

### Rotavirus

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## **Epidemiological studies on rotaviruses**

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Rotavirus infections are common world wide from early years of life to old age and occur repeatedly throughout life. Studies in children show that in the developed countries G1-G4 are the most common rotaviruses. In the developing countries in addition to G1-G4 serotypes, many strains circulate, but remain non-typeable and detected as unusual. Although infections in adults are milder than those in children, deaths due to rotavirus have been reported. Group B rotaviruses were first detected in China during 1982-83 as the causative agent of severe diarrhoea in adults has been recently reported from Kolkata, Bangladesh and western India. In India, limited studies conducted in adults indicate 5-7% prevalence of rotavirus diarrhoea. However, role of rotaviruses as a pathogen in adults has long been under appreciated.

The following studies attempt to delineate the epidemiological aspects of rotaviral infection in different age-groups from India.

### **Pediatric cases**

#### **Objectives**

- To estimate the proportion of rotavirus diarrhoea among hospitalized children <5 years of age.

#### **Achievements**

Fecal specimens were collected from a total of 264 children <5 yrs of age hospitalized for diarrhoea. Nearly 17.80 % specimens were detected positive with a large proportion of samples remaining negative for Group A rotaviruses by ELISA. Rotavirus infection was, therefore assessed for the presence of viral RNA in fecal samples collected from diarrhoea patients using RT-PCR. Since the cases of rotavirus diarrhoea peak in winter, samples collected in the months of November 2004 to February 2005 were investigated. Serum samples from corresponding patients were also tested for Rota viral RNA and anti-rota IgM antibodies.

Among 31 stool samples Rota viral RNA was detected in 21 samples of which 8 were positive in ELISA. Of the 13 Rota RNA positive serum samples 8 serum samples showed presence of anti rota IgM antibodies. The data indicated that RT PCR is a useful assay to detect rotavirus infection that remains undetectable by ELISA probably due to low shedding of virus in the feces. It was also noted that viremia in rota infection is detected by RT-PCR indicating thereby escape of virus from gastrointestinal tract.

Adolescent and adult cases.

## **Objectives**

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

## **Achievements**

The prevalence of Group A rotavirus positivity was detected in 4.5% specimens collected from a population representing the age group 6 -60 years

## **Future Plan**

All faecal specimens will be investigated for characterization of group A and Non-group A rotaviruses.

## **Rotaviral antibodies present in Indian mothers**

Data on the prevalence of rotavirus specific immunoglobulins among mothers at delivery and its relation to development of rotavirus infections and diarrhea among infants are lacking in India. These data in the postpartum serum and milk samples may provide useful information to assess the extent of exposure to rotavirus and subsequent development/maintenance of anti-rotavirus antibody in infants from different socio-economic groups. IgM antibodies that first appear following rotavirus illness is considered as a marker of primary infection. Serum IgA/IgG levels have been correlated with resistance to severe rotavirus illness.

## **Objectives**

- To estimate rotavirus specific immunoglobulin (IgM/IgG/IgA) levels among Indian mothers of higher socio-economic (HSG) and lower socio-economic group (LSG) and occurrence of rotavirus infections among their infants up to six months of age.

## **Achievements**

Cord blood, serum, and colostrum samples were collected from mothers of HSG and LSG following delivery (n=56). Milk samples were collected on days 4 to 6 postpartum (n=56). Seventeen mother-infant pairs were followed up for occurrence of rotavirus infections. Average birth weight for LSG infants was 2.2 kgs (full term low birth weight) and that for the HSG was 3.0 kgs. All HSG mothers were contacted by weekly telephone calls to know feeding regimen and episodes of diarrhea among their infants. None of the LSG mothers possessed telephone; therefore, surveillance could be maintained only by personal home

visits. Stool specimens were obtained from 10 HSG infants of whom 5 had diarrhea. The stool specimens were collected from apparently normal infants in order to detect any excretion of rotavirus in the absence of symptomatic diarrhea. Fifteen infants from LSG had diarrhea. From these 8 stool specimens were available. Thus 18 stool specimens could be collected from HSG and LSG infants between 3-6 months.

