RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 8: 1031-1041 (August 2023)

Presence of Human G Rotavirus Genotypes Amongst Bovine Calves and its Combination with P Genotypes

Vandana Gupta[1], Anju Nayak[1], Madhu Swamy[2], R.V. Singh[3], Vishnu Gupta[4], Ajay Rai[1], Preeti Gupta[5], Megha Pandey[6]

10.18805/IJAR.B-4364

ABSTRACT

Background: Bovine Rotavirus is one of the most important viral etiological agent responsible for causing neonatal diarrhea incurring severe economic loss to farmers. The presence of large genome size, segmented nature and absence of proof reading activity of RNA polymerase leads to frequent reassortment and thus emergence of new G and P types with ability of interspecies transmission.

Methods: During an epidemiological study (July 2016 to July 2019) 200 diarrheic fecal samples were screened for Bovine Rotavirus A using ELISA and RNA-PAGE. Further, twenty two positive samples for RVA were subjected to molecular detection for VP6, VP4 and VP7 genes.

Result: Ten (20/200) and 11(22/200) per cent diarrheic fecal samples were found positive using ELISA and RNA-PAGE respectively. Twenty samples found positive in ELISA were also found positive in RNA-PAGE. Amongst which 22 (100%) samples were found positive for VP6, while 15 (68.18%) samples showed amplification for VP4 and VP7 gene. All Rotavirus A positive samples were genotyped by multiplex RT-PCR assay. G1G3 was found to be most predominant (53.33%) followed by G3 (26.66%), while one sample each showed the presence of G1G5 and G3G8 (6.66%). Ten samples showed mixed genotype (66.66%). One sample was non typeable (6.66%). Among the P types, P[11] was the most predominant (73.33%), while one sample each showed the presence of P[5] and P[5]P[11] (6.66%) and 02 samples were non typeable (13.33%). The G and P genotype combination determined in 12 samples were as follows; G3P [11] found in two samples (16.66%), G3P[5] in 01(08.33%), G1G5P[11] in 01(08.33%), G1G3P[11] in 07 (58.33%), while 01 (08.33%) sample had mixed genotype G1G3P[5]P[11] combination.

Key words: Bovine rotavirus A, G genotype, P penotype, VP4 gene, VP7 gene.

INTRODUCTION

Rotavirus are non-enveloped double-stranded RNA (dsRNA) viruses belonging to the family Reoviridae and it has a triple layered protein capsid of 75 nm, surrounding the viral genome consisting of 11 segments of double-stranded RNA which encodes six structural (VP1, VP2, VP3, VP4, VP6 and VP7) and six non-structural proteins (NS1, NS2, NS3, NS4, NS5 and NS6) (Greenberg and Estes, 2009). The proteins in the mature virus particle determine the host specificity, cell entry and enzymatic functions necessary for the production of viral transcripts and contain epitopes that generate immune responses. Rotaviruses have been classified into nine serological species or groups (A-I) based on the antigenic properties of inner capsid protein (VP6) according to the International Committee on Taxonomy of Viruses (ICTV) (Sharad *et al*., 2018). The RVs belonging to species A, B and C (RVA, RVB and RVC, respectively) are known to infect human and various animals, whereas the species D, E, F, G, H and I (RVD, RVE, RVF, RVG, RVH and RVI respectively) have only been recovered from animals, and mostly birds (Ball *et al*., 2005; Matthijnssens *et al*., 2010). Rotaviruses have segmented double stranded RNA (ds RNA) genome, which can be separated into discrete segments by RNA-PAGE. Viruses classified as Group A rotaviruses (RV-A), including BoRV-A, posses two independent neutralization antigens, namely VP4 and VP7, on the outer capsid, that specify the P type (protease-sensitive protein) and G type (glycoprotein), respectively. It has therefore been proposed that serotypic classification of rotaviruses should account for both VP7 (G) (Hoshino *et al*., Epidemiologically,

[1]Department of Veterinary Microbiology, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.
[2]Department of Veterinary Pathology, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.
[3]Department of Veterinary Public Health, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.
[4]Veterinary Hospital, Barella, Jabalpur-482 001, Madhya Pradesh, India.
[5]Department of Community Medicine, Netaji Subhash Chandra Bose Medical College, Jabalpur-482 001, Madhya Pradesh, India.
[6]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Anjora-491 001, Chhattisgarh, India.

Corresponding Author: Vandana Gupta, Department of Veterinary Microbiology, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.
Email: drvandanagupat4@gmail.com

How to cite this article: Gupta, V., Nayak, A., Swamy, M., Singh, R.V., Gupta, V., Rai, A., Gupta, P. and Pandey, M. (2023). Presence of Human G Rotavirus Genotypes Amongst Bovine Calves and its Combination with P Genotypes. Indian Journal of Animal Research. 57(8): 1031-1041. doi: 10.18805/IJAR.B-4364.

Submitted: 23-11-2020 **Accepted:** 16-03-2021 **Online:** 10-04-2021

RVA is the most important cause of human and animal diarrhea. Specifically, RVA strains have been categorized based on (i) the antigenic properties of VP6, VP7 and VP4 (subgroups, G-serotypes and P-serotypes, respectively); (ii) the migration pattern of the RNA genome segments when subjected to polyacrylamide gel electrophoresis (long, short, super-short or atypical electropherotypes) and (iii) nucleotide sequence analysis (genotypes) (Estes and Kapikian, 2007). To date, 28 G types and 39 P types of RVA have been identified in humans and animals as per the minutes of 7th Rotavirus Classification Working Group (Matthijnssens and Theuns, 2015). Rotavirus is the most common viral pathogens causing diarrhea in young cattle. The study was undertaken to have an update on the prevalence of Group A Rotavirus genotypes amongst bovines from central India thus aiding in better understanding of interspecies transmission, evolution and strategic planning to combat rotavirus infection.

MATERIALS AND METHODS

Sample collection and processing

A total of 200 faecal samples were collected from diarrheic cow and buffalo calves (up to the age of 3 months), from different regions of Jabalpur *viz*. Adhartal Fairy Farm, Military Dairy Farm, Gorakhpur Gausala, Jamtara, Pariyat Dairy Farms, Gaur Dairy Farms, Kaladehi Bargi, Padmaniya Shahdol. The samples were kept on ice and transported to laboratory for processing. 10% suspension of the faecal material was prepared with phosphate buffer saline (PBS, pH 7.2) by dissolving one gram of faeces in 10 ml PBS.

ELISA and RNA-PAGE

All the fecal samples were subjected to Bio X Bovine Rotavirus Sandwich ELISA kit - (Product ID- BIO K 343/2) (Singh *et al*., 2015) and RNA-PAGE analysis for initial screening of all the bovine faecal samples for RV presence by observing the 11 genome segments and their typical migration pattern. For electrophoresis, resolving gel (7.5%) and stacking gel (5%) were prepared as per the method of Laemmli (1970).

RNA extraction and reverse transcription for VP6, VP4 and VP7

Total RNA was isolated using Tri reagent LS according the manufacturers protocol. RNA from 22 positive samples both from ELISA and RNA PAGE were further subjected to RT-PCR for VP6, VP4 and VP7 gene. RNA isolated was reverse transcribed using random primers and reverse transcriptase of Revert Aid First Strand cDNA synthesis kit, Thermo Scientific, USA. The reaction mix was prepared in a 0.5 ml micro centrifuge tube, using template RNA (0.1 ng -5 µg) 1 µl, Random Hexamer 1 µl and Nuclease Free Water 10 µl. After denaturing at 65°C for 5 min, followed by snap cooling on ice, the following reagents were added to the tube: 5X Reaction Buffer 4 µl, RiboLockRNase Inhibitor (20 u/µl) 1 µl, 10 mM dNTP Mix 2 µl, RevertAid M-MuLVRT (200 u/µl) 1 µl and final volume 20 µl. The reaction mixture was kept for 5 min at 25°C followed by 42°C for 1 hr, finally terminated the reaction at 70°C for 5 min. The cDNA (3 µl) thus obtained was used for PCR and the remaining was kept at -20°C until further use.

