Original Research Article

SEQUENCING & PHYLOGENETIC STUDY OF BOVINE PAPILLOMA VIRUS

ABSTRACT

The involvement of different genotype of BPV was reported from different parts of world. In this study a total of 5 BPV-1-positive PCR products were sequenced to determine the type of viral genome. The Delta papillomavirus sp. L1 generating genes showed the most similarity in a blast search (99.66 percent). Furthermore, multiple sequence alignment revealed 99% and 99.7 % identity with Indian sequences KF055288.1 (IVRI, India) and HG918265.1 (IVRI, India) respectively. Phylogenetic analysis of partial sequences from the current study revealed that they are closely connected to MN977321.1 (Italy) and AB626705.1 (Japan), but are distantly related to KX907623.1 (China) and KY372394.1 (Turkey) sequences.

INTRODUCTION

Bovine papillomatosis has been documented in cattle (Bos taurus) in Europe, America, Asia and Oceania (Freitas *et al*., 2004). In India bovine papilloma cases has been reported by a number of workers. Non-human vertebrate genotype diversity is still significantly less well-documented than HPV genotype diversity. In the present study, a total of 5 BPV-1 positive specimens (as determined by PCR) which were selected as representative of BPV for geographical distribution, were sequenced to confirm viral genome type.

MATERIALS AND METHOD

Ethical approval

All samples were collected as per standard sample collection procedure. This type of study does not require any ethical approval.

SAMPLING

For this study, twenty wart tissues were collected from cattle and buffalo of varying ages. The sample collection area includes villages in the Maharashtra districts of Satara and Pune. The samples were collected in sterile containers and transferred with ice packs to the laboratory, where they were maintained at - 20°C until further processing.

MOLECULAR DETECTION & SEQUENCING

The DNA purification kit was used to extract DNA from a wart tissue sample. The extracted DNA was used as a template. Amplification of extracted DNA was carried out by PCR targeting BPV-1genes using published primers. For nucleotide sequencing, of BPV1 genotype total 5 purified PCR product from two different districts (Pune and Satara) were selected.Purified PCR products were sent for nucleotide sequencing . The obtained nucleotide sequences of BPV-1 genotype were subjected to BLAST analysis on the website of NCBI using BLAST tool (http://www.ncbi.nlm.nih.gov/). Amino acid sequences were constructed from obtained nucleotide sequence of BPV- 1 genotype using protein analysis tool on the NCBI website. The obtained sequences were analysed using DNAstar (Laser gene, USA) software .Multiple sequence alignment of the sequences was carried out using Clustal W method in MegAlign (DNAstar). Sequence obtained from Satara and Pune were subjected to establish their phylogenetic relationship with other sequences from NCBI data bank. Phylogenetic tree of the sequences was constructed in DNAstar using neighbor-joining model and 1000 trails in bootstrap analysis.

Histopathology:

BPV suspected wart tissue were collected in 10 % formalin solution for histopathological examination. Paraffin embedding method was carried out as per method described by (Chauhan, 1995). The staining procedure was carried out using routine Haematoxyline (H) and Eosin (E) method of staining. Stained slides with tissue sections were observed under the microscope for specific histopathological changes in wart tissues.

RESULTS AND DISCUSSION

BPV1 genotype sequencing

In the present study, a total of 5 BPV-1 positive specimens (as determined by PCR) which were selected as representative of BPV for geographical distribution, were sequenced to confirm viral genome type. The DNA sequences (250-297bp) of five samples were obtained after trimming the low-quality reads and removal of the overlapping sequence. These partial sequences were analyzed using a BLAST search against non-redundant databases, revealing 147 (90.74 %), reads as Delta papillomavirus , (Fig.No.1). Sequence analysis was done by comparing DNA sequences of current study with the published sequences available at National Center for Biotechnology Information (NCBI, USA) using DNAstar software (USA). Blast search showed the highest similarity (99.66 %) with the Deltapapillomavirus spp. L1 encoding genes (Fig No. 2 and 3). Alignment of the said sequence with the published BPV-1 L1 sequences of Indian origin revealed three mismatches or three

