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Detection of Porcine Rotaviruses Group A in Free-ranging and Farm Pig Population of North East India

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Abstract: *Piglet diarrhea caused by rotavirus is one of the major threats leading to serious economic losses in swine industry of the North East India. Thus, there is a dire need of rapid and sensitive detection techniques in identifying the causative agent for control and containment of the disease. In our study, porcine rotavirus group A was detected using the gold standard method, native RNA PAGE followed by reverse transcription (RT) PCR in 208 fecal samples from diarrheic piglets of 0-6 months of age reared under different management systems in Assam and Tripura states of North East India. The study also indicated that the use of RNA PAGE followed by RT PCR of VP4 as well as VP7 gene is more confirmative over PAGE alone. In addition, the present investigation revealed that the use of RNA PAGE is advantageous in knowing the group prevalent amongst the circulating rotavirus strains of a region whereas RT PCR besides acting as an effective tool in detection of porcine group A rotavirus, comes out as an efficient means for further genetic studies of the same.*
Keywords: *Native RNA PAGE, North East India, Piglet diarrhea, Porcine rotavirus Group A, RT PCR.*

I. Introduction

Gastrointestinal tract infections with acute diarrhea are indeed a cause of concern in the domesticated animals in India in general and North East (NE) India in particular. NE India comprising eight Indian states namely Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Sikkim and Tripura, is a hub for pig rearing. However, diarrheic damage and death of piglet is one of the major setbacks in piggery of this region. Rotavirus, belonging to the family Reoviridae, which is mainly associated with dehydrating diarrhea and gastroenteritis in animals, is one of the important etiological agents causing piglet diarrhea [1-3]. The genome of these non-enveloped rotavirus particles includes 11 segments of double-stranded RNA (dsRNA) encoding 13 proteins, 2 of which are formed by post-translational cleavage. Rotaviruses are classified into seven discrete groups (A-G) based on the antigenicity property which is predominantly determined by VP6 present in the inner capsid of the three layered icosahedral protein capsid. The outer capsid of the virus consists of two major neutralizing antigens, VP4 and VP7 which determines the P (protease-sensitive) and G (glycoprotein) genotypes respectively. Further, on the basis of relative migration patterns of RNA segments in PAGE, rotaviruses are classified into electropherotypes [4]. The electrophoretic pattern of the dsRNA segments by PAGE is constant and unique for a particular rotavirus isolate [5].

Although several studies have been made worldwide [1-3 and 6-8] and in India [9-12] on certain aspects of rotavirus, yet, there is a need of proper surveillance in swine population of NE India as pig rearing is a popular economic activity of the rural and tribal masses besides its importance to public health in containment of the disease. Reports from NE India on porcine diarrhea owing to rotavirus are very scanty [13-15]. On the other hand, extensively sensitive diagnostic tests are required especially in sub-clinically infected cases and chronic shedders of enteric viruses in feces. In order to bridge this gap, the present study has been aimed at for studying the effectiveness of native RNA PAGE and RT PCR in detection of rotaviruses in diarrheic fecal samples of piglets from this region of India. The present study also focused on the isolation and adaptation of rotaviruses from the positive field isolates in MA104 cell line.

II. Materials And Methods

2.1 Sample collection: In the study, a total of 208 freshly voided fecal samples were collected from diarrheic piglets under age group of 0-6 months reared in traditional and intensive management systems in Assam and Tripura states of NE India. Fecal samples were collected in sterile vials and transported on ice to laboratory for further processing. Suspension of each samples were prepared in 0.06M phosphate buffer saline (pH-7.2) by diluting in a ratio of 1:4, followed by centrifugation at 12,000g at 4°C for 30mins. Thereafter, the supernatant was collected and preserved at -20°C until further use.

