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A Study of Interspecies Transmission and Reassortment Events in Rotaviruses from Cattle in Pant Nagar, Uttarakhand, India

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ABSTRACT Most cases of severe acute gastroenteritis (AGE) in neonates and cattle are caused by Group A Rotavirus (RV). Faecal samples were obtained from 150 calves from dairy farms in Pant Nagar, Uttarakhand, India, for the current investigation. RNA-PAGE analysis was used to test them all. In this investigation, amplicons based on the VP6 gene (227 bp) and the VP7 gene (208 bp) were utilised to identify Group A rotaviruses in the positive samples. There is a growing amount of evidence demonstrating that reassortment and transmitting between different species of Rotavirus (RV) occur in the natural world. Recognising RV dynamics and the methods by which RVs develop, pass the species barrier, transfer genes during reassortment, and evolve through point mutations or recombinant genetics requires an examination of the dominance of rotavirus G and P types in human and bovine communities.

INTRODUCTION

The Rotavirus (RV) belongs to *Reoviridae*, consisting of RNA as their genetic material, and is prone to their characteristic high level of genomic mutations (Abad and Danthi 2020). Among the nine RA groups (A- I), Group A is the major causative agent for severe acute gastroenteritis (AGE) in neonatal humans and cattle worldwide (Kapikian et al. 1986). AGE accounts for the death of 1 in 9 children, and its death rose to 2195 daily even after severe prolonging precautions (Alter et al. 2015). Almost 40 percent of these deaths have been putatively linked with RA, and most occur in low-income countries (Changotra and Vij 2017). Mutated strains have an important part in the predisposing of diarrhoea, in addition to societal risk factors such as poverty, malnutrition, limited access to health services, and vitamin deficiencies.

Six structural and six non-structural proteins are encoded by the virus' double-stranded RNA (Bukrinskaya 1982; Saravanan et al. 2022). Except for the 11th segment, all are monocistronic that code for NSP6. This segmented genome aids in

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the reassessment of double-stranded genome generation from individual strands of two virus-infected cells. This assortment capability is the principal reason for RV genetic diversity and viral evolution (Lauring et al. 2013). VP4 and VP7 are expressed by 4 and 9 segments, correspondingly. VP4 is the spike protein that assists in viral entrance into host cells and has been found to contribute in rotavirus haemagglutination, neutralisation, virulence, and protease-enhanced infectivity (Gouvea and Brantly 1995). VP7, on the other hand, determines the antigenic nature of the virus and is a major factor in antigen neutralisation.

Group A rotaviruses are classified as P or G based on their VP 4 and VP 7 genes. The RA strains with more than 89 percent or more similarities in their VP 6 and VP 7 nucleotides are grouped as P and G types, respectively (Hoshino and Kapikian 1996). Molecular biology has uncovered 30 P genotypes and 21 G genotypes (Santos and Hoshino 2005). They have peculiar species distribution and species barrier, but due to the reassortment nature, occasionally, unusual P and G genotypes occurred from the integration of human and animal RA, especially from pigs and cattle (Papp et al. 2013). Hence, the RA of cattle serves as the reservoir for the genetic variability of human rotaviruses. New RA strains have been continuously reported worldwide (Badaracco et al. 2013). The predisposition and continuous surveillance of the strain variation in animals are used to detect the novel circulating genotypes for developing new human vaccines.

Objective

For elucidating the novel strain circulation, the researchers retrospectively investigated the prevalence of the antigenic and molecular relationship that exists among each strain, a combination of typing methods (RNA-PAGE, conventional RT-PCR assay, and ELISA) based on a variety of genes (VP4 and VP7) provide a means of characterising rotaviruses (Esona et al. 2010).

MATERIAL AND METHODS

Specimen Collection and dsRNA Isolation from Bovine RV

All the glassware and plastic materials were procured from Borosil (India) or Corning (USA) and Axygen (USA), and Tarson (India), respectively. Chemicals and reagents were bought from Biolabs (New England), Amresco (USA), SRL (India), Fermentas (USA), and Genei (India). Faecal samples from diarrhoeic bovine (cattle) calves (0-3 months), irrespective of either sex, were the source of the virus in the present study. Calf samples (150) were gathered from dairy farms in Pant Nagar, Uttarakhand, India (Fig. 1). The samples were gathered in a sterile plastic bag for stool collections and sent to the lab on ice for long-term storage at -20 degrees Celsius. To clarify the samples, they were centrifuged at 12,000 rpm for 30 minutes at 4°C after being diluted with phosphate buffer saline (PBS, pH 7.2, 10%). After extracting viral nucleic acid from 1 ml of the supernatant, the remaining volume was frozen at -20 degrees Celsius for later use. Reverse transcription (RT), nested PCR (RT-PCR), and semi-nested PCR (SNP-PCR) are all acronyms for the same thing, that is, polymerase chain reaction (RT-PCR).

