Applied Biological Research 27(3): 439-446; (2025)
DOI: 10.48165/abr.2025.27.01.43

MOLECULAR TYPING AND CHARACTERIZATION OF BOVINE PAPILLOMAVIRUS FROM CUTANEOUS WARTS OF CATTLE AND BUFFALO IN TAMIL NADU (INDIA)

A. Hariharan[1], P. Ponnusamy[2*], K. Sukumar[2], P. Nithya[3], M. Sasikala[3] and M. Selvaraju[4]

[1]Department of Biotechnology, Selvam College of Technology, [2]Department of Veterinary Microbiology,[3]Department of Veterinary Pathology,[4]Dean, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal - 637 002, Tamil Nadu (India)
*****e-mail: *ponsvhl@gmail.com*

(Received 28 February, 2025; accepted 8 September, 2025)

ABSTRACT

Bovine papillomavirus (BPV) is a diverse group of oncogenic viruses and causative agent of bovine papillomatosis which affects the cattle industry by contributing significant economic losses. The present study was aimed to detect and characterize BPV by molecular methods *viz*., polymerase chain reaction and DNA sequencing. The wart samples (n = 10) were aseptically collected from clinically affected cattle and buffalo cases. DNA extraction was carried out directly from the wart samples and then PCR was performed to detect BPV by using BVP specific L1 gene. All wart samples were positive for BPV by PCR targeting the L1 gene with an amplicon size of 480 bp. The histopathological examination of wart samples revealed hyperkeratosis, acanthosis, fusion of rete pegs and characteristic rete pegs with koilocytes. The DNA sequencing of highly conserved L1 gene was carried out for molecular typing of BPV prevailing in Namakkal district of Tamil Nadu (India). The sequences showed 95.9 to 100% identity with BPV type 1 of genus *Deltapapillomavirus*. Phylogenetic analysis of L1 region indicated that all sequences were clustered with BPV 1. It may be concluded that nucleotide sequencing of L1 fragment of BPV showed efficiency in identification and typing of BPV cases. In this study BPV 1 strains were found to be the cause for cutaneous warts in cattle and buffalo.

Keywords: Bovine papillomavirus, molecular typing, nucleotide sequencing, phylogenetic analysis

INTRODUCTION

Papillomaviruses are small tumour causing viruses having circular double-stranded DNA (Zur Hausen, 2009). The virus affects the skin and mucosal epithelia in humans and various animals and triggers benign tumours that usually recede without any serious medical problems in host but occasionally persist (Campo, 2003; de Villiers *et al*., 2004). Bovine papillomavirus (BPV) is a cosmopolitan virus causing endemic infection in dairy and beef cattle. The virus is reported to be highly infectious, highly transmissible, and a causative agent for bovine papillomatosis (Claus *et al*., 2009; Araldi, 2015). BPV is 50-55 nm in diameter with icosahedral symmetrical capsid, non-enveloped virus, and genome size about 8000 bp that belongs to the family *Papillomaviridae* (Munday, 2014). The genome is composed of 5 or 6 open reading frames (ORFs), which are first expressed during the course of infection, and 2 ORFs are manifested in late infection (Howley and Lowy, 2007; Silva *et al*., 2010). Currently, 44 types of bovine papillomaviruses are specified which are

categorized into five genera, namely *Xipapillomavirus*, *Deltapapillomavirus*, *Epsilonpapillomavirus*, *Dyoxipapillomavirus*, and *Dyokappapapillomavirus* (Gilio Gasparotto *et al*., 2024**)**.

The genus *Deltapapillomavirus* primarily induces fibropapillomas whereas the genus *Epsilonpapillomavirus* induces epithelial papillomas as well as fibropapillomas and the genus *Xipapillomavirus* is strictly epitheliotropic and causing squamous papillomas (Jarrett *et al*., 1984; Tomita *et al*., 2007). The disease is characterized by the existence of multiple warts, or papillomas, which relapse spontaneously or develop into malignant tumours. The bovine papillomavirus mainly affects young cattle, but all age groups are susceptible to this disease. The disease causes considerable economic impact due to decreased growth rate, weight loss, and reduced skin value (Munday, 2014; Araldi *et al*., 2017), and its predisposity to secondary bacterial infections leads to mastitis and its associated complications (Borzacchiello *et al*., 2003).

The papillomaviruses are classified phylogenetically based on L1 gene ORF sequence, which is reported to be highly conserved among the various papillomavirus types. The novel virus type and subtype in papillomavirus were determined by differences >10% and between 2 and 10% in open reading frame sequence homology of L1 gene, respectively (Bernard *et al*, 2010; Burk *et al*., 2013).

