



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(3): 1071-1075

© 2018 JEZS

Received: 26-03-2018

Accepted: 27-04-2018

S Das

Department of Veterinary Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

M Medhi

Department of Veterinary Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

M Khaound

North Eastern Region Disease Diagnostic Laboratory, Khanapara, Guwahati, Govt. of Assam, India

P Doley

Department of Veterinary Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

M Islam

Department of Veterinary Pathology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

DP Borah

Department of Veterinary Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Correspondence**M Islam**

Department of Veterinary Pathology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Detection of group a rotavirus infection in diarrhoeic calves by electropherotyping and reverse transcriptase polymerase chain reaction

S Das, M Medhi, M Khaound, P Doley, M Islam and DP Borah

Abstract

In the present study, electropherotyping and reverse transcriptase polymerase chain reaction (RT-PCR) were applied for diagnosis of group a rotaviral infection in diarrhoeic clinical samples of bovine (175) randomly collected from different parts of Assam, India. Electropherotyping analysis revealed that, RNA of 24 numbers of samples showed 4-2-3-2 pattern migration of segments on gel with 13.71% positivity. All the samples were also screened by RT-PCR. For RT-PCR two published primer sets were used which were amplified to produce 309 and 304 bp sized amplicon for VP6 and VP7 gene on 1.7% agarose gel electrophoresis and 39 (22.28%) numbers were found to positive for both VP6 and VP7 genes of bovine rotavirus. Both Electropherotyping and RT-PCR were suitable for detection of group a rotaviral infection though RT-PCR was found to be more sensitive than electropherotyping.

Keywords: Bovine calves, group a Rotaviral infection, electropherotyping, RT-PCR

1. Introduction

The important cause of morbidity and mortality in neonates of various farm animal species throughout the world has been attributed to diarrhoeal diseases [1] among which Rotaviruses (RV) assume a special important etiologic agent of severe diarrhoeal illness in developing countries where malnutrition is common in young animals. Rotaviral diarrhoeas are common in calves, and affected young calves may die as a result of severe dehydration or secondary bacterial infections [2-4] which in turn causes major economic losses. In India, incidence of RV associated diarrhoea in calves below 3 months of age has been reported to be in the range between 4.47% to 43% [5]. In Assam, the overall prevalence of Rota virus was found to be 41.5% where maximum number of positive cases were reported from piglets (46.3%) followed by human (40%) and cattle (37%) [6]. Rota viral infection causes extensive damage to the enterocytes and the virus multiply in the intestinal epithelium of the host which leads to malabsorption which cause severe diarrhoea and toxemia [7]. Rota virus, belongs to the family *Reoviridae*, which are non-enveloped, double-shelled virus with an icosahedral capsid, 75nm in diameter and have a characteristic wheel-like appearance [8]. Rota virus has double stranded RNA divided into 11 segments (16-21 kbp). Segmented genome surrounded by an inner and outer capsid layer [9, 10]. The immune response to rotaviruses is serotype specific. Rotaviruses are classified in to seven groups: A to G and four subgroups based on specificity of VP6 inner shell polypeptide [11]. Group A Rotaviruses contains most of the important pathogens of human beings, cattle, and other animals [12]. Group B viruses rarely affected calves, lambs, piglets and human beings whereas Group C might affect swine and occasionally humans. Group D, F and G mostly affect poultry and Group E might affect swine [13, 14]. However, Group A rotaviruses are the major cause of rotaviral infections in domestic animals. Rotavirus infection is transmitted via faecal-oral route and in respiratory droplets [15]. Rotavirus particles are excreted in diarrhetic faeces in substantial amount. The infected animals excrete up to 10⁷ to 10⁸ infectious particle per gram of faeces [16, 17]. Various methods have been developed to detect Rotavirus in human and animal stool samples and to identify specific genotype. Therefore, a precise laboratory diagnosis is mandatory to discriminate Rota virus from other infectious agent which may cause diarrhoea. Various serological techniques like AGPT, latex agglutination and ELISA are being used for diagnosis of Rotavirus [16, 18]. Various nucleic acid based techniques are used for detection of Rotavirus. Among nucleic acid base techniques, ribonucleic acid-polyacrylamide gel electrophoresis (RNA-PAGE) also called

