The Pharma Innovation Journal 2022; SP-11(2): 1589-1592

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(2): 1589-1592

www.thepharmajournal.com
Received: 19-12-2021
Accepted: 23-01-2022

Neha Singh
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

PR Suryawanshi
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

K Kusum
Department of Veterinary Gynaecology and Obstetrics, MJF College of Veterinary and Animal Science, Chomu, Jaipur, Rajasthan, India

Raghavendra P Mishra
Apollo College of Veterinary Medicine Jaipur, Rajasthan, India

SS Gaikwad
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

Corresponding Author
Neha Singh
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

Prevalence and molecular characterization of cattle and human rotavirus

Neha Singh, PR Suryawanshi, K Kusum, Raghavendra P Mishra and SS Gaikwad

Abstract
Background: Rotaviruses are members of the Reoviridae family and one of the major etiological agents responsible for causing acute, severely dehydrating watery diarrhea. The current study was aimed to fined out prevalence rate and molecular characterization for detection of Rotavirus in cattle and human.
Method: In the present study, 31 cattle faecal and 34 human stool samples were screened for rotavirus by RNA-PAGE and RNA PAGE positive samples were confirmed positive using RT-PCR.
Results: Four cattle samples and 11 human stool samples were found positive by RNA-PAGE. It was observed that 4 cattle calf faecal samples were found positive with a typical 4:2:3:2 electropherotype migration pattern of group A rota virus. In case of human stool samples 11(28.9%) were found positive for rotavirus by RNA-PAGE that too belonged to group A rotavirus. Out of the 15 PAGE positive samples, 2 cattle and 3 human samples were confirmed positive for Rotavirus by RT-PCR.

Keywords: Feces, stool, RNA-PAGE. RT-PCR

Introduction
Rotavirus infection is a major cause of profuse watery diarrhea which leads to severe dehydration and death of millions of children besides causing significant economic losses to the farmers due to affections of the domesticated animals. As the infected animal shed a large concentration of virus in feces, the infectious dose needed is less, can cause widespread infection in calves (Chauhan and Singh, 1990) [3]. Cows or buffaloes excrete virus in feces during late pregnancy can act as a source of infection to the young ones by the second day of infection which continues for 7-8 days, and susceptible calves of 2-3 weeks age may acquire the infection. After 3 months of age calves are usually not affected (Dodet *et al.* 1997) [5] Concurrent infection with secondary pathogens may aggregate the severity of the disease. Rotavirus diarrhea has been attributed to several different mechanisms, including malabsorption, enterocyte destruction; a virus-encoded toxin causes stimulation of the enteric nervous system (ENS), and villus ischemia. Over the past several years, numerous studies have addressed mechanisms of diarrhea induction at the cellular and tissue levels, and lead to a new understanding of the Rotavirus. The factors that induce the severity of disease as well as pathogenesis are the reduced intake of colostrum, age and health status of the calves, immune status of the dam, degree of exposure, virulence of virus, and the presence of secondary pathogens. The age of susceptibility to rotavirus diarrhea is quite different between humans and animals, possibly due to immunological factors and viral receptor differences.

Material and Methods
Sample collection
Faecal and Stool samples
Faecal samples (N=31) were collected from the cattle calf aged between 10 days to 4 month with symptoms of watery diarrhoea suggestive of rotavirus infection from various regions of the Maharashtra state like Marathwada (N=10), Konkan (N=10) and western Maharashtra (N=11) in sterile 50 ml sample collecting vials. The samples were collected from 8th October, 2017 to 28th March 2018. Stool samples from human origin were also collected from, infants and neonates aged between (0-3 yrs) with symptoms of watery diarrhoea during the time period from 26th January, 2018 to 31st March, 2018. Samples were collected in vials from Primary Health Centers, Government and private Hospitals of Marathwada (N=7) and Western Maharashtra (N=27) region.

A 10% faecal suspension was prepared in PBS and clarified by centrifugation at 12000× g for 5 minutes at 4 ºC.. The supernatant was transferred to fresh tube and stored at -20^{0}c until further used (Das *et al*. 2011) [4].