Different diagnostic tests such as southern blot, immunohistochemistry, electron microscopy, and PCR have been used to detect papillomaviruses (Betiol *et al*., 2012). For papillomavirus detection, PCR is considered a key technique due to its high sensitivity and rapidity (Leto *et al*., 2011). However, RFLP analysis of PCR amplicons is useful to categorize the types of papillomavirus but correlates 95% with DNA sequencing. Though real-time PCR can determine the number of viral copies in a sample, the technique has lower reproducibility. For identification and typing of bovine papillomaviruses, PCR followed by DNA sequencing is most commonly used (Crespo *et al*., 2019). In Indian studies, BPV 1 and BVP 10 has been reported from cutaneous warts on the head and neck region and also rice grain warts as well as sessile elevated growths in teats by PCR (Kumar *et al*., 2013; Tumlam *et al*., 2023). There were scanty reports on the molecular typing and characterization of bovine papillomavirus types in India. Some of the BPV types have considerable economic consequences which exhibit secondary as well as tertiary spread from the primary tumours. Further, histopathological examinations of lesions have also lead to the identification of different papillomas including squamous papillomas or fibropapillomas which may be related with the oncogenic potential of the virus. It is crucial for understanding the prevalence of different BPV types in India and identifying the potential risk factors. Understanding the different BPV types and its genetic diversity can aid in developing effective control strategies. Therefore, the present study was aimed to detect and characterize the BPV types associated with cutaneous warts of cattle and buffalo circulating in Namakkal district of Tamil Nadu (India).

MATERIALS AND METHODS

Collection of samples

Ten wart samples (n = 10) were collected from Namakkal region of Tamil Nadu (India) with a history of bovine papilloma cases in the year 2022 during routine clinical diagnosis work and treatment. The consent for sample collection was obtained from the animal owners. The samples were collected from the age group of 11 months to 4 years old cattle belonging to the breeds 'Jersey cross, 'Holstein Friesian cross', 'Kangeyam', and 'Native cattle'. The warts samples were collected in sterile containers with sterile PBS containing antibiotic stock solution of 100 µg streptomycin, 0.25 µg amphotericin B and 100 units of penicillin G (all HiMedia-India) and stored at -80°C till use.

DNA extraction and PCR

The viral DNA was extracted directly from wart samples using QIAamp DNA mini kit (Catalogue No. 51304, Qiagen, Germany). The final eluted DNA was quantified and stored at -20°C for further operations. PCR was performed under thermal conditions of initial denaturation at 94°C for 5 min,

afterwards 45 cycles consisting of denaturation at 94°C for 90 sec, annealing at 62°C for 90 sec, extension at 72°C for 90 sec, and final extension at 72°C for 10 min using the sense and anti-sense primers of 5'-TAACWGTIGGICAYCCWTAT-3' and 5'-CCWATATCWVHCATITCICCATC-3', respectively (Forslund *et al*., 1999). The amplified PCR products were subjected to purification by using the above kit as per the manufacturer's guidelines. The purified PCR products were quantified and captured on ethidium bromide-stained 1.5% agarose gel by Gel Doc TM system (Bio-Rad, USA).

Sequencing and phylogenetic analysis

The purified L1 gene fragment of three BPV field isolates in present study were sequenced by Sanger dideoxy sequencing method in an automated sequencer (Eurofins Analytical Services India Pvt. Ltd., Bangalore). Bioedit software (*http://www.mbio.ncsu.edu/BioEdit/bioedit.html*) was used for sequence assembly and editing. The sequences were deposited at GenBank under Accession No. ON630413, ON630414, and ON30415. Homology was searched using NCBI BLAST with reference sequences available in this database (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*). The multiple sequence alignment (Clustal W) was done by Mega 11 software to generate sequence analysis data (*www.megasoftware.net*). The phylogenetic tree for BPV was constructed by Neighbour Joining (NJ) approach using bootstrap values (1000 replicates) and distance in Mega 11 software in order to assess the relationship between the sequences.

Histopathological examinations

Tissue bits of wart samples were fixed in 10% buffered neutral formalin for regular manual processing. The formalin-fixed tissues were processed by paraffin wax embedding method for tissue sectioning. Tissues of 3-5 µm thickness were cut by an automatic section cutting machine (Rotary Microtome RM 2125, Leica, China), followed by staining with hematoxylin and eosin to identify the histopathological differences in tissues (Bancroft and Stevens, 1996). The H&E stained sections were read under a light microscope (Nikon E100) with the magnification range of 100x to 400x and histopathological changes were recorded using the photomicrography attached with the microscope.

RESULTS AND DISCUSSION

Incidence of bovine papillomavirus

Bovine papillomavirus (BPV) causes endemic infection in cattle associated with benign and malignant tumour-like cutaneous papillomas, fibropapillomas, and cancers in the urinary bladder and esophagus (Lurthu Reetha *et al*., 2020). In present study, BPV infection in cattle and buffalo from Namakkal district (Tamil Nadu, India) was studied from April to May 2022. The warts were found distributed all over the body, face, ear and neck (Fig. 1-2). Ten samples were analyzed for the history

