EPIDEMIOLOGY, SUBGROUPS AND SEROTYPES OF ROTA VIRUS DIARRHEA IN NORTH INDIAN COMMUNITIES

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ABSTRACT

To know prevalence of rotavirus diarrhea subgroups and serotypes, a prospective study was conducted in rural, periurban and urban communities at Chandigarh. Weekly surveillance for diarrheal episodes was carried out in 110 families each from rural, periurban and urban localities constituting 584 children < 5 years of age from October, 1988 to February, 1991. Stool samples of 218 diarrheal episodes occurring in 115 children were subjected to rotavirus dectection by EL1SA. Rotavirus positive samples were further analyzed for subgroups and serotypes using specific monoclonal antibodies. Overall prevalence of rotavirus diarrhea was 4.3% (25/584). Rotavirus constituted 11.5% (25/218) of total diarrheal episodes and 22 % (25/115) among the children affected with acute diarrhea. Among rural, periurban and urban communities, the overall prevalences of rotavirus diarrhea were 7.3%, 3.2% and 2.3% and episode related prevalences of 31.8%, 7.4% and 5%, respectively ($\%^2$ test for trend was highly significant from rural to periurban to urban localities). Forty per cent (10/25 of rotavirus positive samples were subgroup I and 60% (15/25) sub-group II. Of the 25 rotavirus strains, 40% (10) were serotype 2, 24% (n= 6) serotype 3 and 36% (n=9) serotype 4. No definite temporal or seasonal pattern of rotavirus was observed; however, more of rotavirus dirrheal episodes (16%) occurred during winter sesaon. Subgroups and serotypes were observed to cocirdulate during the rotavirus episodes. Demonstration of serotypes in our field study imply that the vaccine to be used in our country must be cross protective to have an effective impact on rotavirus infection. High rotavirus prevalence in the rural community as shown by us in this country should receive major attention through preventive point of view.

Key words: *Rotavirus, Acute diarrhea.*

Rotavirus has been recognized as an important cause of acute diarrhea in infants and young children(l,2). It has been estimated that over 500,000 deaths occur annually due to rotavirus diarrhea(3). The WHO is therefore, actively encouraging development of vaccines to decrease the rotavirus associated mortality and morbidity. Several vaccines have thus passed through the stage of human clinical trials(4). To evolve a correct vaccination strategy to control rotavirus infection, it is necessary to know the magnitude of the disease at population level as well as the antigenic characteristics of the virus prevalent in different communities. Of the five groups of rotaviruses (A-E) identified, Group A is the commonest cause of gastroenteritis in humans(5). Group A has been further classified into two well recognized subgroups and mainly four serotypes(5,6). The clinical and epidemiological

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features of different rotaviruses are variable(7). Although reports of childhood rotavirus diarrhea have been published from our country(8-15), there is paucity of field based epidemiological studies of rotavirus diarrhea, subgroups and serotypes in different communities in India. We therefore, report the results obtained during a prospective community based study from October, 1988 to February, 1991. The objectives of the study were to know: *(i)* the prevalence of rotavirus diarrhea in rural, periurban and urban communities, *(ii)* their subgroups and serotypes, and *(iii)* clinical picture and seasonal pattern associated with various subgroups and serotypes.

Material and Methods

Population and Study Design

Following initial survey of 2 months (July and August 1988) three areas namely rural, periurban and urban were selected. Since rotavirus occurs in children <5 years of age, those families having children below 3 years of age were enrolled at the beginning of the study. As our period of study was 29 months mentioned family selection, enabled us to keep the study group < 5 years of age. The total number of families enrolled for the study in three different areas were 330, constituting a total of 633 children *(Table I)*. Each of the enrolled family was then visited by the team every week from October 1988 to February 1991. Clinical details recorded in any child with diarrheal episode were timing of diarrhea, age at diarrhea, frequency/day, duration, characteristics of stool, vomiting, hydration status and respiratory symptoms. During the diarrheal episode, 2-3 stool samples were collected. A diarrheal episode recurring after an earlier episode with a gap of at least one week of normal stool was considered as a recurrence.