SNP at 83 (A to C); 239 (T to A) and 240 (G to T) in comparison to HG918265.1 (IVRI, India) sequence and one mismatch at 83 (A to C) with KF055288.1 (buffalo, IVRI, India). This nucleotide variation results in amino acid change at 38 (V to L); 116 (K to N) and 117 (A to S) in protein of BPV-1 of current study. The change in amino acid at position116 and 117 is seen in comparison with protein of HG918265.1 whereas change at amino acid position 38 is in regards to proteins of both HG918265.1 and KF055288.1. Furthermore, multiple sequence alignment of all the sequences of current study showed 99 %and 99.7 % identity with KF055288.1 (IVRI, India) and HG918265.1 (IVRI, India) sequences from India, respectively. Also, sequences identity of 95.5 %, 99.3 %, 99.3 % and 99.7% with KY372394.1 (Turkey), AB626705.1 (Japan), KX907623.1 (China) and MN977321.1 (Italy) respectively. Hence it can be opined that there is sequence variation or diversity in sequences of current study as compared to published sequences. Also, within India also there was sequence diversity in BPV-1 sequences, Hamad *et al.* (2016) sequenced the PCR products for the E2, E5, and L2 genes amplified from the collected samples, which all had the same sequence. The majority of the NCBI BLAST-searched BPV-1 sequences had 97 percent identity, confirming the presence of BPV-1. BPV-1 was the predominant genotype detected in all 121 BPV-positive (100 percent) cattle wart tissue samples. Their finding is the first genotyped confirmation of the presence of BPV-1 as a primary causative agent for bovine papillomatosis in Iraqi cattle in the central Iraqi region. The result of the present study were in agreement with the earlier findings regarding BPV-1 as predominant genotype. Similar findings were reported by Timukaran *et al.*, (2017) in their study the aligned sequences showed a high percentage sequence identity to the nucleotide sequence of BPV-1. Peng *et al.*, performed full-length genomic sequencing of all four isolated strains (JX180408, LA150909, HX160815, and BS160810) to explore the molecular basis of currently prevalent BPV strain in Chinese native cattle. The findings of this study are important from epidemiological point of view. Thus, it is essential to characterize genetically all the BPV occurring of different agro-climatic zones of India for effective diagnosis and control measures.

