Haryana Vet. (Dec., 2019) **58(2),** 251-253

Research Article

MOLECULAR SCREENING OF ROTAVIRUS IN BUFFALO CALVES AT AN ORGANISED DAIRY FARM

ISMAILA ALHAJI MAIRIGA*, YUDVIR SINGH RANA, MINAKSHI PRASAD[1] and JAGVEER RAWAT[2]
Department of Veterinary Medicine, [1]Department of Animal Biotechnology, [2]Department of Veterinary Microbiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, India

Received: 01.06.2018; Accepted: 08.01.2019

ABSTRACT

A total of 38 faecal samples were examined for detection of rotavirus using Reverse transcriptase polymerase chain reaction (RT-PCR). RNA PAGE screening of roavirus from faeces of buffalo calves of less than 6 months of age did not reveal positive detection despite the use of specific molecular markers. Further sample screening for detection of rotavirus using specific primers of VP7 gene of group A rotaviruses and Bov9Com5 and Bov9Com3 primer pairs specific for bovine rotavirus to reveal the expected product of 1,013 bp for VP7 gene was also negative. This represents a contrary opinion from that of other workers who reported series of incidences of bovine group A rotavirus in bovine calves in India. It is therefore, concluded that the non-detection of bovine rotavirus from buffalo calves does no precludes the existence of these infections and therefore the need for further research for better surveillance of rotavirus infection for stringent control measures.

Keywords: Buffalo, Faecal, Farm, PCR, Rotavirus

Diarrhoea is the most commonly reported clinical condition in calves and a major cause of calf morbidity and mortality worldwide (Malik *et al.*, 2012). Newly born calves represent an important source of animal production as they serve as replacement heifers for either meat, milk or breeding worldwide (Lorenz *et al.*, 2012). Diarrhoea is one of the very common disease syndromes in the neonatal calves in different countries and this can have severe impacts both economically and in terms of animal welfare (Ozkan *et al.*, 2011).

Rotaviruses are the most commonly identified viral causes of diarrhoea in neonatal food animals. These viruses have also been associated with diarrhoea in adult animals, but their disease incidence in adults is comparably low. However, clinically and sub-clinically infected adults shed the virus and are a source of infection for young animals (Garcia *et al.*, 2000). Viral infections alter cellular function and infected cells are desquamated into the intestinal lumen within a very short period. Functional alterations of the epithelial cells are responsible for abnormal absorption and secretions resulting in an imbalances and fluid accumulation in the lumen of the intestine which contributes to diarrhoea (Ozkan *et al.*, 2011). The present study was aimed at detection of genes of rotavirus that contribute to calf diarrhea.

MATERIALS AND METHODS

Collection of faecal samples and processing : A total of 38 faecal samples were collected from buffalo calves of less than 6 months age maintained at Buffalo farm LUVAS, Hisar. Twenty of these samples were collected from calves with diarrhoea while 18 of the samples were from calves without diarrhoea. A 10% suspension of each faecal sample was prepared in lysis buffer and vortexed for 10 min and centrifugation at 10,000 rpm for 15 min at 4 °C to remove coarse particles and cellular debris. The clarified supernatant was transferred into sterilized vial and stored at -20 °C for RNA extraction.

RNA extraction by TRIZOL^R method: Four hundred µl of supernatant was added to 400 µl of Trizol^R. The mixture was vortexed and kept for 5 minutes and to this 200 µl of chloroform was added and vortexed. It was kept for 15 minutes and centrifuged afterwards at 10,000 rpm for 10 minute at 4 ºC. Aqueous phase (supernatant) was added to 0.5 ml of isopropanol and kept overnight at -20 ºC. The supernatant was decanted and 1 ml of 70% ethanol was added and centrifuged at 10,000 rpm for 10 min at 4 ºC. The pellet was dried and dissolved in 20 µl of nuclease free water (NFW) was added and the product was stored at -20 ºC.

