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Zooanthroponotic transmission of rotavirus in Haryana State of Northern India

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Summary. – Rotaviruses are the major cause of severe gastroenteritis and mortality in young children and animals. Due to segmented nature of dsRNA genome and wide host range, vast genetic and antigenic diversity exists amongst different isolates of rotaviruses. A total of 230 fecal ovine and caprine samples collected from organized farms and villages in Haryana were screened for rotavirus detection. Samples were screened by latex agglutination test and RNA-PAGE followed by RT-PCR and nucleic acid sequencing. The latex agglutination test showed 25 newborn lamb and 4 kid fecal samples positive for rotavirus. However, RNA-PAGE showed only 9 lamb fecal samples positive for rotavirus. All the samples were subjected to RT-PCR employing vp4 and vp7 gene specific primers of group A rotavirus of ovine, bovine and human origin. Only two samples from lamb (Sheep18/Hisar/2013 and Sheep22/Hisar/2013) showed vp4 and vp7 gene specific amplification with human group A rotavirus (GAR) specific primer. However, they did not show any amplification with ovine and bovine rotavirus specific primers. The nucleotide as well as deduced amino acid sequence analysis of vp4 gene of these isolates showed >98/97% and vp7 gene >95/94% nt/aa identity with human GAR from different regions of the world. Based on nucleotide similarity search, Sheep18/Hisar/2013 and Sheep22/Hisar/2013 isolates were genotyped as G1P[8] and G1P[4]. Phylogenetic analysis also confirmed that these isolates were clustered closely with human rotaviruses from different regions of the world. Earlier, higher prevalence of human rotaviruses was reported from the sample collecting area. The amplification of ovine samples with human rotavirus gene specific primers, sequence identity and phylogenetic analysis strongly suggests the zoonotic transmission of human GAR to sheep.

Keywords: ovine rotavirus; RNA-PAGE; RT-PCR; latex agglutination test; vp4 gene; vp7 gene

Introduction

Rotaviruses are the most important etiological agents of acute, dehydrating gastroenteritis in young animals and humans. These pathogens are transmitted via the fecal-oral route with peak disease frequency occurring in cooler and winter months (Estes *et al*., 2007). Rotaviruses belong to the family *Reoviridae* under the genus *Rotavirus*. The infectious virion is non-enveloped. The rotaviral genome is composed of 11 dsRNA segments surrounded by three protein shells i.e. core, inner capsid and outer capsid (Matthijnssens *et al*., 2008). The inner capsid is composed of group specific VP6 protein, encoded by gene segment 6. The outer capsid consists of two main proteins VP4 and VP7. The VP4 protein is a minor component, encoded by gene segment 4 whereas VP7 protein is a glycoprotein, encoded by genome segments 7, 8 or 9 depending upon the strain of the virus. Both VP4 and VP7 proteins are responsible for dual classification of rotaviruses within P and G types (Park *et al*., 2006; Khamrin *et al*., 2007). Rotaviruses have been classified in eight distinct groups (A to H) based on inner viral capsid protein 6 (VP6) (Marthaler *et al*., 2013). Out of which group A rotaviruses (GAR) have been found to be most common etiological agents of diarrhoea in humans and animals. GAR genogroups such as Wa, DS-1 and AU-1 have been widely studied

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Abbreviations: GAR = group A rotavirus

for identification of reassortment as well as interspecies transmission of rotaviruses between animals and humans (Matthijnssens *et al.*, 2008). Based on sequences of genes encoding the serotype antigens the rotavirus strains are now classified into G-types (VP7) and P-types (VP4) (Estes *et al.*, 2007). Recently, a total of 27 G-type and 35 P-type strains have been reported (Matthijnssens *et al.*, 2011).

It has been reported earlier that rotavirus diarrhoea in lambs and kids is highest in 3–7 weeks of age (Khafagi *et al.,* 2010). However, still there is scarcity of information regarding prevalence of ovine rotavirus strains globally. The ovine rotavirus strains mainly belong to serogroup A (Kaminjolo and Adesiyun, 1994) and B (Theil *et al.*, 1995). The most common rotavirus genotypes reported are G6, G8, G10 (in bovine) and G1, G3, G5, G9 (in human) and P types are P1, P11 (in bovine) and P9, P14 (in ovine and humans). The G9 serotype was also reported in ovine (Gazal *et al.*, 2012; Papp *et al.*, 2014).

