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Epidemiology of symptomatic human rotaviruses in Bangalore and Mysore, India, from 1988 to 1994 as determined by electropherotype, subgroup and serotype analysis

Brief Report

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Summary. Epidemiology of symptomatic rotaviruses from Bangalore and Mysore in Southern India was investigated. While serotype G3 predominated throughout the 7-year study period from 1988 to 1994 in Bangalore, serotype G1 was more predominant than serotype G3 in Mysore during 1993 and 1994. Serotype G2 strains were either not detected or infrequently observed in both the cities. However, several strains with subgroup I and 'short' RNA pattern that exhibited high reactivity with typing MAbs specific for serotype 2 as well as other serotypes were detected throughout the period. Among the nonserotypeable strains from both cities, several exhibited dual subgroup (SG I + II) or subgroup I specificity and 'long' RNA pattern indicating their probable animal origin. Notably, a gradual, yet highly significant reduction in rotavirus gastroenteritis, from 45.3% in 1988 to 1.8% during 1994, was observed in Bangalore in stark contrast to the consistently high (about 34%) incidence of asymptomatic infections among neonates by I321-like G10P11 type strains during the same period. Moreover, I321-like asymptomatic strains were not detected in children with diarrhea.

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Rotaviruses are the major causative agents of severe, acute gastroenteritis in infants and young children [27]. Rotaviruses, belonging to the family *Reoviridae*, contain 11 segments of double-stranded RNA (dsRNA) enclosed in

a triple-layered capsid [15, 38]. Rotaviruses have been classified into 7 groups, A to G, among different species, based on the presence of group-specific epitopes on VP6 which constitutes the intermediate capsid [15, 27, 38]. Group A rotaviruses, which are the major pathogens of humans, can be further subdivided into at least four subgroups (SG) i.e., SG I, SG II, SG (I/II) and SG non(I/II) [26]. The outer capsid proteins VP4 and VP7 exhibit two serological specificities; VP4 specifying the P serotype and VP7 specifying the G serotype [15, 27]. To date, at least 14 G serotypes and 18 P types have been identified in humans and animals [14, 25]. The development and use of monoclonal antibodies for serotyping that recognize the serotype-specific epitopes on VP7 of rotaviruses has greatly enhanced the knowledge of the epidemiology of rotavirus infections [25].

Study of the electrophoretic migration patterns of the viral genomic ds RNA segments (electropherotyping) has also been extensively used in epidemiological studies of group A rotaviruses [27, 32, 36, 37, 44]. By this method, all group A rotaviruses can be classified as having either a 'long' or 'short' RNA electropherotype. The 'short' and 'long' patterns of RNA migration seen in human rotaviruses appear to be correlated with the subgroup specificity of the viruses [27]. In general, all human rotaviruses having 'short' or 'super-short' RNA electropherotype exhibit subgroup I specificity [27]. Since subgroup specificity is defined by VP6 encoded by gene 6, the molecular basis for the association of subgroup specificity with 'short' or 'long' electropherotype is not understood. Although electropherotype does not permit identification of the serotype specificity of the strain, in general, rotaviruses with a 'short' electropherotype have been observed to possess serotype 2-specific VP7 [27, 42]. On the other hand, among most animal strains, SG I specificity is associated with 'long' RNA pattern [27].

Of the ten serotypes found in humans (i.e. G1, G2, G3, G4, G5, G6, G8, G9, G10 and G12), only serotypes G1 to G4 are frequently encountered [27]. Epidemiological studies have shown that strains belonging to serotype 1 are predominantly observed in children with diarrhoea in majority of the countries around the world [1, 18, 19, 27, 30, 32, 33, 46]. In addition, seasonal shifts in serotypes as well as predominance of serotype G2, G3 or G4 rotaviruses was observed in many countries [3, 5, 18, 34–36, 43, 47]. Recent studies have demonstrated that monotypes/subtypes of serotypes G1 and G4 exist [8, 9, 16, 21, 44, 48]. Intratypic antigenic variation in serotype G3 human strains has also been reported in epidemiological studies [5]. The existence of a large number of serotypes in nature may hinder the development of a safe and universally effective vaccine.