Results on rotavirus specific serum IgM levels have been described in annual report 2003-04. It was observed that IgM positivity in mothers was in the range of 64%-75%. IgM positivity in infants was significantly higher in LSG than HSG (94.11% vs 52.94%), ( $P < 0.05$ ). Anti-rotavirus IgG levels were determined after delivery in cord / serum/ colostrum and milk samples of mothers from HSG and LSG respectively (Table 1). The IgG GMTs in cord and mothers' sera from HSG at delivery were significantly high as compared to the LSG ( $P < 0.001$  for both specimens). IgG GMTs of colostrum and milk samples at 4-6 days post delivery was not statistically different in both the groups.

**Table 1: Anti-rotavirus IgG GMTs in mothers/infants of HSG and LSG**

Samples (n=56)	GMT in	
	HSG	LSG
Cord serum	1:145162	1:64292
Mothers serum (after delivery)	1:110558	1:49847
Colostrum (at 1-2 days)	1:74558	1:55570
Milk (at 4-6 days)	1:43040	1:29091
Samples of follow-up study (n=17)		
Cord serum	1:115790	1:54781
Infants serum (at 6 months)	1:40000	1:11634
Mothers serum (after delivery)	1:87040	1:36438
Mothers serum (at 6 months)	1:76911	1:45205
Colostrum (at 1-2 days)	1:78145	1:39018
Milk (at 4-6 days)	1:29330	1:20321
Milk (at 3 months)*	1:13049	1:1843
Milk (at 6 months)	1:1619	1:766

\*n=27 for HSG and n=22 and LSG

The IgG GMTs of mothers and infants sera at 6 months were significantly higher in HSG than that of LSG ( $P < 0.05$ , for both). A significant fall in the IgG GMTs of infants' sera was observed at six months from each group; ( $P < 0.01$  for both) indicating decline in maternally acquired antibodies. The IgG GMTs of milk samples in the mothers from HSG were significantly higher at 3 and 6 months ( $p < 0.001$ ,  $p < 0.01$ ) as compared to the LSG mothers. A significant decrease in IgG GMTs in the serial milk samples at 4-6 days, 3 and 6 months was observed in both groups,  $p$  value being  $< 0.01$ , for each.

Anti-rotavirus IgA levels in mothers from high and low socio-economic groups were determined: Cord blood, maternal sera, colostrum and milk samples following delivery were collected from mothers of HSG and LSG ( $n = 56$  from each group).

**Table 2: Anti-rotavirus IgA GMTs in mothers/infants of HSG and LSG ( $n = 56$ )**

Samples	% Anti-rotavirus IgA positivity in		Anti-rotavirus IgA GMTs	
	HSG	LSG	HSG	LSG
Serum (Post delivery)	51.78	58.92	1:75	1:88
Colostrum (at 1-2 days)	42.85	60.71*	1:54	1:105*
Milk (at 4-6 days)	32.14	55.35*	1:40	1:66*
* ( $P < 0.05$ )				

Anti-rotavirus IgA positivity in sera did not differ significantly, however, it differed significantly in colostrum/milk samples from HSG and LSG. Anti-rotavirus IgA GMTs in colostrum and milk samples were significantly high in LSG than those of HSG indicating higher rotavirus infection rates in LSG mothers (Table 2). All cord blood samples from both groups were negative for anti-rotavirus IgA.

### Rotavirus symptomatic/ asymptomatic infections among infants

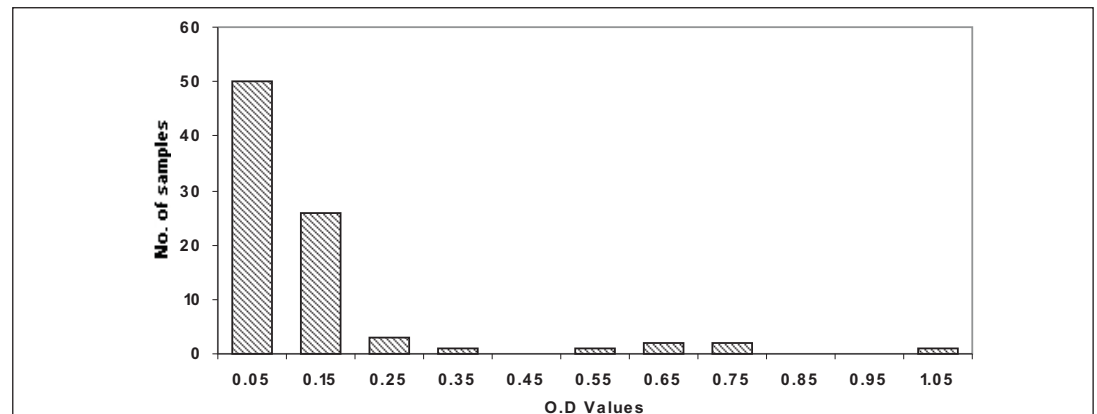
Infants were followed-up for rotavirus infections and diarrhoea upto 6 months of age. The number of infants who suffered from diarrhoeal episodes from the LSG was significantly higher than HSG, ( $P < 0.01$ ). According to surveillance that was conducted on the HSG infants, out of 6 who had diarrhea, stool sample could be obtained from 5, of which 2 were positive for rotavirus by RT-PCR. However, all 6 infants showed presence of rotavirus specific serum IgM, confirming infection/diarrhea with rotavirus. None of these infants were hospitalized. Out of 11 infants from HSG who did not have diarrhoea, 5 showed IgM/IgA seroconversions. Thus, 11 of 17 infants from the HSG had rotavirus infections.