RNA Extraction from the Samples

Using a phenol:chloroform procedure, as reported by Herring et al. (1982), dsRNA of rotavirus was recovered from faecal solution. Alternatively, RNA was extracted using Trizol (SRL), as described by Rio et al. (2010), with certain modifications. Extracted RNA was run on RNA-PAGE according to the methods outlined by Laemmli (1970) and Herring et al. (1982) for the identification of rotavirus' 11-segmented dsRNA (Herring et al. 1982). The samples were analysed by enzyme-linked immunosorbent assay (ELISA) using an ELISA kit purchased from Bio-X-diagnostics (BIO K 343/2). The test kit has a sensitivity of between 77 percent and 97 percent, and a specificity of between 90 percent and 100 percent.

Analysis Using ELISA Kit

Antigen capture ELISA kit (Digestive Bio K 071®, Bio-X Diagnostics, Belgium) was used to analyse faeces. Laboratory tests conducted by the manufacturer found a sensitivity of 77 percent to 97 percent, and a specificity of 90 percent to 100 percent. By use of an ELISA reader (BioStackReady ® BioTek® Power Wave Reader XS), the OD was measured at 450 nm. The absorbance value of the sample was subtracted from the ab-

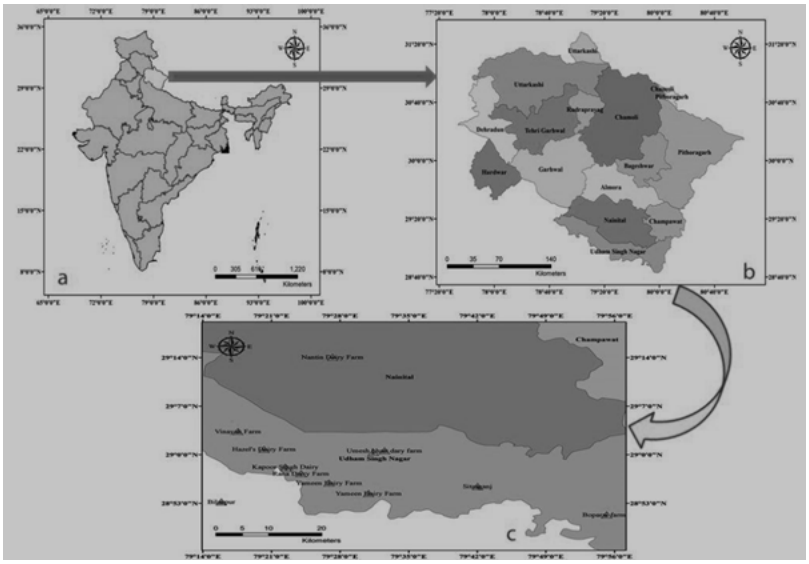

Fig. 1. Sampling site of calves were collected from different dairy farms in Pantnagar, India (a) Map showing Uttarakhand, (b) Map shown the Pantnagar, (c) Sampling sites

sorbance value of the negative control, and samples were considered positive if the resultant value was more than 0.150 Elisa units (EU). All 150 faecal samples were subjected to RT-PCR targeting the VP6 gene. The genes used for RT-PCR analysis and their respective primers are shown in Table 1.

RESULTS

Typical Migration Patterns Showed the Presence of Mixed RV Strains

The present study aimed to isolate the rotavirus strains from 150 cattle calves using multiplex semi-nested PCR assay from Pant Nagar, Uttarakhand, India. Of the 150 samples analysed, 26 (17.33%) were found to be positive for the rotavirus genome on RNA PAGE (Fig. 2a-b). RNA-PAGE has been considered a powerful tool for studying the epidemiology of RVs and genetic variations. With a distinctive migratory pattern of 4:2:3:2 on silver staining, all the positive samples (B1-B26) demonstrated the presence of 11 segmented genomes. Based on the 5th and 6th segments, two different electrophoretic patterns were found in the RVA samples. In the first electrophoretic migration (Type A), the four segments (I - IV) migrated separately, as seen in Fig. 3 (Lane 5, 6, 7, 8, and 9, B3, B20, B14, B10, and B11, respectively). In type B, segments II and III were co-migrated (Fig. 3: Lane 1, 2, 3, 4, and 10; B9, B18, B13, B16, and B7, respectively). In all the samples (Fig. 3), class III segments (7 and 8) moved as a single segment. B9 showed a mixed migration pattern due to the characteristic co-infection of the mixed classes. ELISA was used to confirm the presence of rotavirus in the samples along with RNA PAGE. Thirty-three bovine samples (50.77%) out of 65 samples were positive for rotavirus antigen. Comparatively, the sensitivity was higher in ELISA than RNA PAGE in the present study (Table 2).