Examination of rotavirus serotypes in different geographical locations and at different times, is therefore needed to assess their distribution in the population so as to monitor the effectiveness of a vaccine. This study is aimed towards the assessment of the distribution of rotavirus serotypes in Bangalore and Mysore and to characterize rotaviruses that cause symptomatic infections in young children by electropherotype, subgroup and serotype analyses as well as to

examine the possible involvement of I321-like asymptomatic strains in symptomatic infections.

A total of 694 stool samples from children with diarrhea admitted to different hospitals and clinics over a 7-year period from 1988 to 1994 in Bangalore and 447 samples during the 2-year period from 1993 to 1994 in Mysore, India were collected. Nucleic acids were extracted from all the clarified stool supernatants [39], electrophoresed on 10% polyacrylamide gels and the rotavirus-positive specimens were identified by direct detection of the virus-specific genomic 11 dsRNA segments after staining with silver nitrate [23]. The virus-isolates were further characterized into subgroups and serotypes by enzyme-linked immuno sorbent assay (ELISA) [22, 33, 39]. The following human rotaviruses KU (serotype G1, SGII), S2 (serotype G2, SG I), ST3 (serotype G4, SG II), 69M (serotype G8, SG I), WI61 (serotype G9, SG II) and the asymptomatic neonatal strain I321 (serotype G10, SG I) and animal strains RRV (serotype G3, SG I) and NCDV (serotype G6, SG I) as well as the reassortant strains DXRRV, DS1XRRV, PXUK and ST3XSA11 with serotypes 1, 2, 3, and 4 specificity, respectively, were used as reference strains in serotyping and subgrouping ELISA. All the reference strains were grown in MA104 cells in culture. For serotyping the rotaviral strains. the following VP7-specific MAbs were used: serotype 1, MAbs KU 4 and 5E8; serotype 2, MAbs S2-2G10, IC10 and 2F1; serotype 3, MAbs YO-1E2, 159 and 4F8; serotype 4, MAb ST-2G7; serotype 5, MAb 5B8; serotype 6, MAb 1C3 and serotype 10, MAb B223/N7. The VP6-specific MAbs 255/60 and 631/9 which are specific for SG I and SG II, respectively, were used for determining the SG specificity of the isolates. The source, preparation, characterization and reactivities of the MAbs have been previously described [33].

Of the 694 samples analyzed, 150 samples were found to be positive for rotavirus infection in Bangalore, while 50 specimens out of 447 were positive for rotavirus in Mysore as detected by electrophoretic analysis of viral genomic dsRNA. Although this amounts to 21.6% on the average over a 7-year period in Bangalore and 11.2% in Mysore, a gradual, yet highly significant reduction (from 45.3% in 1988 to 1.8% in 1994) in the number of samples positive for rotavirus infection was observed (Table 1). In contrast, in the city of Mysore, the percentage of rotavirus-positive samples appears to have remained constant and only slightly increased from 8.4% in 1993 to 14.4% in 1994 (Table 1). During 1993 and 1994, large number of samples could not be collected from hospitals in Bangalore for analysis due to lack of admissions of diarrheal cases (Table 1). The cause of decreased admissions is unknown. It was not due to changes in either admission criteria, population served by the hospitals or other community practices that we could identify.

In Bangalore, of the 150 samples positive for rotavirus, 33 samples (22%) were found to show SG I specificity, while 116 (77.3%) samples showed SG II specificity (Table 1). In Mysore, 7 samples (14%) were of SG I and 37 samples (74%) were of SG II (Table 1). Of the total positive samples from both cities, seven strains (3042, MP383, MP269, MP256, MP334, MP386, MP379) exhibited

Table 1. Distribution of rotavirus subgroups and serotypes during 1988 to 1994

Year	No. of samples	No. positive for RV	% positive	Subgroup			Serotype				Nonserotypeable			
				SG I	SG II	Dual SG (I + II)	1	2	3	4	SG I short RNA	SG I long RNA	SG II	Dual SG (I + II)
1988 (B)	86	39	45.3	9	30	–	2	7	14	1	2	–	13	–
1989 (B)	107	34	31.8	9	25	–	3	7	13	2	2	–	7	–
1990 (B)	241	46	19.1	8	38	–	4	4	19	3	4	–	12	–
1991 (B)	68	10	14.7	1	8	1	2	–	2	–	1	–	4	1
1992 (B)	113	19	16.8	4	15	–	4	2	5	1	2	–	5	–
1993 (B)	23	1	4.3	1	–	–	–	–	–	–	1	–	–	–
1994 (B)	56	1	1.8	1	–	–	–	1	–	–	–	–	–	–
1993 (M)	239	20	8.4	–	20	–	8	–	9	–	–	–	3	–
1994 (M)	208	30	14.4	7	17	6	15	–	3	–	5	1	2	4
Total	1141	200	17.5	40	153	7	38	21	65	7	17	1	46	5

