



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2019; 7(6): 373-376

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Received: 01-09-2019

Accepted: 03-10-2019

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## VP6 gene based RT-PCR for detection of rotavirus associated with diarrhea in bovine calves

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DOI: <https://doi.org/10.22271/j.ento.2019.v7.i6g.5935>

**Abstract**

The present study was undertaken on VP6 gene based detection of Rotavirus in feces of diarrheic animals. Bovine fecal samples of cattle calves (54) between the age group of 0-3 months were collected from diarrheic animals from organized and unorganized farms in and around Nagpur. Of them 11 (20.37%) out of 54 samples were found positive for bovine rotavirus by latex agglutination test. The positive sample of calf was subjected to VP6 gene based Reverse Transcriptase polymerase chain reaction which yielded amplicon of 379 bp RT-PCR targeting and confirmed Group A rotavirus. VP6 gene base RT-PCR study confirmed the prevalence of group A Rotavirus in animals and can be used as a sensitive and specific assay for the rapid detection of group A Rotavirus in fecal samples.

**Keywords:** Group a rotavirus, VP 6 gene, diarrhea, RT-PCR, bovine calf

**Introduction**

The calf rearing period is a most crucial period for dairy and beef enterprises as development of healthy calves play major role in making the enterprise profitable. In the first year of life of calves, 50% mortality occurs within the first six weeks. The mortality of neonatal calves in the first month of age is more than 80% of the total mortality (Jenny *et al.*, 1981) [18]. Neonatal diarrhea is one of the most important diseases in calves worldwide causing large economic losses to cattle herds (Stanton *et al.*, 2013) [25].

Various infectious agents like viruses, microorganisms and protozoa are measure accountable for calf diarrhea (Smith, 2009) [24]. From these infectious agents, bovine rotavirus group A and bovine coronavirus act as infective viral agents, some enteric bacteria like *Salmonella* species and *E.coli* K99+ species act as bacterial agents and *Cryptosporidium* species act as protozoan agent (Bhat *et al.*, 2012; Bhat *et al.*, 2013; Singla *et al.*, 2013) [3, 4, 23].

Bovine rotavirus is a very important infectious agent that causes neonatal calf diarrhea (Alferiet *et al.*, 2004) [2] and it belongs to the genus *Rotavirus* of the family *Reoviridae*. It is a non-enveloped virus possessing eleven double-stranded RNA segments (16-21kbp) (Fenner, 2011) [10]. Based on antigenic and genetic similarity of intermediate capsid protein (VP6), there are 7 serogroups (A through G) among rotavirus (Steele *et al.*, 2004) [26]. Major cause of rotaviral infection in domestic animals is Group A rotavirus. It is the Group A where most of the bovine rotavirus (95%) belong to. Groups B and C rotaviruses have conjointly been known to be present in the field cases (Ghosh *et al.*, 2007; Lucchelli *et al.*, 1992; Tsunemitsu *et al.*, 1992) [12, 20, 27].

The rotavirus genome contain six structural and non-structural polypeptides respectively. The VP6 protein genome encodes as segment 6 and is most abundant structural protein forming the inner capsid of the virion. The macromolecules contains epitopes, common for all group 'A' rotaviruses and epitopes for subgroup I or II (Greenberg *et al.*, 1983) [11]. The main role of VP6 protein in transcriptase activity by maintaining the proper conformation of viral core or transcriptional complex (Estes and Cohen, 1989) [8]. This protein is highly antigenic and immunogenic and targeted the VP6 protein in most diagnostic assays for detection of rotaviruses. (He *et al.*, 2002) [15].

The Bovine rotavirus causes diarrhea in 1 to 2 weeks of age group of calves (Chinsangaram *et al.*, 1995) [5]. The calf uptake milk to provides a good environment for the survival of rotavirus at the gastrointestinal pH and infects the intestinal epithelial cell (Dhama *et al.*, 2009) [7]. This may explain the susceptibility of unweaned calves to calf diarrhea than weaned calves.