RT-PCR for VP7 gene to amplify near full length copies of rotavirus gene 9 coding for VP7 protein of rotavirus. The reaction mixture consist of RNA 7 µl, Bov9Com5 (10 µM) 1.5 µl, Bov9Com3 (10 µM) 1.5 µl, this mixture was denatured at 97°C for 5 minutes and immediately chilled on ice. Following reagents were then added RNase inhibitor (10 U) 1.0 µl, 0.1 M Dithiothreitol (DTT) 1.0 µl, 5X RTbuffer 4.0 µl, 100 mM dNTP mix 0.6 µl, Reverse transcriptase (5 U): 0.5 µl, Nuclease Free Water 2.9 µl, with final volume 20 µl. The reaction mixture was mixed well and incubated at 42°C for 1 hour followed by denaturation of RNA-cDNA hybrid at 95°C for 2 minutes and chilled on ice after a brief spin.

PCR for amplification of VP6, VP4 and VP7gene.

All samples positive in Bio X diagnostic ELISA and RNA-PAGE were further subjected to amplification of VP6, VP4 and VP7 gene. The reaction for VP6 and VP4 gene was carried out in a 0.2 ml tube, with 2X PCR Master Mix 12.5 µl, Forward primer (20 pMole/µl) 1 µl, Reverse Primer (20 pMole/µl) 1 µl (Table 1), DMSO 1 µl, cDNA 3.0 µl, Nuclease Free Water 6.5 µl, with final reaction volume of 25 µl. The PCR components were mixed and reaction was set with the amplification condition: Intial denaturation for 5 min at 95°C, followed by 30 cycles at 95°C for 30 sec, 48°C 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 10 mins.

PCR amplification of near full length VP7 gene was carried out in a reaction mixture consisting of reverse transcriptase product 5 µl, 100 mM dNTP 0.2 µl, 10X PCR buffer 2.5 µl, $MgCl_2$ (25 mM) 1 µl, Bov9Com5 (10 µM) Forward primer 0.5 µl, Bov9Com3 Reverse primer (10 µM) 0.5 µl (Table 1), Taq DNA polymerase (3 U/µl) 0.6 µl and Nuclease free water 14.7 µl, final volume 25 µl. Production

Table 1: Primers used for conventional PCR.

Gene	Primers	Sequence 5′ to 3′	Position	Amplicon size (bp)	References
VP6	GARV-D-VP6-F	TTTGATCACTAAYTATTCACC	1130-1150	227	Kattoor *et al*. (2013)
	GARV-F-VP6-R	GGTCACATCCTCTCACTA	1339-1356		
VP4	Bov4Com 5	TTCATTATTGGGACGATTCACA	1064-1085	864	Isegawa *et al*. (1993)
	Bov4Com3	CAACCGCAGCTGATATATCATC	1897-1918		
VP7	Bov9Com5	TGTATGGTATTGAATATACCAC	50-71	1011	Gulati *et al*. (1999)

of 1011 bp product was considered as positive result. PCR reaction was set with the amplification condition: 94°C for 2 min, 30 cycles of 94°C 1 min, 50°C 1 min, 72°C for 2 min with final extension at 72°C for 7 min as per Wani *et al.,* (2004) with slight modifications. The amplicons so generated were resolved on agarose (2%) gelelectrophoresis.

Multiplex-semi-nested P and G genotyping PCR

The first round PCR amplicons of VP4 and VP7 genes were diluted 1:100 with NFW. The resulting diluted PCR products were then used as a template in a semi-nested PCR for P-typing and G-typing, respectively.

G genotyping PCR

The PCR product of the VP7 gene was diluted to 1:20 with NFW and subjected to second round of amplification as described above using generic primer Bov9Com3 and a cocktail of typing primers specific for G1, G3, G5, G6, G8 and G10 (Table 2). The reaction was carried out as follows 2X PCR Master Mix 12.5 µl, Bov9Com3 (10 pMole/µl) 1.0 µl, G1 (10 pMole/µl) 0.5 µl, G3 (10 pMole/µl) 0.5 µl, G5 (10 pMole/µl) 0.5 µl, G6 (10 pMole/µl) 0.5 µl, G8 (10 pMole/µl) 0.5 µl, G10 (10 pMole/µl) 0.5 µl, Template 2.0 µl, Nuclease free water 8.0 µl with Total reaction volume 25.0 µl. PCR reaction was set with the amplification condition 95°C 8 min, followed by 30 cycles of 94°C 45 sec, 52°C 45 sec, 72°C 90 sec, with final extension of 72°C for 10 min. The amplicons so generated were resolved on agarose (2%) gel electrophoresis.

P genotyping PCR

The P genotyping PCR was carried out by using a common forward primer and a set of three different reverse primers (P1, P5 and P11) corresponding to three different P genotypes for bovine RVA. The First round PCR amplicons of VP4 were diluted 1:100 with NFW. The resulting diluted PCR products were then used as a template in a semi-nested PCR for typing. The reaction was carried out as follows: 2X PCR Master Mix 12.5 µl, Forward primer (10 pMole/µl) 1.0 µl, P1 (10 pMole/µl) 0.5 µl, P5 (10 pMole/µl) 0.5 µl, P11 (10 pMole/µl) 0.5 µl, Template 1.0 µl, Nuclease free water 8.0 µl, Total reaction volume 25.0 µl PCR reaction was set with the amplification condition as 95°C 5 min, 30 cycles of 95°C 45 sec, 48°C 30 sec, 72°C 1.5 min with final extension of 72°C 10 min. The amplicons so generated were resolved on agarose (2%) gel electrophoresis.

RESULTS AND DISCUSSION

Rotaviruses are continuously evolving due to lack of proofreading activity in RNA polymerase and segmented nature of genome which leads to frequent reassortment. The global distribution, wide host range and presence of numerous G and P types demands frequent surveillance of rotavirus infection. RT-PCR, ELISA and hybridization assays have been used to detect the G and P types of field strains of group A rotavirus but RT- PCR assay using type specific primers is now-a-days most widely used technique for strain characterization. RT-PCR based assay has been found to be 5000 times more sensitive than hybridization based screening assay (Xu *et al.*, 1990). It has an advantage over ELISA because the later can sometimes yield false positive results (Pedley and McCrae, 1984).

Out of 22 ELISA and RNA-PAGE positive diarrheic samples of calves, in 22(100%), 15(68.18%) samples we could amplify VP6 and VP4 gene segments, respectively. Gene product of VP6 is indicated by 227 bp (Fig 1a, 1b and 1c) amplicon while, 864 bp product showed the presence of partial lengthVP4 gene segment (Fig 2a, 2b and 2c) by RT-PCR. All the eight cow and 14 buffalo samples of total 22 samples subjected for VP6 gene showed 36.36% and 63.63% amplification. For VP4 gene total positive samples were fifteen, out of which 03 (20%) were form cow calf and 12 (80%) were from buffalo calf (Table 3).

