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Detection of VP6 gene of Rotavirus in Feces of Diarrhoeic calves, kids, lambs, piglets, pups and human infants by Reverse Transcriptase–Polymerase Chain Reaction

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Abstract

The present study was undertaken on VP6 gene based detection of Rotavirus in faeces of diarrheic bovine, porcine, caprine, ovine, canine species of animals and human. A total of 44faecal samples from bovine calves, piglets, kids, lambs, pups and human infants (0-1 yr of age) were screened for the detection of group A Rotavirus by VP6 gene based RT–PCR assay. Out of 44 samples total of 43 (97.72%) samples were found positive for Rotavirus. Samples from all other species were found cent percent positive for group A Rotavirus except for the human infants where 17 out of 18 samples were found positive.

VP6 gene base RT-PCR study confirmed the prevalence of group A Rotavirus in animals and human species. RT–PCR assay can be used as a sensitive and specific assay for the rapid detection of group A Rotavirus in faecal samples.

Key words: Group A Rotavirus, VP6 gene, diarrhoea, RT–PCR

Introduction

Neonatal diarrhoea is one of the most important diseases of several species of domestic animals and human and is associated with heavy economic loss and accounts for more than 6 million deaths in infants worldwide (Badaracco *et al*., 2012 and Estes and Kapikian, 2007), specially in developing countries including India (Parasar *et al.,* 2006). Rotavirus is one of the important causative agents of diarrhoea in animals and human (Rajendran *et al.,* 2014,) which account for 25% mortality in young animals (Mukhtar *et al.,* 2016) and causes severe economic losses in livestock sector (Fagiolo *et al*., 2005).

Genus Rotavirus belongs to the family *Reoviridae*. Rotavirus is a trilaminar viral particle with two double capsid layers surrounding the viral core. Based on antigenic specificity Rotaviruses are classified into seven groups (A to G) among which group A to C found in human and animals and D to G only in animals. In swine four antigenic groups of Rotavirus B, C, and E have been detected. There are various G types (*i.e.*, glycoproteins) and P types (protease-sensitive). To date, at least 27 G-types, 35 P-types and 42 different G–P type combinations have been detected. Mondal *et*

al., (2013) reported 26 (12.32%) samples positive for group A Rotavirus by VP6 gene based reverse transcriptase– polymerase chain reaction (RT–PCR) assay from diarrhoeic bovine, porcine and human.

Combined studies on the prevalence of Rotavirus associated diarrhoea in bovine, porcine, caprine, ovine, canine and human species of animals and human have not been much documented in Maharashtra state. In tropical countries like India due to close association of human, and animal there are higher chances of zoonoses and gene segment reassortment among the viruses of human and other host animal is possible (Choudhary et *al.,* 2017). The multiple reassortment have occurred between porcine or human Rotaviruses and co-circulating in human strain (Matthijissens et *al.,* 2010). Therefore it is necessary to evaluate highly sensitive RNA based tools for detection and differentiation of locally circulating types of Rotaviruses, which would eventually help to formulate control strategies.

Materials and Methods

A total of 280 faecal samples comprised of bovine calves (58), piglets (71), kids (25), lambs (26), pups (50) and (50) stool samples from human infants were collected aseptically from different regions of Maharashtra during the year 2016-17.After preliminary screening ,a total 44 diarrhoeicfaecal samples (bovine calves 4 , piglets 7, kids 6, lambs 7, pups 2 and 18 from human infants) were screened for the presence of Rota virus by rapid Rota virus antigen detection kit for the confirmation of group A Rotavirus by VP6 gene based RT–PCR assay.

Detection of Rotavirus

A 10% suspension of the faecal material was prepared in 10mM phosphate buffer saline (PBS; pH 7.2). After thorough vortexing, centrifugation was carried out at 13000 rpm for 15 min at 4^{0}C to remove the coarse debris. Supernatant was collected in other eppendorf tube for extraction of RNA. Total RNA was extracted using Trizol extraction reagent. The viral ds-RNA was subjected to reverse transcription for cDNA synthesis as per the methods described by Jadhav *et al*., (2009) and the synthesized cDNA was stored at –20°C till further use.

For detection of group A Rotavirus, partial length amplification of VP6 gene was carried out by RT-PCR using 379 bp primers and conditions as optimized by Isegawa *et al*., (1993) and Yilmaz *et al*., (2017). The primer sequences and nucleotide position of oligonucleotide primers are shown in Table 1. Amplified PCR products were visualized using agarose gel electrophoresis.

