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Group A Rotavirus Detection from Diarrhoeal Samples from Buffalo Calves and Human Beings by ELISA and RT- PCR

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ABSTRACT

Rotaviruses belong to family *Reoviridae* under the genus *Rotavirus*. It is a major etiological agent of gastroenteritis in buffalo calves. The Mortality due to *rotavirus* infection in buffalo calves reach up to 25%. Rotaviruses cause neonatal diarrhoea in Buffalo calves. The present study was carried out to determine the prevalence of *rotavirus* infection among buffalo calves in Anand and adjacent regions. During the present study, 100 diarrhoeal samples were collected from the organized buffalo farms of villages of Anand, Gujarat. Also 55 diarrheic stool samples from the children were collected. All the samples were screened for the presence or absence of rota virus by Double Antibody Sandwich Enzyme linked Immunosorbent Assay (DAS - ELISA) and Reverse Transcriptase Polymerase Chain Reaction (RT – PCR) out of the 155 faecal samples, 12 samples (7.74%) were found to be positive for *rotavirus* by DAS- ELISA and 3 (1.93%) samples were found positive by RT – PCR. None of the human samples were found positive for *rotavirus*.

Keywords: Rotavirus, Buffalo calves, DAS – ELISA, RT – PCR

Rotaviruses are the most important cause of gastroenteritis in domestic animals and children. In cattle, it is one of the main agents involved in the development of Bovine Neonatal Diarrhea (BND), a disease that mainly affects calves in the first month of life and causes high economic losses, decreased growth averages in calves, and high morbidity and mortality (Pardo-Mora *et al.,* 2018). Rotaviruses are non enveloped viruses of the genus Rotavirus in the family *Reoviridae*. It is a segmented (11 segments) ds RNA virus surrounded by a double icosahedral protein capsid and encodes VP1-VP4, VP6, VP7 and NSP1-NSP6 proteins (Merwad *et al*., 2014). Seven distinct groups (A to G) of viruses have been described. Group A, B and C *rotavirus*es have been found in both humans and animals. The outer shell is composed of a major glycoprotein with a molecular weight of 34,000 (VP7) and a minor, trypsin sensitive protein with a molecular weight of 84,000 (VP4). Both proteins appear to be involved in virus neutralization, but serotype specificity is defined by VP7, which is encoded by gene segment 9 (or segment 8, depending on the strain) Comparative studies on the amino acid sequences of the VP7 proteins of the various serotypes have identified six discrete regions (A to F) with significant amino acid divergence (Prasad *et al*., 2005).

Incidence of *rotavirus*-associated diarrhoea in buffalo calves has been reported between 3% and 64.28%. The mortality due to *rotavirus* infection in buffalo calves may reach up to 25% (Manuja *et al*., 2010). Rotaviruses are generally species-specific, but cross-species transmission is possible, as has been demonstrated experimentally. Several case studies have indicated infection of humans by animal *rotavirus*es. Comparison of genetic sequences of human and animal *rotavirus*es often reveals close identity. Surveillance of circulating *rotavirus*es in the human population has revealed the presence of several uncommon genotypes. Many of these have been found in domestic animals, and it is possible that they arose in the human population through zoonotic transmission (Cook *et al*., 2004).

Present study was undertaken with the aim to identify group A *rotavirus* in faecal samples of buffalo calves and children by ELISA and RT – PCR.

MATERIALS AND METHODS

Samples

Total 100 diarrhoeal samples were collected from buffalo calves of 0 to 3 months age from Buffalo organized farms during the month of November to March from the villages of Anand, Gujarat. 55 stool samples from children of aged under 5 years who had presented with acute watery diarrhoea collected from hospital and Pathology Laboratory of Anand, Gujarat.

Enzyme linked Immunosorbent Assay (ELISA)

All the samples were screened by double antibody sandwich enzymatic immunoassay (DAS) ELISA (Ingezim ROTA DAS 10. RT. K.2 as per manufacturer's instruction). Finally, checked the optical densities in the micro well using a ELISA Reader (Thermo) and using a 450nm filter. Samples with an OD (optical density) higher than 0.3 were considered as positives and if the OD value is lowers than 0.2 the sample were negative.