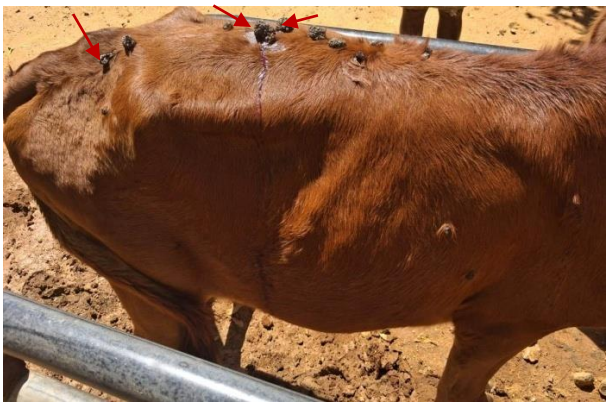

Fig. 1: The cow showing cutaneous warts all over the body

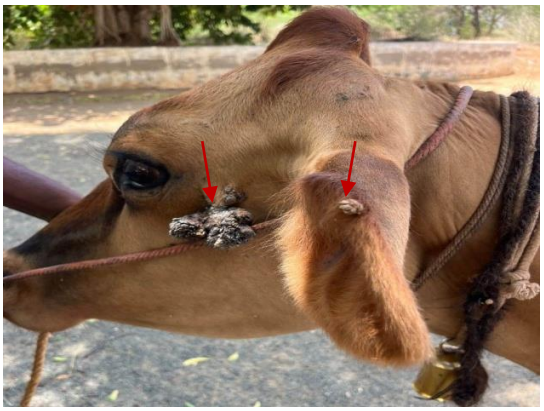

Fig 2: Extensive irregular cauliflower-like growth on the face and ear

of cutaneous warts. The infection was observed in age group ranging from 11 months to 4 years. All age groups were susceptible, but calves and yearlings were commonly affected. These observations are consistent with earlier reports of BPV infection in cattle (Börkü *et al*., 2007). It causes considerable economic impact on the livestock farming community due to decreased growth rate, loss of weight, reduced leather cost, and secondary bacterial complications (Börkü *et al*., 2007). In present study, BPV infection was observed in different cattle breeds, *viz*., 'Jersey cross', 'HF cross', 'Kangeyam', and native breeds. These observations are in agreement with the findings of Lurthu Reetha *et al*. (2020).

Detection of BPV by PCR

After being quantified, the samples with acceptable purity were employed in PCR. The PCR targeting the L1 gene with an amplicon size of 480 bp was used to screen the wart samples for detection of BPV, and a negative template control was also used for comparison. All wart samples collected were found positive for BPV by PCR targeting L1 gene (Fig. 3). PCR is a useful technique for detection of BPV nucleic acid in wart samples by amplifying L1 gene of BPV which is considered to be a major capsid protein. It is considered to be a quick, accurate and more sensitive diagnostic procedure than other BPV detection techniques. In present study, BPV was detected in wart samples by amplifying a portion of L1 gene using gene-specific primers. The L1 gene played a major role in typing and in the identification of various papillomavirus types and was also reported to be highly conserved. Hence, L1 gene was selected for PCR amplification, detection, and typing of BPV (Forslund *et al*., 1999; Bernard *et al*., 2010).

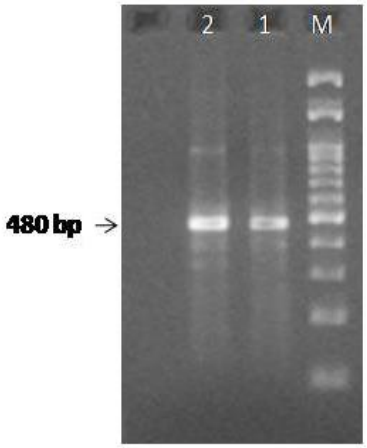

Fig. 3: Agarose gel electrophoresis showing 480 bp amplified PCR product of L1 gene of field BPV; Lane M: 100 bp DNA ladder (100-3000 bp); Lane 1-2: Representative wart samples

In this study, ten wart samples from papilloma cases of cross-bred cattle as well as native breeds were screened by amplifying L1 gene of BPV using gene-specific primers. This is in agreement with Lurthu and Reetha *et al***.** (2020), who studied BPV infection in wart samples collected from papilloma cases in cattle. Similarly, Araldi *et al*. (2014) amplified a 478 bp fragment by using the same set of primers and reported BPV from the wart samples of Angus red cattle in Brazil.

Histopathology

Macroscopically, the cutaneous warts appeared dry, rough and exophytic growths with cauliflower-like appearance on various anatomical sites of skin including head, neck and thoracic regions. Microscopically. cases acanthosis, fusion of rete pegs and characteristic rete pegs with koilocytes (Fig. 4-6). The histopathological examination of BPV

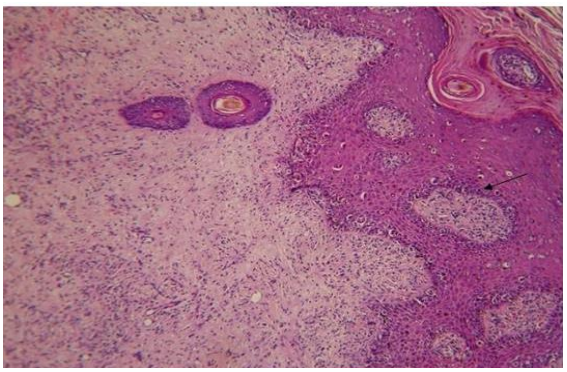

Fig. 4: Papilloma: Skin showing hyper-keratosis, acanthosis and fusion of rete pegs (arrow) (H&E, x100)