Fifteen samples (68.18%), out of twenty two samples screened for VP7 gene were found positive for VP7 (Fig 3a, 3b, 3c). Amongst the total 15 samples 03 (20%) were from cow and 12 (80%) were from buffalo. All the samples which showed positive result for VP4 gene were also found positive for VP7 gene as well.

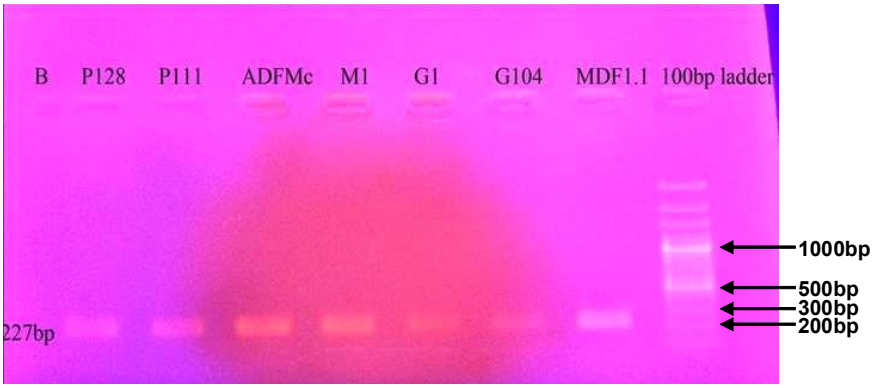
The presence of VP6 gene is demonstrated by other researchers as well which are in accordance with our findings. Basera *et al.* (2010) detected 10 (10/13, 76.9%) samples positive for VP6 antigen. Mukhtar *et al.* (2016) reported the amplification of VP6 gene (33.3%) in 04

Table 2: Detail of primers used for P and G genotyping.

Gene	Genotype	Primers	Sequence (5′ to 3′)	Position	Amplicon size (bp)	References
		Bov4Com5	TTCATTATTGGGACGATTCACA	1064-1085		
	P[1]	GARV-VP4 P[1]	TTAAATTCATCTCTTAGTTCTC	1505-1526	453	
VP4	P[5]	GARV-VP4 P[1]	GGCCGCATCGGATAAAGAGTCC	1704-1725	661	Isegawa *et al.* (1993)
	P[11]	GARV-VP4 P[1]	TGCCTCATAATATTGTTGGTCT	1377-1398	334	
		Bov9Com3	CAACCGCAGCTGATATATCATC			
	G1		CAAGTACTCAAATCAATGATGG	314-335	747bp	Iturriza- Gomara *et al.* (2004)
	G3		CGTTTGAAGAAGTTGCAACAG	689-709	373bp	Gouvea *et al.* (1990)
	G5		GACGTAACAACGAGTACATG	760-799	302bp	Gouvea *et al.* (1994)

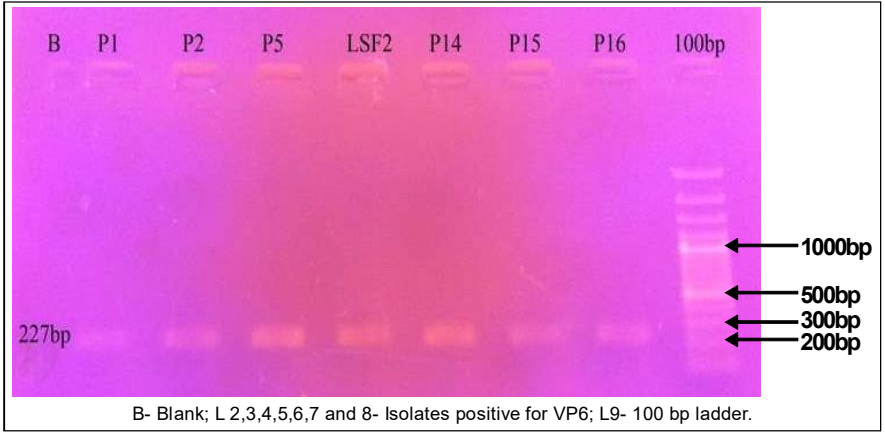
diarrheic samples out of total twelve ELISA positive samples. Singh *et al*. (2015) reported the amplification of VP6 gene in 12/100 (12%) fecal samples from Mathura region.

Multiplex semi nested PCR was performed on VP4 and VP7 amplified products of 15 samples for the P and G typing of Rotavirus. The P genotypes were determined (Fig 4a and 4b) by using 1:100 diluted VP4 amplicons in a multiplex semi-nested PCR assay using cocktail of P typing primers common to bovine RVA. Of the 15 RV positive samples P typed, P [11] was found in 11 (73.33%), P[5] in one (6.66%), mixed P[5]P[11] in one (6.66%) while the 02 samples (13.33%) were untypeable. Out of the 15 samples G typed, G3 was seen in 04 (26.66%) samples, G1G3 in 08 (53.33%), G1G5 in 01 (06.66%) samples, while one (06.66%) sample had G3G8 (Fig 5a and 5b). One sample (06.66%) was non-typeable (Table 4). The G and P genotype combinations as



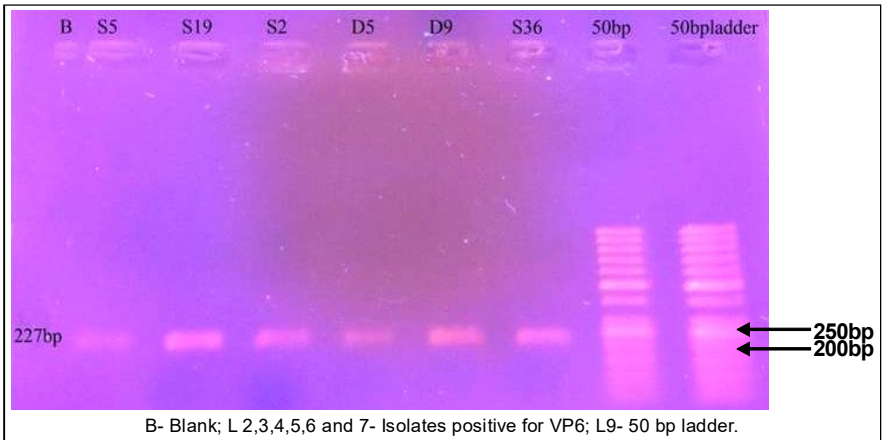
B- Blank; L 2,3,4,5,6,7 and 8- Isolates positive for VP6; L9- 100 bp ladd r.

Fig 1a: PCR amplification of VP6 gene of Bovine Rotavirus A with 227 bp amplicons.



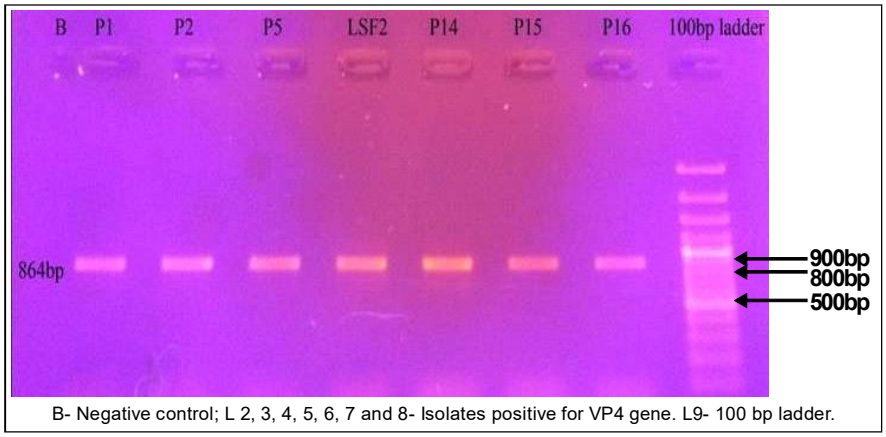
B- Blank; L 2,3,4,5,6,7 and 8- Isolates positive for VP6; L9- 100 bp ladder.