Category A is a prominent human pathogen among the nine rotavirus groups (A-J), and its ratio is a crucial determinant in anticipating human infections. In the current work, the VP6 and VP7 genes were used to determine the presence of Group A rotaviruses among the samples tested, resulting in 227bp (Fig. 4a) and 208 bp (Fig. 4b) amplicons, respectively. The RNA PAGE positive samples were among the 65 total samples that underwent a second ELISA check for the presence of rotavirus antigen (Supplementary Table 1). 33 (50.77%) bovine samples out of 65

Table 1: Primers used for partial length VP6 gene

Gene	*Primers*	*Sequences (5'-3')*	*Location*	*Expected amplicon*	*References*
VP6	VP6-F (GARV-D)	TTTGATCACTAAYTATTCACC	1130-1150	227 bp	Iturriza-Gomara et al. (2002)
	VP6-R (GARV-F)	GGTCACATCCTCTCACTA	1339-1356		
VP6 (Partial Length)	VP7-F	GATATAACAGCTGATCCAACAAC	845-877	208 bp	Gouvea et al. (1990)
	VP7-R	GGTCACATCATACAATTCT	1044-1062		
VP7(Full Length)	VP7-F	GGCTTTAAAAGAGAGAATTTCC GTCTGG	1-28	1062bp	Gouvea et al. (1990); Iturriza-Gomara et al. (2004); Isegawa et al. (1993)
	VP7-R	GGTCACATCATACAATTCTAATCTAAG	1062-1036		
	VP7-G3	CGTTTGAAGAAGTTGCAACAG	689-709	373bp	
	VP7-G6	GATTCTACACAGGAACTAG	481-499	581bp	
	VP7-G8	GTCACACCATTTGTAAATTC	178-197	884bp	
	VP7-G10	ATGTCAGACTACARATACTGG	666-686	396bp	
VP4	VP4-F (Bov4 Com 5)	TTCATTATTGGGACGATTCACA	1064-1085	863	Gentsch et al. (1992); Isegawa et al. (1993)
	VP4-R (Bov4 Com 3)	CAACCGCAGCTGATATATCATC	1897-1918		
	VP4-P1	TTAAATTCATCTCTTAGTTCTC	1505-1526	453	
	VP4-P5	GGCCGCATCGGATAAAGAGTCC	1704-1725	661	
	VP4-P11	TGCCTCATAATATTGTTGGTCT	1377-1398	334	

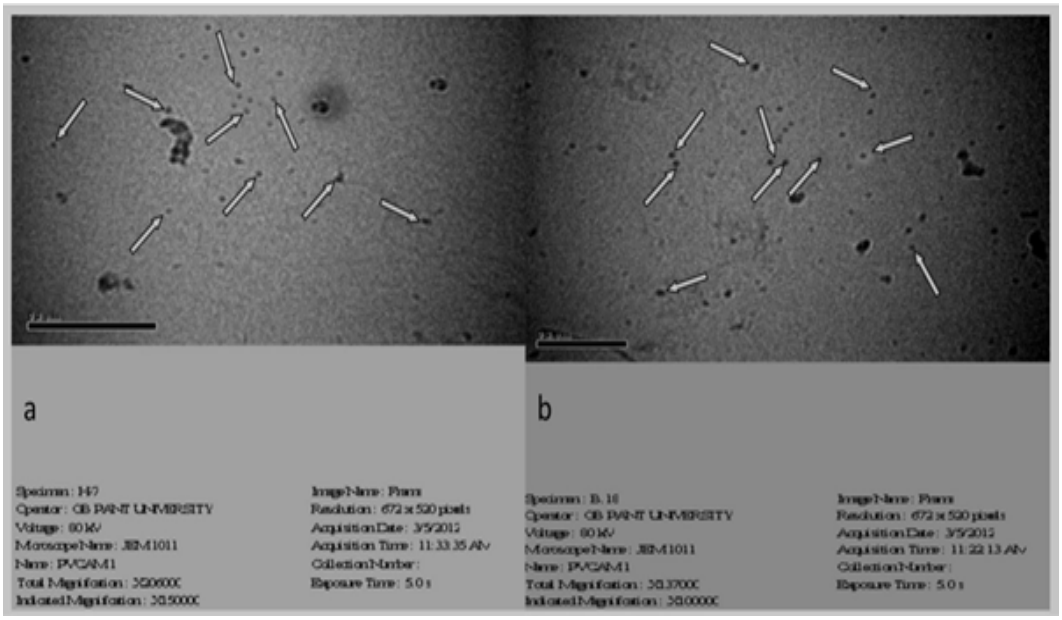

Fig. 2a. TEM of stool suspension of Human sample (H7) positive for rotavirus, 150,000X. b. TEM of faecal suspension of Bovine sample (B18), positive for rotavirus, 150,000X

