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## Molecular characterization and phylogenetic analysis of Bovine and Human Rotavirus

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**Abstract**

Rotavirus infection is a viral disease, caused by Rotavirus belonging from *Reoviridae* family. Young ones of animals and human are most susceptible to this disease. However, adult animals and human with infection may also show similar signs at some extent. The disease is infectious-communicable among buffalo and human, there is a wide range of morbidity and mortality. In the present study, 49 faecal samples from buffalo and 55 stool samples from human, showing the signs and symptoms related to Rotavirus infection were collected. It was observed that out of 49 faecal samples from buffalos, 10 samples and out of 55 human stool samples, 21 samples were found positive for RNA-PAGE. Representative PAGE positive fecal and stool samples, 6 each were selected for RT-PCR and out of 6 fecal samples, 3 samples were found positive and out of six stool samples, 3 samples were confirmed positive for Rotavirus infection. One sample from buffalo and one from human were sent for sequencing. The phylogenetic analysis was carried out and it was confirmed that the positive samples were showing relatedness with the Rotavirus sequences taken from GenBank. As per the sequences brought, it was confirmed that the virus was originating from the eastern part of India (West Bengal). Therefore it is indicative of circulation of virus from state to state in India can be possible. The buffalo sample was showing relatedness with human rotavirus sequence, which is indicative of sharing of Rotavirus between human and animal.

**Keywords:** rotavirus, human, buffalo, phylogenetic

**Introduction**

Rotavirus is the most common cause of severe gastroenteritis in animals and is recognized as the single most significant cause of severe gastroenteritis, malnutrition and diarrhoea, affecting a wide range of mammalian and avian species (Estes MK and Kapikian AZ, 2007) [1]. Rotavirus, a genus within the family Reoviridae, is recognized as the most important viral etiological agent of severe diarrhoeal illness in humans, young animals and birds worldwide (Robert *et al.*, 2001) [2] and Fenner *et al.*, 1993) [3]. Group A rotaviruses are the major pathogens causing acute gastroenteritis in infants and a wide range of animals, including birds. Rotavirus-induced diarrhoea is a serious public health problem worldwide, responsible for more than 600000 child deaths each year (Parashar *et al.*, 2006) [4]. The virus is non-enveloped, icosahedral, double-stranded RNA (ds RNA) with a characteristic 11 segmented genome enclosed in a triple layered protein capsid (Ciarlet and Estes 1999) [5]. Poor hygienic conditions are considered to be one of the predisposing factors of neonatal diarrhea, leading to severe dehydration followed by high mortality rate in developing countries (Alam *et al.*, 2011) [6]. Diarrhea, the third leading killer of children in India today, is responsible for 13% of all deaths in children <5 years of age and kills an estimated 300,000 children in India each year (Bassani *et al.*, 2010) [7]. Rotavirus is the leading cause of severe diarrhea in Indian children under 5, and has been projected to cause 457,000 to 884,000 hospitalizations, 2,000,000 outpatient visits, and 122,000-153,000 deaths annually (Tate *et al.*, 2009) [8].

Therefore, the present study was conducted considering the zoonotic importance of Rotavirus, targeting VP7 gene. The molecular characterization and phylogenetic analysis of the positive PCR samples of buffalo calves and human newborn was performed and thereby confirmed Rotavirus infection.

## Material and Method

### Sample collection

Forty nine fecal samples from buffalo calves suffering with diarrhea were collected in to sterile 50 ml sample collection plastic containers. The area for the sample collection covered was local dairy farms, animal farms and the government animal hospitals in and around Parbhani district of Maharashtra, India. Similarly, 55 stool samples of children suffering with diarrhoea were also collected from civil hospitals, primary health centres and district civil hospital, from December 2017 to June 2018. The samples were transported on ice and stored at -20° C till further processing and were labeled with location & date of collection (Figure no. 01)

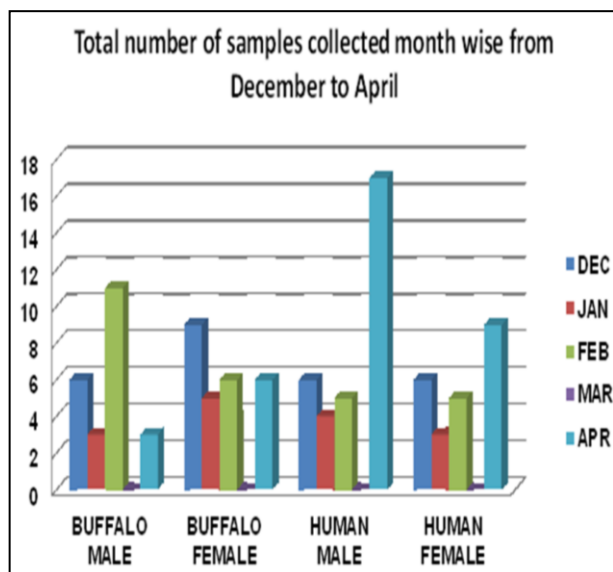


Fig 1: Graphical representation of sample collection (month wise)

### Extraction of Rota viral ds RNA

The dsRNA of Rotavirus was extracted from faecal and stool samples using TRIzol method (Gill *et al.*, 2017) [9] as per the manufacturer's protocol. The isolated RNA was then used for further downstream applications.

