

10. Studies on Viral Pathogens

10.1 Studies on molecular epidemiology, genomic diversity, and interspecies transmission of human and animal rotaviruses

Investigator: Dr. T. N. Naik.

Molecular characterization of bovine group A rotavirus G3P[3] strains

The global scenario of rotavirus infection in humans has been clearly delineated with G1, G2, G3 and G4 as the major human genotypes. On the other hand, G3 strains are rare in calves, and till date, only two bovine G3 strains (J63 and CP-1) have been assessed at molecular level. Similarly the P[3] allele has been reported from humans, but remained undetected in cattle. During a surveillance study, four of 130 group A rotavirus strains, detected from diarrhoeic calves in Eastern India, exhibited G3P[3] specificities. Molecular characterization of VP7 and VP8* genes of one such strain [named as RUBV3 (RU: ruminant and BV: bovine)] revealed genetic relatedness to G3P[3] simian strain, RRV, and RRV related caprine strain, GRV. Strain RUBV3 had VP6, NSP4 and NSP5 genes of bovine origin. Therefore, the present study provides evidence for multiple reassortment events involving ruminant and simian strains, and to our knowledge, is the first report of detection of bovine group A rotavirus strains with G3P[3] specificities. G3 strains (including a G3P[3] strain from Southern India) have been reported in humans from India, and therefore, detection of the G3 VP7 allele in calves with diarrhoea might be indicative of reassortment and/or interspecies transmission events occurring under natural conditions.

Evidence for interstate transmission and increase in prevalence of bovine group B rotavirus strains with a novel VP7 genotype among diarrhoeic calves in Eastern and Northern states of India.

Interspecies transmission of group A rotaviruses between humans and animals have been extensively reported. On the other hand, studies on group B rotaviruses are rare. The present study was designed to probe into the epidemiology, genome nature, and genetic relatedness (to human strains) of group B rotaviruses in animals. During a surveillance study (2003–2005) in a cattle market in Kolkata city, state of West Bengal, Eastern India, 34 (13.0%) of 260 calves with diarrhoea were positive for group B rotaviruses (GBR) by RNA electrophoresis in polyacrylamide gels. Analysis of the partial VP7 gene sequence of 28 of these 34 GBR strains revealed maximum identities (97.7–99.5% at nucleotide level and 97.8–100% at amino-acid level) with the novel bovine GBR ‘Kolkata strains’ reported in a previous surveillance study (1.5%, n=192, 2001–2002) from the same cattle market, and shared low identities of 73.7–78.9% and 80.8–89.6%; 62.6–66.2% and 59.8–65.4%; 58.9–62.2% and 48.6–54.9% at nucleotide and amino-acid level with other bovine, human, and murine GBR. By phylogenetic analysis of VP7, the RUBV GBR strains clustered with the Kolkata GBR strains, corroborating their genetic relatedness to Kolkata GBR strains (Fig. 10.1.1). The GBR-infected calves were traced to districts in neighbouring states of West Bengal. Therefore, the present study reports a rapid increase in prevalence (13.0% in 2003–2005 against 1.5% in 2001–2002) of novel GBR strains among calves with diarrhoea, and provides evidence for interstate transmission of GBRs. In group A rotaviruses, continuous surveillance studies have revealed an increase in prevalence of rare and/or novel VP7

genotypes. A similar situation might exist for group B rotaviruses, and therefore, further surveillance studies should lay emphasis on detection and molecular characterization of group B rotaviruses along with that of group A rotaviruses in humans and animals. To our knowledge, studies tracing the probable route of spread of rotaviruses across states are rare and/or not reported.

Figure 3



Fig. 10.1.1 Phylogenetic analysis of the deduced VP7 amino acid sequences (partial, amino acid 23 - 247) of representative RUBV group B rotavirus strains with that of bovine, human and Murine group B rotaviruses. Phylogenetic tree was constructed by neighbor-joining method (random number generator seed of 111 and 1,000 bootstrap trials). Bootstrap values have been mentioned. The Kolkata strains are DB101, DB176 and DB180. The tree was rooted with cognate sequence of atypical human rotavirus strain J19. Abbreviations used: C-B:- Chhapra-Bihar; P-B:- Purnia-Bihar; D-J:- Dumka-Jharkhand; G-U:- Gorakhpur-Uttarpradesh.