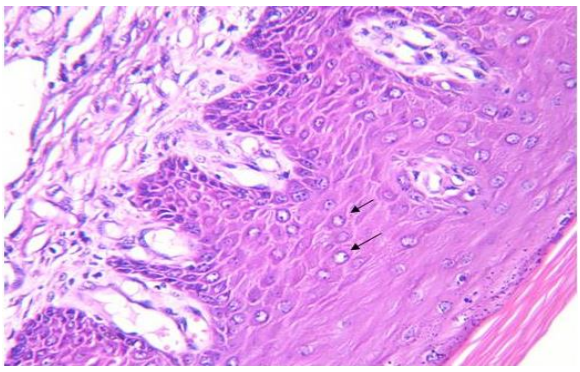

Fig. 5: Papilloma: Skin showing acanthosis and characteristic rete pegs with koilocytes (arrow) (H&E, x400)

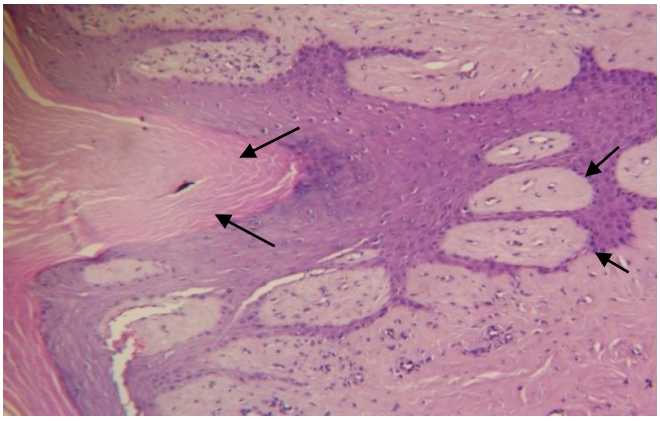

Fig. 6: Papilloma: Skin showing hyperkeratosis and fusion of rete pegs (H&E x100)

cases are in line with Peng *et al*. (2019) who reported the presence of hyperplasia of epidermis and dermis with excessive keratinization of epidermal cells and some formation of granulosa cells showing vacuolation. Further, the cells had a spindle or star-shaped and early nuclear division was visible. Jones *et al*. (1997) and Hargis *et al*. (1995) histopathologically examined the naturally occurring skin papillomas and found hyperkeratosis of epidermis with irregular papillary projections within the skin and viral inclusion bodies which was rarely reported.

DNA sequencing of L1 gene of BPV

Three field BPV positive amplicons were sequenced for L1 gene of BPV using forward and reverse primers. The sequences were edited using BioEdit software, and homology searches were conducted using NCBI BLAST program. BPV reference sequences were obtained from the GenBank database. All sequenced field BPV samples showed more than 95% identity with the reference BPV sequences.

Homology analysis of the L1 gene nucleotide sequences

Homology analysis of L1 gene nucleotide sequences generated in this study was carried out with other reference sequences of BPV. Two of the three BPV Namakkal strains (1 and 2) showed 100% nucleotide sequence homology with each other while strains 1 and 2 had 98.3% homology with strain 3. All the three sequences shared high homology (95.9 to 100%) with BPV type 1 reference sequences. The Namakkal strains showed lower range of nucleotide homology (73.2 to 85.8%) with other types like BPV types 2 and 13 which also belongs to the *Deltapapillomavirus* genus (Fig. 7).