GAR associated with diarrhoea in sheep and goats have been reported from different parts of the world (Lee *et al.*, 2003; Mendes *et al.*, 1994; Pratelli *et al.*, 1999). Due to the segmented nature of genome, rotaviruses can exchange (reassort) genes during co-infections and generate a new, possibly more dangerous virus strain (McDonald *et al.*, 2009). Some rotavirus strains are possibly transmitted to different species as a whole genome, suggesting that interspecies transmission may occur frequently in nature (Nakagomi *et al.*, 1990). The reassortant strain of canine, feline and simian GARs from Korean goat was isolated and vp4, vp7 and nsp4 gene sequences were assigned to P[3], G3 and E3 genotypes respectively (Lee *et al.*, 2003). The vp4 and vp7 genes of GAR of caprine strain from Italy was assigned to P[1] and G6 genotypes respectively (Pratelli *et al.*, 1999). The interspecies transmission of animal rotaviruses to human or *vice-versa* could be possible because of close contact between animals and humans and it may augment interspecies infections and genetic reassortment during co-infection with rotavirus strains from different host species. The reassortment may further result in evolution of novel or atypical rotaviruses (Palombo, 2002). There are several evidences found for the interspecies transmission of rotaviruses (Wani *et al.*, 2003).

Interspecies transmission indicates its zoonotic importance. The data on characterization of rotavirus from small ruminants is very scanty in literature. Therefore in this paper screening of diarrheic fecal samples of sheep and goat for identification and molecular characterization of rotaviruses by RNA-PAGE, latex agglutination test and sequence based studies have been carried out.

Materials and Methods

Fecal samples collection and preparation. A total of 230 fecal samples were collected over a period of nine months (July 2012 to March 2013) from diarrheic sheep, goats, neonatal lambs and kids from organized farms of Lala Lajpat Rai University of Veterinary and Animal Sciences Hisar, Veterinary hospital Danoda, Jind District and village Dhanana, Bhiwani district. A 10% fecal suspension (w/v) was prepared in phosphate buffered saline (0.01 mol/l, pH 7.4; Sigma, USA). The fecal suspensions were centrifuged at 12,000 rpm for 10 min to remove coarse particulate matter and the upper aqueous layer was transferred into a fresh tube. The suspensions were stored at -20°C until further use.

Screening of faecal samples by latex agglutination test. All the faecal samples were screened by commercially available HiRotavirus™ latex test kit (HiMedia, India). The samples were processed and analysed as per the manufacturer's instruction.

Viral nucleic acid extraction and RNA-PAGE. Viral nucleic acid was extracted from processed fecal suspensions by GIT lysis method (Chomoczynski and Sacchi, 1987). All the fecal samples were initially screened by RNA-PAGE using 5% stacking and 10% resolving gels for presence of rotaviral nucleic acid (Laemmli, 1970). The polyacrylamide gel was subsequently stained with silver nitrate (Svensson *et al.*, 1986).

Reverse transcription PCR (RT-PCR). Reverse transcription of viral RNA was carried out in 20 µl reaction volume using random decamer primer (Ambion, USA) and reverse transcriptase enzyme

Table 1. List of oligonucleotide primers for amplification of vp4 gene and P typing of group A human rotavirus (Gentsch *et al.*, 1992)

Primers	Genotype	Sequence	Expected product size
Con3 (11-32)		5'-TGG CTT CGC CAT TTT ATA GAC A-3'	876 bp
Con2 (868-887)		5'-ATT TCG GAC CAT TTA TAA CC-3'	
Type specific			
1T-1 (340-356)	P8	5'-TCT ACT TGG ATA ACG TG-3'	345 bp
2T-1(475-494)	P4	5'-CTA TTG TTA GAG GTT AGA GT-3'	483 bp
3T-1(259-278)	P6	5'-TGT TGA TTA GTT GGA TTC AA-3'	267 bp
4T-1(386-402)	P9	5'-TGA GAC ATG CAA TTG GA-3'	391 bp
5T-1(577-594)	P10	5'-ATC ATA GTT AGT AGT CGG-3'	583 bp

Table 2. List of oligonucleotide primers for amplification of vp7 gene and G typing of group A human rotavirus (Taniguchi *et al.*, 1992)

Primers	Type	Sequence	Expected product size
CC1(1039-1062)		5'-CAC ATC ATA CAA TTC TAA TCT AAG-3'	1062 bp
CC2(1-28)		5'-GGC TTT AAA AGA GAG AAT TTC CGT CTG G-3'	
Type specific			
SS1 (314-335)	G1	5'-CAA GTA CTC AAA TCA ATG ATG G-3'	749 bp
SS2 (406-425)	G2	5'-GAC TAC AAT GAT ATT ACT AC-3'	657 bp
SS3 (481-498)	G3	5'-GAC GCG ACG TTG CAA TTG-3'	582 bp
SS4 (669-688)	G4	5'-TCA AAC GAC AAA TAC AGC TA-3'	394 bp
SS8 (178-198)	G8	5'-GTC ACA CCA TTT GTA AAT TCG-3'	885 bp
SS9 (757-776)	G9	5'-CTA GAT GTA ACT ACA ACT AC-3'	306 bp

