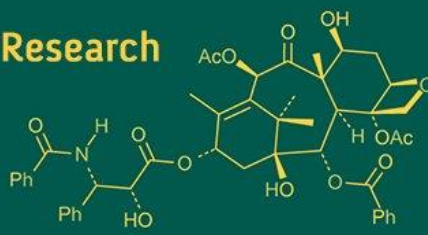


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Cell culture propagation, RNA-PAGE analysis and genotyping of bovine rotavirus group A

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Abstract

The present study was undertaken to isolate, propagate and characterize group A rotavirus (RVA) from diarrhoeic animals in Gujarat, India. A total of 74 faecal samples collected from cattle, buffalo, equine and poultry from different regions of Gujarat were screened using latex agglutination test (LAT), RNA-polyacrylamide gel electrophoresis (RNA-PAGE) and reverse transcription polymerase chain reaction (RT-PCR). Out of 74 samples, 5 (6.76%) samples were found positive by RT-PCR targeting the VP6 gene. Species-wise positivity was highest in cattle calves (16.67%) followed by poultry (3.12%). All positive isolates were successfully propagated in MA-104 cell lines and showed characteristic cytopathic effects within 2-4 passages. RNA-PAGE analysis revealed the typical 4:2:3:2 electropherotype pattern of mammalian group A rotavirus. Molecular characterization based on VP7 and VP4 genes identified G6, G8 and G10 genotypes along with P^[1] and P^[11] types. G6P^[11] was the predominant genotype combination detected among bovine isolates. A poultry isolate exhibited mammalian-type electropherotype suggesting possible interspecies transmission or reassortment events. The study highlights the circulation of genetically diverse bovine rotavirus strains in Gujarat and emphasizes the need for continuous molecular surveillance for effective control and vaccine development against neonatal diarrhoea in livestock.

Keywords: Rotavirus A, bovine calves, RNA-PAGE, genotyping, MA-104 cells, RT-PCR

Introduction

Rotavirus-induced diarrhoea is one of the major causes of morbidity and economic losses in neonatal livestock due to dehydration, reduced growth rate and treatment expenses. Group A rotavirus (RVA) is recognized globally as an important etiological agent of neonatal diarrhoea in calves, piglets, foals, lambs and poultry^[1]. Rotaviruses belong to the family *Reoviridae* and possess an 11-segmented double-stranded RNA genome encoding six structural and six non-structural proteins. Among them, VP6 determines group specificity, while VP7 and VP4 proteins determine G and P genotypes respectively^[2]. In bovines, G6, G8 and G10 genotypes associated with P^[1], P^[5] and P^[11] types are predominantly reported worldwide^[3]. The segmented nature of the viral genome facilitates reassortment and emergence of novel strains that may affect vaccine efficacy^[4]. Therefore, molecular characterization and surveillance of circulating strains are essential for effective disease control.

Several diagnostic methods including latex agglutination test (LAT), RNA-PAGE and RT-PCR are commonly employed for rotavirus detection. RNA-PAGE helps in identifying characteristic electropherotype patterns, whereas RT-PCR provides sensitive and specific molecular detection and genotyping^[5]. Similar diagnostic approaches have also been reported in bovine and avian rotavirus studies^[6, 7].

Recent studies have reported increasing genotype diversity and interspecies transmission events among bovine rotaviruses in India and other countries^[8, 9]. Comparable epidemiological findings have also been documented in recent Indian studies involving bovine calves^[10, 11]. Hence, the present study was undertaken to detect, isolate, propagate and molecularly characterize group A rotavirus from diarrhoeic animals of Gujarat using RNA-PAGE, MA-104 cell culture adaptation and VP4/VP7-based genotyping.

Materials and Methods

Study area and sample collection

The present study was conducted during 2024-2025 at the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India.

A total of 74 diarrhoeic faecal samples were collected aseptically from cattle (n = 24), buffalo (n = 12), poultry (n = 32) and foals (n = 6) from organized and unorganized farms of Anand, Junagadh, Rajkot and Godhra regions of Gujarat. Samples were collected from animals aged ≤2 months. Samples were transported under cold chain conditions and stored at -40 °C until processing.

RNA extraction

Ten percent faecal suspensions were prepared in phosphate-buffered saline (PBS; pH 7.2) and centrifuged at 10,000 × g for 30 min at 4 °C. Viral RNA was extracted using TRIzol™ reagent according to manufacturer's instructions. RNA quality and purity were assessed spectrophotometrically.