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
1		52.6	56.2	48.9	98.1	50.9	53.6	58.8	50.9	51.7	51.3	50.9	57.3	51.1	51.3	53.9	55.4	54.3	54.3	57.1	59.0	51.3	51.5	52.8	50.9	50.9	50.9	59.7	58.2	52.1	58.2	1
2	70.4		60.1	65.7	51.9	68.0	76.6	56.4	68.0	77.9	66.1	68.0	57.5	55.4	65.0	55.4	52.8	65.7	65.7	58.4	56.7	65.0	65.9	77.5	68.0	68.0	67.8	56.7	59.4	76.4	59.4	2
3	62.9	52.6		53.6	55.8	57.1	57.3	76.6	57.1	59.4	57.3	57.1	76.6	53.6	57.3	56.4	58.4	58.4	58.4	72.5	70.2	57.3	57.5	56.9	57.1	57.1	56.7	72.1	74.7	56.7	74.7	3
4	78.3	42.7	65.3		50.4	96.4	63.5	50.9	96.4	60.5	82.8	96.4	55.6	51.3	83.0	52.6	50.4	71.5	71.5	51.7	54.7	83.0	83.0	61.8	96.4	96.4	95.9	54.3	52.8	62.7	52.8	4
5	0.5	70.0	61.7	78.3		50.2	53.2	58.4	50.2	51.5	50.6	50.2	56.7	50.6	51.1	53.2	55.2	53.6	53.6	56.7	58.4	51.1	50.9	52.6	50.2	50.2	51.1	58.8	57.7	51.7	57.7	5
6	77.7	42.6	61.5	0.2	77.4		66.1	54.1	100.0	64.2	85.6	100.0	58.6	53.4	85.8	54.7	53.9	74.2	74.2	54.7	57.7	85.8	85.8	64.8	100.0	100.0	98.3	57.7	55.8	65.2	55.8	6
7	67.5	30.3	59.1	47.2	66.3	46.5		56.0	66.1	76.4	64.6	66.1	57.1	54.5	65.5	54.3	55.6	65.5	65.5	56.2	54.7	65.5	64.8	75.8	66.1	66.1	66.1	55.4	55.4	98.5	55.4	7
8	56.9	61.0	30.2	73.0	55.7	69.1	62.0		54.1	58.2	54.9	54.1	71.9	56.2	55.6	56.7	59.2	55.4	55.4	73.6	70.0	55.6	54.9	56.7	54.1	54.1	54.1	71.7	71.9	55.2	71.9	8
9	77.7	42.6	61.5	0.2	77.4	0.0	46.5	69.1		64.2	85.6	100.0	58.6	53.4	85.8	54.7	53.9	74.2	74.2	54.7	57.7	85.8	85.8	64.8	100.0	100.0	98.3	57.7	55.8	65.2	55.8	9
10	73.3	28.3	53.9	53.7	71.5	50.4	30.6	56.9	50.4		64.4	64.2	58.8	55.6	64.8	54.9	56.4	67.4	67.4	57.1	55.2	64.8	64.2	81.3	64.2	64.2	63.5	56.0	59.0	75.8	59.0	10
11	76.5	46.3	60.8	16.6	76.1	16.8	49.3	67.2	16.8	49.8		85.6	58.2	54.1	89.7	54.3	54.9	74.5	74.5	55.8	57.9	89.7	99.8	65.0	85.6	85.6	84.5	59.4	56.7	64.2	56.7	11
12	77.7	42.6	61.5	0.2	77.4	0.0	46.5	69.1	0.0	50.4	16.8		58.6	53.4	85.8	54.7	53.9	74.2	74.2	54.7	57.7	85.8	85.8	64.8	100.0	100.0	98.3	57.7	55.8	65.2	55.8	12
13	58.9	58.5	28.3	60.1	58.3	57.8	59.7	36.4	57.8	55.4	58.9	57.8		53.9	57.7	55.6	57.3	58.6	58.6	72.3	70.6	57.7	58.4	56.4	58.6	58.6	58.6	78.3	72.3	56.2	72.3	13
14	73.4	62.8	67.1	70.9	72.3	70.2	65.9	60.5	70.2	62.6	68.7	70.2	66.5		54.3	54.5	63.5	54.7	54.7	55.6	53.4	54.3	53.9	53.4	53.4	53.4	52.8	52.1	57.5	53.6	57.5	14
15	76.4	48.3	60.9	16.3	74.7	16.5	47.7	65.5	16.5	49.0	11.6	16.5	60.1	68.2		53.4	53.6	73.8	73.8	56.4	57.5	100.0	89.9	63.9	85.8	85.8	84.5	59.0	58.8	64.8	58.8	15
16	70.1	67.0	61.6	71.6	69.7	70.9	70.3	61.7	70.9	67.9	72.4	70.9	64.1	67.8	74.8		53.0	51.7	51.7	53.4	54.3	53.4	54.1	54.7	54.7	54.7	54.5	55.6	53.4	53.6	53.4	16
17	62.8	73.0	58.8	76.9	61.1	72.1	65.3	56.9	72.1	63.4	69.8	72.1	61.2	49.5	73.4	73.2		52.8	52.8	60.1	58.6	53.6	55.2	53.4	53.9	53.9	53.2	58.6	57.9	54.5	57.9	17
18	68.6	47.1	58.3	33.8	68.2	33.4	47.7	65.6	33.4	44.1	33.1	33.4	57.8	66.8	34.2	80.4	76.2		100.0	56.9	57.9	73.8	74.5	64.4	74.2	74.2	73.2	55.4	58.6	64.2	58.6	18
19	68.6	47.1	58.3	33.8	68.2	33.4	47.7	65.6	33.4	44.1	33.1	33.4	57.8	66.8	34.2	80.4	76.2	0.0		56.9	57.9	73.8	74.5	64.4	74.2	74.2	73.2	55.4	58.6	64.2	58.6	19
20	59.7	56.6	35.7	70.7	58.5	67.6	61.7	33.9	67.6	59.6	65.0	67.6	35.7	62.0	63.1	69.8	54.9	62.0	62.0		68.2	56.4	55.8	54.9	54.7	54.7	54.5	71.0	74.9	55.4	74.9	20
21	55.2	62.1	40.3	63.8	54.6	61.2	67.3	40.5	61.2	65.6	60.5	61.2	37.6	68.8	61.8	68.4	59.2	60.8	60.8	43.0		57.5	58.2	56.2	57.7	57.7	56.9	69.3	70.2	53.4	70.2	21
22	76.4	48.3	60.9	16.3	74.7	16.5	47.7	65.5	16.5	49.0	11.6	16.5	60.1	68.2	0.0	74.8	73.4	34.2	34.2	63.1	61.8		89.9	63.9	85.8	85.8	84.5	59.0	58.8	64.8	58.8	22
23	75.9	46.7	60.3	16.3	75.5	16.5	48.8	67.1	16.5	50.3	0.2	16.5	58.4	69.3	11.3	72.9	69.3	33.1	33.1	65.0	60.0	11.3		65.2	85.8	85.8	84.8	59.4	56.9	64.2	56.9	23
24	70.0	28.8	60.2	50.6	68.3	48.9	31.5	60.5	48.9	23.1	48.3	48.9	61.4	68.5	50.6	68.3	71.2	49.8	49.8	65.4	62.7	50.6	47.9		64.8	64.8	64.6	57.3	56.2	74.7	56.2	24
25	77.7	42.6	61.5	0.2	77.4	0.0	46.5	69.1	0.0	50.4	16.8	0.0	57.8	70.2	16.5	70.9	72.1	33.4	33.4	67.6	61.2	16.5	16.5	48.9		100.0	98.3	57.7	55.8	65.2	55.8	25
26	77.7	42.6	61.5	0.2	77.4	0.0	46.5	69.1	0.0	50.4	16.8	0.0	57.8	70.2	16.5	70.9	72.1	33.4	33.4	67.6	61.2	16.5	16.5	48.9	0.0		98.3	57.7	55.8	65.2	55.8	26
27	76.7	42.2	61.6	1.4	76.8	1.1	45.6	68.1	1.1	50.9	17.5	1.1	56.9	71.1	17.5	70.4	73.0	34.4	34.4	67.2	62.5	17.5	17.2	48.5	1.1	1.1		57.3	55.4	65.2	55.4	27
28	53.5	60.6	35.8	63.4	53.3	59.8	64.1	36.6	59.8	62.2	55.9	59.8	25.9	70.9	56.9	63.8	58.2	65.7	65.7	38.0	39.8	56.9	55.9	59.2	59.8	59.8	59.9		72.3	54.9	72.3	28
29	57.5	54.8	32.2	69.1	56.3	66.2	65.0	36.8	66.2	56.0	63.9	66.2	36.4	58.3	58.5	71.0	61.5	58.9	58.9	32.1	39.2	58.5	63.4	62.9	66.2	66.2	66.4	36.7		54.7	100.0	29
30	71.5	30.7	60.6	48.9	70.4	48.2	1.6	64.2	48.2	31.6	50.2	48.2	61.8	68.1	49.0	72.2	68.0	50.3	50.3	63.8	71.0	49.0	50.1	33.2	48.2	48.2	47.3	65.3	66.7		54.7	30
31	57.5	54.8	32.2	69.1	56.3	66.2	65.0	36.8	66.2	56.0	63.9	66.2	36.4	58.3	58.5	71.0	61.5	58.9	58.9	32.1	39.2	58.5	63.4	62.9	66.2	66.2	66.4	36.7	0.0	66.7		31
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	