Detection of rotavirus by RNA-Page

Extraction of RNA

RNA extraction from calf feaces and human stool samples was carried out by trizol reagent method as per the protocol (Basera *et al.* 2010) [2].

RNA-Page

Screening of faecal samples for rotavirus estimation was done on the basis of number of genome segments and their typical electrophoretic migration pattern in RNA-PAGE as per the method (Prasad *et al*. 2009) [8]. The extracted dsRNA samples were mixed in a PAGE sample running dye and then each dsRNA sample was loaded into separate well and the gel was run at 100-120V for 3 to 4 hrs. It was continued till the dye came out of the gel into a drum containing 1x Tris-Glycine buffer.

Staining of the gel

The gel was stained using Silver staining method (Svensson *et al.* 1986) [11]. Subsequently, the gel was transferred to the staining plate and the fixative solution I (0.5% glacial acetic acid and 10% ethanol) was added to the gel and was kept on shaker for 30 min of constant stirring and gentle shaking. After 30 minutes, the staining plate containing fixing solution (I) was replaced by fixative solution (II) and the step was repeated consequently for 30 min. In the third step fixative solution (II) was replaced by fixative solution (III) silver nitrate solution and was kept on shaker covered with an opaque paper sheet for 30 min under dark condition. Lastly gel was stained with fixing solution (IV) i.e. with developer (0.75M NaOH and 0.1M formaldehyde) for 5-10 min and reaction was stopped with freshly prepared stopper solution (5% acetic acid) solution. The gel was observed for the electropherotypic migration pattern and the stained gel was photographed.

Quantification of RNA

The viral RNA quality was assessed qualitatively and quantitatively by a Nano Drop Spectrophotometer. Each of the extracted RNA with a concentration of 1.8 to 2 nanogram/ul was subjected for PAGE and PCR analysis.

RT-PCR

For RT PCR, initially the RNA was reverse-transcribed into cDNA using the one step cDNA kit (Shepherd *et al.* 2018) [10]. The cDNA was subjected to PCR using the the published primer sequences of genome segment 9 (Shaunak, 2014) as mentioned in Table 01. The RT-PCR thermal cyclic conditions were optimized for VP7 gene as per the table mentioned below. After the completion of RT-PCR cycle the PCR product obtained were subjected to agarose gel electrophoresis on 0.8% agarose gel. The expected amplicon product sizes of 1062 bp was achieved after amplification of the VP7 gene using the primers c1F-1-28, c2R- 1039-1062 specific to the VP7 gene as determined by comparison with 100 bp DNA ladder run on the same gel and were found matching with predicted size 1062 bp.

Table 1: Primers used for RT-PCR

Primer	Primer sequence	Amplicon Size	Region of complete VP7 gene
C1 VP7 (F)	GGCTTAAAAGAGAGAATTTCCGTCTGG	1062 bp	1-28
C2 VP7 (R)	CACATCATACATTCTAATCTAAG		1039-1062

Table 2: Cyclic conditions used for PCR to amplify full length VP7 gene

Step	Temperature	Time	Cycles
Initial denaturation	95 ºC	2 min	1
Denaturation	95 ºC	20 sec	30
Annealing	48 ºC	20 sec	
Extension	72 ºC	30 sec	
Final extension	72 ºC	5 min	1
Holding	10 ºC	5min	-