TABLE I—*Distribution of Families and Children in Different Communities*

Location	No. of families	No. of children	Children/ family ratio
Rural	110	205	1.86
Periurban	110	235	2.14
Urban	110	193	1.75
Total	330	633	1.92

Laboratory Studies

Stool was suspended in phosphate buffered saline (pH 7.2) as a 10% solution and centrifuged at 5000 g at room temperature. Supernatant was used for the detection of rotavirus antigen by enzyme-linked immunosorbent assay ELISA according to the procedure described previously(12,16). Positive samples were further confirmed by blocking ELISA test. Samples positive for rotavirus antigen were further evaluated for subgroups(17). Rotavirus sub-grouping was performed by'ttidirect ELISA using subgroup specific subgroup I or subgroup II monoclonal antibodies (supplied by kind courtesy of Dr. T.H. Flewett WHO, Collaborating Centre of Diarrheal Diseases). Horse raddish peroxidase conjugated rabbit anti-human rotavirus *(Dakopatts,* Denmark) was used as conjugate and the color was developed with O-phneylene diammine. An absorbance difference > 0.300 between subgroup I and subgroup II monoclonal antibody coated wells for each stool sample, was used to assign the subgroup to the rotavirus sample. Serotyping of rotavirus positive samples was essentially done as described for subgroups except that the ELISA plate in the first step was coated with appropriate dilution of the rotavirus specific serotype-l(2F,), serotype-2(5F8), serotype-3(4F8) monoclonal antibodies (gifted by Dr. H.B. Greenberg, Califor-

nia) and serotype-4 (ST3) specific rabbit hyperimmune serum prepared in coating buffer by overnight inbuation at 4°C. The rotavirus sample was assigned a serotype on the basis of a difference of absorbance > 0.300 as compared to the other three serotype-coated wells for each test sample.

During the course of the study, stool samples from 218 diarrheal episodes occurring in 584 children under observation were tested. Statistical analysis was done by z' test for proportions, Chi-square test and Students 't' test.

Results

Of 584 children under observation for acute diarrhea, 25 children were found to have rotavirus positive infection. Overall prevalence of rotavirus diarrhea from October, 1988 to February, 1991 was 4.3% (25/584). Community based prevalence is shown in *Table II.* Prevalence of rotavirus diarrhea were 7.3% in rural, 3.2% in periurban and 2.3 % in urban communities. χ^2 test for trend from rural to periurban to urban communities was highly significant ($p<0.01$).

Of 218 episodes of diarrhea among 115 children, 25 (11.5%) episodes were due to rotavirus. No child with rotavirus related diarrhea during the study period had recurrent of rotavirus positive diarrhea. Among 115 children with acute diarrhea, rotavirus infection constituted 22% (25/115). Community based distribution of diarrheal episodes are shown in *Table II.* Rotavirus positive diarrheal episodes were much higher in rural area (31.8) than in periurban (7.4%) and urban (5 %) (χ^2 test for trend was highly significant; $p<0.01$). The mean duration of each rotavirus diarrheal episode was 5 ± 3.43 days and non-rotavirus episode 4.64 ± 2.41 days. The mean stool frequency/day of rotavirus diarrheal episode was 5.92 ± 2.48 and non-rotavirus 6.73 ± 3.02. These parameters of the two groups did not show significant statistical differences ($p>0.05$). Rotavirus positive diarrheal episodes constituted 17% (2/12), 12% (7/58), 10% (10/99), 12% (4/34) and 13% (2/15) in the age groups of 6-11, 12-23, 24-35, 36-47 and >48 months, respectively. Variance in the age groups and rotavirus diarrheal episodes were unrelated (χ^2 test 0.577; $p>0.05$). However, of the total of 25 children (mean age 27.72 + 13.26 months) with rotavirus diarrhea, 80% (20/25) were below 3 years of age and 20% (5/25) above 3 years.