Reverse Transcriptase Polymerase Chain Reaction (RT – PCR)

Isolation of RNA of *rotavirus* was done from the diarrhoeal samples by Qiagen kit. For quantification of viral RNA, absorbance was read in Nanodrop Spectrophotometer. Qiagen one step RT- PCR kit was used for Reverse Transcriptase polymerase chain reaction for amplification of VP4 and VP7 genes using their specific primers (Table 1).

Table 1: Primers used for RT- PCR (Manuja *et al.,* 2010)

Gene	Primers
VP7	F: GGC TTT AAA AGA GAG AAT TTC CGT CTG G
(1062bp)	R: GGT CAC ATC ATA CAA TTC TAA TCT AAG
VP4	F: TTC ATT ATT GGG ACG ATT CAC A
(876bp)	R: CAA CCG CAG CTG ATA TAT CAT C

The RT- PCR products were resolved by agarose gel electrophoresis using 1% agarose gels containing 0.5 µg/ml ethidium bromide in 1X tris acetate EDTA. The gel was visualized under Gel Documentation System.

RESULTS AND DISCUSSION

Newly born calves represent worldwide an important source in animal production either for milk, meat or breeding so, this all industry has faces many disease syndromes which usually affect dramatically. One of these diseases is diarrhoea which may lead to mortality of calves less than three weeks of age (Kassem *et al*., 2017).

Many methods are used to determine the presence of rota virus antigen. ELISA, Polyacrylamide gel electrophoresis (PAGE), Latex agglutination (LA), Electron microscopy (EM), Immuno fluorescent (IF) and immune peroxidase (IP). Recently, RT – PCR, using the VP4 and VP7 gene primers is much widely for detection of animal *rotavirus*es.

Enzyme linked Immunosorbent Assay

Out of 155 samples, 12 (7.74%) samples were found positive by Double antibody sandwich enzymatic immunoassay (DAS- ELISA). All human samples were found negative by ELISA.

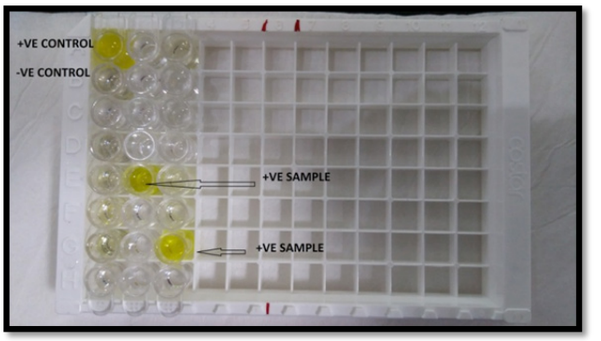

Fig. 1: Double antibody sandwich enzyme linked immunosorbent assay

Jindal *et al.* (2000) studied the prevalence of *Rotavirus* infection by ELISA. He collected faecal samples from seventy eight diarrhoeic cow and buffalo calves. And study found that overall prevalence of infection with *rotavirus* recorded by ELISA was 27.02% which is higher than present study. Manuja *et al*., 2010 evaluated 455

faecal samples of buffalo calves for *Rotavirus* prevalence by ELISA. 7.25% prevalence was noticed which is almost similar to present study. Suresh *et al.* (2013) performed ELISA for 112 diarrhoeic faecal samples from calves having age less than 3 months and he got 24.10% prevalence.

Number of other workers viz. El-Bagoury *et al*. (2014), Yilmaz (2016), Gill *et al.* (2017) and Khalaf and Aldori (2017) did ELISA. They found the prevalence of rota virus by ELISA 30%, 8.93%, 9.59% and 33.13% respectively. Pardo – Mora *et al.* (2018) also found prevalence (19.7%) of rota virus which is higher than present study. He performed ELISA of total 132 samples and 26 samples were found positive.

ELISA was found to be more sensitive than other assays according to present study. Being a rapid and fast test, primary screening test will be done by ELISA. However, the presence of organism could only be established by demonstrating the organism by virus isolation and RT-PCR.