### Polyacrylamide Gel Electrophoresis:

The RNA extracted from the faecal sample was subjected to ribonucleic acid-poly acrylamide gel electrophoresis (RNA-PAGE) as per the procedure described by Fernanda *et al.*, (2012) [10], Laemmli (1970) [11] and Herring *et al.*, (1982) [12]. The concentration of resolving gel and stacking gel was 7.5% was 5% respectively. The gel was constantly run for 15 min at 60V followed by 110 V for approximately 4 hours.

### Silver staining of dsRNA in polyacrylamide gel:

For rapid detection of sample positive for RV, the typical pattern of migration of 11 different segments of rotavirus RNA were observed via silver staining as described by Bassam *et al.*, (1991) [13].

### Primer designing

Common Primers were designed for both human and buffalo samples to amplify VP7 gene (VP7 F:- 5'GGCTTTAAAAGPGAGAATTTCCGTCTGG3', VP7 R:- 5'CACATCATACAATTCTAATTAAG3') using Primer3 online software (Kamel A. Abd-Els alam, 2003) [14].

### Reverse transcriptase-polymerase chain reaction

One step RT-PCR kit (Invitrogen) was used for the amplification of VP7 gene of amplicon size 1062bp. The reverse transcriptase polymerase chain reaction (RT-PCR) was conducted for PAGE positive samples as per manufacturer's protocol and the cyclic conditions were: cDNA synthesis at 50° C for 10 min, Initial denaturation at 98° C for 2 min one cycle respectively followed by 35 cycles of denaturation at 98° C for 10 sec, Annealing at 52° C for 10 sec, extension at 72° C for 30 sec and one cycle of the final extension at 72° C for 5 min. The samples were held at 10° C. The PCR products were stored in fridge at 5° C and run on agarose gel for the check up of the amplicon size and amplicon quality.

### Agarose gel electrophoresis

The PCR amplified products were analyzed on agarose gel electrophoresis (AGE) as per Mohan *et al.*, (2014) [15]. The amplified products were analyzed by electrophoresis on 1% agarose, and analyzed and photographed by a gel documentation system (BIO-RAD, USA). One RT-PCR positive, buffalo sample & one human sample was sent for sequencing. The obtained sequences from the field samples were subjected to BLAST analysis with GenBank database sequences using BLASTn algorithm available at NCBI blast to confirm the presence of sequence specific to Rotavirus.

### Splitstree phylogeny

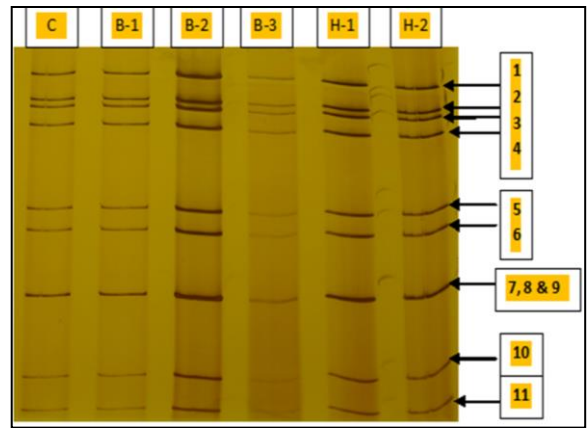
The elucidation of the genetic diversity of different Rotavirus strains was carried out by haplotype network that was inferred with SplitsTree4 Programme (version 4.13.1) using neighbour-net networks (Huson and Bryant, 2006) [16].