electropherotyping and reverse transcriptase (RT-PCR) has been widely used for detection and characterization of RV infection in various species of animals [19, 20]. Reverse transcriptase polymerase chain reaction (RT-PCR) has been found to be a highly sensitive and specific method for diagnosis of RV in faecal samples [21, 22]. This method is advantageous as because it can be used for detection of low level infection and acts as confirmatory method for diagnosis of suspected RV infection. Farmers of Assam are not much aware about rota viral disease in animals and human and such as no systematic vaccination programme are being followed against this disease. Regular monitoring of Rotavirus in animals in a particular geographical area is essential to identify the circulating rotavirus in susceptible population. Considering the importance of the disease and fact that bovine rota viral diarrhoea has not been properly studied from this part of the country, the present study was aimed for the detection of rota viral A infection in diarrhoeic calves by electropherotyping analysis and RT-PCR targeting two main genes *VP6* and *VP7* of group A bovine rotavirus.

2. Materials and Methods

2.1 Collection of faecal samples from diarrhoeic calves

During the period of study, diarrhoeic faecal samples were collected randomly from bovine calves from different districts of Assam for demonstration of Rota viral (RV) antigen and viral nucleic acid. The plan of work was approved by Institutional Animal Ethics Committee (IAEC) of College Of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India. IAEC approval No.770/ac/CPCSEA/FVSc/AAU/IAEC/14-15/261 dated 20/6/2014.

2.2 Preparation of samples

Approximately 4gm of freshly voided faecal samples were collected from bovine calves suffering from diarrhoea or obtained by rectal evacuation in adequately labelled zip locked bag. Collected samples were transported on ice and immediately transferred to the laboratory where they were stored at -20 °C for further processing. All the clinical samples under test were suspended in 20% (W/V) phosphate buffered saline (PBS) solution (pH 7.4). One gram of faecal sample was suspended in 4 volumes of 0.1 M PBS (pH=7.4) and mixed thoroughly and vortexed. The mixed samples were centrifuged in a refrigerated centrifuge machine (Eppendorf, 5430R) at 12000× g for 5 minutes at 4 °C. The supernatant was collected and stored at -20 °C until further use for electropherotyping and RT-PCR.

2.3 Electropherotyping

The presence of rotaviral RNA was checked by horizontal electrophoresis for specific migration pattern of segmented genome was described by the method with slight modification [23]. Briefly, a mixture was prepared with 100µl of faecal suspension and 200µl of 0.1M sodium acetate buffer containing 1% (w/v) sodium dodecyl sulphate (SDS). Further equal volume of 3:2 (vol/vol) phenol: chloroform mixture was added to the faecal suspension and vortexed for one minute. The emulsified mixture was then centrifuged for 10 min at 12000 x g. The resulting clear aqueous layer was collected very carefully in vial and 40µl of this extracted RNA was mixed with 10µl of bromophenol blue indicator containing 1% sucrose and stored at -20 °C until further used. RNA gel electrophoresis was carried out in a gel electrophoresis unit (Biorad, USA). Freshly prepared resolving gel 7.5% and 3%

of stacking gel were poured into the glass mould of size 70×70×0.7mm dimension (Biorad, USA). A comb of 0.7 mm thickness was inserted between the glass plates to form the well. After polymerization of the gel, the comb was removed and the well was washed with distilled water. Excess fluid was soaked with the help of a blotting paper and each well was charged with 30µl of extracted RNA mixed with bromophenol blue. Electrophoresis was carried out using 100-150 volt of constant current 20mA for 2 to 3hour. For staining, the gel was carefully taken out of the glass plates and fixed for 1 hour in fixative solution of ethanol acetic acid. The fixed gel was stained with silver nitrate stain solution for 1 hour. After that, the gel was quickly washed one time in distilled water and then transferred to developer solution containing sodium hydroxide, formaldehyde and sodium borohydride in distilled water. The gel was kept in the solution till clear bands appeared. RNA bands were fixed with fixative solution again for 5 minute. The reaction was stopped by adding stop solution containing 5% glacial acetic acid.