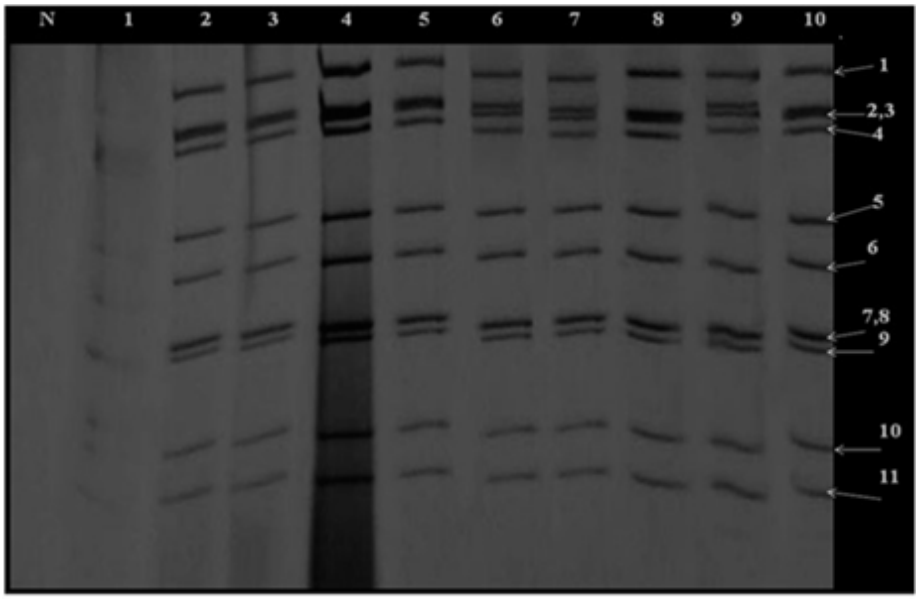

Figure 4: RNA PAGE showing different electropherotypes and migration pattern of BRV.

Lane N : Negative Control
Lane 1-10 : BRV positive samples
Type A : Lane 5, 6, 7, 8 and 9 (segments 1, 2, 3 and 4 migrated separately) (B3, B20, B14, B10 and B11 respectively)
Type B : Lane 1, 2, 3, 4 and 10 (segments 2 and 3 co-migrated) (B9, B18, B13, B16 and B7 respectively)

Fig. 3. RNA PAGE showing different electropherotypes and Migration pattern of Bovie Rota Virus (BRV). Lane N- Negative control, Lane 1- 10 BRV, Type A - Lane 5,6,7,8 and 9 (Segments 1,2, 3 and 4 migrated separately), Type B - Lane 1,2,3, 4 and 10 (segments 2 and 3 co-migrated)

Table 2: Comparative analysis of ELIZA and RNA PAGE of rotavirus samples collected from the cattle farms around Phatnagar, India

S. No.	*Method of analysis*	*Host*	*No. of sample(s) screened*	*Positive sample(s)*	*Prevalence (%)*
1	RNA-PAGE	Cattle	150	26	17.33
2.	ELISA	Cattle	65	33	50.77

were positive for rotavirus antigen (Table 3). Further, they were tested with partial VP7 gene amplification, and only 17 samples (40.47%) showed the amplified product (Table 4). In 33 samples, every four samples (12.90% each) showed G3 and G10 genotypes, respectively. Nine samples (29.03%) showed mixed G genotypes.

For genotyping of P-type, partial length VP4 primers and their cocktails, such as P [1], P[5], and P[11] primers, were used to analyse the P sero-

Table 3: Prevalence G genotypes in bovine samples

G-genotypes		
Genotype	*No. out of 17 bovine samples*	*Prevalence (%)*
G3	4	23.52
G10	4	23.52
G3G10	2	11.7
G6G10	2	11.7
G3G6G10	7	41.17
Untypeable	7 out of 33	22.58

Table 4: Prevalence of P genotypes in bovine samples

	P-genotypes	
Genotype	*No. out of 16*	*Prevalence (%)*
P[1]	5	31.25
P[11]	5	31.25
P[1]P[11]	6	37.50
Non-typeable	17 out of 33	48.38

types among the samples. Of them, 13 (30.95%) samples showed 863 bp amplicon, and the (Fig. 4e) remaining 22 1'8 samples did not produce any PCR product with the primers specific for partial length VP4 gene. Out of 33 bovine samples, P typed, P[1], and P[11] were seen in only 5 (16.12%) samples each, and only six samples (19.35%) showed mixed P genotypes (P[1]P[11]). Among P genotyped 16 samples, both P[1] and P[11] were the most predominant P types, as found in 5/16 (31.25%) samples each. Six samples (37.5%) showed mixed (P[1]P[11]) type, and the rest of them were untypeable. This study conforms with the earlier report by Gulati et al. (1999), who reported P[11] in 94 percent of the samples genotyped. Interestingly, his study also reported P[1] in a single sample out of 36 P-typed samples.