Fig 1b: PCR amplification of VP6 gene of Bovine Rotavirus A with 227 bp amplicons.



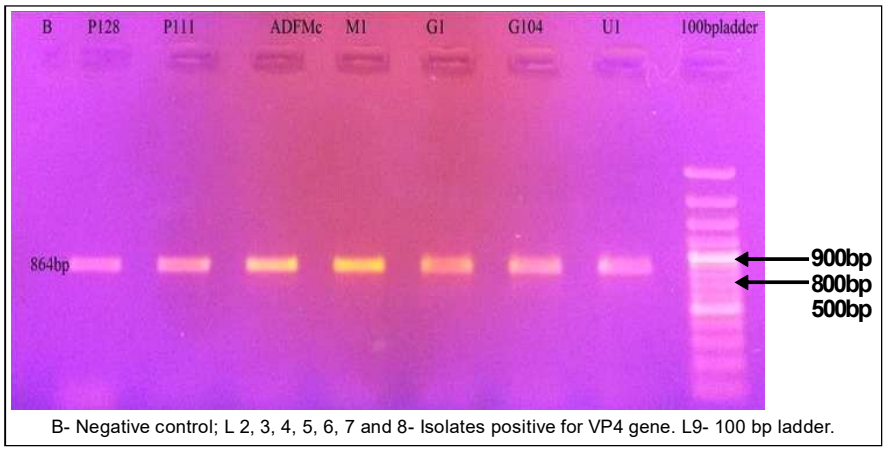
B- Blank; L 2,3,4,5,6 and 7- Isolates positive for VP6; L9- 50 bp ladder.

Fig 1c: PCR amplification of VP6 gene of Bovine Rotavirus A with 227 bp amplicons.



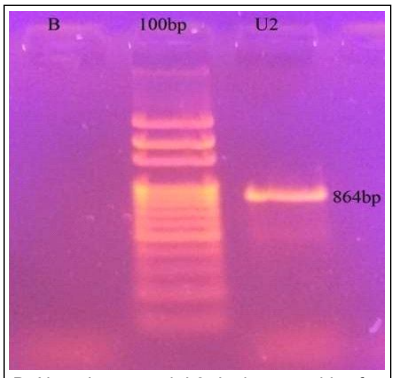
B- Negative control; L 2, 3, 4, 5, 6, 7 and 8- Isolates positive for VP4 gene. L9- 100 bp ladder.

Fig 2a: PCR amplification of VP4 gene of Bovine Rotavirus A with 864 bp amplicons.



B- Negative control; L 2, 3, 4, 5, 6, 7 and 8- Isolates positive for VP4 gene. L9- 100 bp ladder.

Fig 2b: PCR amplification of VP4 gene of Bovine Rotavirus A with 864 bp amplicons.



B- Negative control; L3- Isolates positive for VP4 gene L2- 100 bp ladder.

Fig 2c: PCR amplification of VP4 gene of Bovine Rotavirus A with 864 bp amplicons.

determined by RT-PCR are listed in Table 5. Out of the 12 samples which were G and P genotyped by RT-PCR, G3P[11] was seen in 0 2 (16.66%) samples, G3P[5] in 01 (08.33%) samples, G1G5P[11] in 01 (08.33%) sample, G1G3P[11] in 07 (58.33%) samples, while 01 (08.33%) samples had a mixed G1G3P[5]P[11] combination.

Due to segmented nature of the RNA genome and wide host range, vast genetic and antigenic diversity exists among different isolates of rotavirus. There are many G and P types RVA circulating amongst human and animal population. Human rotaviruses most commonly belong to G types 1-4, 9 and P types [4] and [8] (Gentsch *et al*., 1996) whereas bovine rotaviruses most commonly belong to G types 6, 8 and 10 and P types [1], [5], [6] or [11] (EL-Attar *et al*., 2002). G10 strains commonly occur in combination with P [11], P [5] and P[1] (Pisanelli *et al*., 2005). A G10 P [15] strain was reported for the first time in a lamb infected with rotavirus in China (Shen *et al.,* 1993). In India, so far, bovine diarrhea associated with G10 rotavirus has been seen in combination with P[11] (Beg *et al*., 2010; Minakshi *et al*., 2005; Saravanan *et al*., 2006), P[6] (Gulati *et al*., 2007), P[14] (Ghosh *et al*., 2007) and P[3] (Manuja *et al*., 2008). Despite clear evidence of host range restriction, a number of animal gene segments, mostly those encoding the neutralizing antigens (defining G and P types), have been identified repeatedly in humans in different parts of the world during surveillance studies (Gentsch *et al*., 2005; Santos *et al*., 2005), providing evidence that animals may act as a source of virus and/or of genetic material for evolutionary diversification of human rotaviruses. For example, strains such as G3 (found commonly in species such as cats, dogs, monkeys, pigs,

Table 3: RT-PCR result for amplification of VP6, VP4 and VP7.

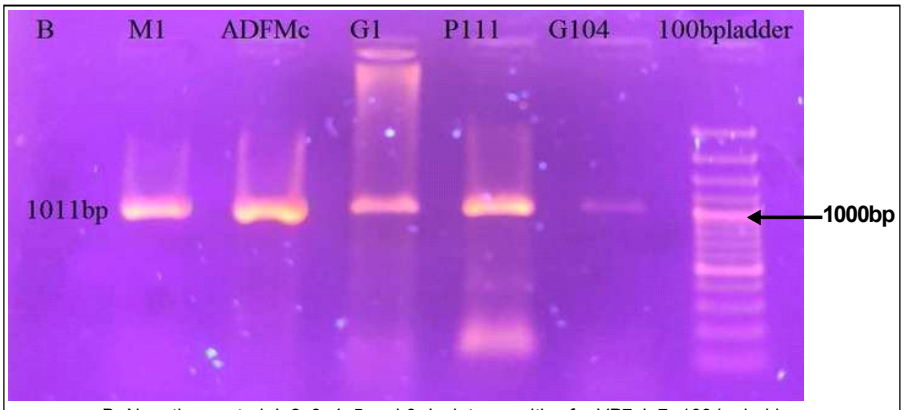
Samples	Cow/buffalo	VP6	VP4	VP7
P1	B	+	+	+
P2	B	+	+	+
P5	B	+	+	+
LSF2	C	+	+	+
P14	B	+	+	+
P15	B	+	+	+
P16	B	+	+	+
P128	B	+	+	+
P111	B	+	+	+
ADFMc	C	+	+	+
M1	C	+	+	+
G1	B	+	+	+
G104	B	+	+	+
MDF1.1	C	+	-	-
S5	B	+	-	-
S19	C	+	-	-
S2	C	+	-	-
D5	C	+	-	-
D9	C	+	-	-
S36	B	+	-	-
U1	B	+	+	+
U2	B	+	+	+

Table 4: Prevalence of P and G genotypes.

