Enteric Viruses

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9 Enteric Viruses

- Epidemiological studies on Rotaviruses
- Full length genome sequencing and characterization of rotavirus strains
- Molecular characterization of rotavirus strains adapted to cell culture
- Development of an ELISA for detection of rotavirus serotype specific antibodies in human sera and its evaluation for diagnosis of acute rotavirus infection
- Preparation of egg yolk antibodies against human rotaviruses
- Hospital based surveillance of non-rota enteric viruses in acute gastroenteritis patients
- Additional Studies
  Investigation of Outbreaks of Acute Hemorrhagic Conjunctivitis (AHC) occurred in Maharashtra and Karnataka states during 2006 and 2007

Epidemiological studies on Rotaviruses

Hospital based surveillance of rotavirus disease and strains among children

SD Chitambar

Rotavirus infections are the major cause of severe dehydrating diarrhea among children. Rotavirus serotypes G1P[8], G2P[4], G3P[8] and G4P[8] are most commonly circulating types globally. In addition to these, uncommon and untypeable rotavirus strains cocirculate and mixed infections with different rotavirus types occur in developing countries. In order to have better understanding of such viruses, epidemiological and molecular studies are required that would help to define the need for and benefits of rotavirus vaccines in India.

Objectives

- To estimate the proportion of rotavirus diarrhea
- To find out prevalent rotavirus types among hospitalized children <5 years of age

Work done

Fecal specimens were collected from a total of 284 children <5 yrs of age, hospitalized for diarrhea. Nearly 38.4% specimens were detected positive for rotavirus by ELISA. Among these specimens, 97.2% were typed for VP7 (G) and 98.1% were typed for VP4 (P) genes. Both G and P types were established in 96.3% of the specimens by multiplex PCR. Each of the two common rotavirus types G1P[8] and G2P[4] represented 57.2% and 17.1% of the strains respectively while none of the specimens showed presence of other two common types-G3P[8] and G4P[8]. G12P[8], G9P[8], G10P[8] and G12P[6] were detected at a frequency of 0.9%, 1.9%, 1.9% and 6.6%, respectively. G and P types in uncommon combinations (G1P[4], G2[8]) and in mixed infections represented 4.7% and 11.4% of the strains respectively (**Fig. 1**). A very few of the strains remained either G or P untypeable (0.9%-1.8%).

Hospital based surveillance of rotaviruses was also carried out in acute diarrhea patients from Jabalpur (n=84). Rotavirus positivity was detected in 10.7% of the patients. G1P [8] was detected in 66.6% whereas G2 P [4], G9 P [4] and G12 P [6] were detected each at 11.1%.

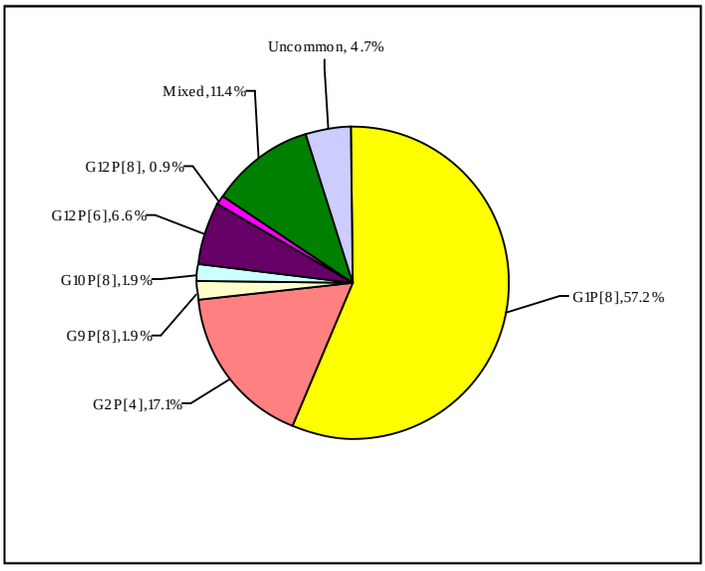

Fig. 1 : Distribution of rotavirus G and P types among typeable specimens

Future Plan

The work will be continued further to analyze the data on rotavirus serotypes and strains and their variations, untypeable strains and seasonal distribution of rotavirus associated hospitalizations.