Combination of P and G Genotypes and Region-Wise Genotypic Distribution

In the bovine samples G3P[11] was seen in 2/13 (15.38%) samples, G10P[1] in 1/13 (7.69%) samples, G3P[1] [11] in 1/13 (7.69%) samples, G10P[1] [11] in 2/13 (15.38%) samples, while 2/13 (15.38%) samples had a mixed G type with P[1], 3/13 (23.07%) mixed G type with P[11] and 2/13 (15.38%) mixed G type with P[1]P[11] combinations. From Uttarakhand, among 17 bovine samples G typed, 4 samples (23.52%) showed mixed G3 and G10, while nine samples (52.94%) showed G3G10 and G3G6G10.

DISCUSSION

The current research aimed to characterise the novel genotypes from G and P groups of the rotaviral family using the RT-PCR method. The present study showed that the presence of non-typeable strains of groups G and P confirmed the possibility of emerging novel genotypes in cattle. The rotaviruses change their genome due to a lack of proofreading activity during their genomic replication. They also attract continuous surveillance and typing to assess their severity in animals and humans (Saran et al. 2015; Saran et al. 2019; Burrell et al. 2017; Vellingiri et al. 2020). Hence, the confirmatory evaluation of cattle rotaviruses is a useful preventive measure against huge economic losses and the livestock industry (Geletu et al. 2021).

Given surveillance of Rotavirus strains, 150 bovine viral samples from different cattle forms around Pant Nagar, India, were analysed and characterised in the present study. The extracted dsRNA from samples were electrophoresed using agarose, and a typical migration pattern, that is, 4:2:3:2 (Class I, II, III, and IV of 11 segments), was absorbed for Group A confirmation. Similarly, Basera et al. (2010) also reported RNA migration patterns from cattle isolates. The present study also showed that nearly half of the many tested samples (13 out of 31 samples) showed mixed G and P genotypes such as G3G6G10P[11] (23.07%) followed by 2/13 (15.38%) each of G3P[11], G10P[1]P[11], G3G10P[1]P[11] and G3G6G10P[1], while 7.69 percent (1/13) samples had G6P[1] and G3 P[1]P[11] combination. Again, this is different from what Gulati et al. (1999) said about the Indi-

Table 5: Prevalence of G and P type combinations in bovine samples

S. No.	*P and G genotype*	*No. out of 13*	*Prevalence (%)*	*Sample Id.*
1	G3P [11]	2	15.38	B1, B2
2	G10P [1]	1	7.69	B6
3	G3 P [1]P[11]	1	7.69	B25
4	G10 P [1]P[11]	2	15.38	B10, B11
5	G3G10P [1]P[11]	2	15.38	B8, B9
6	G3G6G10P [1]	2	15.38	B14, B16
7	G3G6G10P [11]	3	23.07	B17,B20, B20

