Pune, for testing. A total of 489 specimens were collected from children ≤5 years of age.

2) Hospitalized children: Fecal specimens were collected from the Naidu Infectious Diseases Hospital of Pune, which has a diarrheal ward. The patients in this hospital are treated free of charge. Therefore, they generally belong to the low income group. Patients come from Pune city and surrounding areas of the main city on their own or because they are directed by State Government Hospital for hospitalization. The authorities confirmed that approximately 99% of the hospitalized patients for diarrhea were dehydrated at the time of admission.

The reporting period for this communication is from October 1993 to September 1996. During this period, 628 specimens were collected from children in ≤5 years of age.

Diagnosis of rotaviruses

The specimens were tested for the presence of rotaviruses by ELISA, developed at NIV (Kelkar, 1993). All the rotavirus positive specimens, were tested by ELISA for G serotypes. A set of monoclonal antibodies (MAbs) Rota MA, Serotec Laboratory, Ebetsu, Japan was used for serotyping human rotaviruses. The set consisted of serotypes G1, G2, G3 and G4 specific MAbs directed to VP7 and group A specific MAb (YO-156) directed to VP6, the inner capsid protein. ELISA for G serotypes was carried out by the procedure given by the manufacturer of the Serotec kit. The Dynatec plates were coated with MAbs against rotavirus G1, G2, G3, G4 serotypes and YO-156, a group A rotavirus specific MAb. The plates were incubated at 4ºC overnight and saturated with BSA. Ten percent fecal specimens were added. The rotavirus in the specimen was probed with a pool of polyclonal antibodies against G1 to G4 developed in rabbit. Bound antibodies were detected with anti rabbit IgG enzyme conjugate (Sigma). Pooled rabbit hyper immune serum against G1 to G4 serotypes was kindly supplied by Dr S Urasawa, Sapporo, Japan.

RESULTS

Among children treated at the outpatient clinics 76 out of 489 (15.54%) fecal specimens were positive for rotavirus and among hospitalized patients 178 out of 628 (28.34%) specimens were positive during the study period, October 1993 to September 1996. The age and sex distributions of outpatients and inpatients are presented in Table 1. The two age distributions differ and the difference is pronounced in age groups 0-6, 6-12 and 12-18 months. Hence, the crude morbidity rates mentioned above are not comparable. So, to eliminate the effect of age, the age adjusted standardized morbidity rate was computed for outpatients by considering the population of inpatients as standard. This gave standardized morbidity rate for outpatients as 28.30% and the standardized morbidity ratio (SMR) as 54.93% (Lee, 1992).

Mantel-Haenszel test (Schlesselman, 1982)

Suppose the demographic variable 'age' is ignored. Based on the following data; the odds ratio was estimated as 2.15 with 95% confidence interval (CI); 1.58-2.93:

	+ve	-ve	Total
Inpatients	178	450	628
Outpatients	76	413	489

Table 1
Age-sex distribution of outpatients and inpatients with rotavirus diarrhea (October 1993 to September 1996).

Age group (Month)	Total+ve/tested (%)	Male+ve/tested (%)	Female+ve/tested (%)	+ve/total +ve (%)
Outpatients				
≤6	23/226 (10.18)	15/141 (10.64)	8/85 (9.41)	23/76 (30.26)
6-12	36/169 (21.30)	20/105 (19.05)	16/64 (25.00)	36/76 (47.37)
12-18	10/55 (18.18)	6/38 (15.79)	4/17 (23.53)	10/76 (13.16)
18-24	2/15 (13.33)	1/11 (9.09)	1/4 (25.0)	2/76 (2.63)
24-60	5/24 (20.83)	2/9 (22.22)	3/15 (20.00)	5/76 (6.58)
Total	76/489 (15.54)	44/304 (14.47)	32/185 (17.30)	
Inpatients				
≤6	18/75 (24.00)	12/49 (24.49)	6/26 (23.08)	18/178 (10.11)
6-12	106/303 (34.98)	67/178 (37.64)	39/125 (31.20)	106/178 (59.55)
12-18	38/103 (36.89)	26/64 (40.63)	12/39 (30.77)	38/178 (21.35)
18-24	6/35 (17.14)	5/23 (21.74)	1/12 (8.33)	6/178 (3.37)
24-60	10/112 (8.93)	6/66 (9.09)	4/46 (8.70)	10/178 (5.62)
Total	178/628 (28.34)	116/380 (30.53)	62/248 (25.00)	

The stratification of the data according to age has been presented in Table 2. The odds ratio estimate by using Mantel-Haenszel method was 1.96. The chi-square test statistic for homogeneity of age-specific odds ratio was 17.83 ($p < 0.001$). Thus, the hypothesis of homogeneity was rejected. It may be noted that age-specific relative risk as measured by Odds ratio, was maximum in ≤6 months age group for outpatients. That is, mild diarrhea was more prevalent than severe diarrhea in this age group.