The incubation period is very short (12-24 hours) (Steele *et al.*, 2004) [26] and induces per acute diarrhea infected calves. The virus replication occurs in the cytoplasm of epithelial cells of villi (Holland, 1990) [16] and causes destruction of mature enterocytes in the villi. This results in the secretion of a viral enterotoxin (e.g., NSP4) caused by activation of the enteric nervous system by vasoactive components from the damaged cells. This results in maldigestive/ alabsorptive diarrhea. Hence the aim of the study was to targeting and confirmed Group A rotavirus by VP6 gene based Reverse Transcriptase polymerase chain reaction.

## Materials and Methods

### Collection of fecal samples and processing

A total of 54 fecal samples were collected from diarrheic calves of 0-3 months of age from organized and unorganized farms in and around Nagpur. Samples were collected using sterile rectal swabs and kept on ice and transported to laboratory. Samples were then stored at -20 °C.

For the detection of virus, a 10% fecal suspension was prepared in phosphate buffer saline (PBS) (pH 7.2), mixed and centrifuged at 10000 x g for 15 min to remove coarse particles. The clear suspension was transferred to fresh tubes and stored at -20 °C.

### Detection of rotavirus by using Latex Agglutination Test kit

Screening of Collected fecal samples for the presence of rotavirus was carried out by commercially available Latex Agglutination Test. Latex agglutination test was performed using LK08-HiRotavirus Latex Test kit according to manufacturer's instructions. The samples positive for presence of rota virus were used for molecular characterization of Rotavirus.

### Molecular Detection of bovine rotavirus

Extraction of viral RNA was done by TRIzol method. The RNA was quantified and stored at -20 °C. cDNA was synthesized using cDNA synthesis kit according to the instructions. The cDNA was stored for further use at -20 °C. The cDNA synthesized was used for amplification of VP6 gene (379 bp) of bovine rotavirus. The published primer sequences were used for RT-PCR. It was done by the conditions optimized by Isegawa *et al.*, (1993) [17] and Yilmaz *et al.*, (2017) [29]. The primer sequences and nucleotide position of oligonucleotide primers are shown in Table 1.

### Amplification of VP6 gene of rotavirus

The VP6 gene in the c-DNA samples were amplified by RT-PCR using gene specific primers. The sequence of the primer set F5'-GACGGVGCRACTACATGGT-3,R-5'-GTCCAATTCATNCCTGGTGG-3' were used for gene amplification producing 379 bp sized amplicon on 1% agarose gel. For PCR, the reaction mixture was prepared by mixing of 5X buffer 2.0 µl, 25mM MgCl<sub>2</sub> 1.5 µl, 25 dNTP 0.4 µl, Forward primer (10 pmols/ µl) 0.12 µl, Reverse primer (10pmols/ µl) 0.12 µl, Taq DNA polymerase (IU/ µl) 0.4µl, Template (c-DNA) 1.0 µl and Nuclease free water 14.46 µl for both the genes. The properly mixed 20µl reaction mixture was initially denatured at 94 °C for 5 minute. Thirty five cycles at 94 °C (30 sec), 50(1 min), 72 °C for 1 min, Final extension 72 °C for 7 min, for VP6. Both the positive and negative controls were run parallel along with the test samples. To confirm the targeted PCR amplicon which was obtained, the PCR products were subjected to electrophoresis in 1% agarose gel containing ethidium bromide (10 mg/ml) with 1X TAE buffer 100 volts (V) for 60 minutes @ 200 mA. The image was finally captured under gel documentation system.

**Table 1:** Details of the primers used for the detection of VP6 gene of bovine rotavirus

Primers	Sequence (5'-3')	Location	Expected product size (bp)	Reference
VP6-F	5'-GACGGVGCRACTACATGGT- 3,	747-766	379 bp	Falcone (1999) [9]
VP6-R	5'-GTCCAATTCATNCCTGGTGG-3'	1126-1106	379 bp	

## Results and Discussion

In the present study, 11 (20.37%) out of 54 samples were found positive for bovine rotavirus by latex agglutination test as shown in plate no.1. All the samples were subjected for the further detection of group A Rotavirus by VP6 gene based RT-PCR assay in which 11 (20.37%) samples were positive for bovine rotavirus as shown in plate no.2.

