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Nucleotide sequence analysis of the viral envelope protein gene (P32) region of sheep poxvirus from India

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ABSTRACT

Seven local field strains of sheep poxvirus from different districts of Tamil Nadu, India and vaccine strains were characterized by sequencing studies to find out the marker gene in an attempt to differentiate the field and vaccine strains. The full length viral envelope protein gene (P32) of sheep poxvirus was amplified and sequenced. The sequence studies revealed that the local field strains possessed 100% and 99.31% homology with Ranipet and Roumanian-Fanar vaccine strains respectively. Sheep poxvirus could not be differentiated based on the envelope protein nucleotide sequence level in any field outbreaks.

Key words: Basic local alignment search tool, Ranipet strain, Roumaninan Fanar strain, Sheep poxvirus, Viral Envelope protein gene

Sheep pox, a contagious viral disease of economic importance causes heavy mortality in lambs, abortions and mastitis in ewes and skin defects (Singh *et al.* 1979). Sheep poxvirus and goat poxvirus, which are enveloped, double-stranded DNA viruses are classified in the genus *Capripoxvirus* of the family *Poxviridae* (Rao and Bandyopadhyay 2000).

Vaccines for sheep poxvirus had been produced and evaluated under field conditions. Live attenuated virus of sheep pox passaged in primary cultures like lamb kidney or lamb testis was used for vaccination with or without aluminium hydroxide gel (Kitching 2003, Bhanuprakash *et al.* 2004). There are occasional reports of outbreak after vaccination indicating the need for further studies on the field strains of the sheep poxvirus.

P32, one of the structural proteins present in all *Capripoxviruses*, contain major immunogenic determinants, which help in the virus attachment and possibly play a role in virus virulence (Hosamani *et al.* 2004). The amplification of P32 gene followed by restriction enzyme profiling and nucleotide sequence analysis could be used as molecular epidemiological tool in differentiating the strains of sheep and goat poxvirus. Hence, the present study was undertaken with the object to compare the envelope protein gene of the field and vaccine strains of sheep poxvirus to differentiate them using sequence analysis.

Table 1. Details of places of sample collection with their accession numbers.

Place and district	Strain	Year of collection	Accession Number
Ranipet Vaccine strain	Ranipet strain		DQ 1532219
Gollapattikaliyapuram, Coimbatore, Tamil Nadu	CMB 1	2005	DQ 153220
Gollapattikaliyapuram, Coimbatore, Tamil Nadu	CMB 2	2005	DQ 153221
Mallaegoundanpalayam, Coimbatore, Tamil Nadu	CMB 4	2005	DQ 153223
Mallaegoundanpalayam, Coimbatore, Tamil Nadu	CMB 3	2005	DQ 153222
Morappur, Dharmapuri, Tamil Nadu	MOR	2005	DQ 153224
Thevoor, Erode, Tamil Nadu	TVR	2005	DQ 153225
Vandalur, Chennai, Tamil Nadu	BAF	2005	DQ 153226

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MATERIALS AND METHODS

Processing of samples: The suspected scab/skin lesions in full thickness were collected aseptically in buffered

glycerol from different outbreak areas of Tamil Nadu, India. The details of sample collection are presented in the Table 1. The tissue was ground well with sterile sand and tissue culture medium in a mortar and pestle to prepare a 10% suspension (Kitching and Taylor 1985). The suspension was clarified at 3000 rpm for 15 min and the supernatant was used for DNA extraction.

Propagation of sheep poxvirus vaccine strains: The lamb kidney cell culture was prepared for the propagation of sheep pox vaccine strains – Ranipet strain. Lamb kidney cell culture was prepared as per Singh *et al.* (1979). The lamb kidney culture monolayer was inoculated with 1 ml of vaccine virus and incubated for 1h at 37°C. The cultures were observed daily for 72–96 h. After the appearance of cytopathic changes, the cells were frozen thawed three times and coarse centrifugation was done at 5000 rpm for 15 min at 4°C to remove cell debris. The supernatant was subjected for DNA isolation.

