

MOECULAR CHARACTERIZATION OF THE RMO ADAPTED PESTE DES PETITS RUMINANTS VIRUS

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Abstract: This study was carried out to characterize two thermo adapted peste des petits ruminants virus (T PPR - I and T PPR-II) and conventional PPR vaccine viruses developed from a local isolates. RT-PCR amplification of portions of 308 bp of F gene, 317 bp of N gene and 191 bp of M genes were carried out. In the case of F gene nested PCR was done to amplify the inner portion of F gene. PCR amplified F gene, N gene and M gene were sequenced for all the 3 viruses and their sequences were compared. In the F gene sequences of the three viruses, there was an only one nucleotide change (A to T) in the sequence of T PPR- II virus at position 5879 when compared to the other two viruses. However, there was 100 per cent homology in the deduced amino acid sequences. The sequences of N and M gene were analyzed and it was found that there was 100 per cent homology among the three viruses.

Keywords: Thermoadapted PPR virus, RT-PCR, Sequencing.

INTRODUCTION

Peste des petits ruminants (PPR) is an economically important viral disease of goats and sheep. PPR virus (PPRV) is a member of the genus *Morbillivirus* of the family Paramyxoviridae. In India, PPR was first reported in 1987 [8]. Now PPR is considered as a serious threat to the sheep and goat farming in India. A homologous PPR vaccine has been developed by attenuation of local PPRV isolate in Vero cells at TANUVAS in a project funded by National Project on Rinderpest Eradication (NPRE), [5]. However, as with many modified live lyophilized vaccines, the attenuated PPRV vaccine requires refrigeration to prevent thermal inactivation. The present study was carried out to find out whether any molecular changes in thermoadapted PPRV due to prolonged storage. Sequencing of portion of Nucleoprotein (N), Fusion protein (F) gene and Matrix protein (M) genes was done to establish the thermostable nature of the virus at the molecular level.

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MATERIALS AND METHODS

Viruses

Vero cell adapted conventional PPR vaccine virus and two thermoadapted PPR (T- PPR -I and T- PPR -II) were developed and maintained in the Department of Veterinary Microbiology were used in this study.

Revival and titration of PPRV

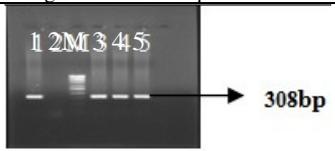
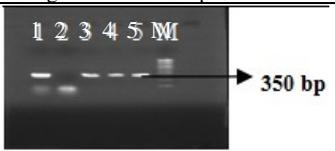
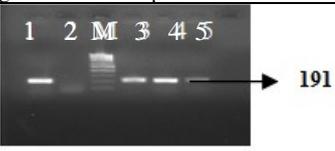
The vero cell monolayer in Roux was infected with 5 ml of 1 in 10 dilution of virus. When the monolayer showed 80 per cent CPE, it was disrupted by freezing and thawing for three times, aliquoted and stored at -80°C .

Reverse Transcription - Polymerase chain reaction

RNA isolation was done by acid guanidium thiocyanate method as described by [2]. PCR was conducted as per the method described by [4] F, N and M gene sequences were amplified using F₁ and F₂, Ind F and Ind R and Mf-morb and Mr -ppr 3 primers respectively. For F gene nested PCR was done. F_{1A} and F_{2A} primers were used to amplify the inner product of F gene. The PCR products of N, M genes and nested PCR products of F genes were sequenced using automated sequencer.

RESULT AND DISCUSSION

In the present study, CPE in Vero cells was observed after 5- 6 days of post infection during the initial passage. In the subsequent passage, CPE developed within 3-4 days. CPE was characterized by cell rounding and aggregation of cells, detachment from the surface, syncytia formation and clock-face arrangement of nuclei was observed. Similar findings were observed in previous studies [1].