Rotavirus viremia in patients with acute gastroenteritis

SD Chitambar

Thirty One pairs of stool -serum specimens collected from hospitalized diarrrheal patients during Nov. 2004- Feb. 2005 were investigated for rotaviruses by RT-PCR using VP6 gene primers and tested in ELISA for rotavirus antigen and anti-rota IgM antibody.

Rotavirus RNA was detected in 80.6% stool and 58.1% sera as against rotavirus antigen in stool (45.2%) and anti-rota IgM antibody (25.8%). All PCR positive specimens were typed for VP7 and VP4 genes by multiplex PCR. Five of 16 pairs could be typed for both the genes. Three of 5 pairs showed correlation between genotypes from stool and serum samples (G2P[4] vs G2P[4]) while two showed discordance (G2P[4] vs G12 P[8]; G2P[4] vs G8P[4]). Though a significant number of sera (56.3%) remained untypeable, data indicating seropositivity of rotavirus in gastroenteritis patients may influence the surveillance studies based on fecally excreted rotavirus strains and contribute to reveal dual/mixed infections in acute gastroenteritis patients.

Detection and characterization of rotaviruses in adolescent and adults cases of acute gastroenteritis

VS Tatte and SD Chitambar

Rotaviruses are the major cause of acute gastroenteritis in children. These viruses are also known to infect adolescents and adults. Recent studies carried out among adults in the developing as well as developed countries have shown rotavirus infections to be on the rise. Limited studies conducted so far in adults from India indicate 5-7% prevalence of acute rotavirus infections, however no data is available on molecular characteristics of rotavirus strains circulating in adolescent and adult cases of acute gastroenteritis.

Objectives

- To characterize the rotavirus strains recovered from adolescent and adult cases of diarrhea.

Work done

Two hundred and fifty three stool specimens were collected during 2004-2007 from adolescent and adult cases visiting OPD of local hospitals for acute gastro enteritis. Fourty four samples (17.4%) positive in ELISA for group A rotavirus were tested in monoclonal antibody based ELISA to determine the subgroups (SGs) of rotavirus strains. Results indicated subgroup diversity with predominance of Non I Non II SG followed by SGI+II, SGI and SGII in rotavirus strains **(Table 1)**. In comparison with the data reported earlier on such strains (AR 2001), a rise in NonI Non II SGs was noted.

Table 1 : Distribution of SGs in rotavirus strains

SG I	SG II	SG I + II	SG II
11.4% (5/44)	4.5% (2/44)	31.8% (14/44)	52.3% (23/44)

Among sixty seven stool specimens collected in the year 2007 from adolescent and adult cases of acute gastroenteritis, 31(46.3%) showed the presence of Group A rotavirus by ELISA. VP7 and VP4 genes of rotavirus strains were further characterized by multiplex PCR. Nearly 20% (6/31) of the specimens were typed for both VP7 and VP4 genes. All strains (n=6) that were typed for both the genes showed unusual combinations of G and P types (3/6 G1P[4],1/6 G9P[4], 1/6 G9P[6] P[4], 1/6 G1,G2 P[4]). Dual P type infections were detected in 6/9 of the P typed specimens. Majority of the strains 15/31 remained untypeable. Unusual / untypeable strains also contributed to subgroup untypeability of rotavirus strains.

Group A rotavirus ELISA negative samples (n=215) obtained during winter months of 1994-95 from adolescent and adult cases of acute gastroenteritis admitted to Naidu Hospital, Pune were screened for groups A, B and C rotavirus RNA by PAGE. Five samples (2.3%) detected positive, showed long RNA migration pattern. Multiplex PCR based characterization showed presence of G3P[8] in 2, G1P[8] in 2 and G nontypeable P[8] in 1 of the specimens. G3P[8] was detected for the first time in adult cases from Pune , western India.

Detection and molecular characterization of rotaviruses from cattle

SD Chitambar and CG Raut

Group A rotaviruses, members of the genus *Rotavirus* within the family *Reoviridae*, are the main pathogens of neonatal calf diarrhea and are classified into G and P genotypes. Among the 19 G and 27 P genotypes, ten G (G1,G2,G3,G5-G8, G10, G11 and G15) and six P(P[1], P[5], P[11], P[14], P[17] and P[21]) genotypes have been associated with diarrhea in calves, and of them, G6,G8 and G10 in conjugation with either P[1], P[5], and P[11] are of epidemiological significance.