RNA-PAGE : The segmented dsRNA genome of the virus was analyzed by RNA-PAGE using the discontinuous buffer system of Laemmli (1970) without SDS. The gel was stained with silver nitrate as described by Svensson *et al.* (1986). Eight percent resolving gel and five percent stacking gel was prepared by adding the reagents sequentially as listed in the Table 1. The resolving gel solution was poured in the gel casting plates assembled in the gel caster. One ml of glass distilled water (GDW) was overlaid on the top of the gel to prevent surface drying. After polymerization of the resolving gel, water layer was removed and the stacking gel solution was overlaid on to the resolving gel. Subsequently the comb was put in the stacking gel solution and was left undisturbed till the gel solidified. The viral dsRNA extracted by Trizol method was dissolved in 2X RNA-PAGE sample buffers by heating at 56 ºC for 5-10 min and the samples were loaded into the wells. The electrophoresis was carried out at a

*Correspondence author: saminuelmairiga74@mail.com

constant voltage of 100 V/cm in 1X Tris-glycine buffer till the dye came out of the gel.

Table 1

Stock solutions for casting the polyacrylamide gel

S.No.	Stock solution	8% Resolving gel	5% Stacking gel
1.	30% Acrylamide/Bisacrylamide	6.7 ml	1.0 ml
2.	1.5 M Tris HCl, pH 8.8	6.3 ml	-
3.	0.5 M Tris HCl, pH 6.8	-	0.75ml
4.	TEMED	15.0 µl	6.0 µl
5.	APS (10%)	250 µl	60 µl
6.	Glass distilled water 11.5 ml 4.1 ml	11.5ml	4.1ml

Silver staining of the gel : The gel was stained by silver nitrate as per the method of Svensson *et al.* (1986). The gel was removed from the plates after the run and placed in fixative solution for 30 min at room temperature (25 ºC) with gentle shaking on the shaker. The fixative was removed and gel was stained for 30 min on gentle shaking platform. The staining solution was drained off and the gel was quickly and thoroughly rinsed with GDW twice to remove the excess silver nitrate to eliminate the chances of background staining. RNA bands were visualized in developer solution by manual shaking. The reaction was stopped by the stop solution in which gel was kept for 15 min and then stored in 10 % ethanol.

RT-PCR : The extracted RNA (2 µl) was added to 0.2-ml thin-walled PCR tubes containing 1.5 µl of dimethylsulfoxide, 30 pmol of primers Bov9com5 and Bov9com3 and 7.9 µl of DEPC-treated water. The RNA was denatured at 65 °C for 5 min and snap chilled on ice. To the denatured RNA, 8 µl of reaction mixture containing 4 µl of 5× reaction buffer, 1µl of RNAse inhibitor (20 U/µl), 2 µl of 10 mM dNTP mix and 1 µl of M-MuLV reverse transcriptase were added. The mixture was incubated at 25 °C for 10 min for annealing. Incubation temperature was raised to 42 °C for reverse transcription for 60 min in thermal cycler. M-MuLV reverse transcriptase was heat inactivated by increasing the incubation temperature to 70 °C for 10 min. The cDNA was stored at -20 °C.

cDNA synthesis protocol: Reverse transcription was carried out in a 20 µl reaction mixture using following protocol: Viral dsRNA 3.0 µl (Approx. 1.2 µg /µl), DMSO 1.5 µl, Random primers 0.5 µl (30pmol), Nuclease free water (NFW) 5.0 µl (Amresco), Total Volume10 µl. The mixture was heated at 99 ºC for 5 min in thermal cycler (Biorad i-cycler and eppendorf master cycler gradient™, Germany), snap chilled on ice and then the following reagents were added: Mo-MuLV-RT, 10 µl (200 units/µl), 5X RT buffer, 5.0 µl, 100mM dNTPs 0.5 µl, NFW, 3.5 µl, total volume, 10 µl.