The present findings are in agreement with findings of Ade *et al.* (2019) [1] who found that out of 288 samples, 35 (12.15%) samples were detected positive for rotavirus by Latex Agglutination test from 5 districts of Amravati region. Jindal *et al.* (2000) reported the incidence of rotavirus ranging from 10% to 52% in cattle calves and 11% to 24% in buffalo calves respectively. Hassan *et al.* (2014) [14] have reported overall incidence of the bovine rotavirus 12.8% (37/290) in calf diarrheic fecal samples by latex agglutination test. Khafagi *et al.* (2010) [19] found that 12.3% of incidence of rotavirus by Latex agglutination test (LA) and Enzyme Linked Immunosorbant Assay (ELISA) for the rotavirus with diarrhea positive lambs and kids in Egypt. Hashem *et al.* (2012) [13] screened 450 fecal specimens by Latex agglutination (LA) and Enzyme Immuno Assay (EIA). In a test, about 94 (20.8%) samples were reactive, 45.7% as non-reactive and 33.3% remained indeterminate. Further all LA

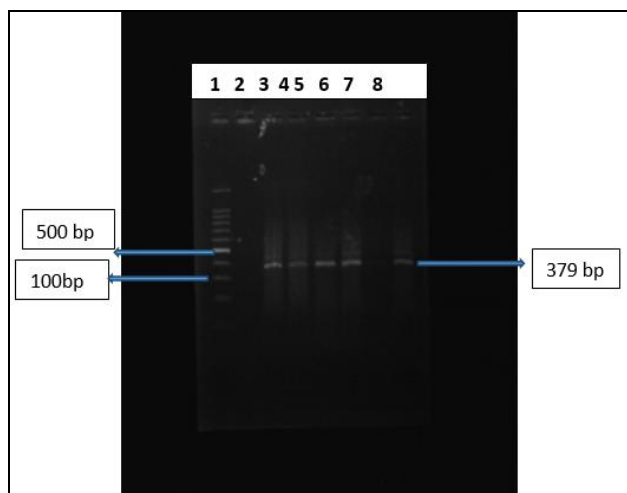
positive also been positive in EIA tests, and 25 LA negative samples were positive by EIA. Paesiet *al.* (2012) [22] reported that the use of Rotavirus Latex Kit (Richmond immunosystems Diagnostics) was more sensitive in 38 samples (11.6%) and found to be positive when compared to PAGE where only 26 samples (7.9%) found positive for detecting rotavirus antigen in diarrheic feces of piglets and human.

Out of 11 samples which were positive for bovine rotavirus by Latex Agglutination Test, 10 samples were found positive by RT-PCR and one sample which was negative by LAT was found positive by RT-PCR. The results of present study confirm that RT-PCR method was more sensitive tool for rapid molecular detection and identification of *Rotavirus*. The present study findings are in agreement with the findings of Das *et al.* (2018) [6] have analyzed that RT-PCR to be more effective method for rotavirus diagnosis. They used two published primer sets which were amplified to produce 309 and 304 bp sized amplicons for VP6 and VP7 gene on 1.7% agarose gel electrophoresis and 39 (22.28%) out of 175 samples were found to be positive for both VP6 and VP7 genes of bovine rotavirus. Similarly Tumlam *et al.* (2018) [28], reported that out of 44 samples screened, 43 (97.72%) samples were found positive for rotavirus. 17 out of 18

samples were found positive by VP6 gene based RT-PCR assay from bovine calves, piglets, lambs, kids and pups were found cent percent positive for group A rotavirus. Mondol *et al.* (2013) [21] revealed that out of 211 samples collected from diarrheic bovine, porcine and human infants (below 6 months of age), 26 (12.32%) samples were found positive for group A rotavirus by VP6 gene based RT-PCR assay. A total of 11.23% (10/89) bovine, 10.97% (10/82) porcine fecal samples and 17.5% (6/40) human stool samples were positive by VP6 based RT-PCR assay. In conclusion, the group A Rotavirus is prevalent in bovine and RT-PCR is used as sensitive and specific molecular method for the detection of bovine rotavirus.