Genotype	No. out of 15	Prevalence (%)
	G-genotypes	
G3	04	26.66
G1G3	08	53.33
G1G5	01	06.66
G3G8	01	06.66
Non-typeable	01	06.66
	P-genotypes	
P[11]	11	73.33
P[5]	01	06.66
P[5]P[11]	01	06.66
Non-typeable	02	13.33

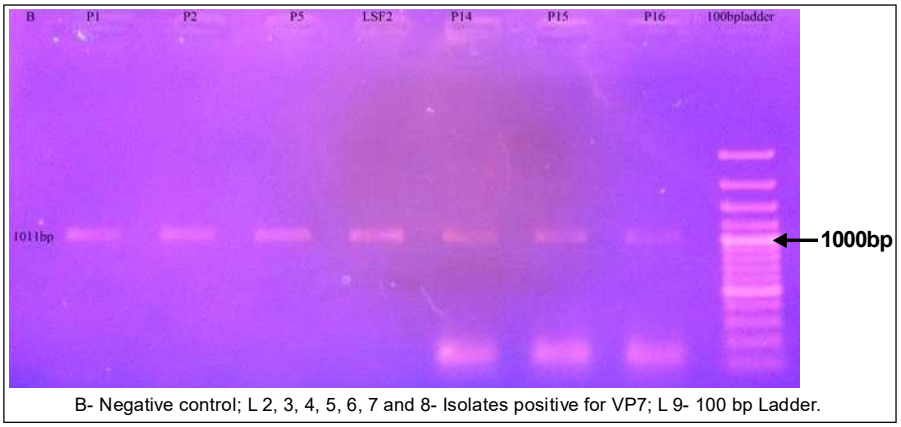
Table 5: Prevalence of G and P type combinations.

P and G genotype	No. out of 12	Prevalence (%)
G3P[11]	02	16.66
G3P[5]	01	08.33
G1G5P[11]	01	08.33
G1G3P[11]	07	58.33
G1G3P[5]P[11]	01	08.33



B- Negative control. L 2, 3, 4, 5 and 6- Isolates positive for VP7; L 7- 100 bp ladder.

Fig 3a: PCR amplification of VP7 gene of Bovine Rotavirus A with 1011 bp amplicons.

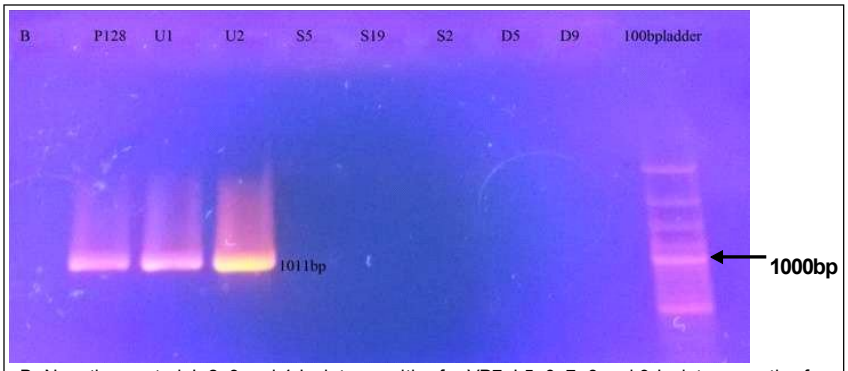


B- Negative control; L 2, 3, 4, 5, 6, 7 and 8- Isolates positive for VP7; L 9- 100 bp Ladder.

Fig 3b: PCR amplification of VP7 gene of Bovine Rotavirus A with 1011 bp amplicons.

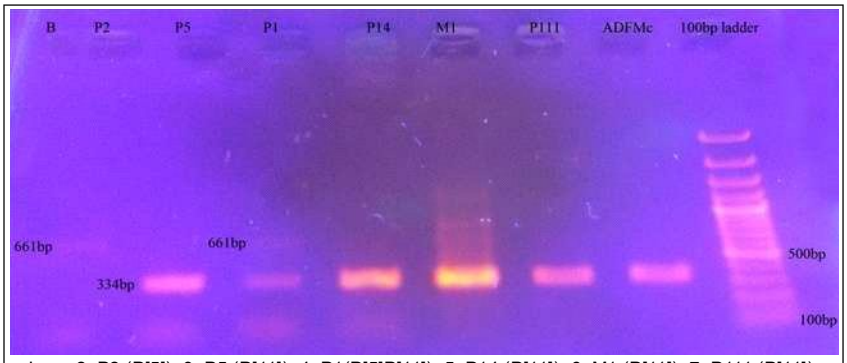
mice, rabbits and horses), G5 (pigs and lambs), G9 (pigs and lambs) and G10 (cattle) have been isolated from the human population throughout the world (Gentsch *et al*., 1996). The G9 strains reported in humans (Cubitt *et al*., 2000) which are found in lambs and pigs (Santos *et al*., 1999), may have been become established in humans during the past two decades through transfer from animals. There are a number of studies reporting rotavirus strain distribution in animals or humans in India but they do not provide any geographic or temporal comparisons of distribution among animals and humans (Kang *et al*., 2001; Nataraja *et al*., 2009). This is also similar to the lack of such reports worldwide with only a few studies that have compared the strains isolated from animals and humans simultaneously in the same region (Steyer *et al*., 2008; Van der Heide *et al*., 2005).

During the present study, 15 samples were genotyped by using multiplex semi-nested PCR assay. The G genotyping PCR assay showed G3 as the most predominant G type found in 04 (26.66%) samples (Table 4), while 10 (66.66%) samples showed mixed G types (two or more G types in same sample), amongst which G1G3 was most predominant 08(53.33%), while G1G5 and G3G8 one each



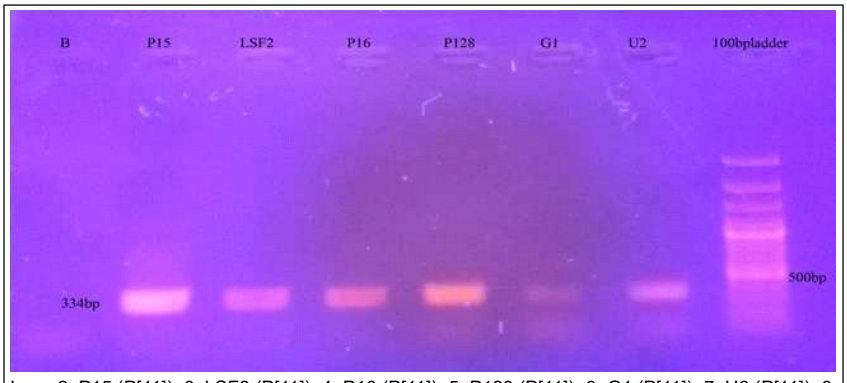
B- Negative control; L 2, 3 and 4-Isolates positive for VP7; L5, 6, 7, 8 and 9-Isolates negative for VP7 and L10-100 bp Ladder.

Fig 3c: PCR amplification of VP7 gene of Bovine Rotavirus A with 1011 bp amplicons.



Lane 2: P2 (P[5]); 3: P5 (P[11]); 4: P1(P[5]P[11]); 5: P14 (P[11]); 6: M1 (P[11]); 7: P111 (P[11]); 8: ADFMc (P[11]) and 9: 100 bp DNA Ladder.