Further more, we compared proportion of positive cases to total positive cases for the two settings, for ≤6 months age group. Using following data, we got odds ratio as 3.86. This again confirms that mild diarrhea is more frequent in ≤6 months age group:

Setting	Age group of cases (month)		
	≤6	>6	Total
Outpatient	23	53	76
Inpatient	18	160	178

The odds ratio computations shown in Table 3 indicate that sex differences for each age group and for each of the two settings were not statistically significant. Thus, in both the settings, rotavirus did not discriminate between the sexes.

Seasonal variation

For outpatients, 65.79% of the total posi-

Table 2
Stratification according to age of rotavirus diarrhea (October 1993 to September 1996).

Age group (Month)	Outpatient	Inpatient	Cases		Odds ratio	CI	p-value
			+ve	-ve			
≤6	-	+	18	57	2.79	1.33, 5.82	<0.05
	+	-	23	203			
6-12	-	+	106	197	1.99	1.26, 3.15	<0.05
	+	-	36	133			
12-18	-	+	38	65	2.63	1.12, 6.30	<0.05
	+	-	10	45			
18-24	-	+	6	29	1.34	0.20, 11.20	>0.05
	+	-	2	13			
24-60	-	+	10	102	0.37	0.10, 1.42	>0.05
	+	-	5	19			

Table 3
Univariate analysis for sex among outpatients and inpatients with rotavirus diarrhea (October 1993 to September 1996).

Age group	Sex		Setting		Cases		Odds ratio	CI	p-value
	Male	Female	Outpatient	Inpatient	+ve	-ve			
≤6	+	-	+	-	15	126	1.15	0.43, 3.11	>0.05
	-	+	+	-	8	77			
	+	-	-	+	12	37	1.08	0.31, 3.84	>0.05
	-	+	-	+	6	20			
6-24	+	-	+	-	27	127	0.65	0.32, 1.30	>0.05
	-	+	+	-	21	64			
	+	-	-	+	98	167	1.40	0.91, 2.15	>0.05
	-	+	-	+	52	124			
24-60	+	-	+	-	2	7	1.14	0.10, 12.26	>0.05
	-	+	+	-	3	12			
	+	-	-	+	6	60	1.05	0.24, 4.77	>0.05
	-	+	-	+	4	42			

tive cases occurred in winter (November-February) whereas the corresponding proportion for inpatients was 61.80%. In rainy season (July-October) 9.21% of the total positive cases occurred in outpatient setting and the corresponding proportion for inpatients was 10.67%. Thus, for both the settings, cases were most frequent in winter and least frequent in rainy season.

Rotavirus serotypes of fecal specimens collected from both settings *viz* outpatients and inpatients are presented in Fig 1. It is clear from the Fig 1 that rotavirus belonging to G2 serotype was the main cause of diarrhea among outpatients, whereas severe diarrhea among inpatients was caused mainly by G1, G2 and to a small extent by G3 and G4.

DISCUSSION

The present study comprises of occurrence of rotavirus cases during the common period from October 1993 to September 1996 in two different settings, outpatient clinics of pediatricians and diarrhea ward of a hospital. The patients included in the study belonged to

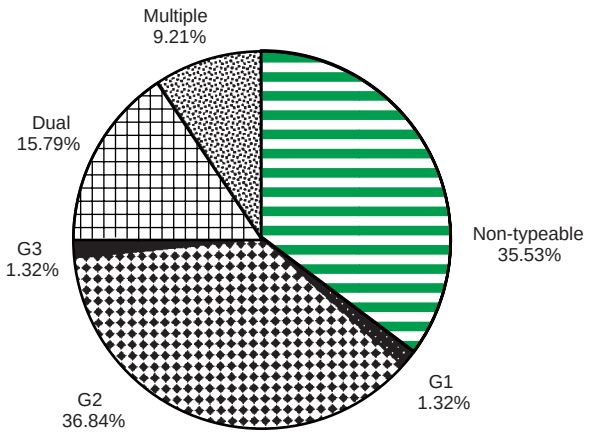

Fig 1a–Rotavirus serotypes among outpatients in Pune, India (N=76).