(Stratagene, USA) as per the manufacturer's instruction. The cDNAs were used for amplification of vp4 and vp7 gene using bovine and ovine GAR specific primers (Isegawa *et al.*, 1993; Khafagi *et al.*, 2010). The PCR was also performed using vp4 (Gentsch *et al.*, 1992) and vp7 (Taniguchi *et al.*, 1992) gene specific primer of human GAR (Table 1 and 2). PCR reactions were carried out in 25 µl volumes containing 2 µl cDNA, 0.75 µl DMSO and 10 µmol of each primer along with 5 µl of PCR mixture consisting of 5 µl 5x Phusion HF buffer, 0.5 µl 100 mmol/l dNTPs, 0.25 µl phusion high-fidelity DNA polymerase (2 U/µl) (NEB, UK). The nuclease free water was added to make final volume 25 µl. The PCR reactions were performed on thermal-cycler (Bio-Rad, USA). The cycling conditions for each primer pair were kept according to published information. The PCR products were visualized in 1% agarose (LifeTech, USA) gel containing ehidium bromide under UV transilluminator (Biorad, USA).

Automated nucleotide sequencing. The PCR products of vp4 and vp7 gene were allowed for nucleic acid sequencing using automated DNA sequencer ABI PRISM™ 3130xL at Department of Animal Biotechnology, LUVAS Hisar (Haryana). Nucleotide sequence data obtained were analyzed using BLASTN 2.3.1+ (Zhang *et al.*, 2000). Multiple sequence alignment and calculation of percent identity of nucleotide as well as deduced amino acid sequences of vp4 and vp7 gene of small ruminant rotaviruses and representative isolates of all well characterized genotypes of GAR isolates were done using Bioedit 7.2.5 (Hall, 1999). The phylogenetic analyses of vp4 and vp7 gene sequences of rotaviruses from various species from different regions of the world were done using neighbor joining method of MEGA 5 software with 1000 bootstrap values (Tamura *et al.*, 2011).

Results

Screening of faecal samples for presence of rotavirus by latex agglutination test

The latex agglutination test showed that out of 230 faecal samples (164 ovine and 66 caprine samples), 25 new-born lamb and 4 kid faecal samples were positive for rotaviruses (Fig. 1).

Detection of rotaviruses from diarrheic faecal samples by RNA-PAGE

All the fecal samples were again screened by RNA-PAGE for rotavirus detection. Out of these, 9 newborn lamb diarrheic fecal samples were found to be suspected for presence of rotaviruses. These samples were among the 25 samples found to be positive by latex agglutination test. RNA-PAGE revealed very faint bands hence characteristic migration patterns (4:2:3:2) of rotavirus were not clearly visualized. However, kid fecal samples did not show any indication for presence of rotavirus.

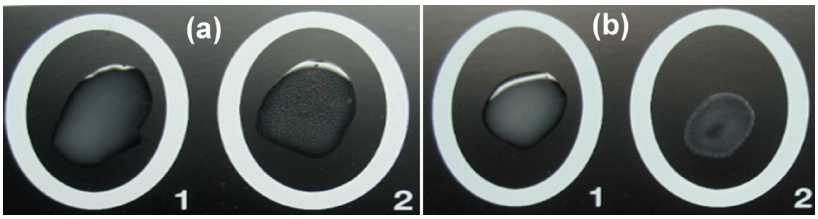

Fig. 1

Latex agglutination test using HiRotavirus™ latex test kit
(a) 1: Negative control; 2: Sheep18/Hisar/2013; **(b)** 1: Negative control; 2: Sheep22/Hisar/2013.

