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Phylogenetic analysis of sheep pox virus (SPPV) Virion Core Protein P4a gene reveled extensive sequence conservation among capripox viruses

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ABSTRACT

In the present study virion core protein P4a gene was PCR amplified from sheep pox virus(SPPV) Jaipur isolate and Roumanian Fanar(RF) vaccine strain adapted and propagated in lamb testis/vero cells. Gene specific primers were designed for amplification of P4a gene. Amplified P4a gene fragment was sequence characterized and 808 bp sequence was compared across SPPV, GTPV and LSDV isolates available in GenBank database which reveled extensive sequence conservation of 97% to 100% within pox virus groups. Sheep pox virus Jaipur isolate was found closely placed with Roumaninan Fanar(RF) and TU isolates. Further, phylogenetic analysis of P4a gene sequence indicated three distinct clusters of Capripox viruses with interestingly GTPV was placed closely to LSDV group.

Key words: Phylogenetic analysis, Sheep pox, Virion core protein P4a gene

Sheep pox virus (SPPV), Goat pox virus (GTPV) and Lumpy skin disease virus (LSDV) are Capripox viruses (CaPVs) responsible for causing severe poxvirus disease in sheep, goat and cattle, respectively. Sheep pox and goat pox are important contagious diseases endemic in Indian sub-continent, Middle East, Central and Northern Africa. Both sheep pox and goat pox can cause wool and hide damage, and reduce the production of mutton and milk, which may result in significant economic losses and threaten the stock breeding. Epidemiological investigations may not be achieved by serological testing due to close antigenic relationship between capripox viruses (Kitching *et al*. 1989). Capripox virus strain identification has relied on the hypothesis that the viruses show well defined host specificity and in the face of cross infections, molecular based techniques are being increasingly used to allow unequivocal strain differentiation. The development of a suitable molecular assay requires the identification of a target gene/locus within the capripox viruses genome which contains species-specific signatures. In this direction, the present study describes the sequence characterization and phylogenetic analysis of the SPPV major core protein P4a (A10L Gene) homologue of Vaccinia that may help in discriminating the animal origin of capripox viruses. Functionally the major core protein P4a is related to viral growth, essential for viral DNA assembly into the nucleoprotein complex to form immature virus particles (Heljasvaara *et al*. 2001), repression of this gene effects up to 20 fold (2 log) decrease in the viral yield.

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MATERIAL AND METHODS

Field isolate "Jaipur" and vaccine strain "Roumanian Fanar" of SPPV were adapted and grown in lamb testis/ vero cell.

Sheep pox virus(SPPV) genomic DNA was extracted from infected vero cell culture by proteinase-k method (Bergalo *et al*. 2006). Quantity and quality was assessed by spectrophotometer. The virion core protein P4a gene specific primers were designed based on SPPV genome sequence -AY077832 (Tulman *et al*. 2002) and DNA template was subjected to PCR amplification using gene specific primers (forward AAATCCCATTGCCGAAAAA and reverse AATGCTTGATGCAGCAAATG). The PCR amplified gene fragment was further gel purified and sequenced by automated DNA sequencer ABI 3100.

Multiple sequence alignments of the nucleotide sequences were performed using ClustalW and MegAlign of DNA Star software package (Lasergene 6.0, DNASTAR Inc. USA). The phylogenetic tree of the SPPV P4a gene homologues was based on the Neighbor-Joining method (Saitou and Nei 1987) using the Molecular evolutionary genetic analysis version 4 (MEGA4) software package version 5.25 with minimum evolution (ME) methods (Tamura *et al*. 2007). The sequences available in GenBank database were used to make comparison and further phylogenetic analysis. The sequences used were three of sheep pox, SPPV-TU (AY077832), sheep pox A strain

(AY077833) and Niskhi strain (AY077834) and other member of capripox virus viz. GTPV Pellor (AY077835), GTPV G20–LKV (AY077836), LSDV Neethling 2490 (AF325528), LSDV Neethling Warmbaths LW 1959 (AF409138) and LSDV Neethling Warmbaths LW (AF409137). Bootstrap values were calculated out of 1000 replicates according to the maximum-likelihood approach (Felsenstein 1985).