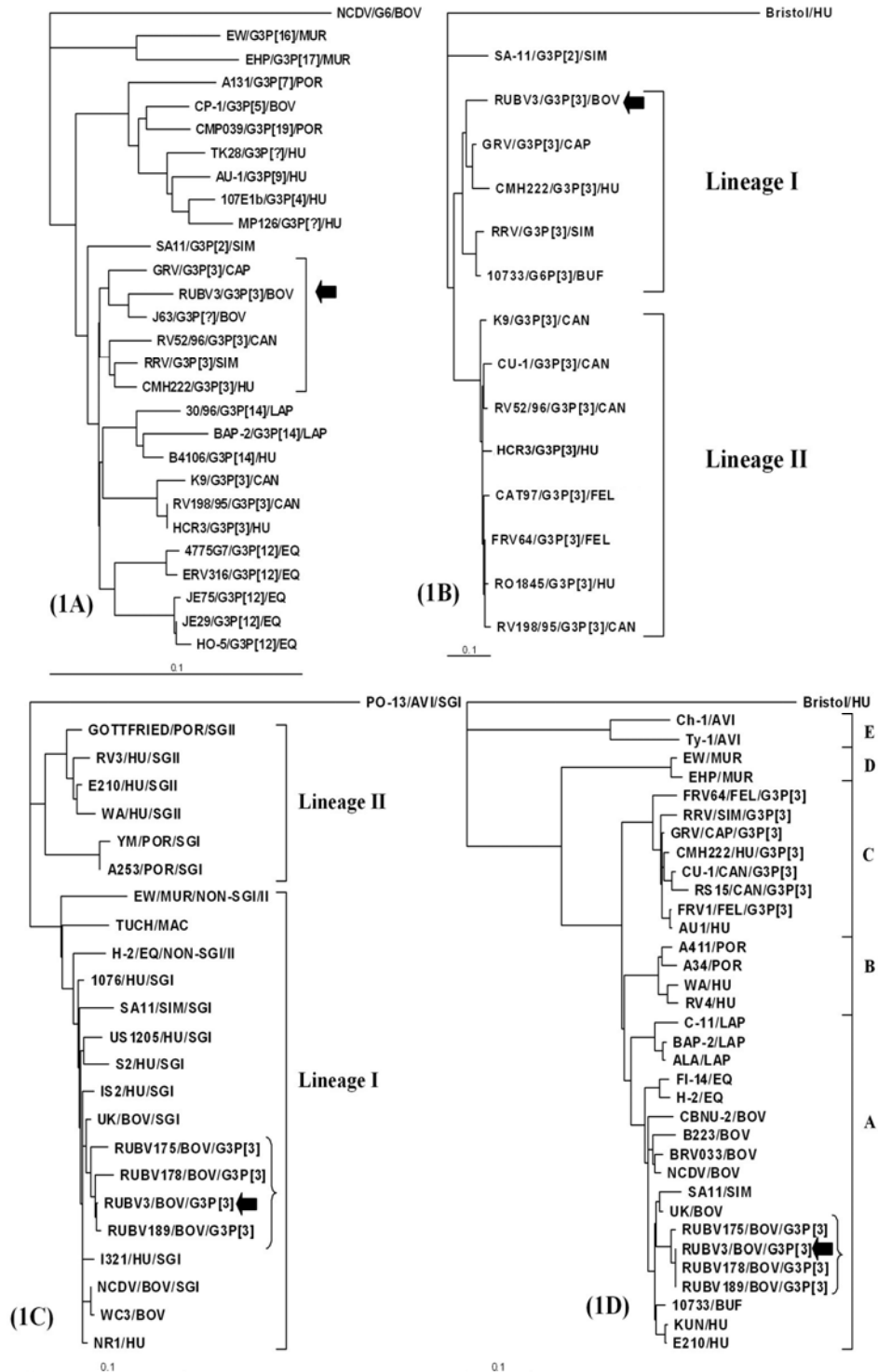


Fig. 10.1.2 Phylogenetic analysis of the VP7 (1A), VP8* (1B), VP6 (1C) and NSP4 (1D) amino acid sequences of bovine group A rotavirus G3P[3] strain RUBV3 with that of other group A rotavirus strains. The position of strain RUBV3 has been indicated (**▀**) in all the four figures.

10.2 Detection and molecular characterization of human caliciviruses, astrovirus and human picobirnavirus

Investigator: T. Krishnan

Studies on human caliciviruses by RT-PCR and sequencing to detect the presence of Norovirus and Sapovirus in Kolkata among acute watery diarrhoea cases

The genus *Norovirus*, a member of family *Caliciviridae*, is an important causative agent of acute gastroenteritis among different age groups, being detected in 95% of non-bacterial gastroenteritis outbreaks throughout the world. Norovirus persist in the environment, are resistant to chlorination and freezing and low dose of inoculum can cause infection. Owing to lack of an animal model, unsuccessful cultivation in a cell line, RT-PCR with specific primers is the chosen method for diagnosis of caliciviruses [*Norovirus* or *Sapovirus*]. As there are two Genogroups of Norovirus [I, II] that causes gastroenteritis, different sets of primers were used to specifically amplify the RNA dependent RNA polymerase (RdRp) gene segment in the viral genome of different strains. The purified PCR products were sequenced and analysis with different softwares showed distinct molecular diversity of the Noroviruses detected in Kolkata from strains reported earlier in different DNA databases. The Kolkata strains formed a new cluster in the phylogenetic tree showing high divergence from the typical strains belonging to each genogroup of Norovirus.

In the phylogenetic tree of Sapovirus one Kolkata strain V-1350 show closer homology with the Manchester type (X86560) whereas two other strains (J-20816, SD-31) formed a separate cluster on the tree with Parkville (U73124) and Stockholm type (AF194182) respectively. Although the two Kolkata strains had low sequence homology amongst themselves, the strain J-20816 showed 8.3% homology with Parkville type and 8% homology with Stockholm type; SD-31 showed only 0.7% homology with Parkville type and 2.8% homology with Stockholm type (Fig: 10.2.1 SLV).