B Bangalore; *M* Mysore; *RV* rotavirus

Table 2. Subgroup analysis of symptomatic rotavirus strains with dual SG specificity

Rotavirus strain	Subgrouping ELISA		SG specificity
	255/60 (I)	631/9 (II)	
Prototype			
S2	661	152	I
RRV	454	110	I
PRICE	122	243	II
WI61	113	302	II
Symptomatic strains			
3042	332	380	I + II
MP256	765	824	I + II
MP269	331	470	I + II
MP334	338	433	I + II
MP379	490	672	I + II
MP383	471	602	I + II
MP386	443	607	I + II

Data are shown as the average $OD_{410} \times 1{,}000$ values of 2 wells each from 2 independent experiments.

MP Patients (symptomatic) samples from Mysore

3042 is a symptomatic sample from Bangalore

dual subgroup specificities (Tables 1 and 2). These strains reacted almost equally with both the SG I and SG II-specific MAbs and thus could not be classified as either SG I or SG II (Table 2). All the specimens with dual subgroup specificity showed a long pattern of RNA migration. Except two isolates (MP346, MP409) from Mysore which showed SG I specificity but a 'long' electropherotype, all the other isolates from Bangalore and Mysore with SG I specificity showed 'short' pattern of RNA migration and all the isolates with SG II specificity showed a 'long' electropherotype. Strains that did not react with either of the SG-specific MAbs were not detected [40].

Serotype analysis of the rotavirus-positive specimens revealed serotype G3 to be more prevalent (35.3%) in Bangalore throughout the 7-year period followed by serotype G2 (14%), serotype G1 (10%) and serotype G4 (4.7%). In Bangalore, serotypes G3 and G2 predominated during 1988 to 1990. Serotypes G3 and G1 were predominant during 1991 and 1992, while serotypes G2 and G4 were either infrequently observed or absent (Table 1). In Mysore, during the two-year period from 1993 to 1994, serotypes G1 and G3 were mostly encountered and serotypes G2 and G4 were absent.

Although serotype G2 strains appear to be either absent or less abundant during the later years of study, several strains with characteristics of serotype G2 viruses i.e., SG I specificity and 'short' RNA pattern were observed during these

Table 3. Serotype analysis of some of the symptomatic rotavirus strains with SG I specificity and 'short' electropherotype

Rotavirus strain	Serotyping ELISA with serotype-specific MAbs (serotype specificity)												
	KU4 (1)	5E8 (1)	S2-2G10 (2)	IC10 (2)	2F1 (2)	159 (3)	ST-2G7 (4)	5B8 (5)	1C3 (6)	B223/N7 (10)	57-B (1, 3, 4)	60 (cross-reactive)	HCV (non-specific)
Prototypes with G type													
DXRRV (1)	1023	>3000	215	157	186	402	203	258	154	127	2105	1718	107
DS1XRRV (2)	146	108	642	483	895	251	191	249	236	182	213	1114	123
S2 (2)	107	126	489	367	471	183	90	145	72	108	85	558	57
RRV (3)	220	215	189	105	82	>3000	139	250	184	225	>3000	>3000	105
ST3 (4)	198	501	106	112	136	203	>3000	280	162	155	>3000	>3000	130
NCDV (6)	210	205	211	153	184	560	261	257	>3000	216	800	>3000	66
I321 (10)	253	516	189	211	162	620	127	221	108	>3000	109	818	58
Clinical samples													
3162	244	138	320	>3000	387	175	140	167	144	129	90	>3000	66
1040	1260	1045	1031	1453	980	1376	885	1281	923	687	544	1392	161
MP291	209	194	186	124	188	127	143	206	191	212	125	156	22
MP312	714	621	675	805	562	455	510	706	432	480	278	972	49
MP415	627	828	465	852	341	288	727	424	638	751	210	1020	52
Cell culture adapted strains													
1040	1365	1127	1241	1685	859	945	849	997	815	680	478	1141	120
MP415	598	502	603	2300	552	490	316	722	364	410	230	1478	75
MP291	762	587	840	1135	612	801	370	730	425	301	462	1200	83