RNA-PAGE analysis

Extracted dsRNA was subjected to RNA-polyacrylamide gel electrophoresis using 8% separating and 5% stacking gels following the methods described by Herring *et al.* and Laemmli [5, 12]. Gels were silver stained and electropherotypes were interpreted according to migration

pattern.

Cell culture propagation

LAT-positive samples were filtered through a 0.22 µm membrane filter and treated with trypsin (10 µg/mL) at 37 °C for 30 min. The inoculum was adsorbed onto confluent MA-104 cell monolayers maintained in Minimum Essential Medium (MEM) supplemented with 2% fetal bovine serum. After adsorption, maintenance medium containing trypsin was added and cultures were incubated at 37 °C in a humidified 5% CO₂ atmosphere. Cytopathic effects were observed daily.

RT-PCR confirmation

Viral RNA extracted from infected cell culture supernatants was subjected to RT-PCR targeting the VP6 gene using previously described primers [13]. Amplified products were analyzed on 2% agarose gel stained with ethidium bromide.

G and P genotyping

VP7 and VP4 genes were amplified using published primers described by Falcone *et al.* [14]. Semi-nested multiplex PCR was performed for G5, G6, G8 and G10 genotyping and P [1], P [5] and P [11] typing. PCR products were analyzed by agarose gel electrophoresis.

Statistical analysis

Descriptive statistics were used to calculate prevalence percentages among species, age groups and regions.

Table 1: Oligonucleotide primers used to amplify a part of the VP6, VP7, and VP4 genes and for the G and P typing PCR

Target gene	Name of primers	Sequences (5' → 3')	Location	Product Size (bp)	References
VP6	VP6 F	GACGGVGCRACTACATGGT	1-19	379	Yilmaz (2016)
	VP6 R	GTCCAATTCATNCCTGGTGG	379-361		
VP7	Bov9Com 5 (VP7-F)	GGCTTTAAAAGAGAGAATTTCGTTTGG	1-28	1062	Falcon <i>et al.</i> (1999)
	Bov9Com 3 (VP7-R)	GGTCACATCATACAACCTCTAATCT	1039-1062		
VP7 for G typing	G5	CATGTACTCGTTGTTACGTC	779-760	779	
	G6	CTAGTTCCTGTGTAGAATC	499-481	499	
	G8	CGGTTCCGGATTAGACAC	273-256	273	
	G10	TTCAGCCGTTGCGACTTC	714-697	714	
VP4	Con3	TTCATTATTGGGACGATTACACA	1064-1085	855	
	Con2	CAACCGCAGCGGATATATCAC	1897-1918		
VP4 for P typing	P [1]	TTAAATTCATCTCTTAGTTCTC	1505-1526	460	
	P [5]	GGCCGCATCGGATAAAGAGTC	1704-1725	659	
	P [11]	TGCCTCATAATATTGTTGGTCT	1377-1398	335	

Table 2: Species-wise Positivity of Rotavirus by RT-PCR

Species	No. of Samples Tested	No. of Samples Positive by RT-PCR	Positivity (%)
Cattle	24	4	16.67
Buffalo	12	0	0.00
Horse	6	0	0.00
Poultry	32	1	3.12
Total	74	5	6.76

Results

Detection of rotavirus

Out of 74 samples tested, 5 (6.76%) were positive for group A rotavirus by RT-PCR. Highest positivity was observed in cattle calves (16.67%) followed by poultry (3.12%). Buffalo and foal samples were negative.

Cell culture propagation

All positive samples were successfully propagated in MA-

104 cells. Characteristic cytopathic effects including rounding, syncytia formation, ballooning and detachment of cells were observed within 48-72 h post-infection.

RNA-PAGE analysis

All isolates exhibited the characteristic 4:2:3:2 electropherotype pattern of mammalian group A rotavirus. The poultry isolate also exhibited mammalian-type electropherotype.

Molecular characterization and genotyping
RT-PCR amplification produced expected amplicons of approximately 1062 bp for VP7 and 855 bp for VP4 genes.

Genotyping identified G6, G8 and G10 genotypes with P^[11] and P^[11] types. G6P^[11] was the predominant genotype combination.