Objectives

- To detect rotaviruses from apparently healthy and diarrheic cattle
- To characterize bovine rotaviruses by RNA-PAGE, RT-PCR and sequencing

Work done

A total of 78 fecal specimens collected from cattle were investigated for rotaviruses. Bovine rotavirus prevalence was 3.8% by ELISA. All ELISA positive specimens (n=3) were amplified in RT-PCR for VP7 and VP4 genes. Sequence analysis of PCR products revealed presence of novel G8P[14] strains closely related to bovine B17 strain of G8 type with 93.9-98.2% and human Hun 5 strain of P[14] type with 94.7-95.4% nucleotide identity. The data suggested the possibility of reassortment between human and bovine group A rotaviruses.

Full length genome sequencing and characterization of rotavirus strains

SD Chitambar and PR Fadnis

The rotavirus strain G2P[4] circulating commonly and G12P[6] emerged recently were undertaken for full length genome sequencing. The sequences obtained from 6 structural and 5 nonstructural genes were compared with reference strains. G2P[4] strain showed 84.4-92.6% and G12P[6] strain showed 96.7-99.0% nucleotide identity with reference strains TB-Chen and Dhaka 12-03 respectively.

Molecular characterization of rotavirus strains adapted to cell culture

Ritu Arora and SD Chitambar

Rotaviruses, members of the family Reoviridae, are the most common cause of severe diarrhea among children. Characterization of rotavirus genes especially of VP7 and VP4 from wild type rotavirus strains recovered from fecal specimens is widely carried out. However**,** limited studies are reported on the genomic changes that occur in human rotavirus during cell culture adaptation**.**

Objectives

- To isolate rotavirus strains from acute diarrhea patients
- To study the genomic changes in rotavirus during adaptation to cell culture

Work Done

Studies conducted earlier on isolation of rotavirus strains from acute diarrhea patients were continued further. Presence of a rotavirus strain in the infected MA-104 cells was confirmed by antigen capture ELISA. RT-PCR followed by sequencing for VP6 gene was carried out at passage 5. The strain showed 98% identity with Matlab13-03 strain (Bangladesh). RT-PCR and sequencing were carried out for all the structural (VP1-VP4, VP6 & VP7) and non structural (NSP1-NSP5) genes of wild type and culture adapted (at passage 5 and passage 10) strains. Sequences were compared with prototype strain, KU from Japan. Substitution was noted only in the deduced amino acid sequence of VP4 gene. All the genes of a culture adapted strain at passages 5 and 10 revealed 100% identity at nucleotide and amino acid levels.

Development of an ELISA for detection of rotavirus serotype specific antibodies in human sera and its evaluation for diagnosis of acute rotavirus infection

SD Chitambar, PG Ray and S. Bhalla

Rotaviruses are recognized as the most important etiologic agents of childhood diarrhea, worldwide. The widespread distribution of rotavirus is indicated by universal acquisition of serum antibodies to rotaviruses at an early age. A high prevalence of rotavirus antibodies in the adults suggest that repeated infections occur.

Objectives

- To develop an ELISA for detection of rotavirus serotype specific antibodies in human sera
- To determine the prevalence of rotavirus serotype specific antibodies in healthy adults and acute diarrhea patients
- To determine the correlation between serotype specific antibodies in serum and infecting genotypes in fecal samples of acute diarrhea patients

Work done

Results on rotavirus serotype specific antibodies in serum samples of healthy adults by a newly developed rapid assay (blocking ELISA) and comparison of the results with that of conventional tissue culture based neutralization assay have already been provided in the previous report (Annual Report 2006-07). Briefly, antibody titers to

different human rotavirus serotypes G1-G4 and animal strains G6 (Bovine) and SA-11 (Simian) were determined in 37 healthy adult sera by blocking ELISA. Fifteen of the 37 sera were subjected to the routine neutralization assay based on tissue culture and ELISA. No significant difference in percent positivity as well as range of titers was observed by both the methods.