**Plate 1:** Latex Agglutination Test for Group A rotavirus 1: Positive control 2: Negative control 3, 4, 5, 6: Positive test samples



**Plate 2:** Detection of VP6 gene of bovine rotavirus by RT-PCR Lane 1: Molecular weight marker (100 bp ladder) Lane 2: Negative control Lane 3: Positive control Lane 4, 5, 6, 8: Positive samples Lane 7: Negative sample

### Acknowledgment

The authors are grateful to the Associate Dean, Nagpur Veterinary College, Nagpur for providing necessary facilities to undertake the present study.

### References

1. Ade MJ, Ingle VC, Tembhurne PA, Tumlam UM, Kadam AS, Bhojne GR *et al.* Incidence and Molecular Characterization of Rotaviruses of Cattle and Buffalo Calves in Amravati Region, Maharashtra, Indian Journal of Veterinary Sciences & Biotechnology. 2019; 14(3):20-23, 4
2. Alfieri AF, Alfieri AA, Barreiros MAB, Letie JPG,

- Richtzenhain J. G and P genotypes of group a rotavirus strains circulating in calves in brazil, 1996-1999. *Veterinary Microbiology.* 2004; 99:167-173
3. Bhat SA, Juyal PD, Singla LD. Prevalence of cryptosporidiosis in neonatal buffalo calves in Ludhiana district of Punjab, India. *Asian Journal of Animal Veterinary. Advice.* 2012; 7(6):512-520
4. Bhat SA, Juyal PD, Singla LD. Bovine cryptosporidiosis: brief review of its distribution in India. *Trends Parasitology. Research.* 2013; 2(2):5-13.
5. Chinsangaram J, Schore CE, Guterbock W, Weaver LD, Osburn BI. Prevalence of group A and group B rotaviruses in the feces of neonatal dairy calves from California. *Comparative, Immunology, Microbiology and Infectious Diseases.* 1995; 18:93-103.
6. Das S, Medhi M, Khaound M, Doley P, Islam M, Borah DP. Detection of group A rotavirus infection in diarrhoeic calves by electropherotyping and reverse transcriptase polymerase chain reaction, *Journal of Entomology and Zoology Studies* 2018; 6(3):1071-1075
7. Dhama K, Chauhan RS, Mahendran M, Malik SV. Rotavirus diarrhea in bovines and other domestic animals. *Veterinary Research Communication.* 2009; 33:1-23.
8. Estes MK, Cohen J. Rotavirus gene structure and function. *Microbiology. Review.* 1989; 53(4):410-449.
9. Falcone E, Tarantino M, Trani LD, Cordioli P, Lavazza A, Tollis M. Determination of G and P serotypes in Italy using PCR. *Journal of Clinical Microbiology.* 1999; 37(12):3879-3882.
10. Fenner. *Veterinary Virology.* 4th ed. Academic Press, Burlington, MA, 2011, 274- 280.
11. Greenberg HB, McAuliffe V, Valdesuso J, Wyatt R, Flores J, Kalica A *et al.* Serological analysis of the subgroup proteins of rotavirus using monoclonal antibodies. *Infection and Immunity.* 1983; 39:91-99
12. Ghosh S, Varghese V, Samajdar S, Manju S, Naik TN, Kobayashi N. Evidence for bovine origin of VP4 and VP7 genes of human group A rotavirus G6P[14] & G10P [14] strains. *Journal of Clinical Microbiology* (Accepted and Published Online ahead of print on 30 May, 2007.
13. Hashem S, Shoman SA, Mohamed AF. Isolation and Molecular Genotyping of Group A Rotavirus Strains Circulating among Egyptian Infants and Children. *Egyptian Journal of Medical Microbiology.* 2012; 21(3):11
14. Hassan. G and P typing and sequence analysis of group A rotavirus in calves and lambs in Kashmir PhD thesis submitted to Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Division of Veterinary Microbiology & Immunology Shuhama Campus, Srinagar, 2014.
15. He KW, Lin JH, Ding ZD, He JH, Jianj JY, Hou JB. Serotyping and antigenic comparison of some animal rotavirus isolated in China. *Zentralbl Vetrinarmed.,* 2002 B; 39:299-300.
16. Holland RE. Some Infectious Causes of Diarrhea in Young Farm-Animals. *Clinical Microbiology Reviews* 1990; 3:345-375
17. Isegawa Y, Nakagomi O, Nakagomi T, Ishida S, Uesugi S, Ueda S. Determination of bovine rotavirus G and P serotypes by polymerase chain reaction. *Molecular and Cellular Probes.* 1993; 7:277-284
18. Jenny BF, Cramling GE, Glaze TM. Management factors