Reverse Transcriptase Polymerase Chain Reaction

Template RNA preparation

Template RNA was extracted by using QIAamp Viral RNA mini kit (Catlog No. 52904, QIAGEN Co. Valencia, CA). The concentration of RNA was measured by Nano Drops pectrophotometer. QIAamp viral RNA extraction kit was proved to be very simple and rapid for template RNA preparation. After RNA extraction, the quantity of RNA from field samples ranged between 69-105 ng/µl. RNA was finally dissolved in RNAse free water in a way that had an average 30 ng/µl concentration. Absorbance ratio at 260/280 nm on spectrophotometer was 1.8-1.9. This ratio indicated that there were no impurities like protein, DNA or salts and the isolated RNA was in pure form.

Since *Rotavirus* has RNA genome, reverse transcription polymerase chain reaction (RT-PCR) is the starting point for most of the molecular technique used to detect and differentiate the virus. Using a reverse transcriptase, the RNA genome is transcribed into a DNA copy, which can subsequently be used as the template in PCR. RT- PCR not only has a diagnostic application, but is also instrumental in molecular characterization of the virus as the obtained product can be utilized for restriction enzyme analysis, probe hybridization and nucleotide sequencing for cleavage site analysis and epidemiological studies. Hence, RT-PCR was used in the present study.

155 faecal samples of *rotavirus*es were screened for the presence of VP4 and VP7 genes by RT-PCR. In present study, primers (VP4 and VP7) were used as suggested by Manuja *et al*., 2010. The primers yielded the products of 876bp (VP4) and 1062bp (VP7) (Fig. 2 & 3) in only three samples indicating the presence of rota virus. All human samples were negative by RT –PCR.

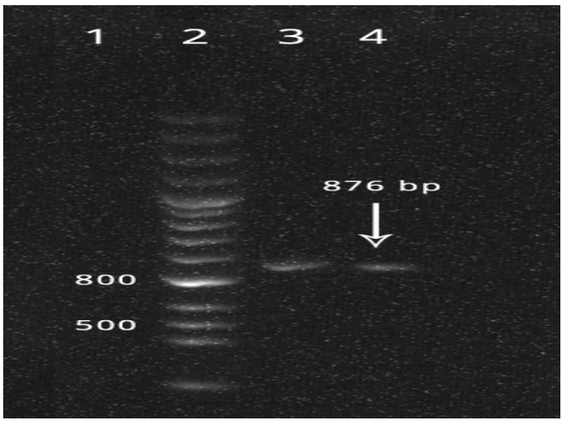

Fig. 2: PCR amplification of VP4 gene

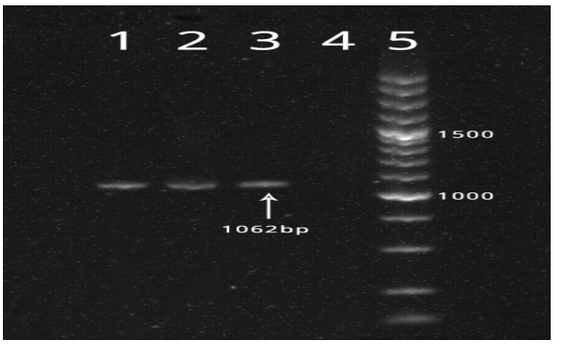

Fig. 3: PCR amplification of VP7 gene

Wani *et al*. (2004) extracted *Rotavirus* ribonucleic acid (RNA) was from ten faecal samples of diarrhoeic calves positive for group A *rotavirus*. RT-PCR was performed to generate the near full length VP7 gene. Only six samples yielded the desired product. This result is higher than present study. Manuja *et al.* (2010) collected 455 faecal

samples from buffalo calves performed RNA extraction and RT–PCR. He found that only 15 (3.3%) samples yielded a specific product of VP4 and VP7 genes.

Present study also agree with the study of other researchers El-Bagoury *et al*., 2014; Yilmaz, 2016; Gill *et al.,* 2017; Khalaf and Aldori, 2017. They found the prevalence of rota virus infection 30%, 8.92%, 7.57% and 5.67% respectively. Same prevalence study was also performed by Kassem *et al.*, 2017. He collected 50 faecal samples from diarrheic calves. He got a higher prevalence i.e. 10% in comparison with present study.

