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Incidence and Molecular Characterization of Rotaviruses of Cattle and Buffalo Calves in Amravati Region, Maharashtra

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Abstract

Fecal samples of diarrheic and non diarrheic cattle calves (198) and buffalo calves (90) were collected during January 2018 to July 2018. Screening of fecal samples for the presence of rotavirus was carried out by commercially available rapid *Rota virus* Ag detection kits.

A total of 35 out of 288 (12.15%) fecal samples were found positive by latex agglutination test. Highest incidence (20.63%) was found in Yavatmal district followed by Amravati (16.00%), Akola (13.33%), Buldana (6.77%) and lowest (3.57%) in Washim. A higher incidence (14.50%) in cattle was observed as compared to buffalo (8.04%) calves of 0 to 6 months of age. Further higher incidence was noted in male and female both in cattle as compared to buffalo calves.

1 out of 35 fecal samples (2.85%) of cattle calves was found positive for bovine group A rotavirus. The positive sample when amplified for VP4 gene by RT-PCR yielded an amplicon of 864 bp base pair indicating the sensitivity of PCR for detection of bovine rota viruses.

Introduction

There are number of infectious and non infectious causes of diarrhea /gastroenteritis e.g. *E. coli*, Salmonella, Coronavirus and Rotavirus etc. Rotavirus is one of the major causes of acute gastroenteritis/diarrhea in human, calves and pigs. (Ahmed *et al.*,2017). Group A rotaviruses comprise the important pathogens of human, cattle and other animals. Group B rarely affects calves, lambs piglets and human beings. Group C may affect swine and occasionally humans. Group D,E,F affects the poultry while G affects swine.

As the virus is excreted in large numbers in the feces, it can be diagnosed easily by electron microscopy which is one of the most specific tests. The other most widely used methods are ELISA, latex agglutination and polyacrylamide gel electrophoresis (PAGE). The detection by PAGE followed by silver staining is a highly specific technique, but lacks sensitivity as a minimum of 3-4 ng of viral RNA is needed for detection.

Continuous surveillance of bovine rotavirus circulating strains is essential for a better understanding of the viral epidemiology within a region and the zoonotic transmission, thereby

improving the implementation of vaccination programs by updating the vaccine strains.

The studies on the prevalence and characterization of bovine rotaviruses have not been undertaken in Amravati Region of Maharashtra, hence the present work is aimed to study incidence of Rotaviruses in Amravati Region of Maharashtra state in bovine and bubalian calves using conventional and molecular tools.

Materials and Methods:

Collection of samples:

A total of 288 fecal samples from diarrheic and non diarrheic cattle calves (198) and buffaloes calves (90) between the age group of 0-1yrs were collected during January 2018 to July 2018 from different districts of Amravati

Detection of rotavirus:

Screening of fecal samples for the presence of rotavirus was carried out by commercially available rapid *Rota virus* Ag detection kits (Hi-Rotavirus Latex Test Kit Himedia,Cat.No LK08-50NO).The tests were performed according to the manufacturer's instructions. The samples positive for presence of rota virus were used for molecular characterization.

RNA-PAGE

The RNA was extracted from the fecal samples by Trizol method (Chomczynski P, Mackey K. ,1995). The segmented RNA genome of the rotavirus was analyzed by RNA-PAGE using the discontinuous buffer system without sodium dodecyl sulphate (SDS) as described by Laemmli (1970).

Amplification of VP4 genes by reverse transcription polymerase chain reaction (RT-PCR)

The cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kits of Applied Biosystems. Polymerase chain reaction was used for partial amplification of the VP4 gene of Rotavirus as mentioned in table.1 (Isegawa *et al*.,1993.)

Table 1. List of oligonucleotide primers used for VP4 gene of cattle calve

Sr. No.	Name of Primer	Sequence of Primer for VP4 gene	Total nucleotides	Amplification size
First round PCR primers				
1	Bov4Com5	5´TTCATTATTGGGAC GATTCACA 3´	1067-1088	864 bp
2	Bov4Com3	5´CAACCGCAGCTGAT ATATCATC 3´	1930-1909	

The presence of VP4 gene of Rotavirus was confirmed by agarose gel electrophoresis.

Results and Discussion

Out of 288 fecal samples, 35 fecal samples (12.15%.) were found positive by latex agglutination test. Our results corroborate with Hassan (2014) who reported overall incidence of the bovine rotavirus to the tune of 12.8% in calf diarrheic fecal samples by latex agglutination test. Similarly Singh and Pandey (1990) also observed 10-52% incidence of rotavirus in diarrheic calves. Al-Khafaji *et al*., (2013) and Al-Robaiee and Al- Farwachi (2013) found rota viral infection in 15.5 % of diarrheic calves fecal samples and 4.5 % from non-diarrheic animals.

In the present study, an incidence of 23.47% was recorded in diarrheic and of 1.20% in non-diarrheic cattle calves. Similarly an incidence of 14.28% was recorded in diarrheic buffalo calves indicating overall 20.73% incidence in diarrheic animals. None of the non-diarrheic buffalo calves showed presence of the rota virus in fecal samples.