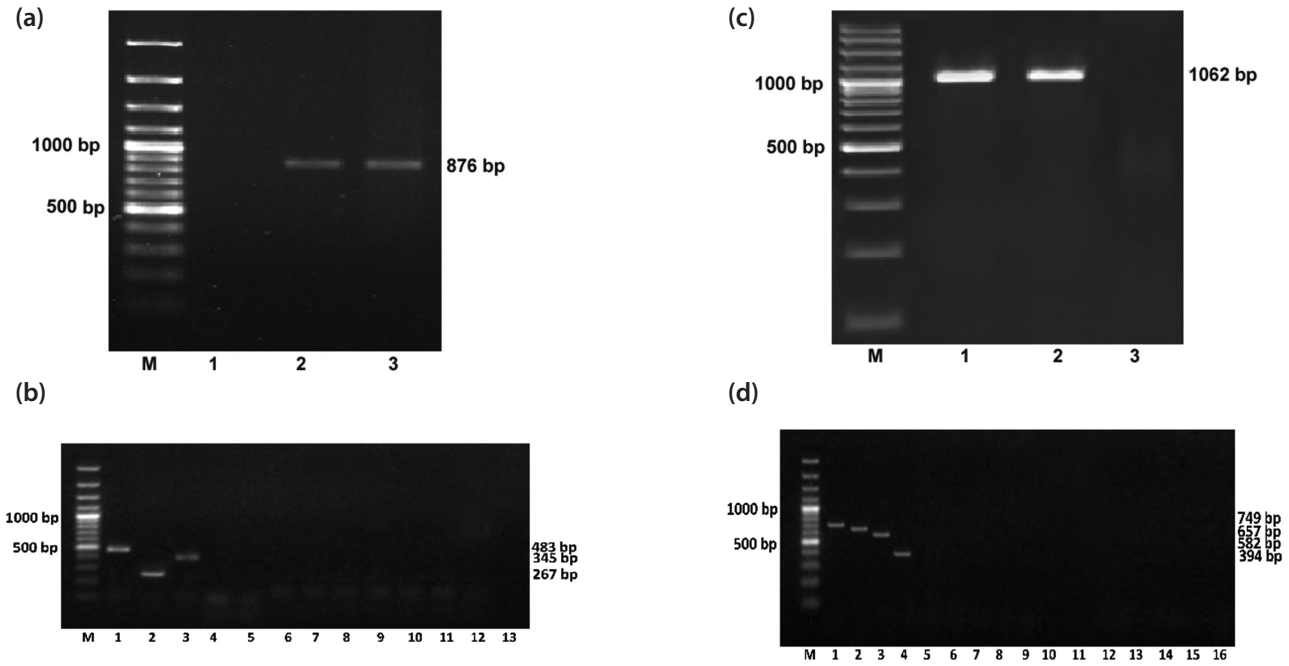

Fig. 2

PCR amplification of ovine rotavirus genes

(a) Vp4 gene amplification of field samples yielding 876 bp products. Lanes: M: 100 bp DNA ladder; 1: negative control; 2: Sheep18/Hisar/2013; 3: Sheep22/Hisar/2013. **(b)** Vp4 genotyping PCR with human GAR primers. Lanes: M: 100 bp DNA ladder; 1–3: positive control human rotaviruses with P4, P6 and P8 genotype primers respectively; 4–8: Sheep18/Hisar/2013 strain with genotype primers P4, P6, P8, P9 and P10 respectively; 9–13: Sheep22/Hisar/2013 strain with genotype primers P4, P6, P8, P9 and P10 respectively. **(c)** Vp7 gene amplification of field samples yielding 1062 bp products. Lanes: M: 100 bp DNA ladder; 1: Sheep18/Hisar/2013; 2: Sheep22/Hisar/2013; 3: negative control. **(d)** Vp7 genotyping PCR with human GAR primers. Lanes: M: 100 bp DNA ladder; 1–4: positive control human rotaviruses with G1, G2, G3 and G4 genotype primers respectively; 5–10: Sheep18/Hisar/2013 strain with genotype primers G1, G2, G3, G4, G8 and G9 respectively; 11–16: Sheep22/Hisar/2013 strain with genotype primers G1, G2, G3, G4, G8 and G9, respectively.

Reverse transcription PCR (RT-PCR)

The ovine and bovine specific vp4 and vp7 gene primer did not show amplification with viral cDNA of any samples. Two samples from newborn lamb (Sheep22/Hisar/2013 and Sheep18/Hisar/2013) yielded a specific PCR product of 876 bp and 1062 bp corresponding to vp4 and vp7 gene with human GAR primers (Fig. 2a,c).

Analysis of nucleotide sequence data

The vp4 gene sequences of Sheep22/Hisar/2013 and Sheep18/Hisar/2013 were deposited to Genbank with accession numbers KJ921616 and KJ921617 and vp7 gene with KJ921619 and KJ921620 respectively. The BLASTN 2.3.1+ search of vp4 and vp7 gene sequences of Sheep22/Hisar/2013 and Sheep18/Hisar/2013 isolates showed maximum identity (>98%) with G1P[4] and G1P[8] genotype respectively with human GAR from different parts of the world. The phylogenetic analysis also revealed the closeness of these isolates with human GAR (Fig. 3 and 4).