### Result and discussion

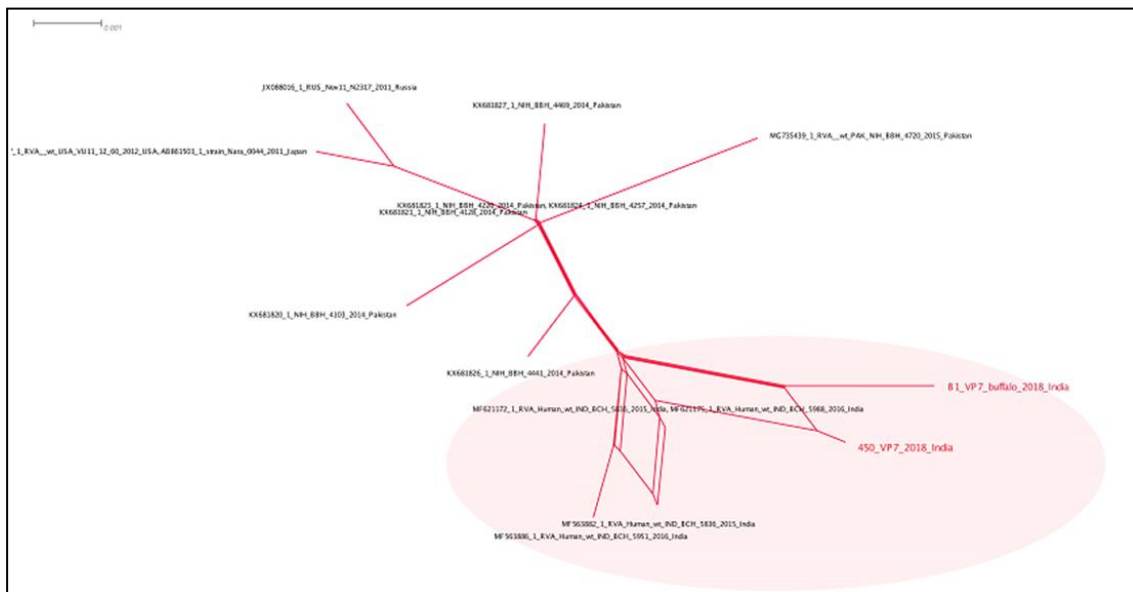
In the present study buffalo fecal samples (n=49) and human stool samples (n=55) were screened for RV using RNA PAGE. It was observed that 10 buffalo samples (20.4%) and 21 (38.18%) human stool samples were found positive for rotavirus by RNA-PAGE. The epidemiological information reveals that rotavirus distribution was associated with some of factors like age, gender, housing type, season and source of water in buffalo calves. The findings in the present study indicate that, the calves less than 3 months of age were infected maximally with rotavirus. Among the rotavirus positive cases, 60% (n= 6) were male buffalo calves and 40% (n= 4) were female buffalo calves. The result reveals a significant association between diarrhea cases and gender of calf i.e. prevalence of rotavirus is maximum in male calves than female calves. All the positive samples showed a typical migration pattern of 4:2:3:2 type., which is of group A (mammalian) rotavirus, with segments 2, 3 and 4 migrating close together, segments 7, 8 and 9 migrating as a triplet and segments 10 and 11 apart (Fig 02). The VP7 genes of three buffalo and three human samples were successfully amplified by RT-PCR. The amplified genes showed the expected size of 1062 bp. The PCR product of one human and one buffalo representative samples were sent for sequencing and sequences were brought. Using BLAST, the sequences of the both samples (one human and one buffalo) obtained were confirmed and compared to the sequences available in the GenBank database. The resulted sequences were also deposited in the GenBank database with the Accession numbers granted MH769747 and MH769746, respectively.

The sequences of this study were aligned with the representative of sequences from human and other animals and phylogenetic analysis was performed to draw the most possible tree. The sequencing reveals in most common recent ancestor of two strains in given data found placed from the eastern part of India (West Bengal).

The Splitstree phylogeny showed that the phylogenetic network obtained for the human and buffalo sample were clustered together. (Fig 03) This is indicative of the closely relatedness among buffalo and human Rotaviruses in the given dataset. It also suggests circulation of virus among species. This interspecies transmission may be facilitated by poor hygienic conditions, close proximity of humans to livestock.



**Fig 2:** page positive buffalo (B) and human (H) samples (1-11 indicates segments of the RNA, C: positive control)



**Fig 3:** Splitstree phylogenetic tree showing clustering of buffalo (B1) and human sample (450)

**Conclusion**

In this study the immense diversity of Rotavirus has been affirmed and there is evidence of the same strain of this viruses circulating in India. From the phylogenetic study it was confirmed that nucleotide sequences of Rotavirus of buffalo and human from Parbhani, India are closely related to each other and show the relatedness with Rotaviruses of other countries too and the most common recent ancestor of two strains in given data found placed from West Bengal, India. The buffalo and human rotavirus samples were clustered together and after sequence analysis, it was found that the buffalo positive sample showed its relation with human rotavirus. This is indicative of trans-species transmission of rotavirus can occur. This work indicates that the migration of rotavirus throughout the country is possible. The increasing demand of purchase of animals for milk production is responsible for the sharing of the strains from the eastern part of country to other, especially in the Maharashtra.

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