Phylogenetic study

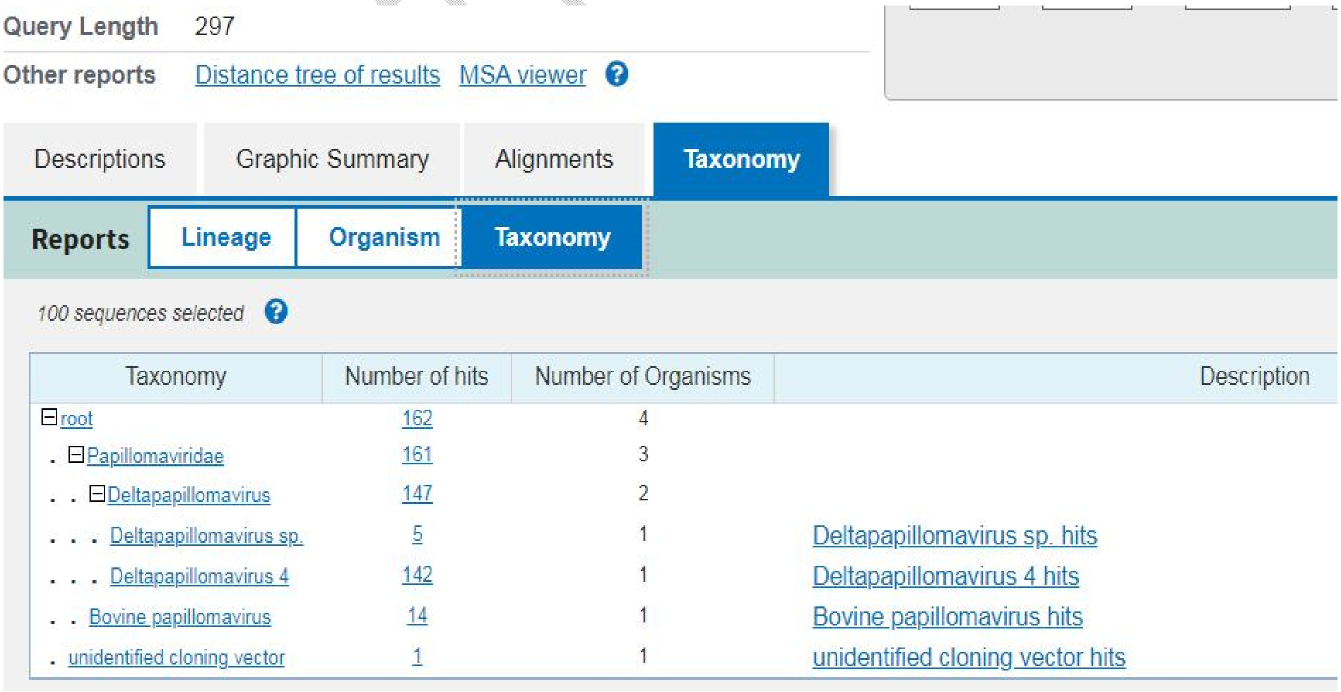
Phylogenetic tree of the sequences was carried out using neighbor-joining model and 1000 trails in bootstrap analysis. Phylogenetic analysis revealed that BPV- 2 L1 sequences (SH1, 2, 3 and 5) of Maharashtra origin formed one cluster with minimum of 88.4 % bootstrap support. SH4 sequence of current study falls in separate branch. Further the nucleotide sequences KF055288.1 (IVRI, India), HG918265.1 (IVRI, India), KY372394.1 (Turkey), AB626705.1 (Japan),KX907623.1 (China) and MN977321.1 (Italy) formed separate clad/ cluster. These two clusters (cluster of SH1, 2, 3 & 5 and cluster of remaining sequences) were separate and this relationship had 50.8% bootstrap support.

Phylogenetic study of partial sequences of current study revealed close lineage with MN977321.1 (Italy) and AB626705.1 (Japan) whereas they are distantly related KX907623.1 (China) and KY372394.1 (Turkey) sequences, respectively (Fig.No.4).Similar findings were reported earlier by Timukaran *et al.* (2017). They reported the presence of BPV-1, with a 97% sequence identity to the BPV-1 sequences.

Histopathological Examination

Histopathological examination of 10 wart tissue was carried out. Microscopically papilloma was diagnosed in 6 cases and fibropapillomas were diagnosed in 4 cases. In papilloma proliferation of squamous epithelial cells (acanthosis), parakeratotic hyperkeratosis& Koilocytes were observed. Koilocytes were characterized by enlarged pale vacuolated cytoplasm which is also a salient findings. In fibropapillomas there was mild, moderate to severe hyperplasia of squamous epithelial cells (acanthosis with papillary projections) multifocal to different hyperkeratosis along with proliferation of fibrous connective tissue in dermis. The fibrous connective tissue proliferation was present in multiple direction interspersed with blood vessels and aggregates of lymphocytes and plasma cells. These findings of prevalence and pathology of cutaneous warts are more or less in accordance with earlier report from K. Pangty *et. al.*2009 .