After allowing the primers to anneal at 25 ºC for 10 min, reverse transcription was carried out at 37 ºC for 60 min in thermal cycler. The reverse transcriptase was heat inactivated at 70 ºC for 10 min. The genome segment 9 (VP7 gene) specific primer sequences were used for RT-PCR amplification. In this study, Bov9Com5 and Bov9Com3 (Mondal *et al.*, 2011) primer pairs were used, because these were specific for amplification of bovine rotavirus and the expected product 1,013bp for VP7 gene (Mondal *et al.*, 2011).

Table 2

Primers for amplification of gene transcripts (Mondal *et al.*, 2011)

Sr.No.	Primer Name	Primer Sequences
1.	Bov9com5 (F)	5'-TGTATG GTATTG AATATA CCAC-3'
2.	Bov9com3 (R)	5'-TCACAT CATACAACT CTAATC T-3'
3.	G6	5'-CTA GTT CCT GTG TAG AAT C-3'
4.	G8	5'-CGG TTC CGG ATTAGA CAC-3'
5.	G10	5'-TTC AGC CGT TGC GAC TTC-3'

Agarose gel electrophoresis (AGE) for PCR product of Rotavirus gene : The PCR products were resolved in 1.0% agarose (LifeTech) gel containing 0.5 µg ethidium bromide (Sigma) per ml in tris-acetate-EDTA (TAE) buffer along with 1kbp DNA ladder (MBI Fermentas). The 3 µl of PCR product was mixed with 1 µl of 6X loading dye and was loaded in the wells. The electrophoresis was carried out at 12 V/cm of gel in 1X TAE running buffer in horizontal electrophoresis unit (Biometra, USA) and power supply (Pharmacia) till the indicator 6X loading dye reached last third of the gel. The gels were visualized under UV transilluminator (Biovis) and photographed. The expected size of PCR products were estimated by comparison with that of standard DNA ladder.

RESULTS AND DISCUSSION

RNA-PAGE analysis : All the 38 examined and analyzed faecal samples from buffalo calves were found negative for rotavirus after RNA-PAGE analysis.

RT-PCR : All the samples were negative as was revealed by RT-PCR and amplification using Bov9com5 and Bov9com3 primers did not yielded an expected product of 1,013 bp as shown in fig. 1.

Rotavirus represents one of the major causes of neonatal mortality in dairy buffaloes in India as reported by series of researchers on this important subject. However, rotaviruses have not been demonstrated in any of the diarrhoeic and non-diarrheic faecal samples of buffalo calves of less than 6 months age group examined

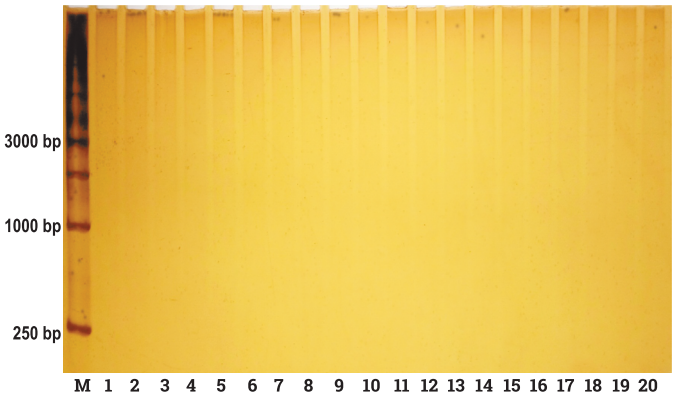

Fig. 1. RNA-PAGE detection of rotavirus positive samples. None of the samples found positive for rotavirus. Lane M ladder for rotavirus, Lane 1 to 20 negative results from the samples.