2.2 Detection of rotavirus in fecal samples

2.2.1 Polyacrylamide gel electrophoresis: Viral RNA was extracted from the fecal suspension by acid-guanidinium thiocyanate-phenol-chloroform extraction method [16] with slight modification in the preparation of the denaturing solution (0.5% SDS as one of the reagent instead of 0.5% sarcosyl). RNA

concentration was assessed by nanodrop spectrophotometer (ND-1000; Thermo Scientific, USA). The extracted RNA is then subjected to native RNA PAGE (without SDS) with slight modifications [17]. The electrophoretic run was carried out at 100-120volts for 2-3 hours using vertical gel electrophoresis apparatus (Biorad, USA). The genomic migration pattern was detected by silver-staining of the gel [17].

2.2.2 VP4 and VP7 gene amplification by RT PCR: Prior to VP4 gene amplification by RT PCR, the viral RNA was extracted from the processed fecal samples by QIAamp Viral RNA Kit (Qiagen) as per the manufacturer's protocol. The primers con2 and con3 were used to reverse transcribe the VP4 gene [18]. One-step RT PCR of VP4 gene was performed [19] but with modifications in the thermal conditions. The RT-1 reaction which consisted of RNA (4µl), 20pmol of each primer and RNase In (40U, Thermo Scientific) was subjected to a denaturing temperature of 95°C for 5 minutes and then immediately chilled on ice followed by RT-2 reaction. The RT-2 reaction was carried out by adding the master mix comprising of 5X RT buffer (Thermo Scientific), 3% DMSO, 1.5mM $MgCl_2$ (Thermo Scientific), 10mM dNTPS (Thermo Scientific), RevertAid H Minus Reverse Transcriptase (100U, Thermo Scientific), Taq DNA Polymerase (2.5U, Thermo Scientific) and nuclease-free water (to make up the final volume up to 20 µl) in the RT-1 reaction tube with thermal conditions as : initially 48°C for 45 minutes; then, 30 cycles of 94°C for 1 minute, 46°C for 2 minutes, 68°C for 3 minutes followed by final extension at 68°C for 10 minutes and hold at 4°C. 5µl from each PCR product was analyzed on 1.2% agarose gel (SRL) and visualized in gel documentation system (Gel Logic 212 PRO).

Full length VP7 gene was amplified with established primers Beg9 and End9 [19, 20]. PCR products (5µl) were confirmed by agarose gel electrophoresis in the same way as in the case of VP4.

III. Result And Discussion

One of the major threats in the piggery sector worldwide is the outbreak of several diseases and viral gastroenteritis leading to dehydrating diarrhea has posed menace to the same. The NE India is no exception in this regard. Thus, the present investigation has been designed for detection of rotavirus induced porcine diarrhea in NE India using native RNA PAGE followed by RT PCR of VP4 as well as VP7 gene. The added advantage of targeting VP7 and VP4 genes of rotavirus is that the G and P genotype of the virus can be determined.

During the present study, 208 fecal samples from diarrheic piglets were collected from two states of NE India viz., Assam (102 samples) and Tripura (106 samples). Further, in this study, continuous RNA PAGE of 7.5% was employed which proved to be a modification over the previously describe protocol [17] yielding a distinct band migration pattern. The RNA PAGE analysis therefore revealed the typical 11 segmented RNA of rotavirus with characteristic band migration pattern of porcine group A rotavirus (4:2:3:2) in several samples (Fig. 1). The RNA PAGE detected 37 (36.27%) and 41 (38.67%) numbers of rotavirus positive cases from the collected samples of Assam and Tripura respectively. High percentage of death in pre-weaning piglets due to diarrheal diseases and rotavirus infection in pig and man has been reported from Assam as well as other parts of India [10-11, 14, 21]. Also, several researchers have revealed rotavirus as one of the major etiological agent causing porcine diarrhea in India (13-15, 22-25) as well as globally [1-3, 8-9].