Result and Discussion

In the present study out of 31 fecal samples of cattle screened by RNA-PAGE, 4 samples were found positive for rotavirus genome. All the positive samples (c17, c131, c136 and c146) from Parbhani showed the presence of 11 segments representative of Rotavirus genome. The overall prevalence of rotavirus was 12.9% (4/31) in cattle. Out of 34 samples of children screened by RNA -PAGE, 11 were found positive for Rotavirus and the prevalence was 32.5% (11/34). All the 15 samples, calves (4) and human (11) found positive by RNA-PAGE showed typical 4:2:3:2 (Class I, II, III, IV) electropherotypic migration pattern representative of group A rotavirus. In a present study all the 4 RNA-PAGE positive samples of cattle Rotavirus showed long electropherotype migration pattern (figure.1). In case of human samples, 6 samples showed long electropherotype and 5 samples were observed with short electropherotype migration pattern (figure. 2). A faster migration of 11th segment relative to 10th segment resulted in characteristic long electropherotype, while slower migration of the same resulted in short electropherotype. (Basera *et al.* 2010) [2] screened 110 diarrhoeic fecal samples of cattle by RNA-PAGE and rotavirus was detected in 13 samples (11.81%) showed the presence of 11 segmented genome, characteristic of rota virus with 4:2:3:2 migration pattern and all the bands were found to be of long electropherotype. In contrast to this, in our study we have observed both long and short electropherotype. In another study (Barman *et al.* 2004) [1] performed RNA-PAGE and got 21 rotavirus positive samples of which 18 showed 4:2:3:2 migration pattern similar to our study and three samples exhibited 4:2:2:3 migration pattern that belonged to group B Rotavirus which was not observed in our study. Both the bovine (Samples: c17, c131, c136 and c146) and human (Samples: Hs1, Hs13, Hs24, R, Hs25, Hs28, Hs31, Hs34) rotavirus positive samples showed migration pattern of 4:2:3:2; among these 11 segments, segment 1,2,3,4, were separate while segments 7, 8 & 9 were co-migrating and segments 10, 11 moved separately. Similarly samples (Hs8, Hs10, and Hs17) showed migration pattern similar to the above mentioned samples but were with short electropherotypes (Figure.2). Similar observations were reported (Malik *et al.* 2013) [6] where they reported all the

samples belonged to group A with segments 7, 8 & 9 migrated in triplet. In contrast to the present study (Rahman *et al.* 2005) [9] found the entire samples negative for group A rotavirus whereas positive for group C rotaviruses with a characteristic 4-3-2-2 RNA migration pattern.

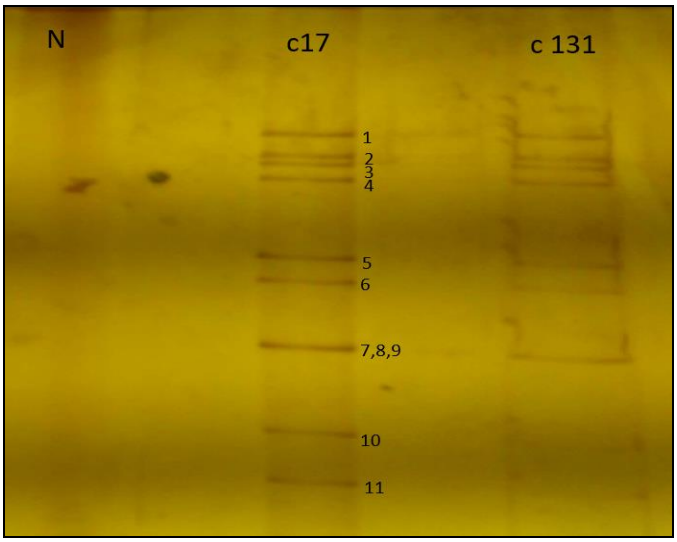


Fig 1: RNA-PAGE of cattle Rotavirus dsRNA in polyacrylamide gel. Sample no- c17 showed long electropherotype with migration pattern of (4:2:3:2) Sample no– c131showed short electropherotype with migration pattern of (4:2:3:2)