In the phylogenetic tree of Norovirus Genogroup-1 eight Kolkata strains formed separate clusters, K-13830 strain showed 67.8% homology with Hualien type (AY903445) and K-8802 formed a new branch in the tree showing 17.9% homology with Saitama type (AB112128) and 28.9% homology with Berlin type (DQ340091). The separate cluster of Kolkata strain in the tree shows new genotype of Norovirus Genogroup-1 (Fig: 10.2.2 NV1).

In the phylogenetic tree of Norovirus Genogroup2 two Kolkata strains K-9422 & K-9335 formed the cluster with Thailand type showing 3.5% and 3.8% homology respectively. The other two Norovirus strains V-1360 & V-518 gives rise to separate branches in the phylogenetic tree. (Fig 10.2.3 NV2)

Molecular characterization of human astroviruses causing acute watery diarrhoea among children detected from Kolkata

Human astroviruses are recognized as another important etiological agent of viral gastroenteritis, after rotaviruses. A human astrovirus strain, detected from faecal samples collected from Dr. B.C Roy Memorial Hospital from Kolkata was selected for its complete genome characterization. Several sets of primers were designed to amplify and sequence the complete capsid gene (ORF2) and the serine protease gene (ORF1a). Upon sequence analysis

of the individual amplicons, it was found that the capsid gene sequence of a Kolkata strain had maximum identity to an astrovirus strain “816/93”, from Japan (Table 1 Astro), except for a small fragment of 171aa (aa192-362, nt574 to 1086 of ORF2) which was highly heterologous and did not show any homology to any known astroviruses. This unique sequence stretch was further verified by SimPlot (Version 3.5.1) (Figure 1 Astro). Molecular characterization of the two other genes (ORF1a and ORF1b) would bring more insight into the nature of the astrovirus strains detected in Kolkata.