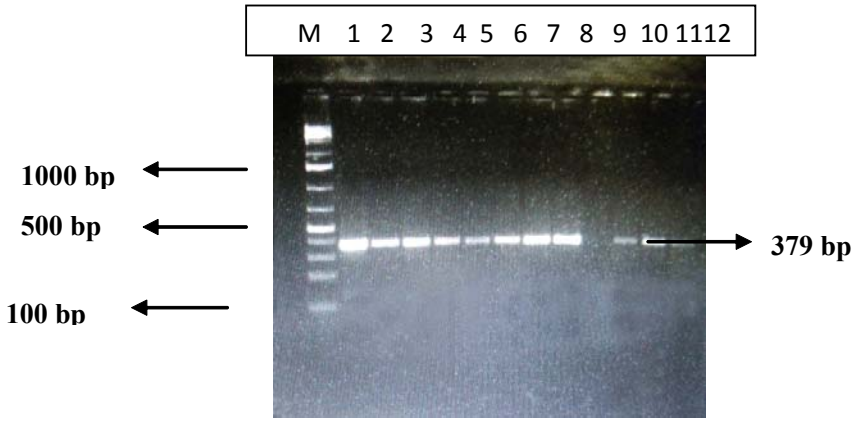

Figure.1:- Screening of VP6 gene of Rotavirus in faecal samples by RT-PCR

Lane M =Molecular weight marker (100bp ladder), Lane-1, 2, 3=Bovine calves, Lane-4, 5=Piglets, Lane-6, 7=Lamb and Kids, Lane-8=Human infants, Lane-9=Negative Control, Lane-10, 11=Pups, Lane-12=Positive Control

Table.1. Primers used for partial length amplification of VP6 gene of Group A rotavirus

Sr. No.	Gene	Primers Sequence (5′– 3′)	Amplicon size (bp)
1.	VP6-F	5'GAC GGV GCR ACT ACA TGG T3'	379bp
	VP6-R	5'GTC CAA TTC ATN CCT GGT GG3'	

Results and Discussion

Out of 44 samples screened, 43 (97.72%) samples were found positive for Rotavirus. Samples from bovine calves ,piglets, lambs, kids and pups were found cent percent positive for group A Rotavirus except for the human infants, where 17 out of 18 samples were found positive by VP6 gene based RT–PCR assay as shown in Table No.2. These findings are in agreement with the findings of Niture et al.(2011) and Mondal et al., (2013) who confirmed group A Rotavirus by VP6 gene based RT–PCRassay.

Table.2 Results of VP6 gene based RT–PCR assay

Sr. No	Species	No. of samples screened	No of sample positive by Vp6 gene RT- PCR
1	Cattle Buffaloes calves	4	4
2	Lambs	7	7
3	Kids	6	6
4	Pups	2	2
5	Piglets	7	7
6	Human infants	18	17
	Total	44	43

The RT–PCR offers many advantages besides high sensitivity and specificity in detection of Rotavirus in faecal samples (Fedorova et al., 2005, Kang et al., 2004). It helps in the detection of viral nucleic acid during initial stages of infection without waiting for higher virus titre and development of immune response in the affected host species (Niture et al., 2010). The RT-PCR can be employed as a sensitive and specific assay for the rapid detection of Rotavirus VP6 gene in cattle, porcine and human faecal samples (Niture et al., 2011). RT-PCR is now being used as confirmatory methods for detecting the Rotavirus from faecal samples.

Yilmaz et al. (2017) also tried for VP6 gene RT-PCR based identification of Rotavirus from sheep faecal samples but they could not get Rotavirus in sheep. However in the present study the presence of Rota virus in the fecal samples of lambs could be confirmed by VP6 gene based RT-PCR. In India, Gulati et al., (1996) first determined the genetic diversity of bovine group A Rotavirus using RT–PCR.

In conclusion, the group A Rotavirus is prevalent in bovine, porcine, caprine, ovine, canine species of animals and human in Maharashtra state. In Indian scenario, human live in close proximity to their livestock, often in poor sanitary conditions which explains possibility of zoonotic transmission of Rotaviruses in human or vice versa. In order to study the circulating genotypes, studies on molecular epidemiological surveillance of group A Rotaviruses in various host species in different geographical region needs to be carried out.

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Conflict of Interest: All authors declare no conflict of interest.

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