Data are shown as the average $OD_{410} \times 1{,}000$ of 2 wells from two independent experiments

MP Patients (symptomatic) samples from Mysore. The other two samples are from Bangalore

years (Table 1). These strains reacted with serotype G2 typing reagents as well as the other serotype-specific MAbs (1040, MP415, MP312, MP354). Some specimens (3138, MP291, MP253) showed very low reactivity with the typing reagents probably reflecting the relatively low amount of the virus in these samples as judged by the genomic viral RNA content by PAGE as well as the low level reactivity observed with the cross-reactive MAb 60. Some of these serotype G2-like strains have been adapted to growth in cell culture and tested in serotyping ELISA. As shown in Table 3, all the cell culture-adapted strains exhibited cross-reactivity with each of the rotavirus serotype-specific MAbs tested but no reactivity when a nonspecific MAb (raised against human hepatitis C virus envelope protein) was used as the capture antibody in serotyping ELISA. One of the strains (3162) having SG I specificity and 'short' RNA electropherotype exhibited high level reactivity with serotype G2-specific MAb IC10, but failed to show significant reactivity with the other two MAbs specific for serotype G2 and was considered as belonging to serotype G2. Though the cell culture-grown isolates MP291 and MP415 showed high reactivity with MAb 1C10, they also exhibited significant cross-reactivity with MAbs specific for the other serotypes. The fact that clinical samples as well as the cell culture-adapted serotype G2-like strains exhibit high degree of cross-reactivity with many typing MAbs suggest that the observed cross-reactivity is not due to interference by some nonspecific components present in the clinical samples or due to the instability of the outer capsid of these strains to proteolytic enzymes of the gut.

Two isolates (1016 and MP402) with 'long' RNA pattern and SG II specificity also showed cross-reactivity with typing MAbs specific for several serotypes. A few strains showed reactivity to MAbs specific for more than one serotype. Three strains (3023, 3028, 650) showed reactivity to MAbs specific for G1 and G4 and one strain (MP213) reacted with MAbs specific for types G1, G3 and G4. Specimens reacting with MAbs specific for more than one serotype have been reported in previous studies but such reactivities have been attributed to mixed infections [27]. In none of the samples with multiple G serotype reactivities could we identify existence of mixed infections as was evident from their single RNA electropherotypes. Reactivities of these strains can be attributed to the presence of shared neutralization epitopes on VP7 [10, 20].

About 36% of the isolates from Bangalore and 30% of the isolates from Mysore could not be assigned to any serotype. These include strains which showed cross-reactivity with MAbs specific for more than one serotype. Of the seven samples showing dual SG specificity, two isolates (3042, MP383) belonged to serotype 1, one isolate (MP269) belonged to serotype 3, while others could not be assigned to any serotype. Of the two isolates from Mysore which showed SGI specificity but have 'long' RNA electropherotype, isolate MP346 had serotype 1 specificity while MP409 could not be assigned to any serotype.

Throughout the study period, serotype 10 viruses similar to the I321-like G10P11 type asymptomatic neonatal strains (11) were not detected in children suffering from diarrhea. However, epidemiological studies on asymptomatic

infections during the same period in Bangalore revealed that a consistently high proportion (about 34%) of neonates had asymptomatic infections exclusively by I321-like G10P11 strains. As was reported earlier in Bangalore [11, 39], I321-like viruses were also found to be exclusively associated with asymptomatic neonates in Mysore.

The long term survey of seven consecutive seasons in Bangalore and two seasons in Mysore has yielded information of epidemiological significance. The persistent predominance of serotype G3 viruses in Bangalore over a 7-year period and their relative prevalence in Mysore as well is unusual in that serotype G1 was reported to be largely associated with diarrhea in majority of the countries [1, 18, 19, 27, 30, 32, 33, 46]. Prevalence of serotype G3, even over a short period, was only rarely observed [5, 43].