For further evaluation of blocking ELISA, 14 acute and 9 convalescent phase serum samples of children hospitalized for rotavirus diarrhea were tested for serotype specific antibodies. Simultaneously, fecal specimens from the respective children were also tested for the infecting G-type by multipex PCR. Two to 4-fold rise in the blocking ELISA antibody titers was observed in all the 9 convalescent phase sera that correlated with the G-type detected in the fecal specimens by multipex PCR. The infecting serotypes detected were G1 (3/9), G2 (3/9), G2, G9 (1/9) and G9 (2/9). Antibody response to the serotypes other than the infecting serotypes was also detected in 7/9 sera by blocking ELISA **(Table 2).**

Ten of the sixteen sera from rotavirus negative children showed absence of anti-rota antibodies. Six sera showed antibody response to G1-G4, or G6, (titers = 1:100) indicating past rotavirus infection. The fecal specimens of these 6 children were negative to all rotavirus serotypes by multiplex G-typing (**Table 3).**

Table 2 : Rotavirus serotype specific antibodies in the sera from rotavirus positive children as detected by blocking ELISA

Sr. No.	Specimen No.	Age/ Sex	Antibody titres against rotavirus serotypes in acute/convalescent sera							G type by PCR (In fecal sp)
			G1	G2	G3	G4	G9	G6 (Bovine)	SA-11 (Simian)	
1	006559	7m/F	-ve/<100	50/100	100/-ve	100/-ve	100/200	100/50	-ve/-ve	G9
2	006583	15m/M	-ve/400	-ve/400	-ve/-ve	-ve/-ve	50/100	-ve/400	-ve/-ve	G2
3	006693	13m/M	-ve/<100	-ve/50	-ve/-ve	-ve/-ve	-ve/50	-ve/50	-ve/-ve	G2, G9
4	006833	1yr/F	-ve/<100	-ve/-ve	-ve/-ve	-ve/50	50/100	-ve/-ve	-ve/-ve	G9
5	006943	6m/Vl	-ve/NA	-ve/NA	-ve/NA	100/NA	100/NA	-ve/NA	-ve/NA	G9
8	022233	5m/Vl	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	G1
7	022235	6m/Vl	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	G1
8	022562	11m/F	ve/<100	ve/ ve	ve/ ve	ve/ ve	ve/ ve	ve/ ve	ve/ ve	G1
9	023257	15m/M	-ve/400	-ve/200	-ve/400	-ve/-ve	-ve/400	-ve/200	-ve/50	G1
10	026237	5m/F	-ve/50	-ve/-ve	-ve/-ve	-ve/-ve	-ve/-ve	-ve/-ve	-ve/-ve	G1
11	026282	15m/F	-ve/50	-ve/200	-ve/-ve	-ve/-ve	-ve/50	-ve/-ve	-ve/-ve	G2
12	026661	18m/M	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	G2
13	026639	10m/M	ve/NA	ve/NA	ve/NA	ve/NA	ve/NA	ve/NA	ve/NA	G2
14	026736	9m/F	-ve/50	-ve/100	-ve/50	100/-ve	100/-ve	-ve/-ve	-ve/-ve	G2

NA: Not available

Table 3 : Rotavirus serotype specific antibodies in the sera from rotavirus negative children as monitored by blocking ELISA

Sr No	Specimen No.	Age/ Sex	Antibody titres against rotavirus serotypes in acute/convalescent sera							G type by PCR (In fecal sp)
			G1	G2	G3	G4	G9	G6	SA-11	
1	006948	11m/M	ve	ve	ve	ve	ve	ve	ve	ve
2	006950	13m/M	ve	ve	ve	ve	ve	ve	ve	ve
3	02540	9m/M	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	02567	1.5yr/M	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5	02628	2yr/M	50	100	100	100	-ve	-ve	-ve	-ve
6	02647	15m/F	-ve	-ve	100	100	-ve	-ve	-ve	-ve
7	02715	8m/F	100	-ve	100	50	-ve	50	-ve	-ve
8	02824	1yr/F	ve	ve	ve	ve	ve	ve	ve	ve
9	02826	4m/F	ve	ve	ve	ve	ve	ve	ve	ve
10	021321	10m/M	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11	021555	14m/F	-ve	-ve	-ve	-ve	-ve	100	-ve	-ve
12	022560	2yr/M	-ve	100	100	100	-ve	-ve	-ve	-ve
13	023221	5m/F	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
14	023223	2yr/M	-ve	-ve	-ve	-ve	-ve	100	-ve	-ve
15	023102	6m/F	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
16	025235	8m/F	ve	ve	ve	ve	ve	ve	ve	ve

Blocking ELISA can be used as a substitute for the neutralization test, which is a tissue culture based ELISA test and requires three days for completion. It can be suitably used with its limitations for the detection of serotype specific neutralizing antibodies in epidemiological studies.