Discussion

Rotaviruses are major cause of diarrhoea in young animals and humans. In this study we have selected 230 fecal samples from small ruminants for rotaviral molecular biology study. The latex agglutination test revealed that 25 (15.24%) and 4 (6.06%) samples from lamb and kid respectively were found positive for rotaviruses. Prevalence of rotaviruses in lambs from 12.3% to 13.2% and in kids 7.9% by latex agglutination test has been reported earlier (Khafagi *et al.,* 2010; Gazal *et al*., 2012). However, only 5.48% (9) of lamb samples and no any kid samples were found positive by RNA-PAGE. Earlier it was reported that prevalence of rotaviruses in lambs was up to 9.8% by RNA-PAGE analysis (Gazal *et al*., 2012). The higher number of positive sample detection by latex agglutination test may be due to the false positive detection in comparison to gold standard, RNA-PAGE analysis (Singh and Jhala, 2011).

RT-PCR has been shown to be the most useful assay for diagnostics of common rotavirus types (Desselberger *et al.,* 2001). The RT-PCR based on vp7 gene for G typing

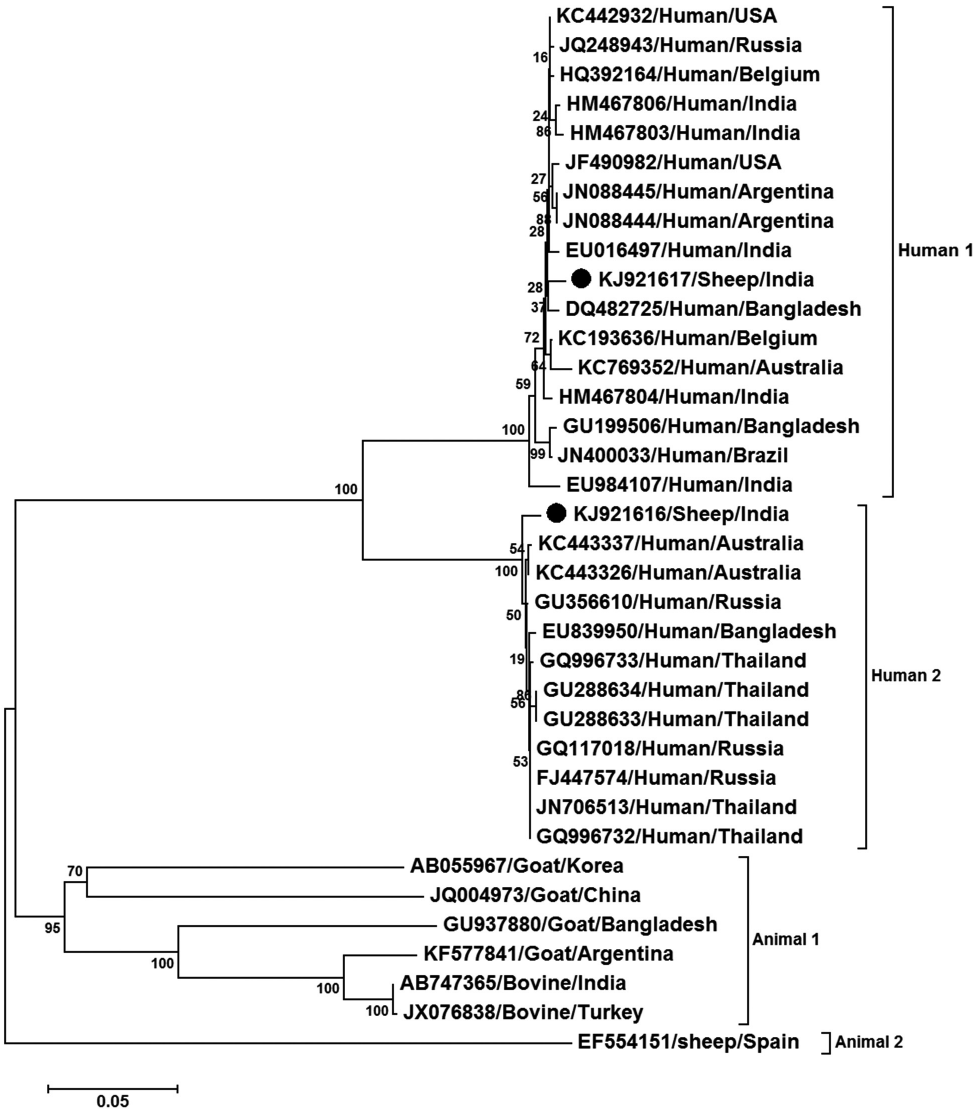

Fig. 3

Vp4 gene nucleotide sequence based phylogenetic analysis of Sheep18/Hisar/2013 and Sheep22/Hisar/2013 isolates along with other global isolates of group A rotavirus (• = isolates used in present study)