Viral DNA isolation: The viral DNA was isolated from suspected scab suspensions and infected tissue culture fluid of vaccine strain by proteinase K method using the DNEasy tissue kit. The DNA isolated from uninfected tissue culture fluid was used as negative control.

Polymerase chain reaction of P32 gene: PCR was carried out as per Hosamani *et al.* (2004). The DNA of sheep poxvirus was amplified by PCR utilizing P32 gene region specific primers. The sequence of the primers already described by Heine *et al.* (1999) was used in this study.

Forward primer 5' CAC GGA TCC ATG GCA GAT ATC CCA TTA 3'

Reverse primer 5' AAC AAG CTT ACT CTC ATT GGT GTT CGG 3'

Ten µl of PCR product obtained from seven SPV strains, Ranipet vaccine strain and 1 µl of 100bp DNA ladder were analyzed in agarose gel electrophoresed and viewed under UV transilluminator and photographed.

Purification of PCR products: The PCR amplicons of P32 gene was purified by running on low melting point agarose and using gel extraction column. as per the manufacturers' protocol.

Nucleotide and aminoacid sequence comparison

The purified P32 gene products of sheep poxvirus obtained from different districts of Tamil Nadu along with the Ranipet strain, IVPM were sequenced in an automated sequencer. The nucleotide sequence of lamb testis adapted Roumanian-Fanar strain of SPV already done by Hosamani *et al.* (2004) was used for comparison.

The envelope protein gene P32 nucleotide sequence data of the SPV field strains and Ranipet strain sequence were written on a single file in FASTA format which was used as input file for the sequence alignment software CLUSTAL W (Thompson *et al.* 1997).

The MEGA 1.02 Molecular Evolutionary Genetic Analysis Software involved in creating multiple sequence alignment of field, vaccine and other SPV strains using alignment command. Then proportion of nucleotide difference between each pair of nucleotide was compared using pair wise command. Finally, phylogenetic tree was constructed by selecting the phylogeny command in the software.

RESULTS AND DISCUSSION

DNA extraction followed by PCR was done for 7 strains and Ranipet vaccine strain using pair of primers for the full length P32 gene region. The expected amplicons size of 1027 bp was observed in all 7 field strains and vaccine strain when tested in 1% agarose gel with 100 bp DNA molecular weight marker (Fig. 1). No amplification was noticed in the negative control samples.

The multiple sequence alignment of nucleotide sequences of all seven field strains with 2 vaccine strains and strains from other countries such as Niskhi, Nigerian strain (NC1), Strain A and Strain TU using commercial programme were compared. The results of comparison of the nucleotide and aminoacid sequence homology between field and vaccine strains were furnished in the Table 2. The phylogenetic analysis of the nucleotides sequence (Fig. 2) showed that the local field strains were very much closer to the Ranipet strain followed by Roumanian – Fanar strain. The strains from other countries were similar to each other and also to the Indian strains at P32 gene sequence.

The serological assay to detect the antigen and to quantify antibodies to sheep poxvirus are difficult to interpret because of existence of common antigen between *Capripoxviruses* (Ireland and Binepal 1998), which necessitates a highly sensitive and specific assay for detection of sheep poxvirus. The genome of sheep poxvirus consist of a single linear double stranded DNA of about 150 kb (Tulman *et al.* 2002) and PCR-based methods to diagnose and characterize was

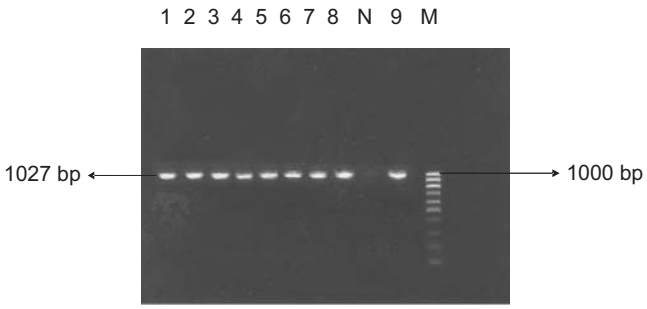

Fig. 1. Agarose gel electrophoresis of full length sheep poxviral P32 gene.