2.4 Reverse transcriptase polymerase chain reaction (RT-PCR)

Presence of RV antigen in the faecal samples was further confirmed by using RT-PCR targeting *VP6* and *VP7* gene as per the method followed by Suresh *et al.* [24].

2.4.1 Extraction of viral RNA

Rota viral RNA was extracted from faecal sample using RNA sure virus kit (Genetix, Asia Biotech Pvt. Ltd. New Delhi) following the manufacturer's protocol. Extracted RNA was quantified by spectrophotometric analysis.

2.4.2 RT -PCR for detection of rota viral nucleic acid

The *VP6* and *VP7* gene were amplified as per method of Suresh [24]. The template RNA solution was thawed on ice. The primer solution, RT buffer, dNTP mixture, and RNase free water were also thawed at room temperature and then stored on ice immediately. Each solution was mixed by vortexing. A primer cocktail was prepared with Random hexamer 5.0 µl, RV (F) primer (10pmol) 1.5 µl, RV(R) primer (10pmol) 1.5 µl and Nuclease free water 1.0 µl. Complementary DNA (c-DNA) was synthesized with 6 µl of the quantified RNA using 1.0 µl primer cocktail and 5µl nuclease free water. The mixture was heated to 70 °C for 5 minutes and snaps cooled on ice for 5 minutes. After spinning down, 5XRT buffer 4 µl, 10mM dNTP mix. 2 µl and RNase inhibitor 1 µl (40U/ µl) were added to incubate at 25 °C for 5min after which immediately snap chilled on ice. Finally M-MuLV RT (200U/ µl) 1 µl added to the previous mixture, spin down and placed in the thermal cycler for incubation at 25 °C for 10 min, 42 °C for 60 min and 70 °C for 10 min.

2.4.3 Amplification of *VP6*, and *VP7* gene of rotavirus

The *VP6* and *VP7* region in the c-DNA samples were amplified by RT-PCR using gene specific primers. The sequence of the primer set F 5' /- AAAGATGCTAGGGACAAAATTC-3', R 5' /- TTCAGATTGTGGAGCTATTCCA3' for *VP6* and F 5' /- GATCCCGAATGGTTGTGTAATCCAAT-3', R 5' /- AATTCGCTACGTTTTCTCTTGG-3' for *VP7* were used for gene amplification producing 309 and 304 bp sized amplicon on 1.7% agarose gel [24].

For PCR, the reaction mixture was prepared by mixing of 10X buffer 2.0 µl, 25mM MgCl₂ 1.5 µl, dNTP 0.4 µl, Forward primer (10 pmols/ µl) 0.12 µl, Reverse primer (10pmols/ µl)

0.12 μ l, Taq DNA polymerase (IU/ μ l) 0.4 μ l, Template (c-DNA) 1.0 μ l and Nuclease free water 14.46 μ l for both the genes. The properly mixed 20 μ l reaction mixture was initially denatured at 95 $^{\circ}$ C for 5 minute. Thirty cycles at 94 $^{\circ}$ C (1min), 52 $^{\circ}$ C (1min) for *VP6* and 55 $^{\circ}$ C (1min) for *VP7* and finally at 72 $^{\circ}$ C (1min). The reaction mixtures were extended at 72 $^{\circ}$ C for 10 minutes. 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 1.7% 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 (DNR Bio imaging system) using UV mode.

3. Results and Discussion

Electropherotyping followed by silver nitrate staining is a highly sensitive method for detection of Rota viral genome in faecal sample [25, 26]. In comparison to ELISA and electron microscopy, RNA-PAGE has been widely used for detection and characterization of RV infection because it is more sensitive and specific. By this method the group of RV can be identified based on the migration pattern of RV genome [23, 19, 6]. Electropherotyping or Polyacrylamide agarose gel electrophoresis (PAGE) of the rotavirus showed 11 segmented RNA genome with a migration pattern 4:2:3:2 [27] however during the present study only 8 bands were distinct. Reduction in number of bands may be due to the positions of the segments 2 and 3, and 7, 8 and 9 which were so closely situated in agarose electrophoresis that the segments cannot be distinguishable. The study showed an agreement with earlier researchers [28, 29].