(Gouvea *et al*., 1990) and vp4 gene for P typing (Gentsch *et al*., 1992) have been in wide use. The Sheep22/Hisar/2013 and Sheep18/Hisar/2013 isolates showed amplification with human GAR vp4 and vp7 gene specific primers only. However, due to unknown reasons, they did not show any amplification in genotype (vp4 and vp7 gene) specific PCR for human GAR.

The pairwise BLASTN similarity search confirmed the genotype of Sheep22/Hisar/2013 and Sheep18/Hisar/2013 isolate as G1P[4] and G1P[8]. The G1P[4] and G1P[8] genotypes of GAR have been well characterized in human children (Abdel-Haq *et al*., 2003; Shintani *et al*., 2012). Previous study also revealed that G1-G4 serotypes and P[8] and P[4] genotypes of GAR are commonly reported from humans (Gentsch *et al*., 1992). The G1P[8] genotype of GAR was also reported from human diarrheic fecal sample in Nigeria (Ianiro *et al*., 2015).

The vp4 gene nucleotide as well as deduced amino acid sequence of Sheep18/Hisar/2013 and Sheep22/Hisar/2013 showed 85.3/86.7% nt/aa identity among them selves. It indicates that vp4 gene of these two isolates are distinct from each other. The vp4 gene of Sheep22/Hisar/2013 showed