NC
MW543422
MW436424
MT974519
MT974515
MK347523
MH220402
MG602223
MG263871
MG252779
MF741676
MF435917
LC514113
KY705374
KX113620
KU519393
KU519391
KR868228
KP276343
KM983393
KF017607
KC256805
JQ798171
DQ098913
BPVNKL2
BPVNKL1
BPVNKL3
AJ620208
AJ620207
AJ620206
AF486184

Fig. 7: Nucleotide sequence identity of different BPV sequences based on L1 gene (Determined by Clustal W method in the MegAlign program of Lasergene (DNASTAR), Upper right triangle is the nucleotide sequence identities, and the lower left triangle is the divergence identities.

Multiple sequence alignment analysis of L1 gene of BPV field isolates

A multiple sequence alignment was performed using three BPV strains generated in this study and 28 published bovine papillomavirus sequences from NCBI using the Cluster W program of Mega11. The results showed a clear demarcation between various BPV types based on amino acid sequences and nucleotide variations. All three BPV L1 sequences generated in this study were identical to BPV 1 isolates or strains based on analysis of sequences at nucleotide and amino acid levels.

In present study, partial L1 gene of BPV sequences was used for molecular genotyping of bovine papillomavirus and also identifying the prevalent types of BPV present in the region. The nucleotide and amino acid sequences of partial L1 gene of BPV were compared with 28 reference strains from various genera including *Deltapapillomavirus, Epsilonpapillomavirus, Dyokappapapillomavirus*, *Dyoxipapillomavirus*, and *Xipapillomavirus*. There was no gene sequence variation found between BPV type 1 reference sequences and the field sequences generated in this study. There were nucleotide and amino acid variations observed between other BPV types with field sequences in the study (Timurkan and Alcigir, 2017). The three field BPV sequences were deposited in GenBank, and the accession numbers obtained were ON630413, ON630414 and ON630415.

Phylogenetic analysis of L1 gene of BPV

Phylogenetic analysis of L1 gene of three field BPV sequences was done with 28 reference sequences retrieved from GenBank NCBI database (*https://www.ncbi.nlm.nih.gov*). The phylogenetic tree was constructed using Neighbour-joining algorithm with bootstrap values along with distance. All the three field BPV sequences were clustered into one major group of *Deltapappillomavirus* genus, specifically with BPV-1 strains from other countries. The finding suggests that field BPV outbreaks were due to type I BPV. Further, grouping is distinct, with groups comprising strains according to the different types belonging to different genera of BPV. This indicates that variation in L1 gene has relevance to the typing of BPV (Fig. 8). Our findings are in line with Timurkan and Alcigir (2017) who reported the molecular characterization of bovine papillomavirus type 1 (BPV-1) isolated from papilloma cases in the north western region of Turkey. Similarly, Silva *et al.* (2010) amplified L1 gene sequences using FAP primers, which are frequently employed for BPV phylogeny and molecular identification. The present study concluded that

