MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF *BOVINE PAPILLOMAVIRUS* IN CATTLE FROM MAHARASHTRA, INDIA

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ABSTRACT

Papillomaviruses (PVs) are epitheliotropic, double-stranded DNA viruses that induce benign and occasionally malignant proliferative lesions in cattle. The present study was undertaken to investigate the occurrence and molecular characteristics of bovine papillomavirus (BPV) in cattle from Maharashtra, India. A total of 14 wart-like cutaneous growth samples were collected from clinically affected cattle. Molecular detection was performed using polymerase chain reaction (PCR) with consensus primers (MY09/11) and bovine papillomavirus-specific primers targeting the L1 gene region. Papillomaviral DNA was detected in 5 (35.71%) samples. Amplification with MY09/11 primers yielded approximately 450 bp products, while BPV L1-specific primers generated amplicons of approximately 850 bp. Sequencing and BLAST analysis of the amplified products revealed 99.19–100% nucleotide identity with previously reported BPV isolates from different parts of the world. Phylogenetic analysis grouped all study isolates within the genus Deltapapillomavirus, indicating a close evolutionary relationship with global BPV strains. Histopathological examination of positive samples demonstrated characteristic papillomatous changes, including epithelial hyperplasia, acanthosis, hyperkeratosis, and koilocytosis. The findings confirm the circulation of bovine papillomavirus in cattle of Maharashtra and underscore the utility of molecular diagnostics and sequence-based characterization for epidemiological surveillance and genetic analysis of BPV infections.

Keywords: Bovine papillomavirus, PCR, L1 gene, phylogenetics, Maharashtra

Introduction

Bovine papillomavirus (BPV) is a member of the family *Papillomaviridae* and comprises a group of small, non-enveloped viruses possessing a circular double-stranded DNA genome of approximately 8 kb (McBride, 2017). The virus exhibits a marked tropism for the basal cells of stratified squamous epithelium and induces benign proliferative lesions of the skin and mucous membranes, commonly known as papillomas or warts. Although these lesions are generally self-limiting, persistent infection with certain BPV types may lead to fibropapillomas and, under specific host and environmental conditions, malignant transformation (Munday *et al*., 2021).

Bovine papillomavirus infection is distributed worldwide and is recognized as one of the most important viral causes of cutaneous tumors in cattle. The disease is more commonly observed in young animals and immunocompromised cattle, and transmission occurs through direct contact with infected animals or indirectly via contaminated fomites and management practices that cause minor abrasions in the skin. BPV infection can lead to significant economic losses due to reduced hide quality, interference with milking, secondary bacterial infections, impaired growth, and decreased market value of affected animals.

To date, multiple BPV genotypes have been identified and classified into several genera, including *Deltapapillomavirus*, *Xipapillomavirus*, and *Epsilonpapillomavirus*. Among these, BPV-1 and BPV-2 are the most extensively studied and are associated with cutaneous fibropapillomas and, in combination with carcinogenic compounds present in bracken fern, urinary bladder tumors and enzootic hematuria (Medeiros-Fonseca *et al*., 2021).

The L1 gene, which encodes the major capsid protein, is the most conserved region of the BPV genome and serves as the standard molecular target for detection, genotyping, and phylogenetic characterization. Molecular techniques such as polymerase chain reaction (PCR), nucleotide sequencing, and phylogenetic analysis have significantly improved the sensitivity and specificity of BPV diagnosis and have facilitated detailed investigations of viral diversity and evolution.

Despite the clinical and economic significance of bovine papillomavirus, information on the molecular epidemiology and genetic diversity of BPV in Indian cattle remains limited. In particular, there is a paucity of sequence-based data from Maharashtra, an important livestock-producing state of India. Comprehensive molecular characterization of circulating BPV strains is essential for understanding viral epidemiology, tracking genetic variation, and designing effective control strategies.

Therefore, the present study was undertaken to detect and molecularly characterize bovine papillomavirus from cutaneous wart lesions of cattle in Maharashtra, India, and to assess the genetic relationship of the detected isolates with previously reported global strains through sequencing and phylogenetic analysis of the L1 gene. Histopathological examination was also performed to correlate molecular findings with the characteristic microscopic lesions associated with bovine papillomatosis.

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Materials and Methods

Ethical approval

All samples were collected as per standard sample collection procedure. The technical approval was taken from institutional ethical committee for conduct of Veterinary Clinical Research of College of Veterinary and Animal Sciences, Parbhani as the samples were collected from clinical cases.

Study location

The study was conducted at the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, MAFSU, Parbhani, with protocol standardization at ACTREC, Tata Memorial Centre, Navi Mumbai.

Sample Collection

A total of 14 wart-like tissue samples were collected from cattle across different districts of Maharashtra. Samples were obtained aseptically, transported under cold chain conditions and stored at -20°C until further processing (Fig. 1 and 2).