RESULTS AND DISCUSSION

For sequence characterization and comparison we selected a virion core protein P4a gene that encodes a homologue of vaccinia virus A10L gene. Primers were designed based on the SPPV-TU (AY077832) genome sequence reported by Tulman *et al*. (2002), to partially amplify the P4a gene fragment of Jaipur and Roumanian fanar strain of sheep pox virus. The P4a gene fragment was PCR amplified and sequence generated for 808 bp for SPPV Jaipur (JP) and Roumanian Fanar (RF) strains, which were compared with other SPPV, GTPV and LSDV isolates from GenBank database. Multiple sequence alignment of nucleotide sequences revealed near identical sequences

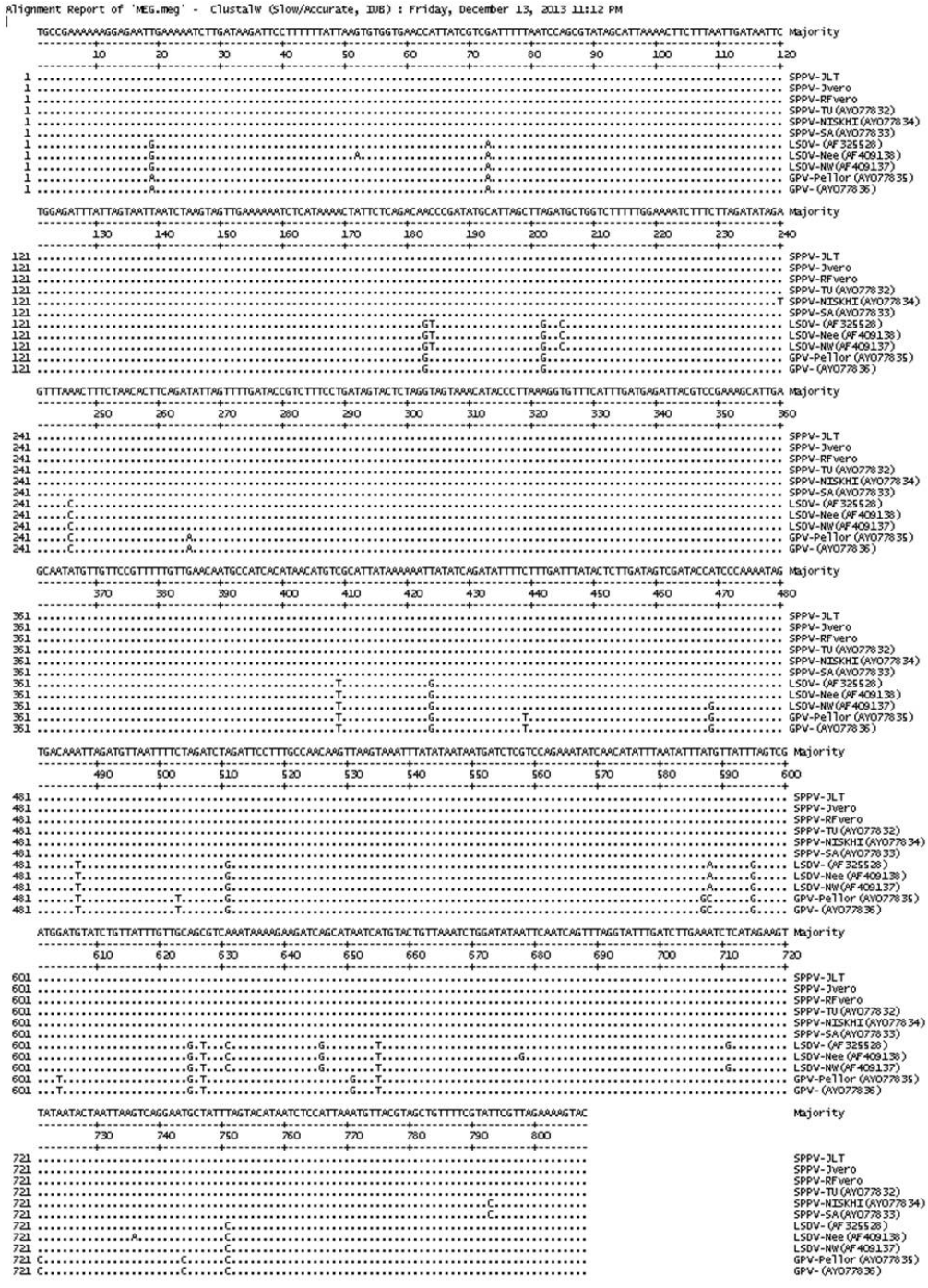

Fig. 1 Multiple sequence alignment of 808bp virion protein P4a gene across SPPV, GTPV and LSDV isolates.