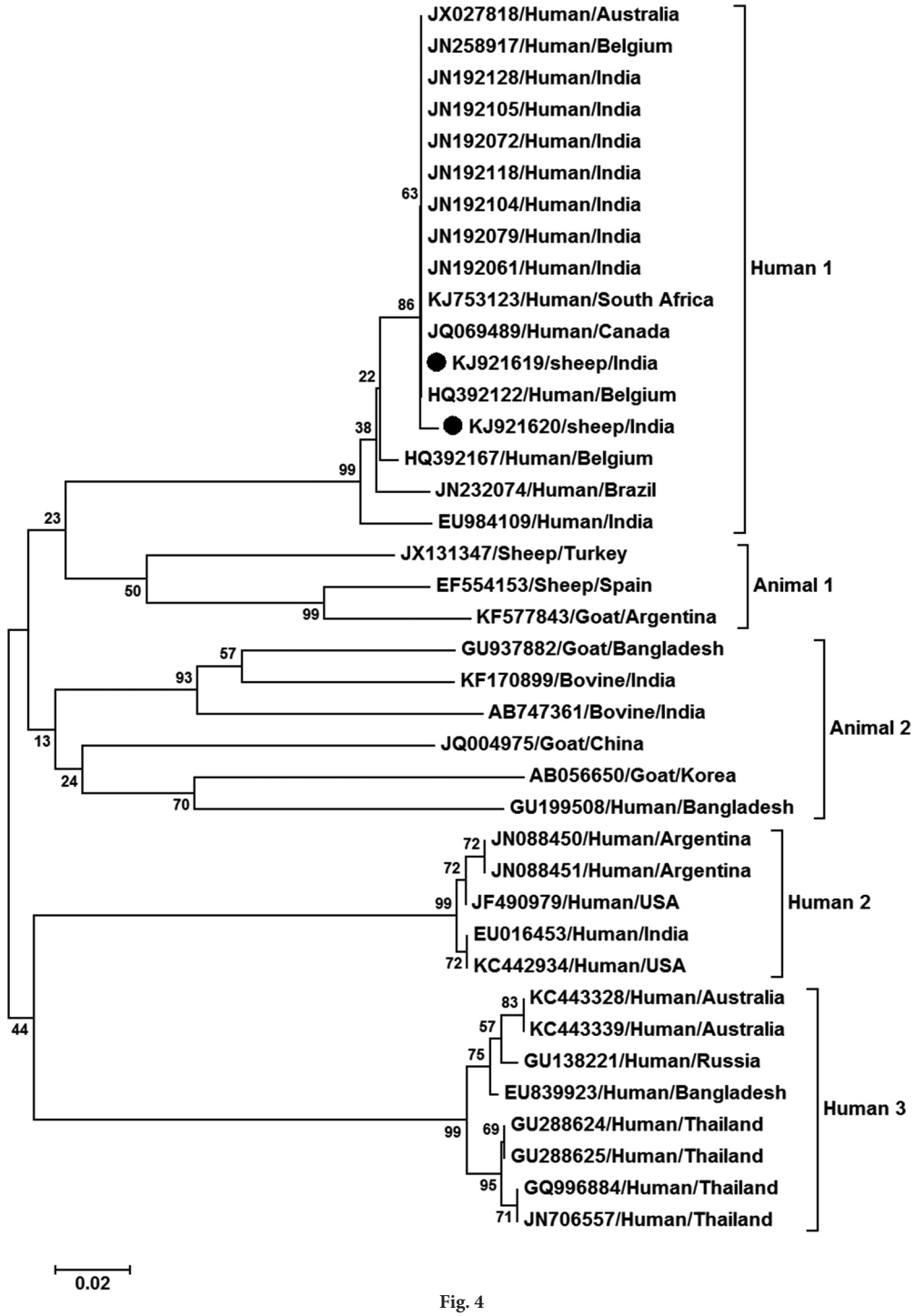

Fig. 4

Vp7 gene nucleotide sequence based phylogenetic analysis of Sheep18/Hisar/2013 and Sheep22/Hisar/2013 isolates along with other global isolates of group A rotavirus (• = isolates used in present study)

98.1–99.1/97.7–98.5% nt/aa identity with human GAR from Russia (strains Hu/RUS/Nov04-H676/2004, Hu/RUS/Nov05-137/2005 and Hu/RUS/Nov05-29/2005), Thailand (strains CU304-NR/08, CU209-KK/08 and CU209-KK/08), Australia (Human-wt/AUS/CK20030/2006), and Bangladesh (MMC6). However, Sheep22/Hisar/2013 isolate showed nt/

aa identity of only 57.1/55.5% with sheep (Spain), 60.9–64.2/56.2–62.1% with goat (Argentina, Korea, Bangladesh and China) and 62–63.3/56.6–57.7% with bovine GAR (India and Turkey).

Similarly, vp4 gene of Sheep18/Hisar/2013 also showed 97.4–99.2/97–98.5% nt/aa identity with human GAR from India (strains AP06-I, 0613158, ISO125, AB06-I and AB06), Bangladesh (Human-wt/Dhaka22/2001 and Human-wt/BGD/Matlab36/2002), Russia (Hu/RUS/Nov09-D210), USA (Human-wt/USA/VU08-09-40/2008 and Vanderbilt/VU08-09-6/2008) and Belgium (Human-wt/BEL/BE00021/2007 and Human-wt/Bel/BE00092/2003). However, Sheep18/Hisar/2013 isolate showed nt/aa identity of 56.3/54% with sheep (Spain), 60.2–64.7/55.8–61.7% with goat (Argentina, Korea, Bangladesh and China) and 60.7–62.1/56.2–57.3% with bovine (India and Turkey) GAR. These results clearly indicated that vp4 gene of Sheep18/Hisar/2013 and Sheep22/Hisar/2013 isolates were of human GAR origin.

The cross species transmission and segmented nature of rotavirus genome lead to evolution of newer strains. In Egypt reassortant strain of human GAR strain having vp4, vp7 and vp8 genes of human GAR and nsp4 and nsp5 genes of ovine and simian origin respectively was isolated (El Sherif *et al.*, 2011). Similarly, an ovine rotavirus strain (762) in Spain was reported as P[14] rotavirus which showed highest amino acid sequence identity with human rotavirus strain from Italy. Strain 762 also displayed a common ruminant G8 genotype of vp7 gene along with bovine like VP6 (genotype I2), NSP4 (genotype E2) and NSP5/NSP6 (genotype H3) indicating the cross species transmission of rotaviruses between human, bovine and ovine (Ciarlet *et al.*, 2008).

The phylogenetic analysis of vp4 gene of Sheep18/Hisar/2013 and Sheep22/Hisar/2013 with global isolates of GAR of sheep, goat, bovine and human origin showed four distinct clusters (2 each in human and animal rotaviruses). The Sheep18/Hisar/2013 and Sheep22/Hisar/2013 isolates were placed in cluster 1 and 2 of human rotaviruses along with several human GARs. The goat and bovine rotaviruses form close cluster and were distinct from sheep rotaviruses.

The experimental oral administration of human rotavirus to gnotobiotic lambs caused diarrhea and virus excretion. This indicates that lamb might be infected with human rotavirus and may spread it to other humans (Snodgrass *et al.*, 1977). The phylogenetic study of P[14] genotype of human rotavirus confirmed the close relationship with sheep, cattle, goat, guanaco and antelope rotaviruses (Matthijnssens *et al.*, 2009). The whole genome constellation based previous study also showed the common evolution of caprine strain of GAR with human and ruminant rotaviruses (Ghosh *et al.*, 2010).