Fig.1 Blast analysis of nucleotide sequence of BPV-1 field sample



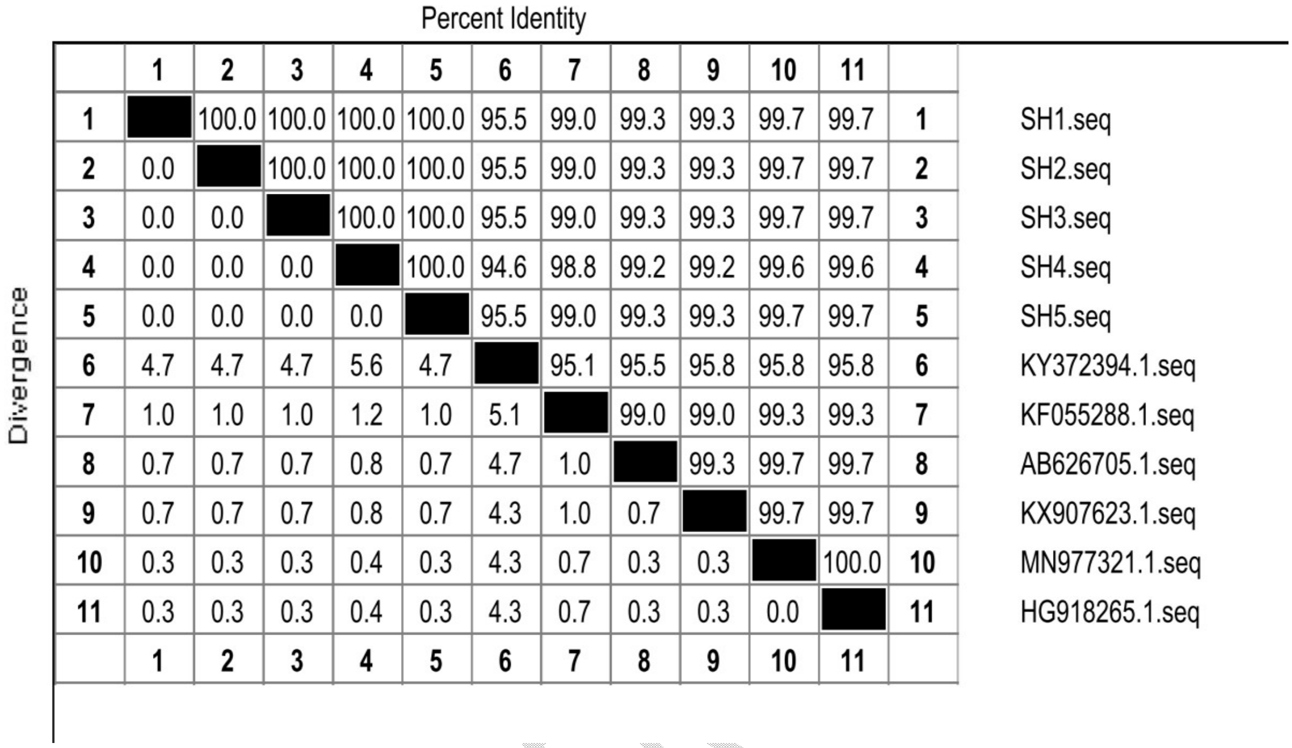
Query Length 297

Other reports Distance tree of results MSA viewer

100 sequences selected

Taxonomy	Number of hits	Number of Organisms	Description
⊟root	162	4	
. ⊟Papillomaviridae	161	3	
. . ⊟Deltapapillomavirus	147	2	
. . . Deltapapillomavirus sp.	5	1	Deltapapillomavirus sp. hits
. . . Deltapapillomavirus 4	142	1	Deltapapillomavirus 4 hits
. . Bovine papillomavirus	14	1	Bovine papillomavirus hits
. unidentified cloning vector	1	1	unidentified cloning vector hits

Fig. 2 Sequence identity and divergence of BPV-1 nucleotide sequenc



Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11		
1	■	100.0	100.0	100.0	100.0	95.5	99.0	99.3	99.3	99.7	99.7	**1**	SH1.seq
2	0.0	■	100.0	100.0	100.0	95.5	99.0	99.3	99.3	99.7	99.7	**2**	SH2.seq
3	0.0	0.0	■	100.0	100.0	95.5	99.0	99.3	99.3	99.7	99.7	**3**	SH3.seq
4	0.0	0.0	0.0	■	100.0	94.6	98.8	99.2	99.2	99.6	99.6	**4**	SH4.seq
5	0.0	0.0	0.0	0.0	■	95.5	99.0	99.3	99.3	99.7	99.7	**5**	SH5.seq
6	4.7	4.7	4.7	5.6	4.7	■	95.1	95.5	95.8	95.8	95.8	**6**	KY372394.1.seq
7	1.0	1.0	1.0	1.2	1.0	5.1	■	99.0	99.0	99.3	99.3	**7**	KF055288.1.seq
8	0.7	0.7	0.7	0.8	0.7	4.7	1.0	■	99.3	99.7	99.7	**8**	AB626705.1.seq
9	0.7	0.7	0.7	0.8	0.7	4.3	1.0	0.7	■	99.7	99.7	**9**	KX907623.1.seq
10	0.3	0.3	0.3	0.4	0.3	4.3	0.7	0.3	0.3	■	100.0	**10**	MN977321.1.seq
11	0.3	0.3	0.3	0.4	0.3	4.3	0.7	0.3	0.3	0.0	■	**11**	HG918265.1.seq
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**		