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In the LSG, out of 15 infants who had diarrhoea, stool samples could be obtained from 8, of which 5 were positive for rotavirus, 3 by ELISA & 2 by RT-PCR. Two infants at the age of 3 and 4 months respectively, were hospitalized with severe diarrhea. Rotavirus specific IgM was detected in all, except one infant who had symptomatic rotavirus diarrhoea. Hence, all the 17 infants from the LSG had rotavirus infections before 6 months of age.

Sixteen additional stool specimens were collected from HSG infants' upto 6 months of age. Of these, six infants had diarrhea and rotavirus was detected in 3 infants; 1 by ELISA & 2 by RT-PCR. Rotavirus was not detected in any stool specimens of the infants who did not have diarrhea.

Anti-rota IgG GMTs in cord sera and sera at 6 months of HSG infants who suffered symptomatic/asymptomatic rotavirus infections, were significantly lower than those without rotavirus infections ( $P < 0.05$ ). HSG mothers of the former group showed significantly lower antirota IgG GMTs in their serum samples at delivery and colostrum as compared to those of the latter group ( $P < 0.05$  for both specimens). LSG mothers of all diarrheal infants showed much lower titers of antirota IgG).



**Figure 1 : Reactivity of healthy donors sera in anti-rota IgM capture ELISA**

Distribution of O.D values in ELISA was in the range of 0.04- 1.02 with average  $\pm$  SD,  $0.14 \pm 0.17$  -fifty four of 90 samples showed O.D values below 0.05.-thirty of 90 samples showed O.D values above 0.15 in capture ELISA.- Six of /90 samples showed O.D values between 0.2-1.05.. Study indicates that subclinical rotavirus infection occurs in apparently healthy population from Pune. Serum samples were collected from 90 healthy donors in a blood donation camp organised by Inlaks Budharani Hospital Blood Bank Pune and tested for anti-rota IgM by ELISA .

## Isolation and analyses of rotaviruses from hospitalized diarrhoea cases.

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Fecal specimens were collected from hospitalized diarrhea cases from Pune, India during the year 1990-1997. Out of 432 rotavirus positive fecal specimens 47.92% could not be serotyped earlier by monoclonal antibody (MAb) against G1-G4, G6, G8 and G10 serotypes, and were reported as nontypeable. Three nontypeable and five group A positive fecal specimens collected from diarrheal children from Navi Mumbai in the year 2000, were processed for further studies.

### Objectives

- To characterize rotavirus isolates by molecular and serological methods.

### Achievements

Three Group A rotavirus positive faecal specimens, nontypeable by MAb (against G1-G4, G6, G8, G10 serotypes) based ELISA were processed for isolation of rotavirus in tissue culture. The characteristics of virus from fecal specimens and tissue culture are shown in (Table 3). ELISA detected rotaviruses at passage 4. Two rotavirus isolates were serotyped as G9 using MAb ELISA and showed a long electropherotype. Both the isolates were genotyped as G9 P[8] by RT-PCR. The partial VP7 gene sequence showed 97% homology with AU32 G9 strains. The third strain on culture adaptation showed presence of dual serotype G9 & G3 by MAb ELISA and genotyped as G9 P[8] and G3P[8] by RT-PCR. Partial VP7 gene sequence of G3 type showed 97% homology with simian G3 SA 11 strain. The sequencing of PCR product specific for G9 is being carried out.