Another important observation was the identification of a large number of strains with characteristics of serotype G2 viruses (i.e., SG I specificity and 'short' RNA electropherotype) but could not be assigned to serotype G2. Instead, these strains reacted with serotype G2-specific typing reagents as well as MAbs specific for the other serotypes (Table 3). Preliminary sequence analysis of the VP7 gene from some of these strains revealed that it was closely related to the corresponding gene of serotype G2 strains of human rotavirus RV5 and S2 but contained point mutations at specific locations. It would not be surprising if a few amino acid changes in the antigenic regions of VP7 could have resulted in the loss of specific reactivity of the conformational epitopes with serotype G2-specific MAbs. Alternatively, a unique VP4 in these strains might be acting to influence the antigenicity of VP7 [6, 7, 13, 28]. Complete sequence analysis of VP7 and VP4 genes from these strains should reveal the molecular basis for the hyper cross-reactivity of these strains with typing MAbs specific for several serotypes. Such highly cross-reacting outer capsid proteins would be ideal candidates for incorporation into reassortant or recombinant vaccines [31].

The ability to determine the serotype of any sample has been observed to be influenced by the number of virus particles as well as their integrity in the specimens. However, in the present study, at least for some of the nontypeable samples, the amount of the virus appears not to be a limiting factor as observed from the reactivity with the cross-reactive MAb 60 as well as the readily detectable amounts of viral RNA in the specimens. It is likely that some of these nontypeable specimens represent viruses having unknown specificity or belong to other serotypes like G8 or G9 for which MAbs were not used in this study due to their nonavailability.

The present studies also revealed the existence in circulation of several strains having characteristics of animal rotaviruses. Rotaviruses with dual subgroup (SG I + II) specificity and 'long' RNA electropherotype possessing G3 or G13 specificity have been previously identified in horses (strain FI–14) and rarely in humans [24, 26, 41, 46]. Thus strains with SG I + II specificity (3042, MP383, MP269, MP256, MP334, MP386, MP379) are likely of animal origin.

The SG I specificity and 'long' RNA electropherotype associated with isolates MP346 and MP409 also suggest that these strains might have been

derived by natural reassortment between SG I and SG II human strains or between human and animal strains. In this context, it should be noted that strains with SG I specificity and 'long' RNA pattern have been isolated from children suffering from diarrhea as well as asymptomatic neonates in different parts of India [17, 39]. While the symptomatic strains from the North-Eastern state of Manipur were of serotype G2 specificity and were shown to have originated by genetic reassortment between two human rotaviruses having SG I and SG II specificities [29], the asymptomatic strains from Bangalore were shown to be reassortants between a G10P11 type bovine virus and a human virus [11]. Neither of these 2 strains (MP346, serotype 1; MP409, nontypeable) showed serotype G2 or G10 specificity. Asymptomatic rotaviruses that were reassortants between human and bovine strains have also been reported from New Delhi [12].

Frequent isolation of reassortants between human and animal rotaviruses in different parts of India point to the close interaction of majority of the Indian population with cattle and other domesticated animals as the cause for the evolution of novel rotaviruses. Understanding the antigenic specificities of rotaviruses circulating in India is essential for formulating strategies towards development of an effective vaccine against rotavirus diarrhea under the Indian context.

The gradual yet highly significant reduction in rotavirus diarrhea in Bangalore from 45.3% in 1988 to 1.8% in 1994 coupled with the predominance of serotype G3 over seven seasons is of epidemiological significance. We have previously shown that the I321-like G10P11 strains are exclusively associated with asymptomatic infections in neonates in Bangalore [11, 39]. It is significant to note that while symptomatic rotavirus infections were hard to detect in Bangalore, in recent years, the asymptomatic infections by I321-like strains remained consistently high (about 34%) throughout the seven-year study period. Moreover, serotype G10 strains were not detected in children with diarrhea either in Bangalore or in Mysore indicating that I321-like asymptomatic strains are most likely not involved in symptomatic infections. One possible reason for the steep fall in rotavirus gastroenteritis in Bangalore is that infection of neonates by asymptomatic I321-like strains is conferring resistance to the disease caused by symptomatic viruses during the later periods of age [2, 4]. It is possible that I321-like asymptomatic strains have spread rapidly through the population in recent years and are naturally vaccinating the children. I321-like strains might be ideal candidates for live rotavirus vaccine. Since any successful vaccine could alter the epidemiology of the disease, it is likely that the reduced incidence of rotavirus diarrhea as well as the prolonged predominance of serotype G3 in Bangalore are the consequence of persistent large scale natural vaccination of new-born children by I321-like asymptomatic strains.

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