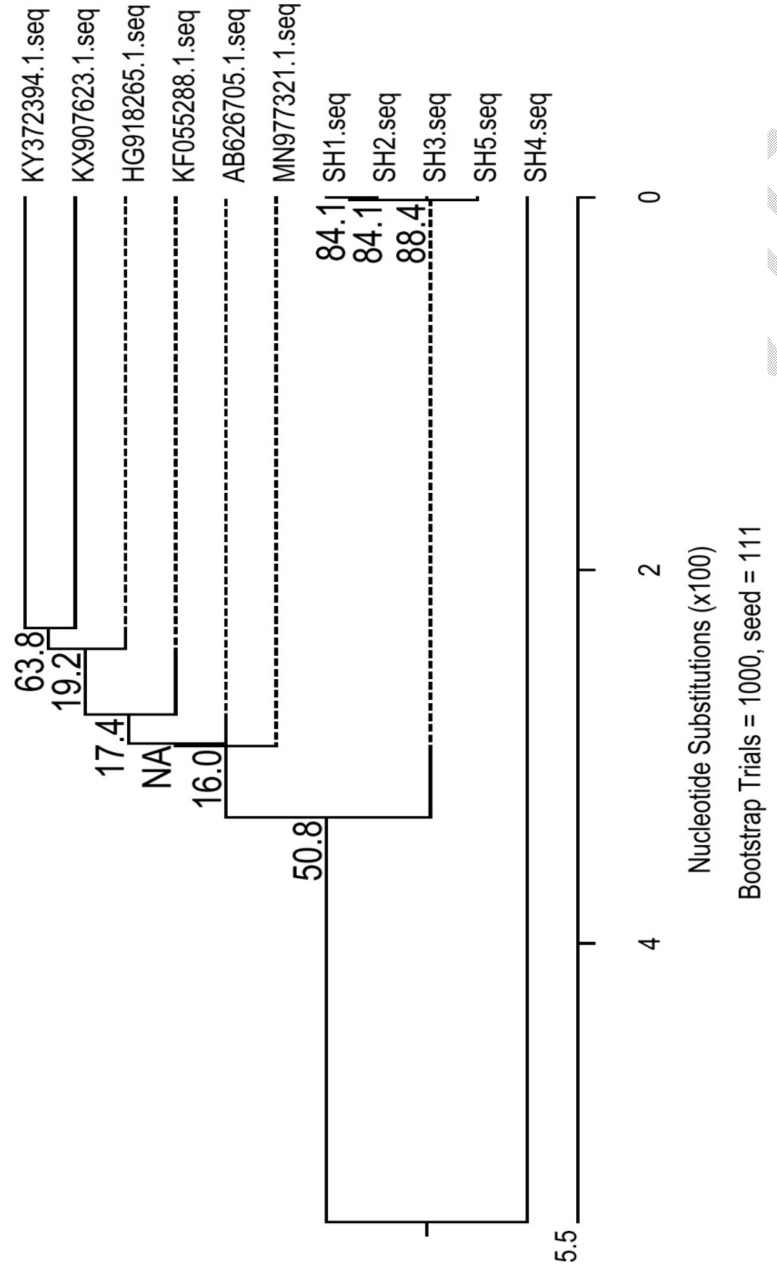


Fig. 3 Phylogenetic tree of BPV-1 nucleotide sequences

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