Table 3: Virus isolation from clinical specimens

Fecal Specimen/ Isolate	Fecal specimen		Tissue culture Isolates			
	Sub Group	RNA Pattern	Sub group	RNA Pattern	Mab ELISA	PCR
9217932	II	Long	II	Long	G9	G9P[8]
9310350	Non I	Non II	ND	BN I&II	LongG9	G9P[8]
934859	II	Long	I&II	Long	G3, G9	G3P[8] G9P[8]

\*First two digits indicate the year of collection of the specimen

ND: Not done

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Five fecal specimens collected from children at Navi Mumbai were inoculated in MA104 cell line. Four cultures were detected positive for rotavirus between passages 6 and 10 by diagnostic ELISA. The isolate I-006881 showed presence of subgroup I & II specific reactions and was serotyped as G9, G3 by MAb based ELISA tests. The presence of dual serotypes in this isolate was also evidenced by RT-PCR indicating presence of G9 P[8] and G3P[8] genotypes. The partial VP7 gene 306bp (G9 specific) and 582bp (G3 specific) products of this isolate showed 97% homology to AU32 G9 strain from Japan and 97% homology to simian G3, SA11 strain. Three isolates yielded G9 strains with subgroup II activity. These isolates showed 97-98% homology to AU32 G9 strain. All the isolates showed long electropherotype (Table 4).

**Table 4: Characteristics of rotavirus strains isolated from gastroenteritis cases from Navi Mumbai**

<b>Fecal Sp. No.</b>	<b>Sub Group</b>	<b>RNA pattern</b>	<b>MAb ELISA</b>	<b>Isolate No.</b>	<b>Sub Group</b>	<b>RNA pattern</b>	<b>MAb ELISA</b>	<b>PCR</b>
FS-006881	I	Long	-ve	I-006881	II&II	Long	G9, G3	G9P[8] G3P[8]
FS-006883	II	Long	-ve	I-006883	II	Long	G9	G9P[8]
FS-006884	II	Long	-ve	I-006884	II	Long	G9	G9P[8]
FS-006887	II	Long	-ve	I-006887	II	Long	G9	G9P[8]

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## **Investigation of gastroenteritis outbreaks**

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A widespread diarrhea outbreak occurred during the year Dec 2000-Jan 2001 at Jawhar, Thane district. Four hundred ninety patients were hospitalized and 27/39 (69.23%) fecal specimens tested were positive for rotavirus by ELISA. Of these, four fecal specimens were processed for isolation and characterization of rotaviruses.

A diarrhea outbreak that occurred in a family from Pune was studied. The rotaviruses involved were culture adapted, characterized by molecular methods and confirmed by serology.

### **Objectives**

- To isolate and characterize rotaviruses associated with diarrhea outbreak in tribal population from Jawhar and in a family from Pune.

### **Achievements**

Three isolates were obtained from four fecal specimens during an outbreak of diarrhea that occurred in Dec 2000-Jan 2001 at Jawhar, Thane district. All three isolates were serotyped and genotyped (G type) as simian G3 SA-11 like strains. The isolates were further P typed as P[8] by RT-PCR. Partial VP4 gene of one of the isolates, I-006964 and its original fecal specimen FS-006964 was sequenced. Comparison of sequences showed 98%-99% identity with G1 P[8] strain OP60, 94% identity with human G3P[8] YO strain, and 80%-82% identity with simian G3P[2], SA-11 strain.

Two fecal specimens collected from children and two fecal specimens, one each from grandparents were culture adapted in MA104 cell line. Rotavirus could be isolated from all four specimens and viral antigen could be detected in cell culture between passage 3 and 4 by ELISA.

## *Antigenic and molecular characterization of the isolates*

MAB based ELISA detected G3 and G9 strains in first and third day specimens of the child respectively and G9 strains from grandparents. All the specimens showed subgroup II except for child's first day specimen which showed change in subgroup, from SG II to SG I & II on virus cultivation. All the isolates showed long RNA pattern. One isolate from child's day one fecal specimen was genotyped as G3P[8] and other three isolates as G9P[8] by RT-PCR (Table 5). The nucleotide sequencing of partial DNA product of the G3 isolate showed 97% homology with simian G3, SA11 strain while G9 isolates showed 97-99% homology with prototype G9 AU32 strain.