within SPPV isolates except variation at position 240 and 793 (Fig.1). Similarly high degree of nucleotide homology was observed within GTPV whereas LSDV isolates showed little variation. The GTPV and LSDV isolates revealed similar sequence except at 13 nucleotide positions different than SPPV isolates (Fig.1). The sequence divergence analysis among SPPV, GTPV and LSDV isolates resulted 97% to 100% identity of the 808 bp P4a gene fragment indicating highly conserved gene sequence among capripox viruses. Similarly comparison of the deduced amino acid sequence of the virion protein P4a gene indicated 96.7% to near identical 100% identity of amino acid sequence among SPPV, GTPV and LSDV isolates. Alignment of deduced amino acid sequence of 269aa revealed only single difference at 190 position from 'S' to 'T' in SPPV-Nishiki strain. Further only 5aa differences were observed between SPPV group and GTPV/LSDV taken combined as a group (Fig.2). The resulting phylogenetic tree (Fig. 3) based on Neighbour joining method revealed three distinct virus clusters consisting of the LSDV group, the GTPV group and the SPPV group. Interestingly, this sequence analysis of P4a gene sequence indicated that GTPVs were more closely grouped to LSDVs than to SPPVs.

Classification within the genus Capripox is based upon the host from which the virus is isolated viz. sheep pox, goat pox and lumpy skin disease from sheep, goat and cattle respectively with the assumption that they are strictly host-specific (Esposito *et al*. 2001). CaPV are antigenically related to each other (Bhanuprakash *et al*. 2006). Further no CaPV-like disease has been reported in sheep or goats in southern Africa, where LSD in cattle is endemic. However, there is one confirmed isolation of a LSDV isolate from sheep, the Kenyan O–240 isolate. Lumpy skin disease has never been reported in Asian countries, where sheep pox and goat pox are endemic. In addition, there are reports of capripox outbreaks in which both sheep and goats were involved (Diallo and Viljoen 2007). In view of this it is very important to identify the causal virus and study its phylogenetics through molecular approaches. For this the sequence characterization of genes and their phylogenetic analysis would be necessary to address evolution of these isolates belonging to various groups under capripox viruses. The present study revealed close relatedness of LSDV with that of GTPV as compared to SPPV which is in sync with Hosamani *et al*. 2004 and Gubser *et al*. 2004 who concluded the same using phylogenetic study on different sheep pox virus genome segments. Tulman *et al*. (2002) study was based on the genes with virulence and host range function suggesting that small ruminants pox virus may have emerged from common LSDV like ancestor. Studies have established the approach for differential identification of capripox virus genetically Stram *et al*. 2008 suggested most variable region in the left termini of capripox virus genome to be ideal for distinguishing between LSDV, GTPV and SPPV and Le Goff *et al*. (2009) used variable gene GPCR for genetic discrimination between small ruminants pox virus. The phylogenetic study of viral core protein P4a gene showed highly conserved nature and does not show any variation within the species, may act as a locus to differentiate between capripox virus with high boot strap value. Among the sequences analyzed Nisikhi and Roumanian Fanar are the vaccine strain and the nucleotide