Subgroups and Serotypes

Subgrouping and serotyping was done in all the 25 children with rotavirus diarrhea. Ten (40%) children belonged to subgroup (SG) I and 15 (60%) to SG II. Serotypes (ST), 2,3 and 4 were found in 10 (40%), 6(24%) and 9 (36%) cases, respectively. In none of the children serotype 1 was detected. Distribution of rotavirus subgroups and serotypes in relation to different communities, are shown in *Table III.* For each of the communities SG as well as ST were compared separately and no significant differences were* found ($p>0.05$). Mean duration and frequency/day of diarrheal episodes of SG I and II were not significantly different. The same statistical observations were noted for these parameters comparing serotypes 2, 3 and 4 *(Table III).* The mean age of children with different SG and ST are shown in *Table III.* Comparative analysis did not show any statistical significance.

Temporal Pattern and Seasonal Variation

There was no uniform distribution of rotavirus diarrheal episodes from October, 1988 to February, 1991 *(Fig).* The monthly prevalence varied from 0.17% to 1.02%. Rotavirus infection exhibited only one defi-

TABLE II- *Prevalence and Percentage of Rotavirus Related Diarrheal Episodes in Different Communities*

	No. of children observed (n=584)	No. of children with diarrhea (n=115)	No. of children with rotavirus (n=25)	Prevalence* of rotavirus diarrhea (%)	No. of episodes of diarrhea (n=218)	Rotavirus positive episodes (n=25)	% of ** rotavirus positive diarrheal episodes
Location							
Rural	191	33	14	7.3	44	14	31.8
Periurban	217	38	7	3.2	94	7	7.4
Urban	176	44	4	2.3	80	4	5.0

* Calculated as no. of children with rotavirus diarrhea/number of children observed x 100.
** Calculated as no. of rotavirus diarrheal episodes/number of diarrheal episodes x 100.

TABLE III - *Subgrouping and Serotyping of Rota virus Positive Children (n=25)*

	No. of children					Duration	Frequency
	Total (%)	Rural	Peri-urban	Urban	Age (mo) Mean±SD	of diarrhea (days) mean:tSD	of stools/day Mean±SD
Subgroups							
I	10 (40)	7	1	2	27.2 ±15.76	5.4 ±3.47	5.9 ±1.52
II	15 (60)	7	6	2	28.0 ±11.90	4.73 ±3 .51	6.00 ±3.03
Serotypes							
1	-	0	0	0	-	-	-
2	10 (40)	4	5	1	23.7 ±10.43	5.20 ±3.64	5.6 ±1.64
3	6 (24)	5	0	1	32.5 ±16.20	4.66 ±2.06	7 ±2.68
4	9 (36)	5	2	2	29.0 ±14.22	5.0 ±4.21	5.88 ±3.10

Above given community distribution and other parameters for different subgroups and serotype were statistically compared ($p > 0.05$)

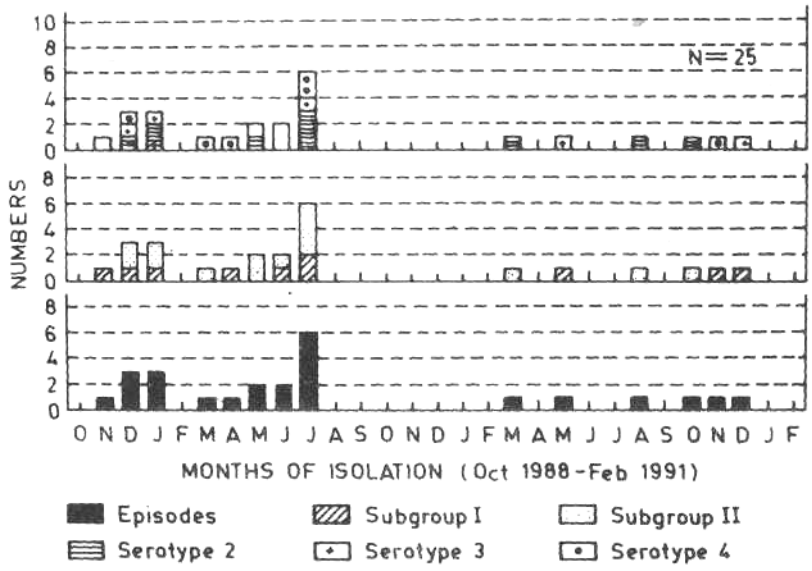

Fig. Monthly pattern of rotavirus diarrheal episodes, subgroups and serotypes.