Table 3: Overall type combinations and Relative Frequency of G and P Types Circulating in the Bovine Population

Genotype	Cattle Isolates-P ^[11] (Positive Samples)	Positive (%)	Cattle Isolates-P ^[11] (Positive Samples)	Positive (%)	Total (Cattle) Positive Samples	Positive (%)	Poultry Isolate (Non- typeable) Positive Samples	Positive (%)
G6	0	00.00	2	50.00	2	50.00	0	00.00
G8	1	25.00	0	00.00	1	25.00	0	00.00
G10	1	25.00	0	00.00	1	25.00	0	00.00
Non-typeable	0	00.00	0	00.00	0	00.00	1	100.00
Total	2	50.00	2	50.00	4	100.00	1	100.00

Table 4: Details of Samples Positive by RT-PCR for VP6, VP7, VP4 Genes and G-P Genotyping of Rotavirus A Isolates Propagated in Cell Culture

Sr. No.	Sample Code	RT-PCR VP6	RT-PCR VP7	RT-PCR VP4	G6	G10	G8	P ^[11]	P ^[1]	G-P Type Combination
1	C-27	Positive	Positive	Positive	-	-	Positive	-	Positive	G8P ^[1]
2	C-30	Positive	Positive	Positive	-	Positive	-	-	Positive	G10P ^[1]
3	C-35	Positive	Positive	Positive	Positive	-	-	Positive	-	G6P ^[11]
4	C-36	Positive	Positive	Positive	Positive	-	-	Positive	-	G6P ^[11]
5	P-27	Positive	Positive	Positive	Non-typeable	Non-typeable	Non-typeable	Non-typeable	Non-typeable	Non-typeable

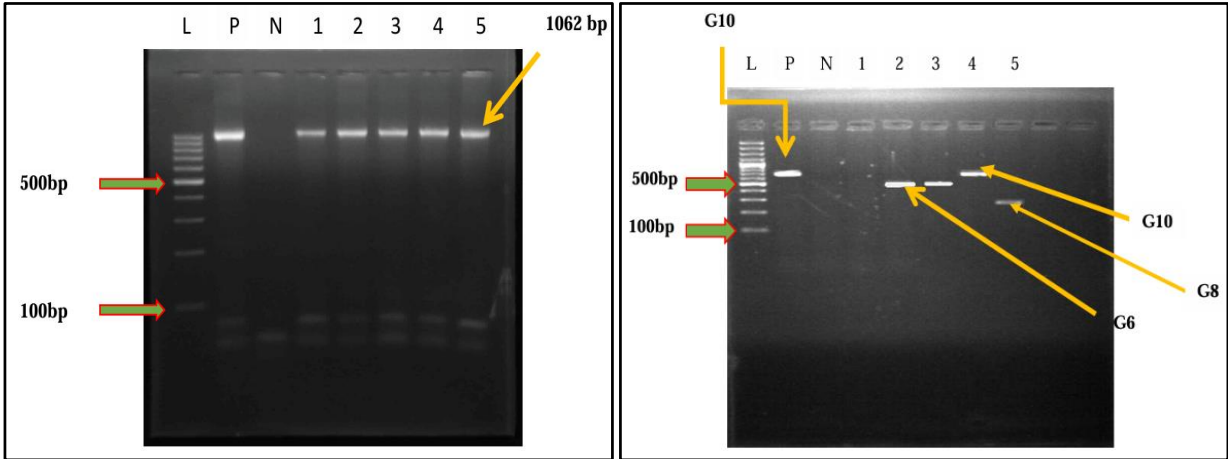


Fig. 1: Agarose gel electrophoresis showing (left) ~1062 bp VP7 gene amplicons of Rotavirus A isolates and (right) G-genotyping results. Left panel: L-100 bp ladder, lanes 1-5-isolates, P-positive control, N-negative control. Right panel: lane 1—poultry isolate, lanes 2-3—bovine isolates G6 (499 bp), lane 4—bovine isolate G10 (714 bp), lane 5—bovine isolate G8 (273 bp).