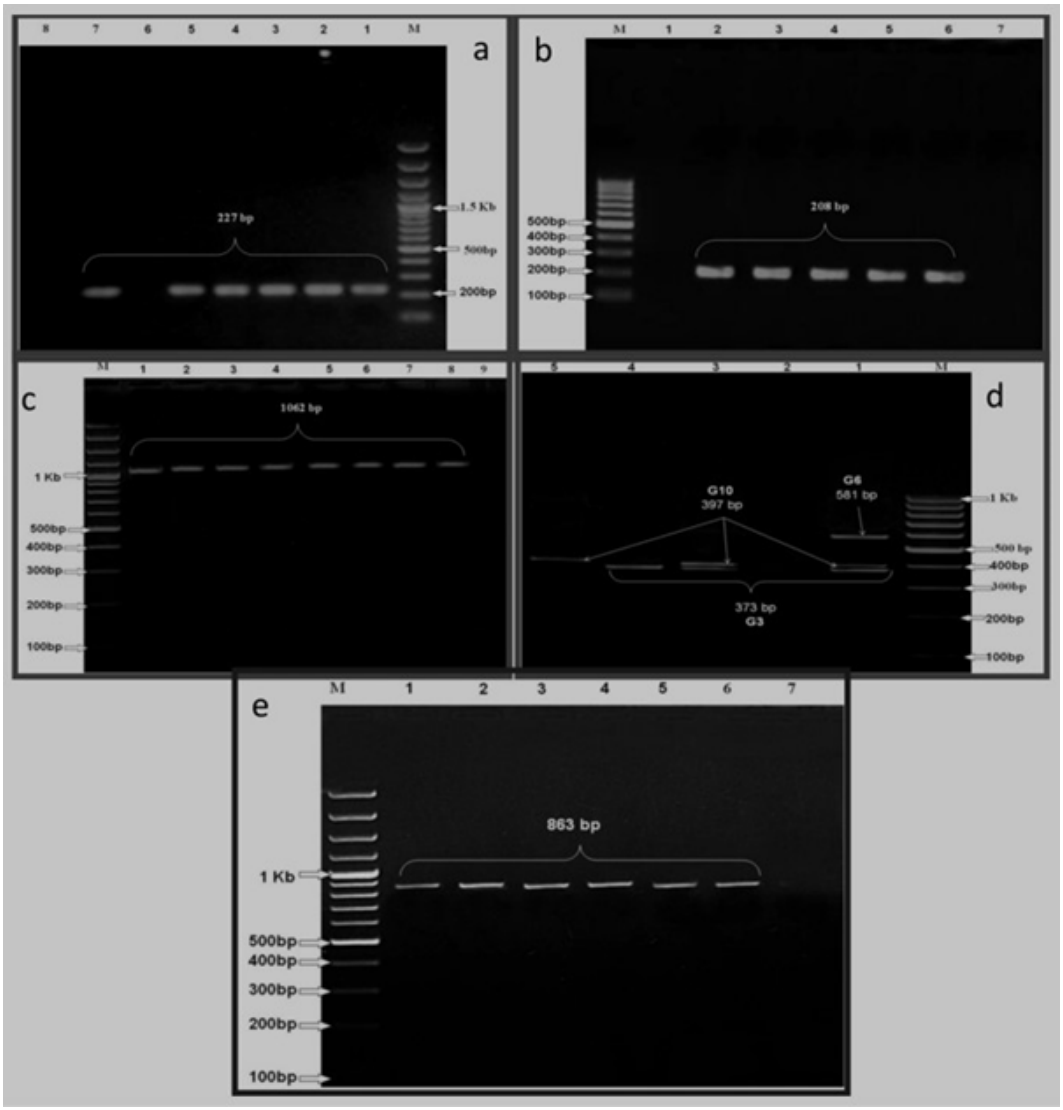

Fig. 4. a. RT-PCR of partial length VP6 gene of Bovine rotavirus. Lane M: GeneRulerTM 1Kb plus DNA Ladder (MBI, Fermentas); Lane 1:- B1; Lane 2:- B2
Lane 3:- B3; Lane 4:- B4; Lane 5:- B20; Lane 6:- Negative; Lane 7:- B25; Lane 8:- Negative Control
b. RT-PCR of partial length VP7 gene of Bovine rotavirus. Lane M: GeneRulerTM 100 bp plus DNA Ladder (MBI, Fermentas); Lane 1:- Negative; Lane 2:- B14; Lane 3:- B15; Lane 4:- B16; Lane 5:- B17; Lane 6:- B18; Lane 7:- Negative Control
c. RT-PCR of full length VP7 gene (Bovine and Human). Lane M: GeneRulerTM 100 bp plus DNA Ladder (MBI, Fermentas); Lane 1:- B14; Lane 2:- B15; Lane 3:- B17; Lane 4:- B18; Lane 5:- H1; Lane 6:- H2; Lane 7:- H7; Lane 8:- H8; Lane 9:- Negative Control
d. Multiplex PCR for genotyping of VP7 gene in Bovine rotavirus. Lane M: GeneRulerTM 100 bp plus DNA Ladder (MBI, Fermentas); Lane 1:- B14; Lane 2:- Negative; Lane 3:- B8; Lane 4:- B25; Lane 5:- B18
e. Figure 4e: RT-PCR of partial length VP4 gene of bovine rotavirus; Lane M: GeneRulerTM 100 bp plus DNA Ladder (MBI, Fermentas); Lane 1:- B5; Lane 2:- B6; Lane 3:- B14; Lane 4:- B16; Lane 5:- B23; Lane 6:- B25; Lane 7:- Negative Control

an bovine population. They said that G10P[11] (81%) was the most common combination of G and P types, followed by G6P[1] (3%) and G6P[11] (3%). The RNA-PAGE positive, positive samples were again analysed using ELISA, and comparatively, ELISA appeared to be more effective in detecting RVs than RNA PAGE. Similarly, the published works also suggested the effectiveness of ELISA over the RNA-PAGE analysis (Manuja et al. 2010; Dash et al. 2011; Soltan et al. 2016; Lorestani et al. 2019). However, the present study contradicts the early findings where RNA PAGE detected more positive samples than ELISA. In the meanwhile, research by Dash et al. (2011) showed strong correlations between RNA PAGE and ELISA, and all RNA PAGE-positive samples were also ELISA-positive. Because calves and children were sampled between the ages of 0 and 3 months, it is possible that the reduced prevalence of rotavirus identified in the current research is attributable to the age factor. It has been shown that calves younger than one month are most vulnerable to BRV infection. All of the 70 samples taken from clinically asymptomatic calves were negative for RVs, which may be related to the fact that they were taken in the early winter.