- associated with calf mortality in South Carolina dairy herds. *Journal of Dairy Science*. 1981; 64:2284-2289.
19. Khafagi MH, Mahnoud MA, Habashi AR. Prevalence of rotavirus Kobayashi N, Lintag LC, Urasawa T, Taniguchi K, Sanial MC, Urasawa S. Unusual human rotavirus strains having subgroup I specificity and “long” RNA electropherotype. *Archive of Virology* 1989, 2010; 109:11-23.
  20. Lucchelli A, Lance SE, Bartlett PB, Miller GY, Saif LJ. Prevalence of bovine group A rotavirus shedding among dairy calves in Ohio. *American Journal of Veterinary Research*. 1992; 53:169-74.
  21. Mondal A, Sharma K, Malik YPS, Joardar NS. Detection of Group A Rotavirus in Faeces of Diarrhoeic Bovine Porcine and Human Population from Eastern India by Reverse Transcriptase–Polymerase Chain Reaction *Advances in Animal and Veterinary Sciences*. 2013; 1(1S):18-19
  22. Paesi S, Rosa CE, Dornelles PRM, Luz F, Schenkel CA, Zampieri D. Evaluation of a Latex Agglutination Kit for Detecting Rotavirus in Piglets. *Acta Scientiae Veterinariae*, 2012; 40(1):1014.
  23. Single LD, Gupta MP, Singh H, Singh ST, Kaur P, Juyal PD. Antigen based diagnosis of *Cryptosporidium parvum* infection in cattle and buffalo faeces. *Indian J Anim. Sci*. 2013; 83:37-39.
  24. Smith GW. Treatment of calf diarrhoea: oral fluid therapy. *Veterinary Clinical North American Food Animal Practitioner*. 2009; 25:55-72.
  25. Stanton AL, Kelton DF, LeBlanc SJ. Effects of tulathromycin on incidence of various diseases and growth of young heifers. *Journal of American Veterinary Medicine Association*. 2013; 243:267-276.
  26. Steele A, Geyer A, Gerdes G. Rotavirus infections. In: coetzer, J.A.W. and Tustin, R.C(eds). *Infectious diseases of Livestock*, Oxford University Press, Southern Africa, 2004, 1256-1264.
  27. Tsunemitsu H, Jiang B, Saif LJ. Detection of group C rotavirus antigens and antibodies in animals and humans by enzyme-linked immunosorbent assays. *Journal of Clinical Microbiology*. 1992; 30:2129-34.
  28. Tumlam UM, Ingle VC, Tembhurne PA, Kurkure NV, Chaudhari SP, Chitambar SD *et al*. Detection of VP6 gene of Rotavirus in Feces of Diarrhoeic calves, kids, lambs, piglets, pups and human infants by Reverse Transcriptase–Polymerase Chain Reaction. *The Indian Journal of Veterinary Sciences & Biotechnology*. 2018; 13(4):12-16.
  29. Yilmaz V. Investigation of Rotavirus Infection in Calves with Diarrhea in Northeast Turkey. *Animal and Veterinary Sciences*. 2016; 4(1):1-4.  
doi: 10.11648/j.avs.20160401.11