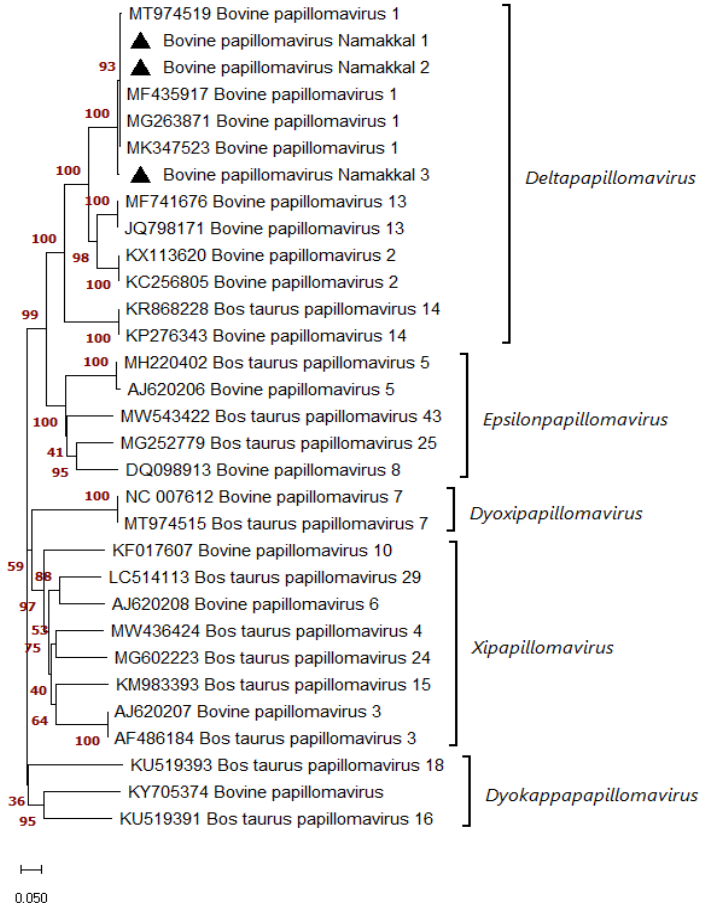

Fig. 8: Phylogenetic analysis based on nucleotide sequence of partial L1 gene of BPV. The tree was drawn by Neighbour-joining algorithm using MEGA 11 software with distance and bootstrap value of 1000 replicates.

molecular characterization of L1 gene fragment of BPV showed efficiency in genotyping of BPV cases. BPV 1 strain, belonging to *Deltapappillomavirus* genus, was found responsible for cutaneous warts in cattle and buffalo in this region. Further, more samples need to be analyzed for identifying other BPV genotypes prevailing in this area.

Conflict of interest: The authors declare no conflict of interest in the present research work.

Ethical statement: The wart samples were collected during routine clinical diagnosis work and treatment purposes and the consent for sample collection was obtained from the animal owners.

REFERENCES

Alcigir, M.E., Atalay Vural, S. and Timurkan, M.O. 2016. Investigation of E2, E5 and E6 gene expression and DNA *in situ* fragmentation findings associated with progressive and regressive changes in benign neoplastic cutaneous lesions arising naturally from bovine papillomavirus-1 infection. *Medycyna Weterynaryjna*, **72**: 549-557.

Araldi, R. 2015. *Bovine papillomavirus: What We Know and What We Should Know.* Lambert Academic Publishing, Saarbrücken, Germany.

Araldi, R., Carvalho, R., Melo, T., Diniz, N., Sant'ana, T., Mazzuchelli-De-Souza, J., *et al.*, 2014. Bovine papillomavirus in beef cattle: First description of BPV-12 and putative type BAPV8 in Brazil. *Genetics and Molecular Research*, **13**: 5644-5653.

Araldi, R.P., Assaf, S.M.R., Carvalho, R.F., Carvalho, M.A.C.R., Souza, J.M., Magnelli, R.F., *et al.*, 2017. Papillomaviruses: A systematic review. *Genetics and Molecular Biology*, **40**: 1-21.

Bancroft, J.D. and Stevens, A. 1996. *Theory and Practice of Histological Techniques* (4th edn.). Churchill Livingstone, London, UK.

Bernard, H.U., Burk, R.D., Chen, Z., van Doorslaer, K., Hausen, H. and de Villiers, E.M. 2010. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology*, **401**: 70-79.

Betiol, J.C., Kignel, S., Tristão, W., Arruda, A.C., Santos, S.K.S., Barbieri, R. *et al.*, 2012. HPV 18 prevalence in oral mucosa diagnosed with verrucous leukoplakia: Cytological and molecular analysis. *Journal of Clinical Pathology*, **65**: 769-770.

Börkü, M., Atalay, O., Kibar, M., Cam, Y. and Atasever, A. 2007. Ivermectin is an effective treatment for bovine cutaneous papillomatosis. *Research in Veterinary Science*, **83**: 360-363.