Plate 1:Agarose gel electrophoresis of F gene nested PCR products	Plate 2:Agarose gel electrophoresis of N gene nested PCR products	Plate 2:Agarose gel electrophoresis of N gene nested PCR products
		
Lane-1-PC, Lane-2-NC, M- 100 bp DNA ladder, Lane-3- Conventional PPR, Lane-4-T PPR-I and Lane-5-T PPR-II	Lane-1-PC, Lane-2-NC, Lane-3- Conventional PPR, Lane-4-T PPR-I, Lane-5-T PPR-II and M- 100 bp DNA ladder	Lane1-PC, Lane-2-NC, M- 100 bp DNA ladder, Lane3- Conventional PPR, Lane-4-T PPR-I and Lane-5-T PPR-II

The portion of F gene was amplified using F₁ and F₂ primers. This outer F gene PCR product was used as template for nested PCR using F_{1A} and F_{2A} internal primers. The amplified product size is 308 bp.(Plate 1) [4]. The portion of N gene of PPR vaccine viruses were amplified using Ind F and Ind R primers. The size of the product is 317 bp.(Plate 2) NP₃-NP₄

primers and amplified the 350 bp product of N gene of PPRV [3]. Using Mf-morb and Mr-ppr3 primers the portion of M gene was amplified. The size of the amplified M gene product was 191 bp.(Plate 3)

In the F gene sequences of three viruses, there was a only one nucleotide change in the position of 5879 of sequence of T- PPR-II virus. The change was not seen in T PPR-I virus and conventional vaccine virus. The amino acid sequences were deduced and showed that 100 per cent homology between the three viruses. Three base changes were observed at positions 1075, 1084 and 1085 in the thermostable PPR virus (MIB 187(T) strain) and one change was noticed at position 1068 with the another strain (MIB 197(T) strain) [6]. Six nucleotide changes in the F gene of thermostable Arasur- PPR virus [7]. These changes also could not be obtained in this study.

The sequences of N and M gene were analyzed and it was found that there was no change in nucleotide and amino acid sequences. They showed 100 per cent homology among three viruses. However 36 nucleotide changes in the N gene of thermostable Arasur- PPR virus [7].

Phylogenetic analysis of F gene of thermostable as well as conventional PPR virus showed close relation with Nigerian strain of PPRV (Fig1)

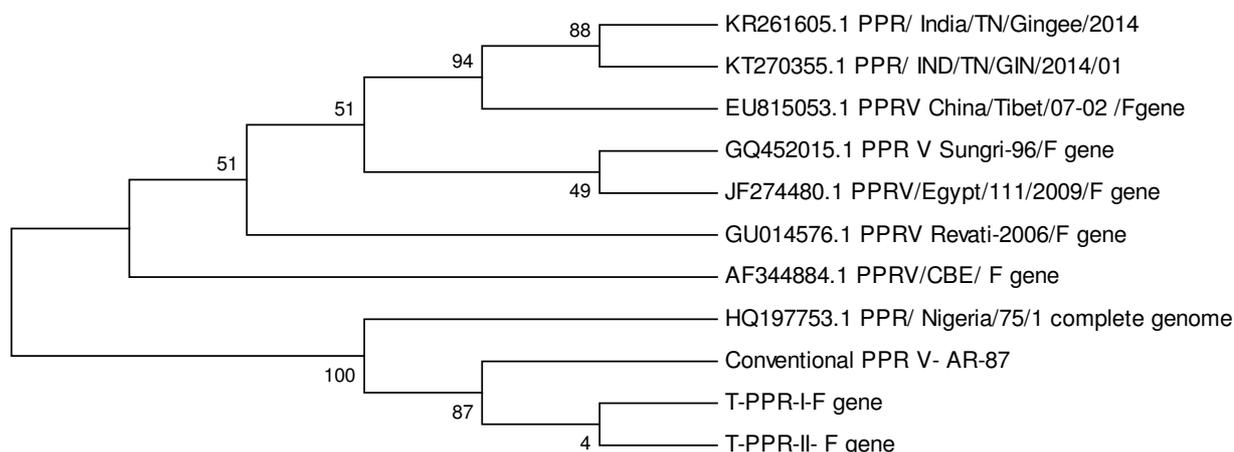


Fig 1. Phylogenetic analysis of F gene of conventional and thermostable PPRV by Neighbor –Joining tree method using 1000 boot straps of MEGA 5.0

The thermostability of the two viruses is not a permanent property retained over several consecutive passages. The resistance of thermostable virus to higher temperature was much less in the present analysis compared to the earlier reports. It becomes difficult to correlate

this change with thermostability. Another point to be noticed is that only a small portion of the F, N and M genes were sequenced and compared in this study. It is also possible for nucleotide changes to occur in the other motif of gene. Complete sequencing of all the three genes would give more insight for meaningful conclusion.

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