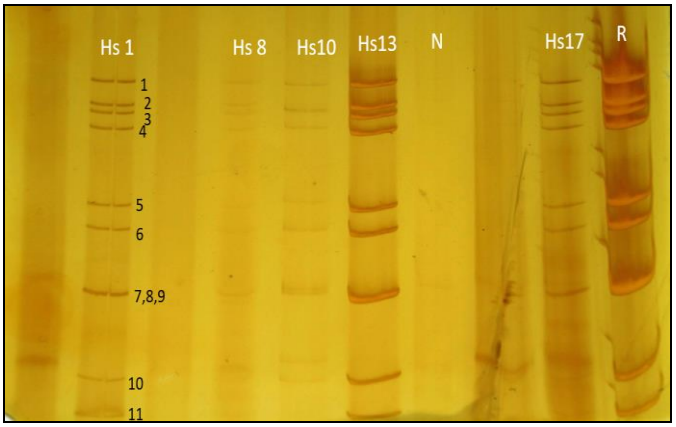


Fig 2: RNA-PAGE of human Rotavirus in polyacrylamide gel. Sample No.-Hs1, Hs13, R, showed long electropherotype with migration pattern of (4:2:3:2) Sample no – Hs8, Hs10, HS17 showed short electropherotype with migration pattern of (4:2:3:2)

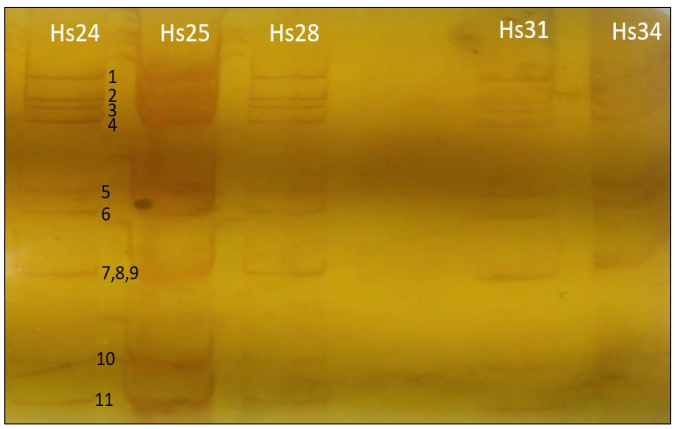
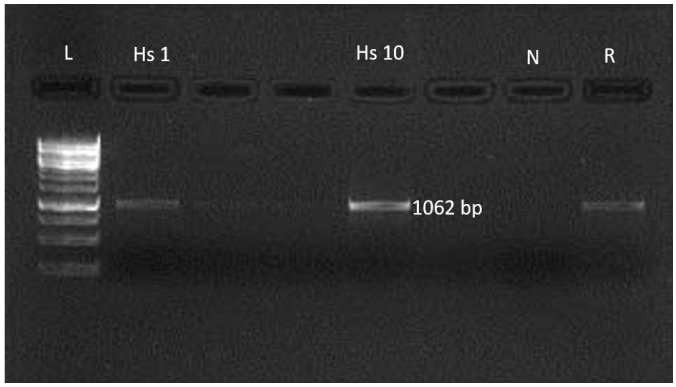


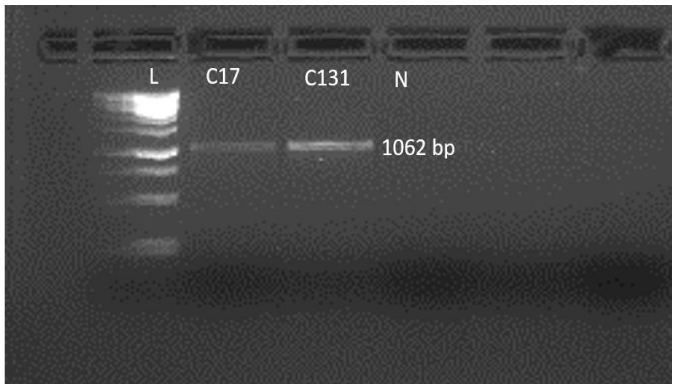
Fig 3: RNA-PAGE of human Rotavirus in polyacrylamide gel. Sample No-Hs24, Hs25, Hs28, Hs31, Hs34 showed long electropherotype with migration pattern of (4:2:3:2)