In the present study, extraction of genome of RV from the faecal suspension was done using sodium acetate buffer (pH 5.0) containing sodiumdeodecyl sulphate and phenol chloroform mixture [30] used phenol guanidium thiocyanate chloroform extraction method and Hasegawa [31] used the phenol chloroform acid mixture method. The extracted genome was then subjected to electrophoresis in a discontinuous gel solution of 7.5% and 3% which was a modification over the Herring method. The gel solution that was being used in the present study yielded a distinct clear band of 4:2:3:2 (Fig: 1) and arranged in 4 different cluster (I, II, III, IV) suggesting group A RV and this finding was in accordance with the finding of several workers [32, 33, 6, 34]. Prevalence of Group ARV in Assam was confirmed earlier in pigs [32] and was confirmed circulation of group A RV among man and animals of Assam by RNA-PAGE [6]. Other workers have reported association of group B and C in animal [35] and human [36, 37] based on RNA electropherotype. Overall incidence of RV during the study was found to be 8% in faecal samples of bovine calves. The result of RV detection by electropherotyping corroborated with the findings of others [6], where he reported similar occurrence of RV in cattle (14.28%), pigs (22.85%) and human (22.01%). During the present study, the result of electropherotyping from the faecal samples of calves were similar with the findings of other several workers [38, 33, 39], who also detected 22%, 16.83% and 13.3% RV respectively in diarrhoeic samples of calves. RV infection in bovine was 17-19% positive by others [40, 41]

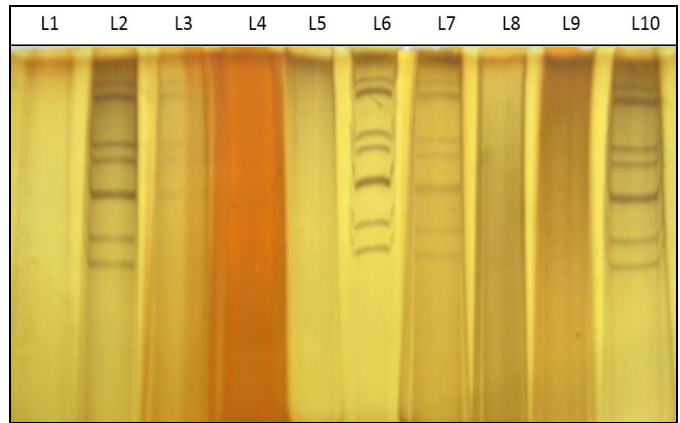


Fig 1: RNA Electrophoretic pattern of bovine Rotavirus

L1= Negative control L2= Positive control

L3, L4, L5, L8 & L9=Faecal sample of cattle (non infected)

L6, L7 & L10= Faecal sample of cattle (infected)

The PCR techniques are used throughout the world for detection and typing of strains of Group a rotavirus directly extracted from diarrhoeic faecal suspension. Reverse transcriptase-polymerase chain reaction (RT-PCR) has been used targeting *VP6* and *VP7* genes for detection of RV in field samples. The antigenicity of *VP7* gene expressed the major glycoprotein (G genotype) and the group and sub group specificity was confirmed by *VP6* protein [24].

In the present study, viral RNA was extracted directly from 10% faecal suspension using commercial kit (RNASure VIRUS Kit, Nucleopore Genetix brand). About 6 μ l of extracted RNA was used for preparation of c-DNA with a primer cocktail containing gene specific primer and random hexamur. Primers used for amplification for the segments of *VP6* and *VP7* gene were procured from company active oligos.

Amplification of *VP6* and *VP7* gene of RV was done from the extracted rotaviral RNA using gene specific primers which were previously published by others [24]. They amplified a targeted sequence of both the genes and 309bp and 304 bp amplicon were produced (Fig 2 and fig. 3). Out of 175 samples, 37(21.14%) were found to be positive in RT-PCR. The present study showed slight similarities with the result of the research conducted by Suresh [24] where they found out of 112 samples 27(24.11%) were positive for RT-PCR.