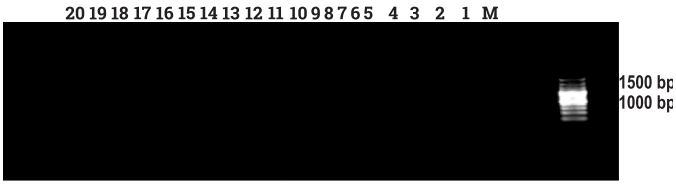

Fig. 2. RT-PCR revealing negative detection of rotavirus (Lane 1 to 19). Lane M 100bp plus RNA ladder; Lane N - negative control.

using conventional and modern molecular tools of RNA-PAGE and RT-PCR in the present study. This result is different from the findings of Sagar (2008) who reported 20% and 2.7% incidence of bovine group A rotavirus in bovine calves in India. However, in a study of rotavirus in Kolkata, Nataraju *et al.* (2009) showed 10.52% (10/95) samples with group A rotavirus like long-type electropherotype (e-type) pattern and 4.21% (4/95) samples showed the characteristic group B rotavirus long-type electropherotype pattern in buffalo calves in Kolkata, Eastern India. Similarly, Niture *et al.* (2011) detected rotavirus in 7.22% in buffalo calves, 7.40% in poultry and 19.75% in human faecal samples in Western India.

Although, samples were RNA-PAGE negative they were subjected to RT-PCR for amplification of VP7 gene of group A rotaviruses. Bov9Com5 and Bov9Com3 primer pairs used in this study was because they are specific for bovine rotavirus to reveal the expected product 1,013 bp for VP7 gene. Although negative in our study, such an expected size of 1,013 bp of VP7 gene amplicon was obtained by Mondal *et al.* (2011).

As there were reports of positive detections of rotavirus infection in India by other researchers (Niture *et al.*, 2011), although from different locations using different sets of animals, the non detection of this virus in the present study does not therefore preclude the prevalence of rotavirus infection in the LUVAS farm. It is therefore suggested that further research efforts should be intensified in screening of LUVAS farm for detection of rotavirus using buffalo calves of similar age category towards augmenting and validating the earlier claims of positive detection of rotavirus. Furthermore, this will also serve as a better surveillance exercise towards achieving stringent control measures.

REFERENCES

Garcia, A., Ruiz-Santa-Quiteria, J.A., Orden, J.A., Cid, D., Sanz, R., Gomez-Bautista, M. and de la Fuente, R. (2000). Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. *Comp. Immunol. Microbiol. Infect. Dis.* **23**: 175-183.

Laemmli, U.K (1970). Cleavage of non-structural proteins during the assembly of the head of the bacteriophage. *Nature.* **227**: 680-685.

Lorenz, I., Fagan, J. and More, S.J. (2011). Calf health from birth to weaning. II. Management of diarrhea in pre-weaned calves. *Ir. Vet. J.* **64(1)**: 9.

Malik, S., Verma, A.K., Kumar, A., Gupta, M.K. and Sharma, S.D. (2012). Incidence of calf diarrhea in cattle and buffalo calves in Uttar Pradesh, India. *Asian J. Anim. Vet. Adv.* **7**: 1049-1054.

Mondal, A., Majee, S.B. and Bannalikar, A.S. (2011). Molecular characterization of VP7 gene of Bovine Group A Rotavirus by Reverse Transcription-PCR. *Anim. Sci. Reporter.* **5(1)**: 27-33.

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. *Eur. Rev. Med. Pharmacol. Sci.* **13(1)**: 7-11.

Niture, G.S., Karpe, A.G., Prasad, M. (2011). Characterization of buffalo, poultry and human rotaviruses in Western India. *Vet. Arhiv.* **81**: 307-319.

Özkan, C., Altuo, N., Yüksek, N., Kaya, A. and Akgul, Y. (2011). Assessment of electrocardiographic findings, serum nitric oxide, cardiac troponins and some enzymes in calves with hyperkaliemia related to neonatal diarrhea. *Revue Med. Vet.* **162**: 171-176.

Svensson, L., Uhnoo, I., Grandien, M. and Wadeli, G. (1986). Molecular epidemiology of rotavirus infections in Upsala, Sweden, 1981, disappearance of a predominant electropherotype, *J. Med. Virol.* **18**: 101-111.

Sagar, P. (2008). RNA-PAGE analysis of rotavirus from calves and children. M.V.Sc. thesis submitted to Bombay Veterinary College, MAFSU, Nagpur.