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Comparative nucleotide sequence analysis of the phosphoprotein gene of peste des petits ruminants vaccine virus of Indian origin

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Abstract

The nucleotide sequences of the phosphoprotein (P) gene of peste des petits ruminants (PPRV) vaccine virus (PPRV Sungri/96) belongs to Asian lineage have been determined and the deduced amino acid sequences were compared with another vaccine strain PPRV/Nigeria75/1 and with those of the other morbilliviruses. The 1652 nucleotides of the P gene encode a phosphoprotein of 509 amino acid residues (from nucleotide numbers 60 to 1587), which is 91% identical to that of PPRV/Nigeria75/1. The C protein consists of 177 amino acid residues and is 91% identical with that of PPRV/Nigeria75/1. The conserved mRNA editing site (5′TTAAAAGGGCACAG) was present at positions 742–756 in the P gene, which is conserved in all other morbilliviruses. The CTT trinucleotide sequence is present at the N/P and P/M intergenic region, which is totally conserved in morbilliviruses. This will be the third sequence for the P gene of PPRV since that of the vaccine strain and a wild-type Turkish isolate has been published already.

Keywords: Morbillivirus; Peste des petits ruminants virus; Vaccine virus; Phosphoprotein gene; Nucleotide sequence; Amino acid sequence

1. Introduction

Peste des petits ruminants (PPR) is a viral disease of goats and sheep with a widespread distribution across sub-Saharan Africa, the Arabian peninsula and the Indian subcontinent (Nanda et al., 1996; Dhar et al., 2002). PPR is considered to be one of the main constraints to improving productivity of small ruminants in the regions where it is endemic (Stem, 1993). The causative agent peste des petits ruminants virus (PPRV) is classified in the morbillivirus genus of the family Paramyxoviridae (Lamb and Kolakofsky, 2001). This genus also includes Rinderpest virus, Canine distemper virus, human Measles virus and viruses of marine mammals, Phocine distemper virus of seals and the Cetacean morbillivirus isolated from dolphins and porpoises (Barrett et al., 1993a; Dhar et al., 2002). The virus contains a single stranded negative sense RNA of ~16 kb in length, encoding six structural proteins namely: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin protein (H) and large polymerase protein (L), and two nonstructural proteins V and C.

The polycistronic phosphoprotein (P) gene is second proximal to the 3′ end of the genome, and is flanked by the nucleoprotein (N) and matrix (M) protein genes. A common feature of Paramyxovirus P gene is the synthesis of two virally encoded non-structural proteins, C and V, in addition to the P protein, from the P gene transcription unit (Mahapatra et al., 2003).

The sequence for this gene was deduced for the African lineage vaccine strain PPRV/Nigeria75/1(named here as PPRV-N) (Mahapatra et al., 2003). In this paper we report the Phosphoprotein gene sequence of PPRV vaccine virus

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PPRV Sungri/96 (named here as PPRV-S) belonging to Asian lineage (Sarkar et al., 2003; Dhar et al., 2002) for which the sequence data is not available.

2. Materials and methods

2.1. Cells and virus

Vero cells at passage level of 131 were used for propagation of the vaccine seed virus developed at Rinderpest Laboratory; IVRI-Mukteswar campus using an indigenous isolate of PPR virus (''PPR Sungri/96''). The vaccine virus was propagated as described previously (Sarkar et al., 2003) and aliquots of 250 μl were stored at −80 °C until use.