Subsequently, all the PAGE positive and a few suspected PAGE negative samples when subjected to RT PCR of VP4 gene, successful amplification of 876bp (Fig. 2) were observed. This led to detection of more number of cases as positive in comparison to those detected in PAGE alone. As such, 43 numbers of positive cases (42.15%) came out from Assam and 49 positive cases (46.22%) from Tripura. The same number of positive cases on rotavirus induced porcine diarrhea was detected by VP7 gene amplification which yielded the expected product size of 1062bp (Fig. 3). Efficiency of various techniques has been demonstrated for detection of porcine group A rotaviruses in Assam. Mention may be made of detection of rotavirus specific antibodies in serum samples of pigs by indirect ELISA [13]. Also, rotavirus was detected in feces of infected pigs by sandwich ELISA and PAGE [15]. Hence, the present investigation emphasized the detection of porcine rotavirus in Assam and Tripura using RNA PAGE followed by RT PCR of VP7 and VP4 genes.

IV. Figures

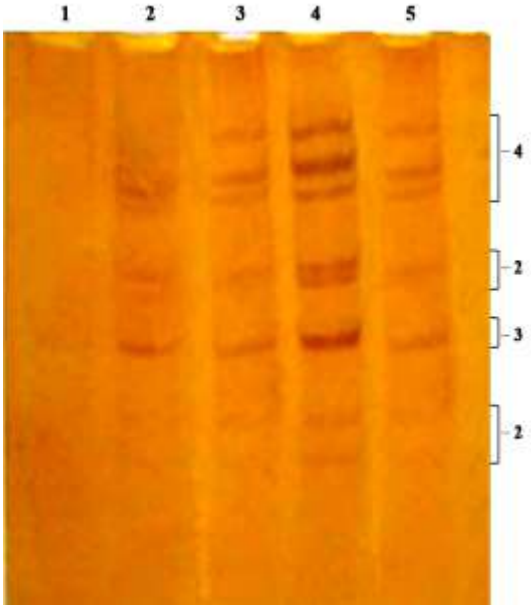

Fig. 1 RNA-PAGE showing 11 segments of dsRNA of porcine rotavirus exhibiting typical cluster of 4:2:3:2 pattern characteristic of group A rotavirus.
Lane 1: Negative control,
Lane 2 & 3: Rotavirus positive samples from piglets of Assam,
Lane 4 & 5: Rotavirus positive samples from piglets of Tripura.

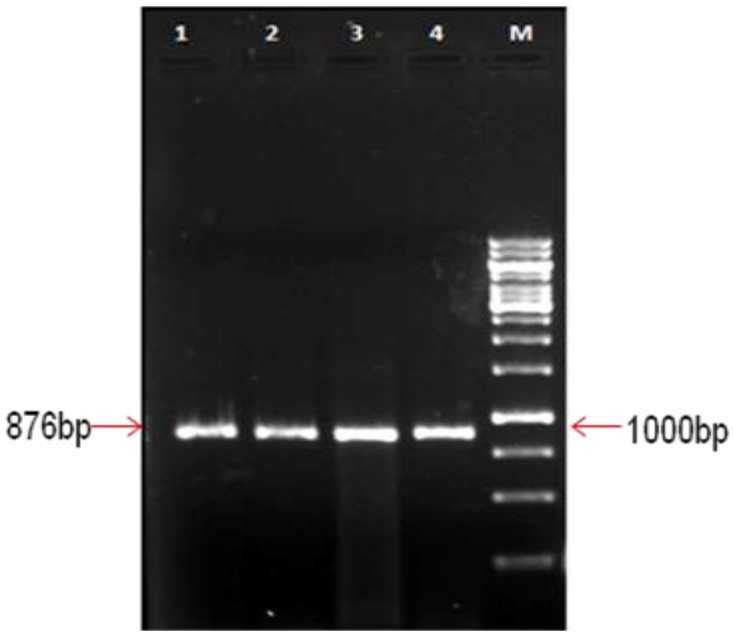

Fig. 2 Amplification of partial VP4 gene (876bp) of porcine rotavirus.
Lane 1 & 2: PCR products of porcine rotavirus from Assam,
Lane 3 & 4: PCR products of porcine rotavirus from Tripura,
Lane M: DNA ladder (1kb, Thermo Scientific)