Preparation of egg yolk antibodies against human rotaviruses

Manika Burgohain, Ganesh Dhale, SD Chitambar

Egg is a complete diet for the developing embryo and a supplement for the first few days of life of a chicken. The birds vaccinated against human/poultry pathogens produce eggs having yolks with high level of antibody protein, IgY.

Objectives

- To prepare immunoglobulins against human rotaviruses in egg yolk and evaluate their efficacy by *in vitro* and *in vivo* tests.

Work done

Five month old specific pathogen free (SPF) White Leghorn hens were immunized against commonly circulating human rotavirus (HRV) strains KU(G1P[8]), S2(G2P[4]), YO(G3P[8], ST3(G4P[8]) and F45(G9P[8]). The birds were found to generate anti-rota antibodies, with ELISA titers ranging from 1:50,000 to 1:1,00,000 after third booster dose.

Eggs were collected from the birds and processed for purification of IgY and its protein estimation and anti rota antibody titers. The protein content of the purified IgY was in the range of 2.4-3.5 mg/ml and antibody titers varied from 1:64,000 to 1:256,000 in various lots.

Purified IgY antibodies from different lots of immnune eggs were tested for its neutralizing activity against homologous and heterologous virus strains by cell culture based neutralization assay. Neutralization titers were observed to be higher against homologous viruses (=1: 6400) while the same were lower against heterologous viruses (=1: 800) (**Table-4).**

Table 4 : Neutralizing antibody titers against rotaviruses

IgY	Neutralizing antibody titers				
	HRV-1	HRV-2	HRV-3	HRV-4	HRV-9
IgY HRV-1	>6400	200	800	200	400
IgY HRV-2	400	6400	400	200	400
IgY HRV-3	200	200	>6400	400	400
IgY HRV-4	400	200	200	>6400	400
IgY HRV-9	800	200	400	800	6400

To test *in-vivo* efficacy of the antirota IgY antibodies, an infant mouse model was developed. Four to five days old BALB/c infant mice were inoculated orally with HRV-3(YO) and a single diarrheal dose of 50 µl having 1:2000-1:4000 EEP infectious titer was able to produce diarrhea in 70-90% of animals expressing loose yellow faeces. Maximum numbers of animals were observed to be diarrheic at 48 hours of post infection.

Hospital based surveillance of non-rota enteric viruses in acute gastroenteritis patients

SD Chitambar and V. Gopalkrishna

Acute gastroenteritis is one of the most common diseases in humans and continues to be a significant cause of morbidity and mortality worldwide. Among enteric viruses, rotavirus is the leading viral agent associated with severe diarrhea especially in infants and young children. However, some patients develop diarrhea with non-rotavirus infections indicating involvement of other enteric viral or bacterial pathogens. Recently, association of other enteric viruses such as Calici, Astro, Adeno, Entero viruses have been reported in sporadic and outbreak cases of diarrhea in Asian and European countries and US. In India, limited studies are reported on other enteric viral pathogens. The causative agents in such cases are rendered unidentified in the absence of concerted efforts in most of the episodes of gastroenteritis. It is essential to study the spectrum of unknown viruses in sporadic and outbreak cases of gastroenteritis.

Objectives

- To determine the proportion of diarrhea cases attributable to Calici, Astro, Adeno and Entero viruses in sporadic infections and outbreaks of gastroenteritis in India.

Identification and molecular characterization of Norovirus strains in acute gastroenteritis patients from western India

Preeti Chhabra and SD Chitambar

A total of 830 fecal specimens were collected from acute gastroenteritis patients aged = 7 years, admitted to the hospitals or visiting OPDs in different cities of western India during July 2005 - June 2007. These included 570 specimens (520 IPD and 50 OPD) from Pune, 70 (49 IPD and 21 OPD) from Aurangabad and 190 specimens (IPD) from Nagpur. All specimens were tested for norovirus RNA by RT-PCR using RNA polymerase region primers specific to genogroup I and II.

Of 570 specimens collected from Pune, noroviruses were detected in 72 specimens (12.8%), which included 66/530 (12.4%) IPD and 6/50 (12%) OPD patients. While 70/72 (97.2%) specimens showed positivity for GII noroviruses, 1/72 (1.3%) was positive for GI and 1/72 (1.3%) specimens showed mixed infection with both GI and GII noroviruses. In comparison, 5/70 (7.1%) specimens from Aurangabad were found positive for GII noroviruses including 4/49 (8.1%) IPD and 1/21(4.7%) OPD cases. Twelve of 190 (6.3%) specimens from Nagpur showed norovirus positivity that included 10 (83.3%) GII infections while 2 (16.6%) of GI. Overall, in western India norovirus positivity varied from 6.3 to12.8% with GII being predominant genogroup (98%).