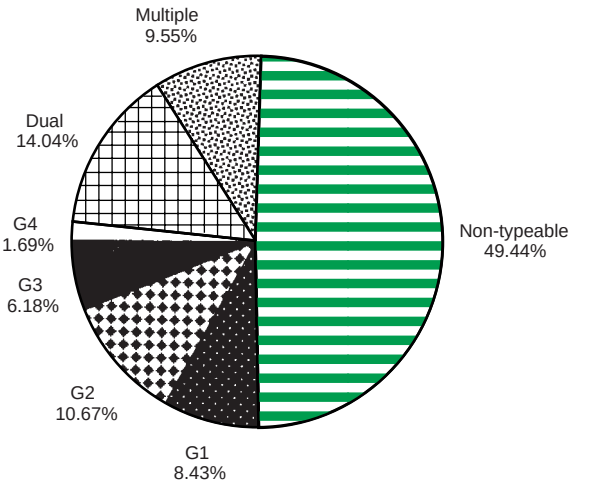

Fig 1b–Rotavirus serotypes among inpatients in Pune, India (N=178).

the age group of ≤5 years. In both the settings, children from less affluent area were predominant.

Although the crude prevalence of rotavirus diarrhea in hospitalized cases was nearly double of that in outpatient clinics, the age adjusted standardized morbidity rates for the two settings were approximately the same. In general, potential etiological agent is less likely to be identified in the fecal samples when the diarrhea is mild (Kotloff *et al*, 1988). There also is a possibility that rotavirus remained undetectable in number of specimens due to limitation of ELISA among the outpatients.

It has been suggested that the etiology of community enteric illness can be reasonably inferred from the etiology of inpatient disease in children in the same geographic area (Pitson *et al*, 1986). This is only because often it is difficult to obtain fecal specimens from children attending outpatient clinics. The strains of rotaviruses causing mild diarrhea have been shown to have the same electropherotype and serotype as strains responsible for severe diarrhea, requiring hospitalization in the same community. Thus, it was concluded that for knowing the rotavirus strains circulating in the particular community, etiological agents of rotavirus diarrhea can be obtained from hospitalized cases. However, results of the present study are different and interesting. It was found that G2 was the major serotype which caused disease among outpatients, whereas among inpatients, G1 and G3 serotypes were also responsible. Whether G2 strains causing mild diarrhea and severe diarrhea are genetically different needs to be studied in depth. Efforts are being made to isolate G2 strains from the fecal specimens which are positive for G2 strain from outpatients and inpatients.

Rotaviruses are known to cause symptoms ranging from mild to severe, life threatening diarrhea. Children attending outpatient clinics normally have mild diarrhea which can be treated at home.

Age distributions in both the settings were compared. It appears that cases of rotavirus mild diarrhea are more frequent in ≤6 months age group. This fact could be related to the partial protection given by the presence of maternal antibody. Presence of high titerd antibodies to rotaviruses among Indian mothers has been shown (Kelkar *et al*, 1996).

It was found that seasonal incidence of rotavirus diarrhea followed similar patterns in both the settings. It has been reported earlier that there was a close correspondence between

the seasonal peaks of rotavirus gastroenteritis in outpatients and inpatients (Cunningham *et al*, 1988). Winter is the peak season in either of the settings. The importance of this finding is, if any kind of immunoprophylaxis for the prevention of rotavirus diarrhea needs to be introduced, it can be started before the onset of winter so that the effect of immunoprophylaxis will last during the peak period of rotavirus diarrhea. Such a mode for prevention could be introduced with emphasis on low birth weight babies and children, undergoing immunosuppression whether congenital or acquired because rotavirus often leads to chronic diarrhea in such cases (Kotloff *et al*, 1988; Cunningham *et al*, 1988; Sausbury *et al*, 1980; Pedly *et al*, 1984; Engleberg *et al*, 1982).

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