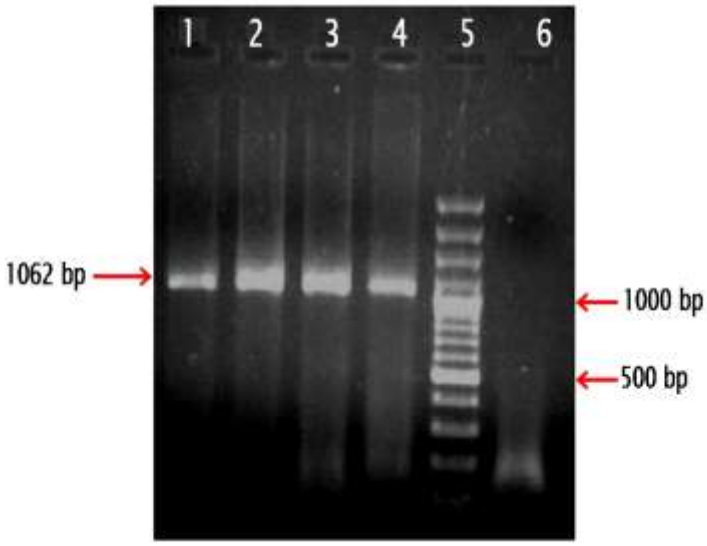

Fig. 3 Amplification of full length VP7 gene (1062bp) of porcine rotavirus.
Lane 1 & 2: PCR products of porcine rotavirus from Assam,
Lane 3 & 4: PCR products of porcine rotavirus from Tripura,
Lane 5: DNA ladder (100bp plus, Thermo Scientific),
Lane 6: Negative template control

V. Conclusion

In this study, it has been revealed that the two molecular techniques, RNA PAGE and RT PCR of VP4 and VP7 genes vividly detected group A rotaviruses in clinical samples with piglet diarrhea. Further, the added advantage with the two techniques is that, RNA PAGE helps in determination of the electropherotypes of the virus while, the PCR products so obtained by amplification of VP7 and VP4 genes will lead to genotypic determination of the rotavirus strain circulating in a particular region. Also, RNA PAGE can be considered as a highly sensitive and economical test for detection of rotavirus in feces and also for molecular epidemiological studies as the relative migration patterns of RNA segments will be affected by genetic drift, shift or rearrangements.

Efficiency of various techniques has been demonstrated for detection of porcine group A rotaviruses in Assam. Mention may be made of detection of rotavirus specific antibodies in serum samples of pigs by indirect ELISA [13]. Also, rotavirus was detected in feces of infected pigs by sandwich ELISA and PAGE [15]. However, reports from other parts of India and abroad on detection of rotavirus, made use of molecular techniques like RNA PAGE and RT PCR [9, 12, 18, 23-26] rather than ELISA. Hence, the present investigation emphasized the detection of porcine rotavirus in Assam and Tripura using RNA PAGE followed by RT PCR of VP4 and VP7 genes. Moreover, this is the first report on rotavirus detection from pig population of Tripura. Detection made by RNA PAGE and RT PCR of VP4 and VP7 genes on the contrary to previous reports by ELISA technique is more beneficial as PAGE can determine the electropherotypes while, RT PCR of the two genes can bring out the G and P genotypes prevalent among the rotavirus strains circulating in the NE India. Thus, the work can be considered as a baseline study for exploring the genotype of the circulating rotavirus strain in pig population reared in NE India. The findings therefore confirmed that group A rotaviruses are circulating among the swine population in Assam and Tripura. However, in order to know the genotypes of the circulating rotavirus strains and their zoonotic implications further investigation is required.

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