Analysis of clinical severity scores indicated very severe (4.8%), severe (57.3%), moderate (35.3%) and mild (2.4%) disease in hospitalized patients. In comparison to this, 28.5% experienced severe, 57.1% moderate and 14.2% mild disease in outpatients. Nearly 32% and 43% patients did not experience vomiting in hospitalized and OPD cases, respectively.

Norovirus positivity rates were found to be high in children ≤ 2 years (0-36 months) of age (85.7%). Positivity rate decreased in children with higher age groups and were lowest in children 6-7 years of age **(Fig. 2).**

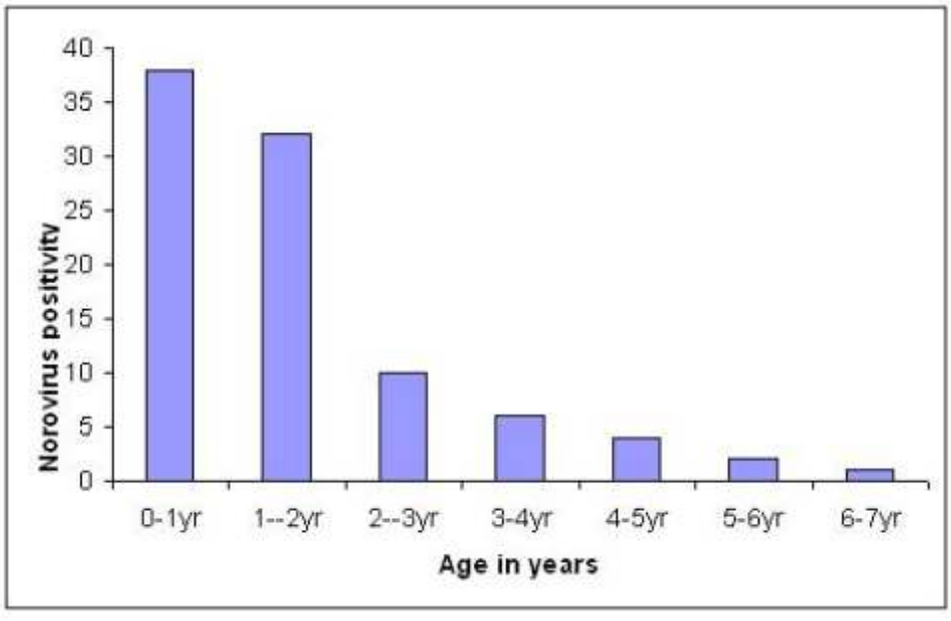

Fig. 2 : Age dependent distribution of norovirus infections in sporadic cases of acute gastroenteritis

Norovirus infections were observed throughout the year. However, norovirus positivity gradually increased in January and was highest in March in both the years 2005-07. Summer month seasonality supported norovirus infections in western India **(Fig. 3)**