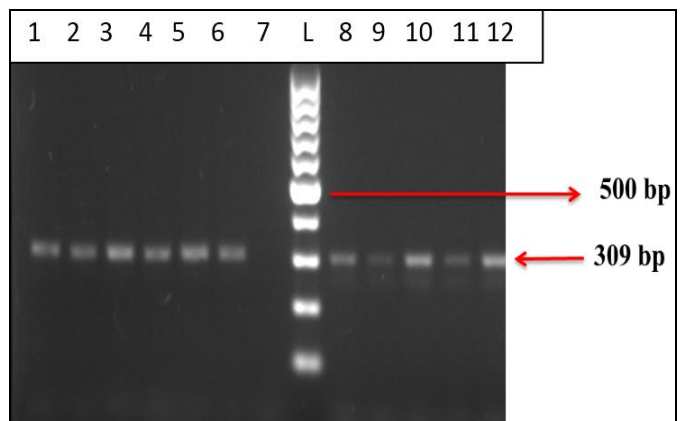


Fig 2: Gel photograph showing PCR amplification of *VP6* gene

L= 100bp DNA ladder, 7 = negative control, 6= positive control 1, 2, 3, 4, 5, 9, 10, 11, 12, 13 = RT-PCR amplified products (309 bp) of *VP6* gene

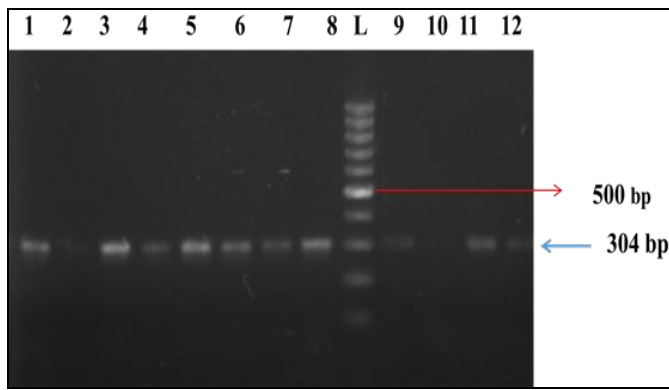


Fig 3: Gel photograph showing PCR amplification of VP7 gene

L = 100bp DNA Ladder plus, 10= negative control, 1 = positive control 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12 = RT-PCR amplified products (304 bp) of VP7 gene

4. Conclusion

In the present study, RT-PCR was found to be specific technique where the sample could be detected by this test and can be used as a valuable tool to complement the routine diagnostic procedures for RV virus diagnosis. Thus, the work can be considered as a baseline study for exploring the genotype of the circulating rotavirus strain in calf population in Assam. However, in order to know the genotypes of the circulating rotavirus strains and their zoonotic implications, further investigation is required.

5. Acknowledgement

The authors are thankful to the DBT twinning project on molecular epidemiology of RVA infection in north eastern region, Director of Research, Head of the Department of Veterinary Microbiology and Pathology, College Of Veterinary Science, AAU, Khanapara, Guwahati-22 for providing necessary facilities to conduct the present research work. We also acknowledge the field veterinarians from the study area for their co-operation in this study.