This bovine group A rotavirus prevalence is lesser than what was initially reported (Fukai et al. 1999). Joshi et al. (2019), on the other hand, revealed that after RNA-PAGE analysis of 36 diarrheic faecal specimens from calves, there was no RV infection from Maharashtra. All 26 BRVs found by RNA PAGE throughout the investigation were known A rotaviruses, with a migration pattern of 4:2:3:2 (class I-IV), and all samples had a lengthy electropherotype as shown by migration of fragment 10th and fragment 11 of the genome. Contrary to Jindal et al.'s (2000) results, which exclusively found short electropherotypes in bovine calves, none of the isolates had the short electropherotype (Jindal et al. 2000). According to a recent research by Manuja et al. (2010), who used viral isolation as the gold standard, RNA-PAGE and RT-PCR were respectively 66.7 percent and 71.4 percent accurate.

Group A rotavirus was identified using RT-PCR of the VP6 gene partial length with VP6-F and VP6-R primers. In the current study, amplification of the VP6 gene fragment yielded the predicted amplicon size of 227 bp. Of 42 ELISA-positive samples, 39 (92.85%) samples showed an amplicon of 227 bp in 1 percent agarose gel. Three samples (B7, B12, and H4) did not produce any PCR product with the primers specific for the partial length VP6 gene. Ten percent (12/128) of samples from dairy and beef calves were positive for group A rotavirus when tested with an immunocard that detects group A rotavirus VP6 (Rodriguez-Lomas et al. 2011). Untypable G and P strains were found in this research, which may indicate the emergence of novel genotypes. Since protection against infection and severe illness is thought to be type specific, rotavirus monitoring and typing are still critical.

CONCLUSION

Overall, the present study's results indicated a changing pattern of circulation of bovine RVA in India. During the investigation, the detection of human RVA G1 and G3 types as well as bovine RVA G3 and G10 types provide crucial information about the rising trend of these G serotypes circulation in the examined locations.

RECOMMENDATIONS

The findings add additional evidence of interspecies RV transmission and reassortment in the wild. Understanding RV ecology as well as how RVs grow, cross species boundaries, transfer genes during reassortment, and evolve as a result of single-point mutations or genetic recombination, it is crucial to examine the distribution of rotavirus G and P in bovine and human communities. The research comes to the final conclusion that creating a unique vaccine to prevent human and bovine RV-caused diarrhoea will be essential to combine them into growing G types, increasing protection. It is now becoming clear that in order to keep up with the ongoing development of these viruses, the reagents and techniques utilised must be periodically reviewed and improved.

REFERENCES

Abad AT, Danthi P 2020. Recognition of reovirus RNAs by the innate immune system. *Viruses*, 12.

Alter SJ, Bennett JS, Koranyi K, Kreppel A, Simon R 2015. Common childhood viral infections. *Current Problems in Pediatric and Adolescent Health Care*, 45(2): 21-53.

Badaracco A, Garaicoechea L, Matthijnssens J et al. 2013. Phylogenetic analyses of typical bovine rotavirus genotypes G6, G10, P [5] and P [11] circulating in Argentinean beef and dairy herds. *Infection, Genetics and Evolution*, 18: 18-30.

Basera SS, Singh R, Vaid N, Sharma K, Chakravarti S, Malik YPS 2010. Detection of rotavirus infection in bovine calves by RNA-PAGE and RT-PCR. *Indian Journal of Virology*, 21: 144-147.

Bukrinskaya AG 1982. Penetration of viral genetic material into host cell. *Advances in Virus Research*, 27: 141-204.

Burrell CJ, Howard CR, Murphy FA 2017. Pathogenesis of virus infections. *Fenner and White's Medical Virology*, 77-104. doi: 10.1016/B978-0-12-375156-0.00007-2

Changotra H, Vij A 2017. Rotavirus virus-like particles (RV-VLPs) vaccines: An update. *Reviews in Medical Virology*, 27: e1954.

Dash S, Tewari A, Kumar K, Goel A, Bhatia A 2011. Detection of Rotavirus from diarrhoeic cow calves in Mathura, India. *Veterinary World*, 4: 554.