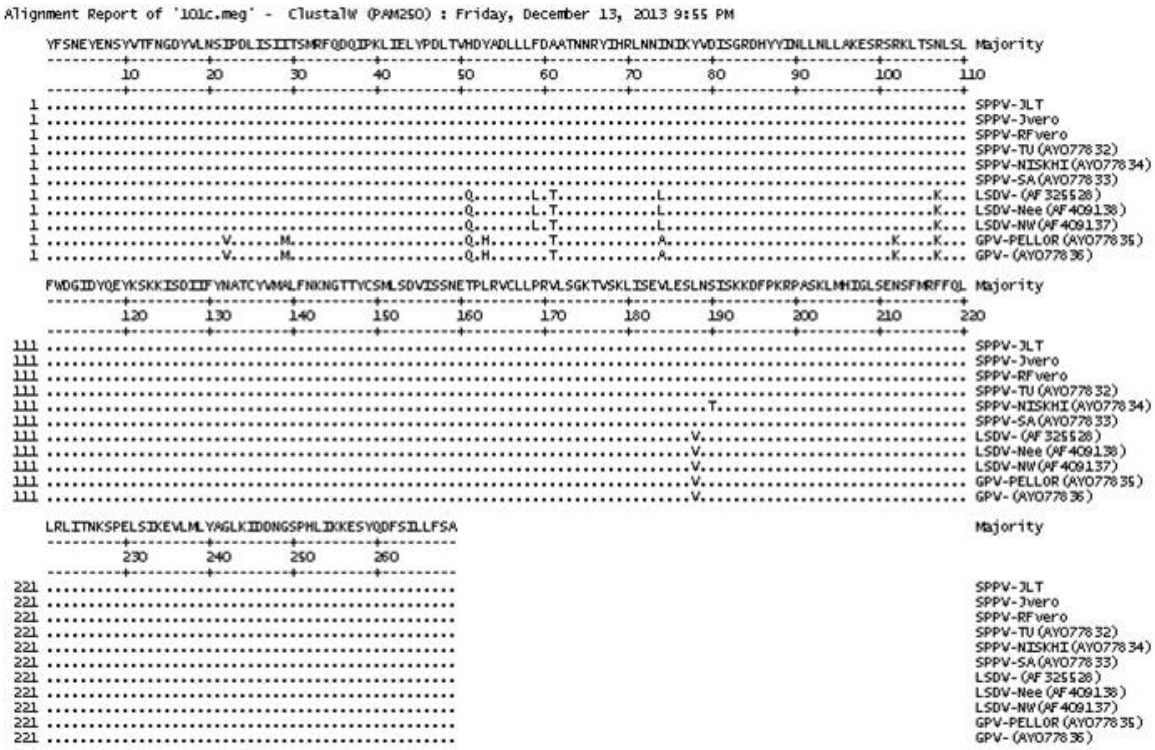

Fig. 2. Multiple deduced amino acid sequence alignment of virion protein P4a gene across SPPV, GTPV and LSDV isolates.

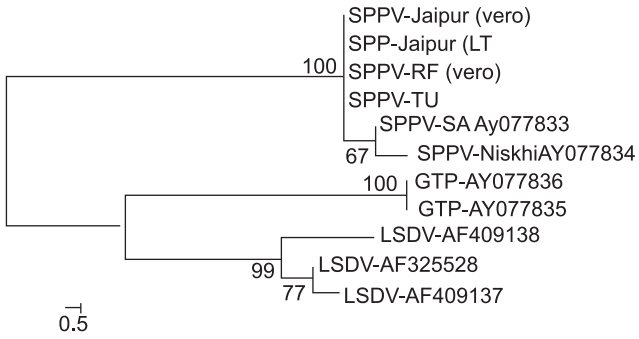

Fig. 3. Unrooted phylogenetic tree of P4a gene showing three distinct clusters as SPPV, GTPV and LSDV. The SPPV Jaipur isolate is placed closely with RF vaccine strain and TU isolates. Tree was constructed based on nucleotide sequence by neighbor joining method in MEGA-4 using 1,000 replicates of the dataset. The bootstarp confidence values of major clusters are indicated against each node of tree.

sequence identities with the virulent strain indicate that the attenuation process of these vaccine strains did not involve the viral core protein p4a gene. There was also no change detected in the nucleotide sequence of P4a gene during *in vitro* passaging of sheep pox virus in vero cells up to 30 passages further indicating the stability of the gene.

Figure 1: Multiple sequence alignment of 808bp virion protein P4a gene across SPPV, GTPV and LSDV isolates.

Figure 2: Multiple deduced amino acid sequence alignment of virion protein P4a gene across SPPV, GTPV and LSDV isolates.

Figure 3: Unrooted phylogenetic tree of P4a gene showing three distinct clusters as SPPV, GTPV and LSDV. The SPPV Jaipur isolate is placed closely with RF vaccine strain and TU isolates. Tree was constructed based on nucleotide sequence by neighbor joining method in MEGA–4 using 1,000 replicates of the dataset. The bootstarp confidence values of major clusters are indicated against each node of tree.

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