2.2. RNA extraction, cDNA synthesis and PCR

Total RNA was extracted by the acid-guanidinium–thiocyanate–phenol–chloroform method using TRIZOL reagents (Invitrogen, USA) essentially as described earlier (Dhar et al., 2002; Forsyth and Barrett, 1995). The Oligonucleotides used in RT-PCR to generate overlapping fragments covering the entire Phosphoprotein are shown in Table 1 and were designed from the published sequences (Mahapatra et al., 2003; Haffar et al., 1999; Diallo et al., 1994) using the software DNASIS version 2.6 (Hitachi, Japan) and were obtained from M/S Metabion (GmbH, Germany). Reverse transcription was performed on 1–5 μg of total RNA using MMLV reverse transcriptase (200U) and random hexamers (75 μg) at 37 °C for 1 h and subsequent PCR amplification was carried out using 5 μl of the RT product. The PCR cycling conditions were as described previously (Shaila et al., 1996; Forsyth and Barrett, 1995). Briefly, the cDNAs were subjected to a 30-cycle amplification (denaturation at 95 °C, annealing for 1 min at 50 °C and primer extension for 2 min at 72 °C) using 10 pmol of each primer and 1.25 U of Taq DNA polymerase enzyme (Promega, USA).

2.3. P gene editing

To analyze the P gene editing, a method of Locke et al. (2000)) is followed with slight modifications. Briefly, cDNA was synthesized from the mRNA isolated using PolyATtract® mRNA Isolation System II (Promega, USA) using the primer pprp_rev4 and the PCR was performed using the primer pair pprp_rev4 and pprp_fr4 (Table 1). The RNA was extracted from PPR virus purified on a 30–60% discontinuous sucrose density gradient (Singh et al., 2004) using TRIZOL reagents according to the manufacturer's instructions with necessary modifications (Chomczynski and Sacchi, 1987). The genomic sequence was confirmed using the PPR viral RNA as template and RT-PCR was performed using Access RT-PCR system (Promega, USA) with the above-mentioned primers. The amplicons were cloned and sequenced on both the strands as mentioned later (Locke et al., 2000).

2.4. Cloning of PCR products and sequencing

The PCR amplicons were checked for its correct size in 1% agarose gel and purified using Wizard® PCR Purification system (Promega, USA). The purified amplicons were cloned in to pGEM-T vector and the recombinant plasmid DNA was isolated from representative clones and checked for its correct size as described previously (Dhar et al., 2002). These cloned amplicons were sequenced on both the strands using fmol DNA cycle sequencing kit (Promega, USA) and Cy5 labeled M13 forward and reverse primers in an automated sequencer ALF express II (Amersham Pharmacia Biotech, UK).

2.5. Sequence analysis and phylogenetic tree construction

The sequenced fragments of the gene were assembled using the Megalign software of the DNASTAR package. The portions overlapping and the primer sequences were eliminated appropriately. For comparison, the following sequences (GenBank Accession numbers within parenthesis) were taken from NCBI sequence databases; Vaccine virus PPRV Nigeria/75/1 (AJ298897), Rinderpest virus (Z30697), Dolphin Morbillivirus (NC 005283) Phocine Distemper Virus (X75717), Canine Distemper Virus (AF305419), and Measles virus (AF266289). The phylogenetic tree was constructed using the Neighbour Joining method with Kimura-2-parameter model available in the program MEGA version 2.1 (Kumar et al., 2001). The alignment gaps were excluded from pairwise distance estimations. The robustness of the predicted tree was statistically evaluated using the bootstrap method (Felsenstein, 1985; Hedges, 1992). The bootstrap p values are obtained

Table 1
Primers used for amplification of P gene of PPRV-S in the study

Sl. No.	Name of the primer	Sequence (5′ to 3′)	T_A (°C)	Binding location in the gene	Expected product size (bp)
1	pprn-fr3	GAGTCTAGTCAAAACCCTCG	50	1479–1488 in N gene	465
	pprp_rev1	GTCTGATCCGATTGCTGGTG	50	271–252	
2	pprp_for1	TAGGACCCAGGTCAAGCAAC	50	03–22	767
	pprp_rev2	GACTGAGTTCCCGTCTGTGC	50	769–750	
3	pprp_fr4	TTCAATGGAGGAGAGGATGG	50	479–499	1110
	pprp_rev4	GTTACGGCTGCTTGGCAAGA	50	1589–1570	
4	pprp_for2	ATGATGTCTCTAGGTCAACA	50	578–598	1312
	ppr-5852-rev	GGGAGACAGGGGGTCGTTATCC	50	212–233 in M gene	