Borzacchiello, G., Ambrosio, V., Roperto, S., Poggiali, F., Tsirimonakis, E., Venuti, A., *et al.*, 2003. Bovine papillomavirus type 4 in oesophageal papillomas of cattle from the South of Italy. *Journal of Comparative Pathology*, **128**: 203-206.

Burk, R.D., Harari, A. and Chen, Z. 2013. Human papillomavirus genome variants. *Virology*, **445**: 232-243.

Campo, M. 2003. Papillomavirus and disease in humans and animals. *Veterinary and Comparative Oncology*, **1**: 3-14.

Claus, M., Lunardi, M., Alfieri, A., Sartori, D., Fungaro, H. and Alfieri, A. 2009. Identification of the recently described new type of bovine papillomavirus (BPV-8) in a Brazilian beef cattle herd. *Pesquisa Veterinária Brasileira*, **29**: 25-28.

Crespo, S.E.I., Lunardi, M., Otonel, R.A.A., Headley, S.A., Alfieri, A.F. and Alfieri, A.A. 2019. Genetic characterization of a putative new type of bovine papillomavirus in the Xipapillomavirus 1 species in a Brazilian dairy herd. *Virus Genes*, **55**: 682–687.

De Villiers, E.M., Fauquet, C., Broker, T.R., Bernard, H.U. and ZurHausen, H. 2004. Classification of papillomaviruses. *Virology*, **324**: 17-27.

Forslund, O., Antonsson, A., Nordin, P. and Hansson, B.G. 1999. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. *Journal of General Virology*, **80**: 2437-2443.

Gilio Gasparotto, P.H., Ribeiro dos Santos, I., Viera Dantas Filho, J., Soares da Silva, M., Dos Anjos Souza, F., De Macedo Sousa, J.C., *et al.*, 2024. Characterization of bovine papillomavirus types detected in cattle rumen tissues from Amazon Region, Brazil. *Animals,* **14**: 2262.

Hargis, A.M. and Ginn, P.E. 2012. The integument. pp. 1009-1146. **In**: *Pathologic basis of Veterinary Disease* (eds. J.F. Zachary, ad M.D. McGavin). Elsevier/Mosby, St. Louis, United States.

Howley, P.M. and Lowy, D.R. 2007. Papillomaviruses. pp. 2299-2354. **In**: *Field's Virology* (eds. D.M. Knipe and P.M. Howley). Lippincott Williams and Wilkins, Philadelphia, Pennsylvania, USA.

Jarrett, W.F., Campo, M.S., O'Neil, B.W., Laird, H.M. and Coggins, L.W. 1984. A novel bovine papillomavirus (BPV6) causing true epithelial papillomas of the mammary gland skin: A member of a proposed new BPV subgroup. *Virology*, **136**: 255-264.

Jones, D.L., Thompson, D.A. and Münger, K. 1997. Destabilization of the RB tumour suppressor protein and stabilization of p53 contribute to HPV type 16 E7-induced apoptosis. *Virology*, **239**: 97-107.

Kumar, P., Jangir, B.L., Saikumar, G. and Somvanshi, R. 2013. Co-infection of bovine papillomavirus type-1 and -10 in teat warts of dairy cattle. *Veterinarni Medicina*, **58** (12): 605-608.

Leto, M., Santos Júnior, G.F., Porro, A.M. and Tomi-mori, J. 2011. Human papillomavirus infection: Etiopathogenesis, molecular biology and clinical manifestations. *Anais Brasileiros de Dermatologia,* **86**: 306-317.

Lurthu Reetha, T., Manickam, R. and Puvarajan, B. 2020. Detection of bovine papillomavirus in cutaneous lesions by polymerase chain reaction (PCR) in cattle. *International Journal of Current Microbiology and Applied Sciences*, **9**: 1611-1616.

Munday, J.S. 2014. Bovine and human papillomaviruses. A comparative review. *Veterinary Pathology*, **51**: 1063-1075.

Peng, H., Wu, C., Li, J., Li, C., Chen, Z., Pei, Z., *et al.*, 2019. Detection and genomic characterization of bovine papillomavirus isolated from Chinese native cattle. *Transboundary Emerging Diseases*, **66**: 2197-2203.

Silva, M.S., Weiss, M., Brum, M.C., Dos Anjos, B.L., Torres, F.D., Weiblen, R. *et al.*, 2010. Molecular identification of bovine papillomaviruses associated with cutaneous warts in southern Brazil. *Journal of Veterinary Diagnostic Investigation*, **4**: 603-606.

Tomita, Y., Literák, I., Ogawa, T., Jin, Z. and Shirasawa, H. 2007. Complete genomes and phylogenetic positions of bovine papillomavirus type 8 and a variant type from a European bison. *Virus Genes*, **35**: 243-249.

Tumlam, U.M., Desai, D.N., Muglikar, D.M., Pawade, M.M. and Mhase, P.P. 2023. L1 gene based molecular characterization of bovine papillomavirus type 1 (BPV1) isolated from cutaneous warts of cattle, Maharashtra. *Agricultural Science Digest*, [*DOI: 10.18805/ag.D-5754*].

Zur Hausen, H. 2009. The search for infectious causes of human cancers: Where and why. *Virology*, **392**: 1-10.