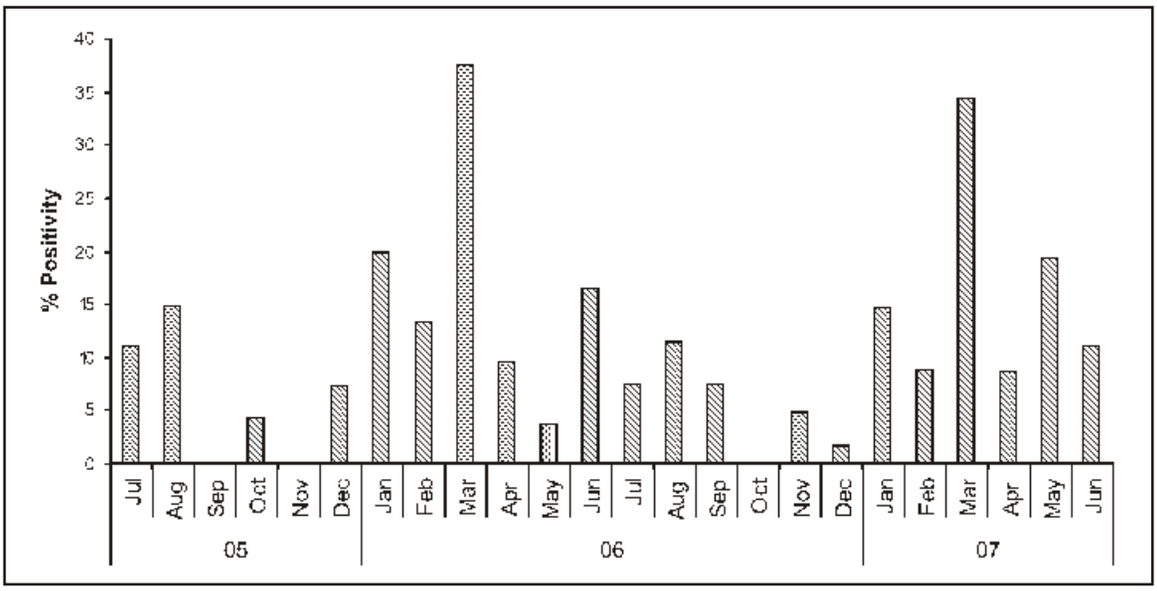

Fig. 3 : Monthwise positivity of norovirus infections in western India

Hospital based detection and characterization of enteric adenovirus and astro virus strains in acute gastroenteritis patients

Harsha Verma, SD Chitambar and V. Gopalkrishna

A total of 228 fecal samples were collected from June 2006 to August 2007 from children with acute gastroenteritis hospitalized at Govt. Medical College, Nagpur, India. Fifty-seven samples were also received from local hospitals of Pune, in January - December 2007. All samples were tested for the presence of adenovirus by PCR using Hexon gene primers. Adenovirus DNA was detected in 7.5% of Nagpur samples and 12.3% of Pune samples respectively. Sequencing of PCR products showed presence of serotype 31 in 10, serotype 40 in 6 and serotype 41 in one of the specimens from Nagpur. The serotype 41 was predominantly detected in Pune samples.

Fecal samples collected from Aurangabad (n=100, May 2005-Feburary 2006), Nagpur (n=228, June 2006-August 2007) and Pune (n=131, January-December 2007) were tested for the presence of astrovirus by RT-PCR using ORF 1a gene primers. Total astrovirus prevalence in Aurangabad, Nagpur and Pune was found to be 4%, 3.5% and 2.3% respectively. Sequencing of PCR products revealed predominance of astrovirus serotype 8 in these regions.

Hospital based detection and characterization of enterovirus strains in acute gastroenteritis patients

PR Patil, SD Chitambar and V. Gopalkrishna

A total of one hundred and eighty fecal specimens were collected from sporadic acute diarrhea cases, admitted at Govt Medical College, Aurangabad (n=95, May 2005-February 2006) and Govt. Medical College, Nagpur (n=85, June 2006-Feb 2007). All samples were tested for the presence of enterovirus RNA by RT-PCR using 5' non-coding region primers. Nearly, 22 and 8.9% of the fecal specimens showed the presence of enterovirus RNA from Aurangabad and Nagpur respectively. Sequencing and phylogenetic analysis showed distribution of enterovirus as- CA-1(n=3),CA-19(n=1),CA-22(n=1),CA-20(n=1),CA-10(n=7),CB-2(n=4),EV-90\91(n=1),EV-89\76(n=1),HEV-B(n=2) at 90-98% level in Aurangabad and EV-89\76(n=3), EV-90\91(n=1),CA-1\CA-22 (n=2) at 94-98% level in Nagpur. In addition, the 119 fecal samples collected from acute diarrhea cases from Pune hospitals during 2006

were also tested for enterovirus. Fifteen (12.6%) samples were found to be positive for EV-RNA by RT-PCR.

Additional Studies

Investigation of Outbreaks of Acute Hemorrhagic Conjunctivitis (AHC) occurred in Maharashtra and Karnataka states during 2006 and 2007

V. Gopalkrishna, PR Patil and SD Chitambar

An outbreak of acute hemorrhagic conjunctivitis was reported during October-November 2006 in Pune, India. Thirty eye swabs collected from the patients were subjected to virus isolation in HeLa cell line. Enterovirus and adenovirus detection was carried out by RT-PCR (5'NCR) and DNA PCR (Hexon region) respectively. Fourteen of the 30 cultures inoculated with clinical specimens showed CPE after blind passaging. All 14 isolates were found positive for enterovirus RNA by RT-PCR. Sequencing of PCR products showed 98-99% nucleotide homology with DSO isolate of Coxsackie A24 from Singapore. Further, eleven of the 14 isolates showed amplification in the VP3-VP1 region using CA-24 specific primers and revealed 96.9-98.0% homology with Singapore strain, 95.8-97.4% with China strain and 94.9-96.7 % with Spanish strain. PNI within isolates was observed to be 98.9-100%. None of the isolates showed presence of adenovirus.