nite peak (July, 1989). Based on seasonal patterns *i.e.,* Winter months (November-February), summer (March-June) and rainy season (July-October) the percentage of rotavirus positive diarrhea! episodes were 16.4% (9/55), 7.8% (8/103) and 13.3% (8/60). There was a significant difference ($p < 0.05$) as observed between summer with rainy season and rainy with winter season. No definite seasonal pattern was observed between SGI and II and also among various serotypes *(Fig).*

Discussion

Overall prevalence of rotavirus diarrhea was 4.3 % in our field study. Rotavirus constituted 11.5% of the total diarrheal episodes and 22% among the children affected with acute diarrhea. Hospital based frequency studies of rotavirus diarrhea from our country show considerable variation between 5-70%(9-13). Prevalence of 11.5**%** (episode related) observed by us was less than the most of the studies reported from this country; because of obvious difference in the study design (population based). Results of hospital based studies are not comparable with field studies. Field studies give the true reflection of the disease in the population; therefore, these observations may be more meaningful for formulating national health policies. In the rural rotavirus diarrhea as well as higher positivity of rotavirus diarrheal episodes (31.8%) when compared to periurban and urban localities. This observation may be due to relative underdevelopment of rural area in our country resulting in certain host environmental factors contributing to the infection. Indian population below 5 years of age is 114.4 million(18). Applying the figures from the present study, the projected diarrheal episodes below 5 years in India would be 19.2 million and of these, projected rotavirus diarrheal episodes in India/

year would be 2.1 million. This is when we are talking of Chandigarh which is one of the cleanest areas of our country. The magnitude of the problem may be much higher in other less developed areas of the country.

Diarrheal episodes due to rotavirus were no different from non-rotavirus episodes in terms of duration and frequency/day. Our results shows no age specific prevalence of rotavirus diarrhea. These observations were similar to those reported in a rural cohort study from Delhi(14).

In the present study, infection with SGII (60%) rotavirus was encountered more frequently than SGI (40%). Our findings are in agreement with other reports from our country(10,12). We did not find any difference in the prevalence and severity between SGI and SGII rotavirus infections in the three communities. Similar severity of the illness in the two subgroups has also been reported by other workers(7). Study from Vellore(10), the only report available on serotypes from our country showed STI (46.9%), ST2 (9.3%), ST3 (6.3%) and ST4 (37.0%), Serotype isolate pattern in the present study was somewhat different. In our study STI was not isolated in any of our cases and the frequency of ST2 (40%) and 3 (24%) was higher. Variance in the frequency and pattern of serotypes are well known to occur in different countries as well as different regions of a country depending upon geographical and environmental factors. ST specificity was neither prevalent in any of the communities nor was it correlating with parameters of age, duration or frequency of diarrhea.

Temporal and seasonal patterns of rotavirus diarrhea have been described. The variance depends upon multiple factors including nature of the community and the climatic conditions. In general, studies in temperate climates have described in winter peak, whereas those in tropical areas have described a more uniform distribution throughout the year(9,10). We did not observe any definite pattern of rotavirus diarrhea during 29 months of study. Overall analysis based on seasonal patterns revealed more of rotavirus diarrheal episodes occurring in winter months (16.4%). Lower temperature and relative humidity during winter have been hypothesized to facilitate rotavirus transmission^), perhaps by prolonging virus survival. There appears to be no seasonal pattern of any specific SG or ST as they were observed to cocirculate during the rotavirus episodes *(Fig.)*. Currently, all over world there is tremendous thrust in the application of rotavirus vaccine to prevent diarrhea resulting from this infection(20). The immunity is thought to be predominantly serotype specific. Demonstration of serotypes of our field study imply that the vaccine to be used in our country must be cross-protective to "have an effective impact on rotavirus infection. High rotavirus prevalence in the rural community as shown by us in this country should receive major attention through preventive point of view.