6. References

- Kapikian AZ, Hoshino Y, Glass RI, Midthunk K, Goorziglia M, Chanock RM. Rotaviruses the major etiological agent of severe diarrhoea may be controlled by Jennerian approach to vaccination. *J Infect. Dis.* 1986; 153:815-825.
- Holland RE. Some infectious causes of diarrhea in young farm animals. *Clinical Microbiology Review.* 1990; 3:345-375.
- Saif LJ, Theil KW. Comparative aspects of enteric viral infections, In *Viral diarrheas of man and animals.* CRC Press. Boca Raton, Florida, 1990, 9-31.
- Chauhan RS, Singh NP. Epidemiology of rota virus infection in calves in India. *International J Ani. Sci.* 1996; 11:221-223.
- Singh A, Pandey R. Molecular biology and vaccinology mammalian group a rotavirus. *Indian J Microbiol.* 1990; 31:233-263.
- Sharma R, Bora DP, Chakraborty P, Das S, Barman NN. Circulation of group a rotaviruses among neonates of human, cow and pig: study from Assam, a north eastern state of India. *Indian J Virol.* 2013; 24:250-255.
- WHO. Manual of rotavirus detection and characterization method. Department of immunization, vaccine biological. WHO/IVB/08-17, 2009.
- Van Doorn LJ, Kleter B, Hoefnagel E, Stainier I, Poliszczak A, Colau B. Detection and genotyping of human rotavirus VP4 and VP7 genes by reverse transcriptase PCR and reverse hybridization. *J Clin Microbiol.* 2009; 47:2704-2712.
- Murphy FA, Gibbs FPJ, Morzinek MC. In *Veterinary Virology.* Edn 3, Academic Press, USA, 1999, 391-404.
- Desselberger U. Rotaviruses. In: BWJ. Mahy and VT. Meulen (eds), *Topley and Wilson's microbiology and microbial infections.* ASM press, USA, 2005, 946-958.
- Paul PS, Stevenson GW. Rotavirus and Reovirus. In: *Diseases of Swine,* 7th Edn. Leman, AD. Straw, BE. Mengeling, WL. Allaire SD. and Taylor DJ. (eds.), Wolfe Publishing Ltd. London, 1992, 31-348.
- Matthews REF. Classification and nomenclature of viruses. *Intervirology.* 1979; 12:129-296.
- Steele AD, Geyer A, Gerdes GH. Rotavirus infections. In: Coetzer JAW, Tustin RC, editors. *Infectious diseases of Livestock.* Southern Africa: Oxford University Press, 2004, 1256-1264.
- Villarreal LYB, Uliana G, Valenzuela C, Chacon JL, Saldenberg ABS, Sanches AA *et al.* and Ferreira AJP. Rotavirus detection and isolation from chickens with or without symptoms. *Revue Brasilia Ciencia Avicola.* 2006; 8:187-191.
- Ansari SA, Springthorpe VS, Sattar SA. Survival and Vehicular spread of human rotaviruses: possible relation to seasonality of outbreaks. *Rev. Infect. Dis.* 1991; 13:448-461.
- Woode GN, Bridger JC, Hall GA, Jones JH, Jackson G. The isolation of reovirus like agent (rotavirus) from acute gastroenteritis of piglets. *J Med. Microbiol.* 1976; 19:203-209.
- Broor S, Ghosh D, Mathur P. Molecular epidemiology of rotavirus in India. *Indian J Med. Res.* 2003, 118:59-67.
- Dhama K, Chauhan RS, Mahendran M, Malik SSV. Rotavirus diarrhoea in bovines and other domestic animals. *Vet. Res. Commun.* 2009; 33(1):1-23.
- Wani SA, Bhat MA, Qureshi S, Samanta S, Ishaq SM, Buchh AS *et al.* Epidemiology of diarrhoea caused by rotavirus and Escherichia coli in calves in Kashmir Valley. Paper presented in XXI Conf. Indian Assoc. Vet. Microbiol. Immunol. Infect. Dis. Feb. 7-9, Umiam, Meghalaya, India, 2003.
- Chinsangaram J, Akita GY, Castro AE, Osburn BI. PCR detection of group A bovine rotaviruses in feces. *J Vet. Diagn. Invest.* 1993; 5(4):516-521.
- Taniguchi K, Wakasugi F, Pongsuwama K, Urasawa T, Ukae S, Chiba S *et al.* Identification of human and bovine rotavirus serotype by polymerase chain reaction. *Epidemiol. Infect.* 1992; 109:303-312.
- Hermann J, Braclow NR. Gastroenteritis Viruses. In: *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections.* Lennette EH, Lennette DA, Lennette ET. (eds.), 7th Edn. Washington DC, American Public Health Association, 1995.
- Herring AJ, Inglis NF, Ojelt CK, Snodgrass DR, James D. 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.
- Suresh T, Rai RB, Dhama K, Sawant PM, Kumar D, Bhatt P. Detection of group A bovine Rotavirus in diarrhoeic calves by reverse transcriptase polymerase chain reaction (RT-PCR) and electrophoresis. *Indian Vet. Pract.* 2011; 2:134-137.