The vp7 gene nucleotide as well as deduced amino acid sequence analysis revealed that Sheep18/Hisar/2013 and Sheep22/Hisar/2013 showed 99.1/100% nt/aa identity among them selves. The vp7 gene of these two isolates showed only nt/aa identity of 68.6–79.5/75–86.8% with sheep (Spain, Turkey), 75.6–80/82.8–88.1% with goat (Argentina, Bangladesh, China, and Korea) and 76.9–77.8/86.8–88.1% with bovine (India) GARs. The phylogenetic and sequence analysis revealed that Sheep18/Hisar/2013 and Sheep22/Hisar/2013 isolates showed maximum closeness with human GAR (95.6–100/94.7–100% nt/aa identity) from India (strains NIV-0717148, NIV-083374, NIV-0717154, NIV-0717150, NIV-083515 and 0613158-CA), Australia (Human-wt/AUS/CK00083/2008), Belgium (Human-wt/BEL/BE00017 and Human-wt/BEL/BE00021/2007), Canada (Human-wt/CAN/RT092-07/2007), Brazil (R321-2004) and South Africa (Human-wt/ZAF/MRC-DPRU2489/2008). All these isolates formed a separate close cluster Human 1. However, other human isolates from different parts of the world formed two more clusters (Human 2 and 3) and sheep, goat and bovine rotaviruses formed separate clusters (Animal 1and 2). The sequence identity and phylogenetic analysis confirmed the human GAR origin of vp7 gene of Sheep18/Hisar/2013 and Sheep22/Hisar/2013. Similarly, a very close relationship between G8 serotype of GAR of humans and bovine has been reported previously (Adah *et al.*, 2003).

Positive samples (Sheep18/Hisar/2013 and Sheep22/Hisar/2013) were collected from Dhanana village of Bhiwani district. This is a remote village area where sheep farming is major livelihood for farmers. Most of the farmers are poor and stay in small mud houses. Here, close contact between children and livestock, including sharing of common shelter can be easily observed. Many of the children with severe diarrheic conditions have been reported from that geographical area. Some of the local medical officials have used latex agglutination test for preliminary examination and confirmation of rotaviruses in affected human kids for assistance in treating such conditions. However, proper genotypic surveillance of rotaviruses in human kids has not been conducted there. It may explain the probable route of zoonotic transmission of human GAR to sheep.

The complete genome sequence based study of rotaviruses also revealed the common origin of human and porcine and human and bovine rotaviruses (Matthijnssens *et al.*, 2008). The nsp1 gene sequences based previous study also indicated interspecies relatedness of human, porcine, bovine, feline and equine rotaviruses (Kojima *et al.,* 1995). These results substantiate the potential for transmission of human rotaviruses to animals including ovine. The incidence of mixed infection of rotavirus in humans as well as animals is significantly higher in developing countries than in developed countries. More intimate contacts between humans and animals are conceivable in developing countries. A large number of mixed infections of rotaviruses were observed in Bangladesh after a devastating flood (Dey *et al.,* 2007).

Conclusions and future perspectives

Rotaviruses are one of the major etiological agents of acute viral gastroenteritis in newborns and young of the large number of animal species and humans. Due to close contact between human population with animals and unrestricted movement of different species of animals appear to facilitate inter-species transmission of rotaviruses and evolution of novel strains in India. Two rotavirus strains of sheep origin were found to originate from human GAR. In rural areas people are economically dependent on small scale farming of small ruminants such as sheep and goat. Animals usually reside with humans especially with children. This provides an opportunity for mixed infection and cross species transmission of GAR from humans to animals or *vice-versa*. Moreover, segmented nature of the rotaviral genome allows reassortment in mixed infection leading to emergence of new strains of the virus. Because of this reason many of the human rotaviral strains are now reported from animals or *vice-versa*. Thus, it indicates the potential threat for evolution of newer zoonotic strains of rotaviruses in coming future, which might be difficult to control. Therefore, continuous monitoring is required at state and national level for screening of small ruminants for presence of rotaviruses. Such monitoring should include RT-PCR and nucleic acid sequence based genotyping of rotavirus strains to know their possible zoonotic potential. Further studies on rotaviral genetic diversity should be done through complete genome sequencing and suitable bioinformatics analysis. It will assist in surveillance of GAR strains circulating in a geographical area, identification of their zoonotic origin, development of sensitive diagnostic assays and selection of strains for future vaccine designing to control the disease.

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