Present study revealed that in comparison with ELISA, RT–PCR gave less prevalence. The reason for this may be that non-specific inhibitors of the PCR reaction, present in the faecal samples, were carried through the extraction procedures. Also one disadvantage of RT–PCR is that virus isolation is a labour-intensive and time consuming procedure.

CONCLUSION

The present study was aimed Group A *rotavirus* detection from diarrheic samples from Buffalo calves by ELISA and RT- PCR In present study ELISA was found to be more sensitive than RT-PCR. Prevalence of group A *rotavirus* infection in buffalo calves in Anand area of Gujarat state was 7.74% by DAS- ELISA. Three samples were positive by RT-PCR. ELISA being simple, fast and sensitive assay can be used as routine laboratory test for the diagnosis of group A *rotavirus* and field prevalence studies. No human samples were found positive by ELISA and RT-PCR.

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REFERENCES

Cook, N., Bridger, J., Kendall, K., Gomara, M.I., El-Attar, L. and Gray, J. 2004. The zoonotic potential of *rotavirus*. *J. Infect.,* **48**(4): 289-302.

El-Bagoury, G.F., El-Nahas, E.M, Sharaf El-Deen, S.S. and Salem, S.A.H. 2011. Frequency of *rotavirus* detection by a sandwich ELISA in faeces of diarrheic bovine calves from Qalubia province, Egypt. *Benha Vet. Med. J.*, **27**(2): 341-347.

Gill, G.S., Kaur, S., Dwivedi, P.N. and Gill, J.P. 2017. Comparative Prevalence and Molecular Characterization of Group A *Rotavirus* in Cow Calves of Punjab, India, *J. Anim. Sci.,* **7**(5): 927-933.

Kassem, I.K., Magouz, A.F., Desouky, A.Y. and Hagag M. F. 2017. Isolation and identification of rota virus infection in diarrheic calves at EI Gharbia Governoratw. *Glob. Vet.,* **18**(3): 178 – 182.

Khalaf, A.S. and Aldori, A.A. 2017. Genomic diversity and prevalence of *Rotavirus* in cow buffalo calves in middle area of Iraq. *J. Entomol. Zool. Stud.*, **5** (6):1206 – 1211.

Manuja, B.K., Prasad, M., Gulati, B.R., Manuja, A. and Prasad, G. 2010. Comparative efficacy of immunological, molecular and culture assays for detection of group A *rotavirus* from faecal samples of buffalo (Bubalus bubalis) calves. *Trop. Anim. Health Prod.*, **42**(8), 1817-1820.

Merwad, A., Abdallah, F. and Saber, T. 2014. Close relationship of group A *rotavirus*es between bovine and human based onVP7 gene sequence in Egypt. *Pak Vet. J.*, **34**(3): 391-393.

Pardo-Mora, D., Vargas-Bermúdez, D.S., Oliver-Espinosa, O. and Jaime-Correa, J. 2018. Molecular characterization of *rotavirus*es isolated from calves with bovine neonatal diarrhea (BND) in Colombia. *Infectio.*, **22**(2): 99-104.

Prasad, G., Malik, Y. and Pandey, R. 2005. G and P genotyping of bovine group A rotaviruses in faecal samples of diarrhoeic calves by DIG-labelled probes. *Indian J. of Biotechnol.*, **4**: 93-99.

Suresh, T., Ral, R.B., Wani, M.Y. Damodaran. T. and Dhama, K. 2013. Detection of Bovine *Rotavirus* in neonatal calf diarrhoea by ELISA, FAT and Transmission Electron microscopy. *Int. J. Curr. Res.,* **5**(7): 1935 – 1939.

Wani, S.A., Bhat, M.A., Ishaq, S.M. and Ashrafi, M.A. 2004. Determination of bovine *rotavirus* G genotypes in Kashmir, India. *Rev. sci. tech. Off. int. Epiz.,* **23**(3): 931-936.

Yilmaz, V. 2016. Investigation of *Rotavirus* Infection in Calves with Diarrhea in Northeast Turkey. *Ani. Vet. Sci.*, **4**(1): 1-4.