**Table 5: Characteristics of rotavirus strains cultivated from fecal specimens of diarrhea patients (P)**

Subject Code/ Age/Sex	Fecal Specimen No.	Faecal Specimen			Tissue culture Isolates					
		ELISA OD	Sub Group	RNA pattern	MAB ELISA	ELISA OD	Sub Group	RNA pattern	MAB ELISA	PCR
P1/11m/F	*016916-1	0.308	II	Long	G3,G9	0.203	I&II	Long	G3	G3P[8]
P1/11m/F	016916-2	0.631	II	Long	G9	0.304	II	Long	G9	G9P[8]
P2/61y/M	017245	-ve	-ve	Long	-ve	0.263	II	Long	G9	G9P[8]
P3/58y/F	016306	0.795	II	Long	G9	0.500	II	Long	G9	G9P[8]

Footnote: m=months, y=years, M=male, F=female

\*First two digits of specimen no. indicate year of collection.

# *Rotavirus*

## *Serological Studies*

Acute serum samples from the child and her mother were collected on day 5 after child's hospitalization. Serum sample from grandmother collected in the year 1987, was available in National Institute of Virology (NIV) repository. Convalescent serum samples were collected three and a half months following the diarrhea episode from all the three patients and from contacts, viz. child patient's mother (C1) and paternal uncle (C2). Serological studies revealed that patients P1 & P3 showed at least fourfold seroconversion for NAb against G9 serotype while seroconversion to simian G3, SA11 was not observed in P1. Significant level of rotavirus specific IgM was observed in convalescent serum samples of all three patients. Rotavirus specific IgA developed in P1, P2 and C1 but not in P3. It appears that contact C1 had subclinical infection with G9 whereas C2 remained unaffected.

The study showed that (a) Reassortants, of simian and human origin can potentially cause diarrhea in humans. (b) Rotavirus G9 serotype has potential to cause diarrhea not only in children but also in adults if the protective immunity is low.

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## **Molecular characterization of Group A and non Group A rotaviruses isolated from India**

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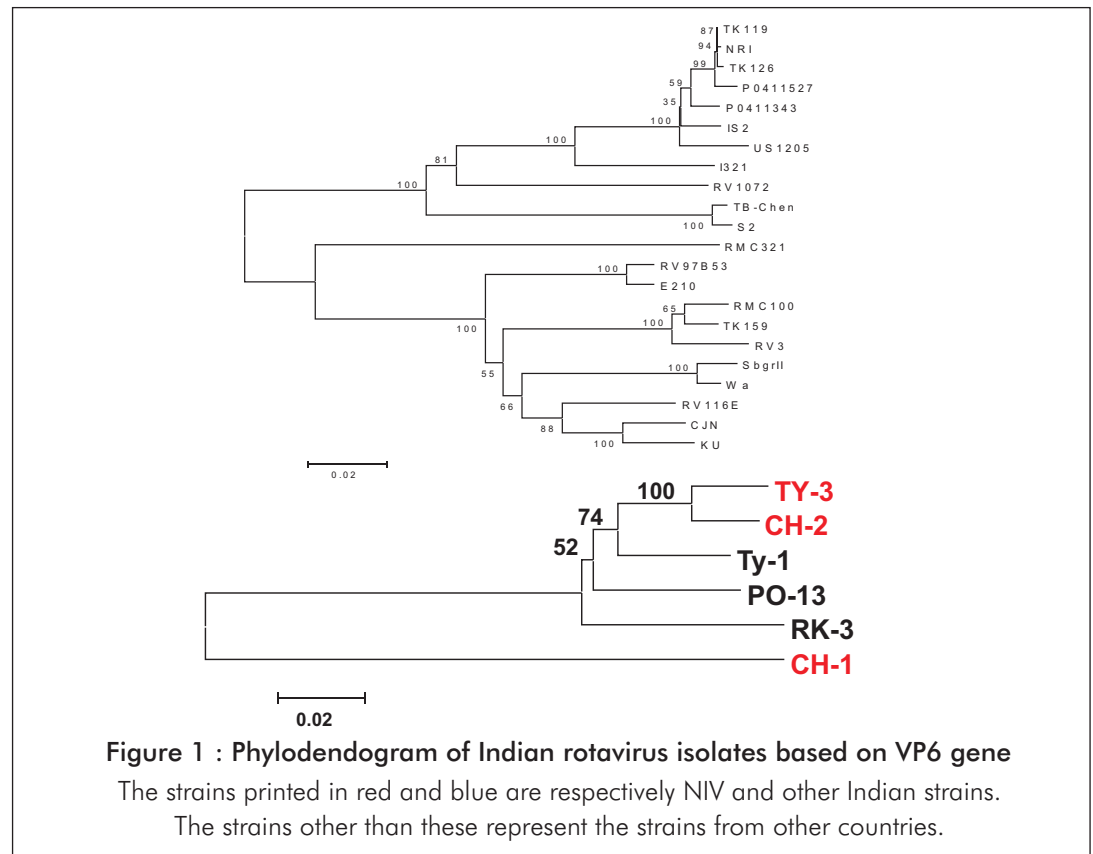
The VP6 is known to comprise subgroup specific regions of rotaviruses. The amino acids at position 172, between 296 and 299, at 305 and 315 contribute for subgroup determination by Mabs 255/60 and 631/9.