Esona MD, Armah GE, Duncan Steele A 2010. Rotavirus VP4 and VP7 genotypes circulating in Cameroon: Identification of unusual types. *Journal of Infectious Diseases*, 202: S205-S211.

Fukai K, Sakai, T, Hirose M, Itou T 1999. Prevalence of calf diarrhea caused by bovine group A rotavirus carrying G serotype 8 specificity. *Veterinary Microbiology*, 66: 301-311.

Geletu US, Usmael MA, Bari FD 2021. Rotavirus in calves and its zoonotic importance. *Veterinary Medicine International*, 6639701.

Gouvea V, Brantly M 1995. Is rotavirus a population of reassortants? *Trends in Microbiology*, 3: 159-162.

Gulati BR, Nakagomi O, Koshimura Y, Nakagomi T, Pandey R 1999. Relative frequencies of G and P types among rotaviruses from Indian diarrheic cow and buffalo calves. *Journal of Clinical Microbiology*, 37: 2074-2076.

Herring AJ, Inglis NF, Ojeh CK, Snodgrass DA, Menzies JD 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. *Journal of Clinical Microbiology*, 16: 473-477.

Hoshino Y, Kapikian AZ 1996. Classification of rotavirus VP4 and VP7 serotypes BT - Viral Gastroenteritis. In: S Chiba, MK Estes, S Nakata, CH Calisher (Eds.): *Viral Gastroenteritis*. Vienna: Springer Vienna, pp. 99-111.

Jindal SR, Maiti NK, Oberoi MS 2000. Genomic diversity and prevalence of Rotavirus in cow and buffalo calves in northern India. *Revue scientifique et technique (International Office of Epizootics)*, 19: 871-876.

Joshi MS, Walimbe AM, Dilpak SP, Cherian SS, Gopalkrishna V 2019. Whole-genome-based characterization of three human Rotavirus C strains isolated from gastroenteritis outbreaks in Western India and a provisional intra-genotypic lineage classification system. *Journal of General Virology*, 100: 1055-1072.

Kapikian AZ, Flores J, Hoshino Y, Glass RI et al. 1986. Rotavirus: The major etiologic agent of severe infantile diarrhea may be controllable by a Jennerian approach to vaccination. *The Journal of Infectious Diseases*, 153: 815–822.

Laemmli UK 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

Lauring AS, Frydman J, Andino R 2013. The role of mutational robustness in RNA virus evolution. *Nature Reviews Microbiology*, 11: 327-336.

Lorestani N, Moradi A, Teimoori A, Masodi M et al. 2019. Molecular and serologic characterization of rotavirus from children with acute gastroenteritis in northern Iran, Gorgan. *BMC Gastroenterology*, 19: 100.

Manuja BK, Prasad M, Gulati BR et al. 2010. Comparative efficacy of immunological, molecular and culture assays for detection of group A rotavirus from faecal samples of buffalo (Bubalus bubalis) calves. *Tropical Animal Health and Production*, 42: 1817-1820.

Papp H, László B, Jakab F, Ganesh B et al. 2013. Review of group A rotavirus strains reported in swine and cattle. *Veterinary Microbiology*, 165: 190-199.

Rio DC, Ares M, Hannon GJ, Nilsen TW 2010. Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harbor Protocols*, 6: 5439.

Rodríguez-Limas WA, Tyo KEJ, Nielsen J et al. 2011. Molecular and process design for rotavirus-like particle production in Saccharomyces cerevisiae. *Microbial Cell Factories*, 10: 33.

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

Saran N, Bupesh G, Magesh S, Vennila S et al. 2015. Epidemiological studies and molecular characterization of Herpes Simplex Virus among urban population in Chennai, Tamilnadu. *Epidemiology*, 5: 2.

Saran N, Anandharaj B, Bupesh G, Vasanth S, Beulah JP, Balachandar V 2019. Molecular docking analysis of a secondary metabolite with the glycoprotein receptors of HSV 1 and HSV 2. *Bioinformation*,15(12): 887.

Saravanan KM, Zhang H, Senthil R, Vijayakumar KK et al. 2022. Structural basis for the inhibition of SARS-CoV2 main protease by Indian medicinal plant-derived antiviral compounds. *Journal of Biomolecular Structure and Dynamics*, 40: 1970-1978.

Soltan MA, Tsai YL, Lee PYA, Tsai CF et al. 2016. Comparison of electron microscopy, ELISA, real time RT-PCR and insulated isothermal RT-PCR for the detection of Rotavirus group A (RVA) in feces of different animal species. *Journal of Virological Methods*, 235: 99-104.

Vellingiri B, Jayaramayya K, Iyer M, Narayanasamy A et al. 2020. COVID-19: A promising cure for the global panic. *Science of the Total Environment*, 725: 138277.

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