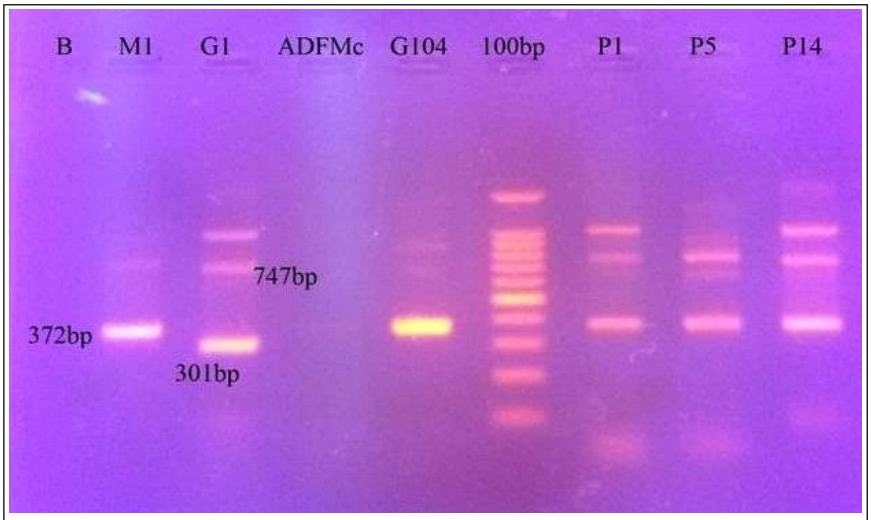
Fig 4a: PCR based P genotyping.



Lane 2: P15 (P[11]); 3: LSF2 (P[11]); 4: P16 (P[11]); 5: P128 (P[11]); 6: G1 (P[11]); 7: U2 (P[11]); 8: 100 bp DNA Ladder.

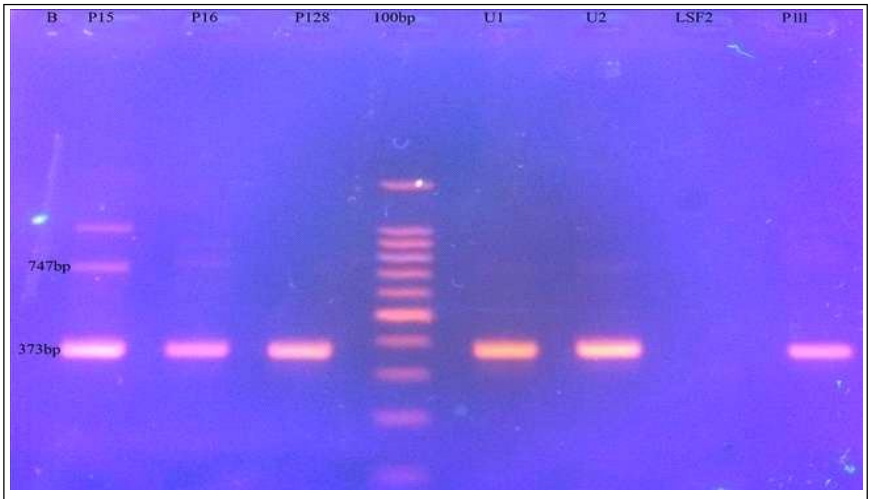
Fig 4b: PCR based P genotyping.

(6.66%). This study was in contrast to the earlier reports which detected G10 as the most predominant G-type in India (Varshney *et al.*, 2002; Saravanan *et al.,* 2006). Gulati *et al.* (1999) detected G10 (83%) as the most predominant G type in India followed by G6 (6%). Minakshi *et al*. (2005) also reported a frequency of G10 and G6 genotypes to be 86.61 and 13.39%, respectively, in Indian bovine rotavirus isolates. Reidy *et al.* (2006) reported G6 as most prevalent, followed by G10. Our findings are in accordance with the findings of Sravani *et al.* (2014), who detected G3 as most predominant with 43.75% and combination of G3G8 as 50%. They also recorded that prevalence detected by RNA-PAGE is higher (19.16%) as compared to RT- PCR (13.3%) thus underlying the benefit of simultaneous use of atleast two test for improved diagnostic potential. Malik *et al*. (2012) also found 52.9% of G3 and 47% of mixed G types in the samples, with G3G8 and G3G10 60.6%. Among the mixed genotypes we found G1G3 the most predominant one which is supported by the findings of Das *et al.* (2002) who reported the presence of G1G3 genotype in human fecal sample reported the presence of 1% G1G3 genotype. Castillo *et al.* (2000) also reported 02 diarrheic samples of children from Mexico having G1G3 genotype. Ezung *et al.* (2014) also reported the presence of G1G3 genotype in human diarrheic sample from Uttarakhand region. This is probably the first report of depicting G1G3 from bovine diarrheic samples as per our knowledge. G1 and G3 genotypes are most commonly reported from human diarrheal cases, the presence of these genotypes in bovines suggest the transmission of rotavirus strains from human to bovines. This might be because of close proximity between human and bovine.



Lane 1: Negative control; 2: M1 (G1G3); 3: G1 (G1G5); 4: ADFMc (G3); 5: G104 (G8G3); 6: 100 bp DNA Ladder; 7: P1(G1G3); 8: P5 (G1G3); 9:P14 (G1G3).

Fig 5a: PCR based G genotyping.



Lane 1: Negative control; 2: P15 (G1G3); 3: P16 (G1G3); 4: P128 (G3); 5: 100 bp DNA Ladder; 6: U1 (G3); 7:U2 (G1G3); 8:LSF2 non-typeable; 9: P111 (G1G3).

Fig 5b: PCR based G genotyping.

As per our findings, among the G type one sample was non typeable which is supported by the findings of Klingenberg *et al.* (1999) where they found 07 samples non typeable for any of the G types. Reidy *et al.* (2006) also found 03 (3.2%) samples non typeable. Niture *et al.* (2011) could not type any buffalo sample for G genotype. The high proportion of non typeable strains is a common feature of rotavirus characterization in most developing countries, where up to 30% of strains may remain un typed (Aminu *et al.*, 2010). These strains may represent common strains with accumulated point mutation in the currently used primer binding sites, animal rotaviruses including G5, G6, G10 and G12 or new unidentified rotavirus strains. Sequence analysis and the constant monitoring of genetic drift and shift would aid researchers in the developing world in the typing of circulating rotavirus strains.

The P genotyping PCR assay showed P[11] as the most predominant P type as it was found in 11 (73.33%%) samples (Table 4), while one (6.66%) sample each showed P[5]P[11] mixed P type and P[5] whereas two (13.33%) sample was un typeable. Although P[1] typing primers were incorporated in the multiplex semi-nested typing PCR assay, none of the isolates was typed as P[1]. This study is in conformity with the earlier report by Gulati *et al.* (1999), who reported P[11] in 94% of the samples genotyped. Falcone *et al.* (1999) reported 55% of P[11] and 42.3% of P[5], interestingly he also reported none sample positive for P[1], similar to our findings. Reidy *et al.* (2006) found P[5]P[11] combination in 11% *i.e.* six out of 54 samples positive for this combination.