### **Objectives**

- To study the variability of VP6 gene of human and avian strains of rotaviruses.

### **Achievements**

The VP6 gene of rotavirus strains recovered from two patients suffering from diarrhea was amplified by RT-PCR and sequenced. Phylogenetic analysis of nucleotide sequences (1012) showed clustering of the strains with TK119 and TK126 (Kolkata strains) with 96-97% identity. Percent nucleotide identity was 97.5% with each other. Deduced amino acid sequences showed presence of alanine at position 305 indicating specificity of subgroup (Fig 1) VP6 gene of avian rotavirus strain CH-2 was sequenced. Analysis of the sequence showed 97% identity with turkey rotavirus strain TY-3, 84% identity with chicken rotavirus strain CH-1 and remarkably low (65-70%) identity with mammalian counterparts. (Fig 1)



### Typing and characterization of group A rotaviruses

Serotyping of rotaviruses using monoclonal antibodies can be carried out by two procedures. In **Method A** ELISA plates are coated with monoclonal antibodies and antigen is detected by polyclonal antibodies while in **Method B** ELISA plates are coated with polyclonal antibodies and antigen is detected by monoclonal antibodies. Normally, **Method A** is used for serotyping. It was noticed earlier that some fecal specimens which could not be serotyped by **Method A** were positive for G2 serotype by **Method B**. Such specimens (911838, 9218037, 9218621, 932588, 932631 and 933140) were tested by nested RT-PCR. The product of nested PCR from five specimens was sequenced and all specimens were confirmed as G2 serotypes.

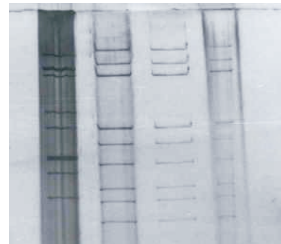
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Simultaneously, optimization of the conditions of multiplex PCR for group A rotavirus serotypes was initiated using primers specific to gene 9 encoding VP7 glycoprotein and tissue culture derived reference serotypes G1-G4, G8, G9 of human origin and G5, G6, G10, SA-11(G3) of animal origin (Fig 2.). Phylogeny analysis of different rotaviruses based on Gene 4, Gene 5 and Gene 8 is shown in fig. 3

## *Non Group A rotaviruses*

Three fecal specimens from adult diarrhoea cases that occurred in Surat were received at NIV, Pune. Specimens were negative for Group A rotavirus by ELISA and hence tested by RNA-PAGE and RT-PCR. Typical group B RNA pattern was observed on PAGE in all three specimens.

The comparison of partial sequences derived for the genes 4, 5 and 8 showed nucleotide identity 91%, 95% and 94% with ADRV respectively. The highest identity (97%) was noted with Bangladesh 373 (except for gene 8), NIV 2000 and CAL-1 strains for all 3 genes.



RNA Pattern typical of group B Rotavirus  
Lane 1: SA-11 (group A rotavirus)  
Lane 2-4: Surat specimens 04622,04623,04624

**Figure 2 : Polyacrylamide gel electrophoresis pattern of group B rotavirus**

## **Future plan**

Characterization of nontypeable/multireactive fecal specimens will be continued further.