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REFERENCES

1. Kapikian AZ, Wyatt RG, Greenberg HB, *et al.* Approaches to immunization of infants and young children against gastroenteritis to rotavirus. Ren Infec Dis 1980, 2: 459-469.

2. Davidson GP. Viral diarrhea. Clin Gastroenterol 1986, 15: 39-53.

3. Vesikari T, Isolauri E, Delem A, *et al.* Immunogenicity and safety of live oral attenuated bovine rotavirus vaccine strain RIT 4237 in adults and young children. Lancent 1983, I: 807-811.

4. Green KY, Taniguchi K, Mackow ER, *et al.* Homotypic and heterotypic epitope-specific antibody responses in adult and infant rotavirus vaccinees: Implications for vaccine development. J Infect Dis 1990; 161: 667-679.

5. Anonymous. Puzzling diversity of rotaviruses. Lancet 1990, 335: 573-574.

6. WHO Memorandum. Nomenclature of human rotaviruses: Designation of sub groups and serotypes. Bull WHO 1984, 62: 501-513.

7. L, Perez M, *et al.* Relative frequency of rotavirus subgroups 1 and 2 in Venezuelan children with gastroenteritis as assayed with monoclonal antibodies. J Clin Microbiol 1984, 19: 516-520.

8. Sammantray JC, Mohapatra LN, Bhan MK, *et al.* Study of rotavirus diarrhea in North Indian Community. Indian Pediatr 1982, 19: 761-765.

9. Paniker CKJ, Mathew S, Mathan M. Ro tavirus and acute diarrhoeal disease in a South Indian coastal town. Bull WHO 1982, 62: 123-127.

10. Brown DWG, Mathan MM, Mathew M, *et al.* Rotavirus epidemiology in Vellore, South India: Group, subgroup, serotypes, and electropherotype. J Clin Microbiol 1988, 26: 2410-2414.

11. Sen D, Saha MR, Niyogi SK, *et al.* Etiological studies in hospital patient's with acute diarrhea in Calcutta. Trans R Soc Trop Med Hyg 1983, 77: 212-214.

12. Singh V, Broor S, Mehta S, *et al.* Clinical and epidemiological features of acute gastroenteritis associated human rotavirus subgroups 1 and 2 in Northern India. Indian J Gastroenterol 1989, 8: 23-25.

13. Maiya PP, Mathan SM, Mathan M, *et al.* Etiology of acute gastroenteritis in infancy and early childhood in southern India. Arch Dis Child 1977, 52: 482-485.

14. Raj P, Bhan MK, Prasad AK, *et al.* Elec-trophoretic study of the genome of human rotavirus in rural Indian community. Indian J Med Res 1989, 89: 65-68.

15. Chakravarti A, Kumar S, Mittal SK, *et al.* Comparison of latex agglutination and polyacrylamide gel elctrophoresis with enzyme linked immunoabsorbent assay rotavirus in stool specimen. Indian Pediatr 1991, 28: 507-510.

16. -Yolken RH, Kim HW, Clem T, *et al.* Enzyme linked immunosorbent assay (ELISA) for detection of reovirus like agent of infantile gastroenteritis. Lancet 1977, II: 263-267.

17. Yolken RH, Wyatt RG, Zissis G, *et al.* Epidemiology of human rotavirus types 1 and 2 as studied by enzyme linked immunosorbent assay. N Engl J Med 1978, 299: 1156-1161.

18. Demographic Indicators. *In:* The State of the World's Children. UNICEF, University Press, 1992, p 80.

19. Brandt CD, Kim HW, Rodriguez WJ, *et al.* Rotavirus gastroenteritis and weather. J Clin Microbiol 1982, 16: 478-482.

20. Farthing MJG. Enteric infections. *In:* Recent Advances in Gastroenterology. Ed Pounder RE. New York, Churchill Livingstone, 1990, pp 21-39.