25. Khatter S, Pandey R. Epizootiology of rotavirus infection in neonatal cattle and buffalo calves as revealed by some antibody mediated tests and electrophoresis of genomic RNA. *Indian J Virol.* 1986; 2:164-175.
26. Ramos AP, Stefanelli CC, Linhares RE, de Brito BG, Santosh N, Gouvea V *et al.* The stability of porcine rotavirus in faeces. *Vet. Microbiol.* 2000; 71:1-8.
27. Kalica AR, Sereno MM, Wyatt CA, Mebus Chanock RM, Kapikian AZ. Comparison of human and animal rotavirus strains by gel electrophoresis of RNA. *Virology.* 1978, 87:247-255.
28. Chudzio T, Kasatma S, Irvine N, Sankar-Mistry P. Rapid screening test for the diagnosis of rotavirus infection. *J Clin Microbiol.* 1989; 27:2394-2396.
29. Psikal I, Dvoaak R, Franz J, Snponek J. Rapid identification of bovine rotavirus by electrophoresis in agarose gel. *Acta vet. Brno.* 1991; 60:253-281.
30. Sambrook J, Russel DW. *Molecular cloning*, 3rd Edn. Old Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 2001; 1:74-78.
31. Hasegawa A, Mukoyama A, Suzuki H, Inouye S, Chearskul S, Thongkrajai P. Rotavirus infection of Thai infants: antigen detection, RNA electrophoresis and virus cultivation. *J Diarrhoeal Dis. Res.* 1987; 5:165-170.
32. Bora DP, Barman NN, Bhattacharyya DK. Isolation of rotavirus in MA104 cell line from diarrhoeic piglets of Assam. *Indian J Virol.* 2007; 18(1):40-43
33. Dash SK, Tewari A, Kumar K, Goel A, Bhatia AK. Detection of rotavirus from diarrhoeic cow calves in Mathura, India. *Vet. World.* 2011; 4:554-556.
34. Yadav AK, Saini HM, Khobrai S. Screening and identification of human rotavirus by ELISA and RNA-PAGE method. *Int. J Current. Res.* 2013; 5:1262-1265.
35. Chang KO, Parwani AV, Smith D, Saif LJ. Detection of group B rotaviruses in fecal samples from diarrhoeic calves and adult cows and characterization of their VP7 genes. *J Clin. Microbiol.* 1997; 35:2107-2117.
36. Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B *et al.* Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *J Clin. Microbiol.* 1990; 28:276-282.
37. Rahman M, Matthijnssens J, Nagar S, Podder G, Sack DA, Azim T *et al.* Characterisation of novel P [25], G11 human Group A rotavirus. *J Clin. Microbiol.* 2005; 43:3208-3212.
38. Ghosh S, Varghese V, Sinha M, Kobayashi N, Naik TN. Evidence for interstate transmission and increase in prevalence of bovine group B rotavirus strains with a novel VP7 genotype among diarrhoeic calves in Eastern and Northern states of India. *Epidemiol. Infect.* 2007; 135:1324-1330.
39. Sravani GVD, Kaur G, Chandra M, Dwivedi PN. P and G genotyping of bovine rotavirus and its prevalence in neonatal calves. *Veterinarski Arhiv.* 2014; 84:475-484.
40. Hammami S, Castro AE, Osburn BI. Comparison of polyacrylamide gel electrophoresis, an enzyme linked immunosorbent assay and an agglutination test for the direct identification of bovine rotavirus from faeces and coelectrophoresis of viral RNA'S. *J Vet. Diagn. Invest.* 1990; 2:184-190.
41. Dighe V, Grover YP, Pandey R. Epidemiological studies on bovine rotavirus in neonatal diarrhoeic calves at organized dairy farms in Haryana and adjoining areas using RNA-PAGE. *Indian J Anim. Sci.* 2003; 73(60):623-626.