As shown in (Table 5) the most predominant G and P type combination as determined by PCR assay was G1G3P[11] (58.33%) followed by G3P[11] (16.66%) and G3P[5], G1G5P[11] and G1G3P[5]P[11] one each (8.33%). This again is in contrast with the earlier report by Gulati *et al.* (1999) who reported G10P[11] (81%) as the most predominant G and P type combination among Indian bovine population, followed by G6P[1] (3%) and G6P[11] (3%). Saravanan *et al.* (2006) also reported G10P[11] from Tamil Nadu as the most predominant genotype combination. Beg *et al.* (2010) also reported from Kashmir G10P[11] as the most predominant (80.6%) combination followed by G8P[11] (7.7%). However, Manuja *et al.* (2008) reported unusual G10P[3] as the most predominant (73.3%) combination from north India followed by G6P[11] (26.7%). Ezung *et al.* (2014) reported predominant G and P type combination in bovine samples was 23.07% G3G6G10P[11] followed by 15.38% each of G3P[11], G10P[1]P[11], G3G10P[1]P[11] and G3G6G10P[1], while 7.69% had G10P[1] and G3 P[1]P[11] combination. In HRV, most predominant G and P type combination was G1G3P[8] (28.57%) followed by G1P[4] (14.28%) from Uttarakhand and G3P[8] (14.28%) from Nagaland. Malik *et al.* (2012) detected G3P[11] as most prevalent genotype and mixed genotypes as G3G10P[11], G3G8P[11], G3G8P[1]P[11] and G3G10 P[1]P[11]. Mixed genotypes have been commonly reported in bovine RVA. Minakshi *et al.* (2005) reported two isolates with extra-genomic bands in RNA-PAGE, with mixed G and P types (one isolate with G6G10 and another with P[1]P[11]). Mixed infection has also been reported in bovines from India by Sharma *et al.* (2009) and Beg *et al.* (2010). Mixed genotype infections can pave a way for reassortment between co-infecting RV strains and emergence of novel genotypes.

Knowledge of G and P types of group A rotavirus and their combinations in a particular geographical location is very important prerequisite as far as the prophylactic and control measures against the rotavirus infection are concerned. Genotypic strains prevalent in and around Jabalpur had been determined first time.

CONCLUSION

The presence of such large number of combinations of G and P types suggests genetic reassortment amongst the circulating strains. The presence of G1 and G3 genotypes are commonly found in humans, its presence in bovine samples indicate interspecies transmission. There should be continuous monitoring of rotavirus A strains circulating in different species.

ACKNOWLEDGEMENT

We would like to thank, The Dean, College of Veterinary Science and Animal Husbandry, Jabalpur for providing all needed assistance and facilities for conducting the above work.

REFERENCES

Aminu, M., Page, A.N., Ahmad, A.A., Umoh, U.J., Dewar, J. and Steele, D.A. (2010). Diversity of Rotavirus VP7 and VP4 genotypes in North western Nigeria. Journal of Infectious Diseases. 202(Suppl): S198-S204.

Ball, J.M., Mitchell, D.M., Gibbons, T.F. and Parr, R.D. (2005). Rotavirus NSP4: A multifunctional viral enterotoxin. Viral Immunology. 18: 27-40.

Basera, S.S., Singh, R., Vaid, N., Sharma, K., Chakravarti, S. and Malik, S.P.Y. (2010). Detection of rotavirus infection in bovine calves by RNA-Page and RT-PCR. Indian Journal of Virology. 21(2): 144-147.

Beg, S.A., Wani, S.A., Hussain, I. and Bhat, M.A. (2010). Determination of G and P type diversity of group A rotaviruses in faecal samples of diarrhoeic calves in Kashmir, India. Letters in Applied Microbiology. 51(5): 595-599.

Castillo, R.A., Villa, A.V., Gonzalez, J.R., Pimentel, E.M., Munuia, M.M., Jesus, B.D., Diaz, O.H. and Lozano, G.H. (2000). VP4 and VP7 genotyping by reverse transcripition- PCR of human rotavirus in Mexican children with acute diarrhea. Journal of Clinical Microbiology. 38(10): 3876-3878.

Cubitt, W.D., Steele, A.D. and Iturriza, M. (2000). Characterisation of rotaviruses from children treated at a London hospital during 1996: Emergence of strains G9P2A[6] and G3P2A[6]. Journal of Medical Virology. 61(1): 150-154.

Das, S., Sen, A., Uma, G., Vargese, V., Chaudhuri, S., Bhattacharya, S.K., Krishnan, T., Dutta, P., Dutta, D., Bhattacharya, M.K., Mitra, U., Kobayashi, N. and Naik, T.N. (2002). Genomic diversity of Group A Rotavirus strains infecting Humans in eastern India. Journal of clinical Microbiology. 40(1): 146-149.

El-Attar, L., Dhailwal, W., Gomara, M.I. and Bridger, J.C. (2002). Identification and molecular characterisation of a bovine G3 rotavirus which causes age-independent diarrhoea in cattle. Journal of Clinical Microbiology. 40: 937-942.

Estes, M., Kapikian, A., (2007). Rotaviruses. In: Fields Virology, [Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B. and Straus, S.E. (eds)], 5th edition. Kluwer Health/Lippincott, Williams and Wilkins; Philadelphia; p. 1917-1974.

Ezung, N.Z., Singh, R., Singh, P.S., Kumar, N. and Malik, S.Y. (2014). Occurrence of multiple combination of G and P types of group A bovine and human rotavirus in Uttarakhand and Nagaland States, India. Indian Journal of Animal Sciences. 84(8): 851-854.

Falcone, E., Tarantino, M., Trani, L.D., Cordioli, P., Lavazza, A. and Tollis, M. (1999). Determination of G and P serotypes in Italy using PCR. Journal of Clinical Microbiology. 37(12): 3879-3882.

Gentsch, J.R., Laird, A.R., Bielfelt, B., Griffin, D.D., Banyai, K. and Ramachandran, M. (2005). Serotype diversity and reassortment between human and animal rotavirus strains: Implications for rotavirus vaccine programs. Journal of Infectious Diseases. 192(1): S146-59.

Gentsch, J.R., Woods, P.A., Ramachandran, M., Das, B.K., Leite, J.P., Alfieri, A., Kumar, R., Bhan, M.K. and Glass, R.I. (1996). Review of G and P typing results from a global collection of rotavirus strains: Implications for vaccine development. Journal of Infectious Diseases. 174(Suppl.): 30-36.

Ghosh, S., Varghese, V., Samajdar, S., Sinha, M., Naik, T.N. and Kobayashi, N. (2007). Evidence for bovine origin of VP4 and VP7 genes of human group A rotavirus G6P[14] and G10P[14] strains. Journal of Clinical Microbiology. 45(8): 2751-2753.

Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B. and Fang, Z.Y. (1990). Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. Journal of Clinical Microbiology. 28(2): 276-282.

Govuea, V., Satos, N. and Timentsky, M.R. (1994). Identification of bovine and porcine rotavirus G types by PCR. Journal of Clinical Microbiology. 32(5): 1338-1340.

Greenberg, H.B. and Estes, M.K. (2009). Rotaviruses: From pathogenesis to vaccination. Gastroenterology. 136(6): 1939-1951.

Gulati, B.R., Deepa, R., Singh, B.K. and Rao, C.D. (2007). Diversity in Indian equine rotaviruses: Identification of genotype G10, P6[1] and G1 strains and a new VP7 genotype (G16) strain in specimens from diarrheic foals in India. Journal of Clinical Microbiology. 45(3): 972-978.

Gulati, B.R., Nakagomi, O., Koshimura, Y., Nakagomi, K. and Pandey, R. (1999). Relative frequencies of G and P types among rotaviruses from Indian diarrhoeic cow and buffalo calves. Journal of Clinical Microbiology. 37(6): 2074-2076.

Hoshino, Y., Wyatt, R.G., Greenberg, H.B., Flores, J. and Kapikian, A.Z. (1984). Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque reduction neutralization. Journal of Infectious Diseases. 149: 694-702.

Isegewa, Y., Nakagomi, O., Nakagomi, T., Ishida, S., Uesugi, S. and Uedea, S. (1993). Determination of bovine rotavirus G and P serotypes by polymerase chain reaction. Molecular and Cellular Probes. 7: 277-284.