The district wise incidence was highest in Yavatmal district 20.63% followed by Amravati 16.00%, Akola 13.33% Buldana 6.77% and

Washim 3.57%. The higher incidence in cattle calves from Yavatmal and Amravati districts and in buffalo calves from Akola district could be attributed to presence of infective foci, prevailing environment and managemental practices being followed. The results are in accordance with the earlier studies of Sravani *et al*., (2014) who reported that diarrheic calves below 20 days of age were found to be more susceptible to rotavirus infection. Sharma (2004) reported that susceptibility of rotavirus infection is more upto 2 months of age. The higher susceptibility to infection in the calves of below 6 months may be due to improper management and absence of maternal antibodies against rotavirus.

The incidence of 14.50% and 8.04% was recorded in the cattle and buffalo calves of 0 to 6 months age respectively. Whereas, no positive case was recorded in calves above 6 months. It was noticed that cattle and buffalo calves of age group of 0-6 months suffered most.

Sex wise, the incidence of 22.61% and 13.51% was recorded in the cattle and buffalo male calves and 7.89% and 3.77% in female cattle and buffalo calves respectively. The higher incidence of rota virus infection in males calves may be due to improper care of male calves as compared to female calves which were reared for breeding and milk purpose.

1 out of 35 fecal samples (2.85%) of cattle calf of 0-6 month was found positive for rotavirus, Typical RNA-PAGE with 11 segments having typical migration pattern 4:2:3:2 distribution depending on the relative migration of the 10th and 11th segments and the close migration of segments 7, 8 and 9 as a triplet characteristic of bovine group A rotaviruses indicated that positive sample belonged to group A rotavirus with long electrophoretic migration pattern in polyacrylamide gel electrophoresis (Fig. 1). All fecal samples from buffalo calves were negative for the rotavirus by RNA-PAGE. In a study Berman *et al*. (2005) found typical migration pattern 4:2:3:2 of bovine Group A rotavirus in 18 out of 175 (10.28%) diarrheic fecal sample of bovine between the age group of 0-6 months. Niture *et al*. (2009) reported that out of 48 buffalo samples 6 (12.5%) were positive for rotavirus by RNA-PAGE.

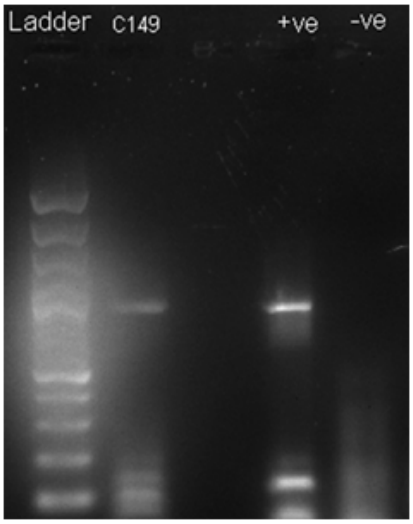

Fig.1. Amplification of VP4 genes by Reverse Transcriptase PCR (RT-PCR)

In this study one diarrheic fecal sample from cattle calf which was positive in RNA-PAGE was also positive for RT–PCR of VP4 gene. An amplicon of 864 bp resolved on agarose gel along with molecular marker (100 bps) indicated the sensitivity of PCR for detection of bovine rota viruses (Fig. 2). Similar findings were reported by Malik *et al*. (2014) who screened 87 fecal samples of calves positive for group A rotavirus, out of these only 7(8.04%) samples were found positive for the amplification of partial length VP4 gene 864 bp. Minakshi *et al*. (2015) found that out of

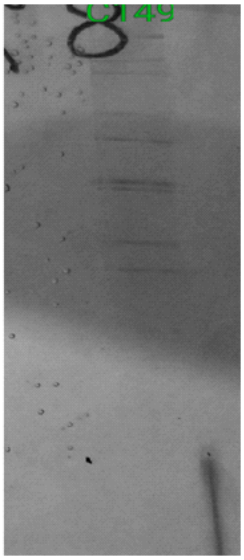

Fig.2. Detection of rotavirus isolates by Ribonucleic acid-Polyacrylamide Gel Electrophoresis (RNA-PAGE)

total 103 fecal samples from diarrheic calves of 0 - 1 month of age, only 11 samples (10.67%) showed amplicons of 864 bp of VP4 gene by RT-PCR. Gill *et al*. (2017) studied prevalence of group A rotavirus in bovine calves in Punjab by RT-PCR and reported 7.57% (15/198) positive incidence . Deswal *et. al.* (2015) found that one out of 11 samples (B29 isolate) showed amplification 864 bp in VP4 gene specific RT-PCR. However, other isolates did not produce any amplification with primers specific to VP4 genes. In the present study also, other isolates did not produce any amplification with primers specific to VP4 genes. This might be due to the presence of inhibitory substances in the fecal samples or mismatches in primer binding sites (Manuja *et al*., 2008).

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Conflict of Interest:

All authors declare no conflict of interest.

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