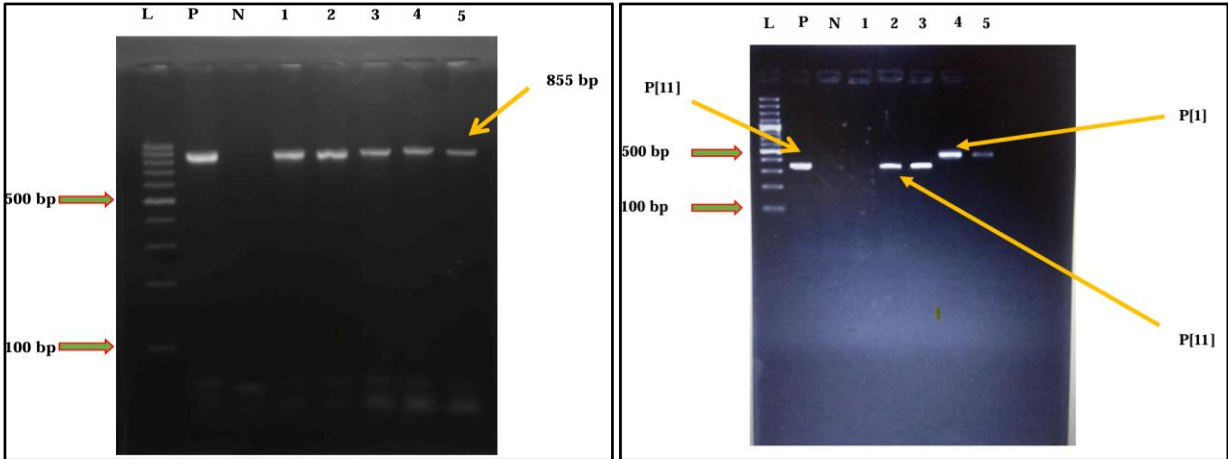


Fig 2: Agarose gel electrophoresis showing (left) ~855 bp VP4 gene amplicons of Rotavirus A isolates and (right) P-genotyping results. Left panel: L—100 bp ladder, lanes 1-5—isolates, P—positive control, N—negative control. Right panel: VP lane 1—poultry isolate, lanes 2-3—bovine isolates P^[11] (335 bp), lanes 4-5—bovine isolates P^[1] (460 bp).

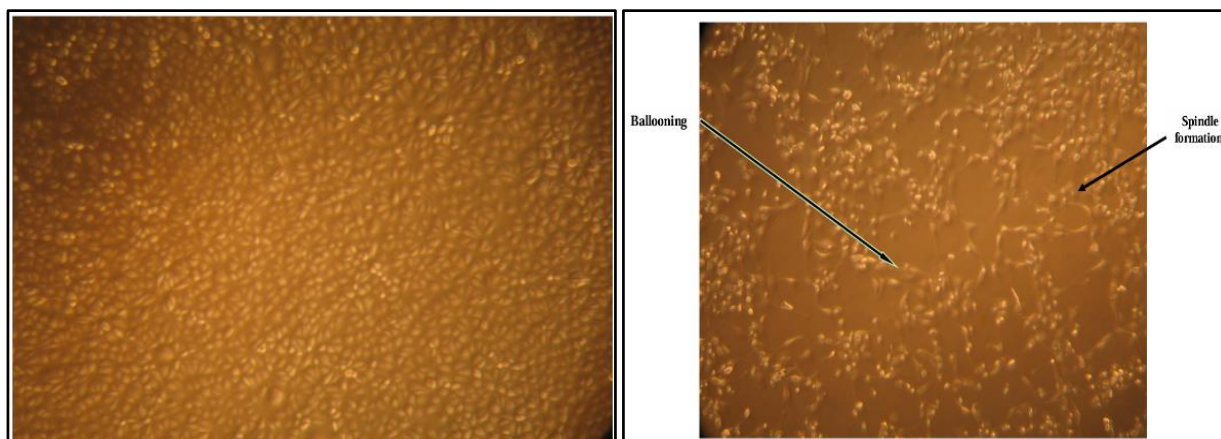


Fig 3: Cytopathic effects of Rotavirus infection in MA-104 cells.

Left panel: Non-infected MA-104 cell line showing normal morphology (10×).

Right panel: Infected MA-104 cells exhibiting characteristic ballooning and spindle-shaped formations after 44 hours post-infection (20×).