Iturriza-Gómara, M., Kang, G., Gray, J. (2004). Rotavirus genotyping: Keeping up with an evolving population of human rotaviruses. Journal of Clinical Virology. 31(4): 259-265.

Kang, G., Raman, T., Green, J., Gallimore, C.I. and Brown, D.W. (2001). Distribution of rotavirus G and P types in north and south Indian children with acute diarrhoea in 1998-99. Transactions of the Royal Society of Tropical Medicine and Hygiene. 95(5): 491-492.

Klingenberg, K.V., Nilsson, M. and Svensson, L. (1999). Rotavirus G-type restriction, persistence and herd type specificity in Swedish cattle herds. Clinical and Diagnostic Laboratory Immunology. 6(2): 181-185.

Laemmli, U.K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature. 227 (5259): 680-685.

Malik, S.Y., Sharma, K. and Vaid, N., Chakravati, S., Chandrashekar, K.M., Basera, S.S., Singh, R., Minakshi, G., Gulati, R.B., Bhilegaonkar, N.K. and Pandey, B. (2012). Frequency of Group A rotavirus with mixed G and P genotypes in bovines: Predominance of g3 genotype and its emergence in combination with G8/G10 types. Journal of Veterinary Science. 13(3): 271-278.

Manuja, B.K., Prasad, M., Manuja, A., Gulati, B.R. and Prasad, G. (2008). A novel genomic constellation (G10P[3]) of group A rotavirus detected from buffalo calves in northern India. Virus Research. 138(1-2): 36-42.

Matthijnssens, J. and Sebastiaan, T. (2015). Minutes of 7th RCWG Meeting. Working Group (RCWG) Meeting 9 October, 12th International Double Stranded RNA Virus Symposium at Goa, India.

Matthijnssens, J., Heylen, E., Zeller, M., Rahman, M., Lemey, P. and Van Ranst, M. (2010). Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. Molecular Biology and Evolution. 27(10): 2431-2436.

Minakshi Prasad, G., Malik, Y.P.S. and Pandey, R. (2005). G and P genotyping of bovine group A rotavirus in faecal samples of diarrhoeic calves by DIG- labeled probes. Indian Journal of Biotechnology. 4: 43-55.

Mukhtar, N., Yakub, T., Masood, A., Javed, H., Nour, J., Aslam, A., Ali, A., Javed, M., Nadeem, A., Hussain, T., Tahir, Z. and Aslam, B.H. (2016). Molecular characterization of Bovine Rotavirus in Pakistan, Jundishpur. Journal of Microbiology. 9(12): e41001.

Nataraju, S.M., Chattopadhyay, U.K. and Krishnan, T. (2009). A study on the possibility of zoonotic infection in rotaviral diarrhoea among calves and buffalo calves in and around Kolkata, India. European Review of Medical and Pharma -cological Sciences. 13(1): 7-11.

Niture, S.G., Karpe, G.A. and Prasad, M. (2011). Characterization of Buffalo, Poultry and Human Rotaviruses in Western India.

Pedley, S. and McCrae, M.A. (1984). A rapid screening assay for detecting individual RNA species in field isolates of rotaviruses. Journal of Virological Methods. 9: 173-181.

Pisanelli, G., Martella, V., Pagnini, U., De Martino, L., Lorusso, E. and Iovane, G. (2005). Distribution of G (VP7) and P (VP4) genotypes in buffalo group A rotaviruses isolated in Southern Italy. Veterinary Microbiology. 110(1-2): 1-6.

Reidy, N., Lennon, G., Fanning, S., Power, E. and O'Shea, H. (2006). Molecular Characterization and analysis of bovine rotavirus strains circulating in Ireland 2002-2004. Veterinary Microbiology. 117: 242-247.

Santos, N. and Hoshino, Y. (2005). Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Review in Medical Virology. 15(1): 29-56.

Santos, N., Lima, R.C., Nozawa, C.M., Linhares, R.E. and Gouvea. V. (1999). Detection of porcine rotavirus type G9 and of a mixture of types G1 and G5 associated with Wa-like VP4 specificity: Evidence for natural human- porcine genetic reassortment. Journal of Clinical Microbiology. 37(8): 2734-2736.

Saravanan, M., Parthiban, M. and Ramdass, P. (2006). Genotyping of rotavirus of neonatal calves by nested- multiplex PCR in India. Veterinarski Arhive. 76: 497-505.

Sharad, S., Sircar, S., Kattor, J.J., Ghosh, S., Kobayashi, N., Banyai, K., Vinodkumar, R.O., De, K.U., Sahoo, R. N., Dhama, K. and Malik, S.Y. (2018). Analysis of structure - function relationship in porcine rotavirus A enterotoxin gene. Journal of Veterinary Science. 19(1): 35-43.

Sharma, G., Anil, T., Rajesh, C. and Bhat, M.A. (2009). Prevalence of bovine rotaviral diarrhoea in Jammu. Indian Journal of Virology. 20: 7-12.

Shen, S., Burke, B. and Desselberger, U. (1993). Nucleotide sequences of the VP4 and VP7 genes of a Chinese lamb rotavirus: Evidence for a new P type in a G10 type virus. Virology. 197(1): 497-500.

Singh, T., Singh, R., Singh, P.A., Malik, S.P.Y. and Prasad, M. (2015). G and P typing of bovine group A rotavirus in Northern India. Indian Journal of Animal Research. 49(6): 814-818.

Snodgrass, D.R., Hoshimo, Y., Fitzgerald, T.A., Smith, M., Browning, G.F. and Gorziglia, M. (1992). Identification of four VP4 serological types (P serotype) of bovine rotavirus using viral ressortants. Journal of General Virology. 73: 2319-2325.

Sravani, V.D.G., Kaur, G., Chandra, M. and Dwivedi, N.P. (2014). P and G genotyping of Bovine Rotavirus and its prevalence in Neonatal calves. Veterinarski Arhive. 84(5): 475-484.

Steyer, A., Poljsak-Prijatelj, M., Barlic-Maganja, D. and Marin, J. (2008). Human porcine and bovine rotaviruses in Slovenia: Evidence of interspecies transmission and genome reassortment. Journal of General Virology. 89(7): 1690-1698.

Van der Heide, R., Koopmans, M.P., Shekary, N., Houwers, D.J., Van Duynhoven, Y.T. and Van der Poel, W.H. (2005). Molecular characterizations of human and animal group a rotaviruses in the Netherlands. Journal of Clinical Microbiology. 43(2): 669-675.

Varshney, B., Jagannath, R.M., Vethanayagam, R.R., Kodhaandharaman, S., Jagannath, V.H., Gowda, K., Singh, K.D. and Rao Durga, C. (2002). Prevalence of and antigenic variation in, serotype G10 rotaviruses and detection of serotype G3 strains in diarrheic calves: Implications for the origin of G10P11 or P11 type reassortment asymptomatic strains in newborn children in India. Archives of Virology. 147(1): 143-165.

Wani, S.A., Bhat, A.M., Ishaq, M.S. and Ashrafi, A.M. (2004). Determination of bovine rotavirus G genotype in Kashmir, India. Rev. Sci. Tech. Off. Int. Epiz. 23(3): 931-936.

Xu, L., Harbour, D. and McMcrae, M.A. (1990). The application of polymerase chain reaction to the detection of rotaviruses in faeces. Journal of Virological Methods. 27: 29-38.