Similar AHC outbreaks were also reported from Pune, Mumbai, Nagpur (Maharashtra) and Banglore (Karnataka) cities in October-November 2007. A total of 60 eye swabs collected from conjunctivitis patients from Banglore (25), Pune (10), Mumbai (10) and Nagpur (15) were tested for enterovirus and adenovirus. Out of sixty clinical samples analyzed, twenty-two (36.6%) were found to be positive for enterovirus by RT-PCR. None of the clinical samples showed the presence of EV70 and adenovirus. Sequencing and phylogenetic analysis of the 5'NCR of enterovirus showed close homology with DSO isolate of CA-24 strains from Singapore.

Publications

- Gopalkrishna V, Patil PR, Kolhapure RM, Bilaiya H, Fulmali P, Deolankar RP. An Outbreak of Acute Hemorrhagic Conjunctivitis caused by Coxsackie-A 24 variant in Gujarat and Maharashtra states in India**.** **J Med Virol** 2007: 79(6): 748-753.
- Gadgil PS, Fadnis RS, Joshi MS, Rao PS, Chitambar SD. Seroepidemiology of hepatitis A in Voluntary blood donors from Pune, Western India 2002 and 2004-05. **Epidemiol Infect.** 2008 Mar; 136(3): 406-9.

Workshops / Conferences / Seminar / Meetings attended

SD Chitambar

- Investigators meeting of the Multicentric project on Hospital Based Rotavirus Surveillance in India held at EVRC, Mumbai, on 14th May, 2007.
- Task force meeting on Hospital Based Surveillance Network for Rotavirus Disease and Strains organized by ICMR at Mumbai on 17th September, 2007
- Workshop on Norovirus detection and typing organized by ICMR and CDC, Atlanta, USA at ERC, Mumbai on 18th-21st Sept. 2007
- Invited lecture on “Rotavirus viremia in acute diarrhea patients: Variance in strains detected in stools and sera” at International Conference on Emerging & Re-Emerging Viral Diseases of the Tropics & Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

- Task force meeting on Genomics and Molecular Medicine for presentation on the project entitled Genomic analysis of Hepatitis A virus isolates from different geographic locations of India, at ICMR New Delhi on 1st February 2008.
- Advanced training in Characterization of untypeable rotavirus strains at CDC, Atlanta from 20th February 2008 3rd April 2008.

V Gopalkrishna

- Molecular characterization of an etiological agent that caused outbreaks of Acute Hemorrhagic Conjunctivitis in Maharashtra and Karnataka states of India, 2006-2007. International conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

VS Tatte

- Uncommon and Mixed Rotavirus infections in Adolescent and Adult cases of acute gastroenteritis from Pune, western India". 5th World Congress of The World Society For Pediatric Infectious Diseases, held in Bangkok, Thailand between 15th-18th November 2007.

Ritu Arora

- Workshop on Norovirus detection and typing organized by ICMR and CDC, Atlanta, USA at ERC, Mumbai on 18th-21st Sept. 2007.
- Sequence analysis of VP4 and VP6 genes of human rotavirus G1P [8] after serial passages in MA104 cells. International Conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

Preeti Chhabra

- Workshop on Norovirus detection and typing organized by ICMR and CDC, Atlanta, USA at ERC, Mumbai on 18th-21st Sept. 2007.
- Contribution of noroviruses in causing acute gastroenteritis in Pune, Western India. International Conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

MM Yadav

- Workshop on "Knowledge Discovery in Life Sciences: Modeling Small Molecules to Macromolecules". Organized by Department of Bioinformatics, University of Pune from 11 17th Dec. 2007.

Harsha Verma

- Detection and molecular characterization of enteric adenoviruses among infants and children hospitalized for acute gastroenteritis. International Conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

Shilpa Bhalla

- Genomic Analysis of Hepatitis A virus isolates from different geographic locations of India“ by, International Conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

Patent filed

A patent is filed on the “Use of IgY antibodies against rotavirus infection in children and poultry” jointly by National Institute of Virology and Venkeys (India) Limited.