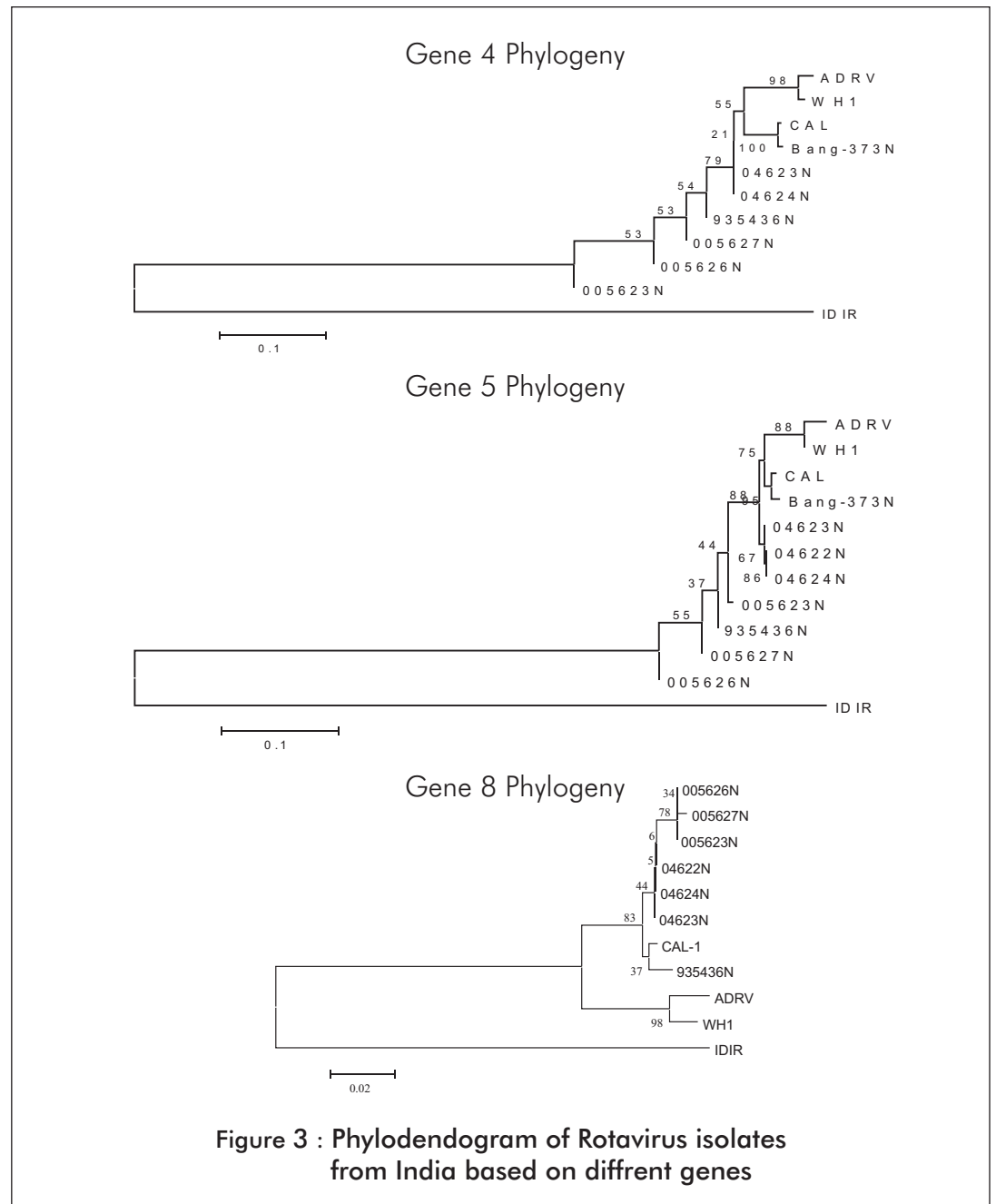


Figure 3 : Phylogenetic tree of Rotavirus isolates from India based on different genes

# *Rotavirus*

## **Preparation of egg yolk antibodies against rotaviruses for passive immunization of humans and poultry**

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

### **Objectives**

- To develop an ELISA specific for detection of avian rotavirus and its antibodies.
- To prepare immunoglobulins against human rotaviruses in egg yolk.

### **Achievements**

An indirect sandwich ELISA system that can be used for detection of avian rotavirus antigen and antibody was standardized by using hyper immune rabbit anti avian (CH2) rotavirus antiserum and rabbit anti CH-2 IgG horse raddish peroxidase conjugate

A series of ELISA tests was carried out to check the quality of standard rotavirus stocks (HRV1-4 and HRV-9) that are proposed for immunization of birds. The stocks that have very low virus titre are being passaged in MA104 cell line. Besides, contamination with other routinely used standard viruses is being ruled out by testing virus stocks by multiplex PCR.