Detection of Human picobirnavirus with large and small profile in Kolkata

Human picobirnaviruses with bisegmented double stranded RNA genome of ‘large’ electropherotype profile were found to be associated with acute watery diarrhoea, even in a 60 year old adult in Kolkata.

Human picobirnaviruses with bisegmented double stranded RNA genome of ‘small’ electropherotype profile were also found to be associated with acute watery diarrhoea. (Fig 10.2.4 PBV)

10.3 Surveillance of circulating respiratory viruses in Kolkata with special focus on Influenza Virus

Investigators: Mamta Chawla Sarkar, T.N. Naik

Influenza virus is a major human pathogen that causes epidemics and pandemics with increased morbidity and mortality, especially in the elderly and those with pre-existing medical conditions. In recent years, outbreak of highly pathogenic avian influenza has been reported worldwide, among poultry and other animals, with >150 human cases. The major problem in fighting influenza is the high genetic variability of the virus, resulting in the rapid formation of variants that escape the acquired immunity against previous virus strains. Moreover, the importance of viruses causing respiratory tract infection has not been studied systematically in Kolkata, West Bengal. This surveillance study will provide crucial information regarding circulation of Influenza strains in Kolkata and also the laboratory setup will be useful in case of outbreak of Avian Influenza in Eastern India.

A total of 720 throat/nasal swabs were collected from children and a few adults visiting outpatient ward presenting with respiratory illness at Dr BC Roy Memorial Hospital and R G Kar Medical College and Hospital, Kolkata. The samples were collected from the symptomatic patients who fulfill the WHO case definition of Influenza virus infection. The samples are screened for a panel of respiratory viruses such as Influenza A, B and C viruses, Respiratory Syncytial Virus (RSV) and Metapneumo Virus by multiplex seminested PCR and genotyped by type specific PCR and confirmed by sequencing. Sixteen percent of the 720 samples were identified as Influenza A and B, 12% as RSV and 3% as metapneumo virus during 2006-2007.

Table 1. Comparison of the deduced amino acid sequence homology of the six amplicons of capsid gene of Kolkata strain, V1347 with other hitherto reported astrovirus strains of different serotypes.

Primer pairs used in RT-PCR experiments to amplify the different fragments of capsid gene	S1 Japan_816/93_BAE97448	A88/2 Newcastle S1 AAC60723	Oxford S1 CAA81032	Oxford S1 AAC34717	Dresden S1 AAW51882	Oxford S2 AA62427	Berlin S3 AAD28540	Ehime S4 BAA93441	Dresden S4 AAW51879	Oxford S4 CAA83947	Brazil S4 AAY84779	Oxford S5 AAA56750	Brazil S5 AAY46274	China S5 BAA90310	Oxford S6 CAA86616	Oxford S7 AAK31913	Mexico S8 AAF85964
PRECAP, Mon270 [743bp; 247aa] [Includes 31aa of ORF1b+ 216aa of ORF2]	92	88	88	90	89	83	84	82	82	77	82	83	83	84	83	82	83
RB1, NC1 [512bp; 171aa]	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
NCAP1, C4 [190bp; 63aa]	100	100	98	98	100	83	91	81	81	81	81	86	83	84	86	86	81
CAP4, C5 [449bp; 149aa]	100	95	96	93	95	54	71	50.3	60	50	50	52	52	44	60	66	63
CAP5, CAP3 [675bp; 225aa]	93	90	90	88	89	47	53	34	35	34	32	86	47	NC	42	51	48
AstS1, CAP3 [210bp; 69aa]	100	93	92	88	94	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC

NC: Non-comparable