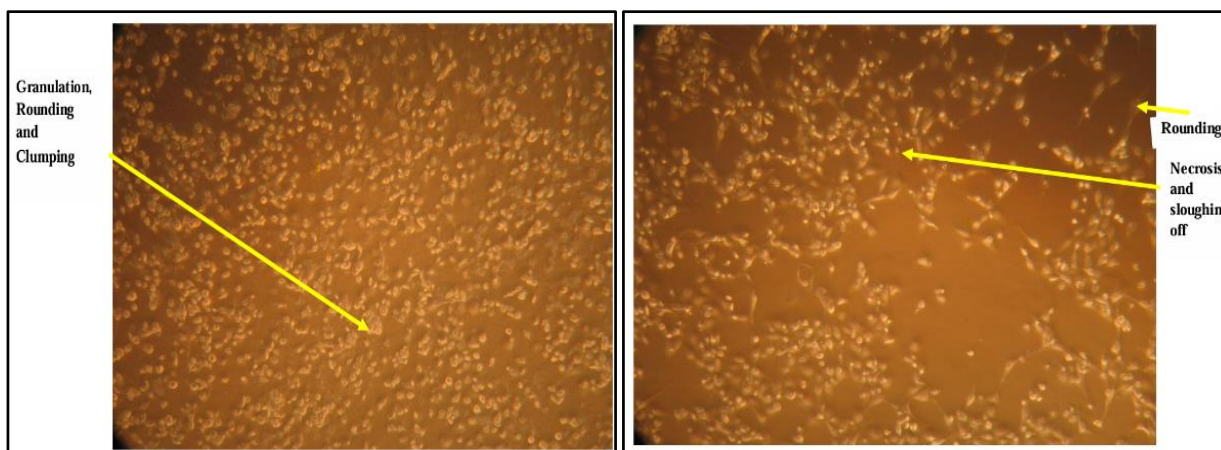


Fig 4: Progressive cytopathic effects of Rotavirus infection in MA-104 cells.

Left panel: MA-104 cells showing characteristic granulation, rounding, and clumping at 36 hours post-infection (10×, phase-contrast microscopy).

Right panel: MA-104 monolayer exhibiting pronounced rounding, necrosis, and sloughing off at 48 hours post-infection (10×).

Table 1: Species-wise positivity of rotavirus by RT-PCR

Species	No. of samples tested	No. positive	Positivity (%)
Cattle	24	4	16.67
Buffalo	12	0	0.00
Horse	6	0	0.00
Poultry	32	1	3.12
Total	74	5	6.76

Table 2: Overall type combinations and relative frequency of G and P types

Genotype	P ^[1] Positive	P ^[11] Positive	Total bovine positive	Poultry positive
G6	0	2	2	0
G8	1	0	1	0
G10	1	0	1	0
Non-typeable	0	0	0	1

Table 3: Details of genotyping of rotavirus isolates

Sample code	VP6	VP7	VP4	G type	P type	G-P combination
C-27	Positive	Positive	Positive	G8	P ^[1]	G8P ^[1]
C-30	Positive	Positive	Positive	G10	P ^[1]	G10P ^[1]
C-35	Positive	Positive	Positive	G6	P ^[11]	G6P ^[11]
C-36	Positive	Positive	Positive	G6	P ^[11]	G6P ^[11]
P-27	Positive	Positive	Positive	Non-typeable	Non-typeable	Non-typeable

Discussion

The overall prevalence of rotavirus A observed in the present study indicated moderate circulation of the virus among neonatal livestock in Gujarat. Similar prevalence rates have been reported earlier from different regions of India^[1, 8, 10]. Kumar *et al.*^[11] also reported considerable genotypic diversity among bovine rotaviruses circulating in India.

Higher infection in calves aged 16-45 days may be associated with decline in maternally derived antibodies during this age, thereby increasing susceptibility to infection. Comparable age-related susceptibility has been reported previously by Maes *et al.*^[15].

Successful propagation of all isolates in MA-104 cell lines confirmed the suitability of this cell culture system for rotavirus isolation. The observed cytopathic effects including cell rounding, syncytia formation and detachment were comparable with earlier findings reported by Herring *et al.*^[5] and Parwani *et al.*^[16].

RNA-PAGE analysis revealed the characteristic 4:2:3:2 electropherotype pattern of mammalian group A rotavirus. Similar electropherotype patterns were also described by Svensson *et al.*^[17]. Detection of mammalian-type electropherotype in a poultry isolate may indicate possible interspecies transmission or reassortment events as suggested by recent reports^[9].

Molecular characterization revealed predominance of G6P^[11] genotype which is in agreement with earlier studies conducted in bovine populations^[3, 18]. Detection of multiple genotypes further supports the dynamic nature of rotavirus evolution and reassortment. Rao *et al.*^[19] also reported emergence of novel G and P genotypes among bovine rotaviruses due to genomic reassortment.

The findings of the present study are also supported by previous epidemiological investigations conducted in India by Kaur^[20] and Golaviya^[21]. Continuous molecular surveillance and genomic studies are therefore necessary to monitor emerging strains and to improve vaccine strategies against neonatal diarrhoea.

Conclusion

The present study demonstrated successful detection, isolation and propagation of group A rotavirus from diarrhoeic animals using MA-104 cell culture. Molecular characterization identified G6P^[11] as the predominant circulating genotype among bovine isolates in Gujarat. RT-PCR proved to be a sensitive diagnostic tool for rotavirus detection. Detection of mammalian-type electropherotype in poultry suggests possible interspecies transmission. Continuous surveillance is essential for monitoring genotype diversity and supporting effective vaccine development.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

Not required.