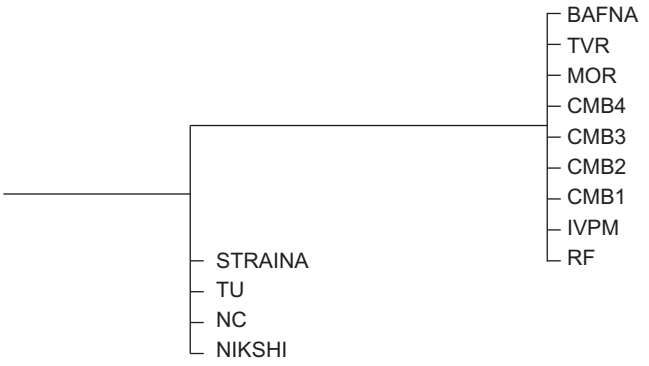

Fig.2. Phylogenetic analysis of Indian and foreign strains of sheep poxvirus.

devised by several workers (Markoulatos *et al*. 2000, Hosamani *et al.* 2004). The crude scab suspensions and infected tissue culture fluid were subjected to DNA extraction followed by PCR assay.

The extracted DNA from scab suspensions and tissue culture fluid of vaccine strain, when subjected to PCR using primers specific for full length P32 gene yielded expected amplicons of size 1027 bp as observed by Hosamani *et al.* (2004). No amplification was obtained in negative controls as reported by Parthiban *et al.* (2005) indicating that the primers are highly specific for the detection of sheep poxvirus.

Different sheep poxvirus strains could not be differentiated by analysis of their proteins and serological methods (Davies 1981). The strain variation within sheep pox is not feasible based on the PCR technique (Rao and Bandyopadhyay 2000). Hence restriction endonuclease and cross hybridization were done to differentiate the strains of sheep poxvirus (Black *et al*. 1986, Gershon *et al.* 1989a). The whole genome of different strains of sheep poxvirus are closely related (Black *et al.* 1986), to find out the pinpoint variation in the genome, nucleotide sequencing of envelope protein gene was done in this study.

Table 2. Comparison of nucleotide and aminoacid sequence homology between field and vaccine strains of sheep poxvirus

	Nucleotides		Aminoacid	
	Ranipet strain	Roumanian-Fanar strain	Ranipet strain	Roumanian-Fanar strain
TNSPV CMB1	100%	99.31%	100%	98.63%
TNSPV CMB2	100%	99.31%	100%	98.63%
TNSPV CMB3	100%	99.31%	100%	98.63%
TNSPV CMB4	100%	99.31%	100%	98.63%
TNSPV MOR	100%	99.31%	100%	98.63%
TNSPV TVR	100%	99.31%	100%	98.63%
TNWSPV BAF	100%	99.31%	100%	98.63%
SPV NISKHI	96.22%	96.0%	96.22%	94.85%
SPV NC	96.22%	96.0%	96.22%	94.85%
SPV TU	96.22%	96.0%	96.22%	94.85%
SPV STRAIN A	96.22%	96.0%	96.22%	94.85%

The sequences of the full length gene of local field strains of sheep poxvirus and the Ranipet strain of IVPM possessed 100% homology at the nucleotide level. The similar finding was made by Parthiban *et al.* (2005) using a part of the P32 gene. The sequences of the local sheep poxvirus strains and Roumanian-Fanar vaccine strain possessed 99.31% homology and so the strains from other countries. Hosamani *et al.* (2004) reported that the local Indian strains of sheep poxvirus and Roumanian strain, one of the vaccine strain used in this country, possessed very high level of homology at the P32 region. On phylogenetic analysis, the local field strains were very much closer to the Ranipet strain as well as the Roumanian-Fanar strain of sheep poxvirus.

In the present study it is concluded that strains of sheep poxvirus cannot be differentiated based on the envelope protein gene sequence level in any field outbreak. Hence, deletant mutant vaccine may be the better choice for differentiation of field and vaccine strains of sheep poxvirus in the countries where vaccination is done regularly.

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