In the present study all the 15 PAGE positive samples were subjected to RT-PCR using the primers specific for the VP7 gene of group A rotavirus for the confirmatory diagnosis of group A rotavirus. Initial standardization was done using primers c1F-(1-28) and c2R- (1039-1062) for bovine and human rotavirus strains. The PCR products were observed under UV illuminator using Biorad Gel documentation system to capture the image. It was confirmed that a total of 5 samples, three of human (figure.4) and two of cattle (figure.5) were found positive for group A rotavirus by RT-PCR . In the present study we found RNA-PAGE to be more sensitive than RT-PCR for the confirmation of rotavirus. The above results were found in similar in collaboration with the study (Manuja *et al.* 2008) [7] in which they found that 4.61% prevalence was observed for bovine rotavirus by employing PAGE, whereas only 3.29% prevalence was observed by employing RT- PCR, therefore advocating use of PAGE for surveillance studies.



L: Ladder
Hs1: Positive sample of 1062 bp
Hs10: Positive sample of 1062 bp
N: Negative control
R: Positive sample of 1062 bp

Fig 4: Agarose gel showing PCR amplified product of human Rotavirus dsRNA.



L: Ladder of 100bp
C17: Positive sample of 1062 bp
C131: Positive sample of 1062 bp
N- Negative control

Fig 5: Agarose gel showing RT- PCR amplification product of VP7 gene

Conclusion

In the present study, RNA-PAGE was found very sensitive, specific and accurate methods for the molecular detection of rota viral diarrhoea in calves and human infants and can be used as a valuable tool to complement the routine diagnostic procedures for rotavirus diagnosis.

Acknowledgement
We are thankful to the Associate Dean, College of Veterinary and Animal Sciences Parbhani for providing all the facilities for the research work.

References

1. Barman S, Gosh S, Das V, Varghese S, Chaudhari S, Sarkar T, *et al*. Sequencing and Sequence Analysis of VP7 and NSP5 Genes Reveal Emergence of a New Genotype of Bovine Group B Rotaviruses in India. Journal of Clinical Microbiology. 2004;42(6):2816-2818.
2. Basera SS, Singh R, Vaid N, Sharma K, Chakravarti S. and Malik, YPS. Detection of Rotavirus Infection in Bovine Calves by RNA-PAGE and RT-PCR. Indian Journal of Virology. 2010;7:144–147.
3. Chauhan RS, Singh NP. Rapid diagnosis of rotavirus infection in calves by dot immunobinding assay. Veterinary Records. 1992;130(17):381.
4. Das SK, Tewari A, Kumar K, Goel A, Bhatia AK. Detection of Rotavirus from diarrhoeic cow calves in Mathura, India. Veterinary World. 2011;4(12):554-556.
5. Dodet S, Heseltine B, Mary E, Salioup C. Rotaviruses in human and veterinary medicine 1997 May-Jun;7(3):195-9.
6. Malik Y, Singh S, Vaid K, Batra N, Prasad M, Meenakshi Baldev GP, *et al*. Designing and validation of new primers for efficient genotyping of animal G3rotaviruses. Veterinarsk Arhives. 2013;83(6):112-119.
7. Manuja BK, Prasad M, Manuja A, Gulati BR. Prasad G. A novel genomic constellation G10P [3] of group A rotavirus detected from buffalo calves in northern India. Virus Research. 2008;138(1-2):36-42.
8. Prasad MG, Grover YP. Occurrence of dual infection of bovine group A rotavirus in diarrhoeic calf in Haryana, India. Indian Journal of Animal Sciences. 2009;79(12):611-622.
9. Rahman M, Banik S, Ukalyani SG, Faruque KT, David A, Sack MVR, *et al*. Detection and Characterization of Human Group C Rotaviruses in Bangladesh Journal of Clinical Microbiology. 2005;16:4460-4465.
10. Shepherd FK, Ibata DMH, Porter E, Homwong N, Hesse R, Bai J, *et al*. Whole Genome Classification and Phylogenetic Analyses of Rotavirus B strains from the United States, 2018. doi:10.3390.
11. Svenson L, Uhnoo I, Grandian M, Wadell G. Molecular epidemiology of rotavirus infections in Uppsala, Sweden,1981: disappearance of a predominant electropherotype. Journal of Medical Virology. 1986;18:101-111.