Funding

The study did not receive any external funding.

Authors' Contribution

D.K.L. and R.A.M. designed the study. D.K.L. conducted the experiments. F.A.P. prepared the manuscript. A.V.G. assisted in manuscript revision. All authors approved the final manuscript.

References

1. Dhama K, Saminathan M, Karthick K, Tiwari R, Shabbir MZ, Singh KR. Avian rotavirus enteritis-an updated review. *Vet Q.* 2015;35(3):142-158.
2. Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Banyai K, Brister JR, *et al.* Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group. *Arch Virol.* 2011;156:1397-1413.
3. Fukai K, Sakai T, Kamata H. Distribution of G serotypes and P genotypes of bovine group A rotavirus isolated in Japan. *Aust Vet J.* 1998;76(6):418-422.
4. Papp H, Laszlo B, Jakab F, Ganesh B, De Grazia S, Matthijnssens J, *et al.* Review of group A rotavirus strains reported in swine and cattle. *Vet Microbiol.* 2013;165:190-199.
5. Herring AJ, Inglis NF, Ojeh CK, Snodgrass DA, Menzies JD. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver stained polyacrylamide gels. *J Clin Microbiol.* 1982;16(3):473-477.
6. Kattoor JJ, Malik YS, Sharma K. Molecular evidence of group D rotavirus in commercial broiler chicks in India. *Avian Biol Res.* 2013;6(4):313-316.
7. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970;227:680-685.
8. Mishra N, Sharma A, Singh SK, Mishra B. Epidemiology and molecular characterization of bovine rotavirus strains circulating in India. *Trop Anim Health Prod.* 2021;53(2):1-9.
9. Sharma R, Gupta VK, Malik S, Dhama K. Emerging trends in bovine rotavirus infections: molecular epidemiology, interspecies transmission and vaccine challenges. *Front Vet Sci.* 2023;10:1189456.
10. Gill GS, Kaur S, Dwivedi PN, Gill JPS. Comparative prevalence and molecular characterization of group A rotavirus in cow calves of Punjab, India. *J Anim Res.* 2017;7(5):927-933.
11. Kumar A, Singh S, Kumar R, Kumar P. Molecular characterization and genotypic diversity of group A rotavirus in bovine calves in India. *Vet Microbiol.* 2022;267:109356.
12. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970;227:680-685.
13. Yilmaz V. Investigation of rotavirus infection in calves with diarrhea in northeast Turkey. *Anim Vet Sci.* 2016;4(1):1-4.
14. Falcone E, Tarantino M, Di Trani L, Cordioli P, Lavazza A, Tollis M. Determination of bovine rotavirus G and P serotypes in Italy by PCR. *J Clin Microbiol.* 1999;37(12):3879-3882.
15. Maes RK, Grooms DL, Wise AG, Han C, Ciesicki V, Hanson L, *et al.* Evaluation of a human group A rotavirus assay for on-site detection of bovine rotavirus. *J Clin Microbiol.* 2003;41(1):290-294.
16. Parwani AV, Munoz M, Tsunemitsu H, Luchelli A, Saif LJ. Molecular and serologic characterization of group A bovine rotavirus with a short genome pattern. *J Vet Diagn Invest.* 1995;7:255-261.
17. Svensson L, Uhnoo I, Grandien M, Wadell G. Molecular epidemiology of rotavirus infections in Uppsala, Sweden. *J Med Virol.* 1986;18(2):101-111.
18. Suresh T, Rai RB, Dhama K, Sawant PM, Kumar D, Bhatt P. Determination of G and P type diversity of group A rotaviruses in diarrhoeic calves in India. *Res J Vet Pract.* 2012;13(1):1-8.
19. Rao CD, Gowda K, Reddy BS. Sequence analysis of VP4 and VP7 genes identifies novel P and G genotypes in bovine rotaviruses. *Virology.* 2000;276:104-113.
20. Kaur S. Prevalence and molecular epidemiology of group A rotavirus among children and animals in North India. PhD thesis. Izatnagar: Indian Veterinary Research Institute; 2011.
21. Golaviya A. Isolation, electropherotyping and molecular characterization of bovine rotavirus from diarrhoeal samples of calves. M.V.Sc. thesis. Anand: College of Veterinary Science and Animal Husbandry; 2019.