Fig. 1: Exophytic papillomas observed on the left hind limb of the animal.

Fig. 2: Rice-grain like papillomas observed near the base of the tail.

DNA extraction

Approximately 1 g of excised tissue was transferred into sterile nuclease-free micro-centrifuge tubes containing tissue digestion buffer and Proteinase K, followed by overnight incubation at 55 °C for complete tissue digestion. Viral DNA was subsequently extracted using the phenol–chloroform–isoamyl alcohol method. Briefly, the digested homogenate was sequentially treated with Tris-saturated phenol, phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform, with centrifugation after each step to obtain a clear aqueous phase. DNA was precipitated using sodium acetate and ice-cold isopropanol, washed with 70% ethanol, air-dried and re-suspended in TE buffer or Milli-Q water. The extracted DNA samples were finally stored at -20 °C until further use for PCR analysis.

PCR amplification

PCR assays were performed using consensus primers (MY09/11) and species-specific primers targeting BPV L1 gene (Table 1). Reactions were carried out in a 25 µl volume using a thermal cycler under standardized cycling conditions. Amplified products were resolved using 1.5% agarose gel electrophoresis and visualized under UV illumination.

Table 1: Sequence and amplicon size of the oligonucleotides (Primers)

Primer	Sequence (5'-3')	Amplicon Size	Reference
MY09	GCMCAGGGWCATAAYAATGG	450 bp	Silva *et al.* (2010)
MY 11	CGTCCMARRGGAWACTGATC		
BPV L1 F	AGGAGGGTCATGCTTTGCTC	847 bp	Hamad *et al.* (2016)
BPV L1 R	GCTGTTCGGAGTGGTGTGTA		

Sequencing and analysis

PCR-positive samples were sequenced, and nucleotide sequences were analyzed using BLAST (NCBI). Multiple sequence alignment and phylogenetic analysis were performed using MEGA 11 software.

Histopathology

Tissue samples were fixed in 10% neutral buffered formalin, processed routinely, sectioned, and stained using hematoxylin and eosin for microscopic examination.

Results and Discussion

Clinical observations

Lesions varied from exophytic papillomas to rice-grain-like growths, commonly observed on the neck and dewlap regions.

Molecular detection

Papillomavirus DNA was detected in 5 (35.71%) samples. MY09/11 primers amplified a 450 bp fragment, while BPV L1 primers produced ~850 bp amplicons (Fig. 3 and 4).

Distribution analysis

All positive cases were observed in Holstein Friesian crossbred cattle. Age-wise distribution revealed that all infected animals were within the 1–3-year age group. A slightly higher prevalence was observed in females compared to males. Geographically, positive cases were restricted to Satara, Solapur and Parbhani districts.

Sequence analysis

Multiple sequence alignment of the obtained sequences demonstrated that all five sequences showed clear homology with the L1 gene region of Bovine Papillomaviruses, confirming

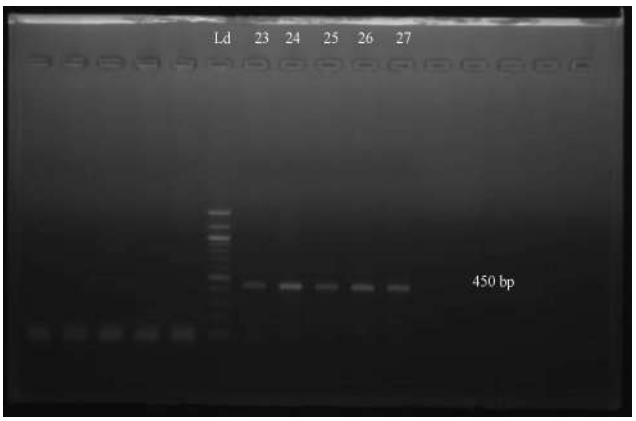

Fig. 3: Polymerase chain reaction (PCR) used for determination of Bovine Papillomavirus (BPV) with amplicon size 450 bp of L1 gene using MY09/11 Primers

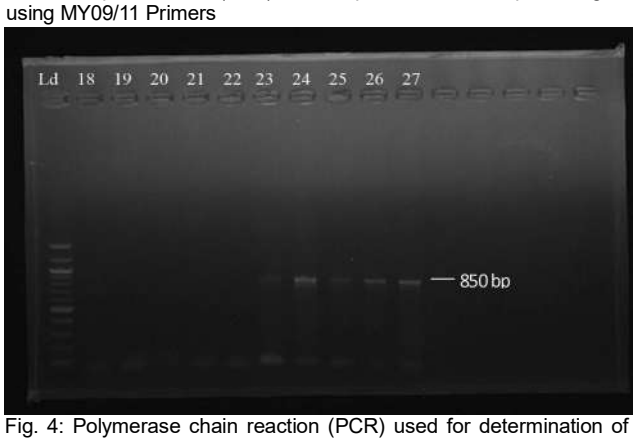

Fig. 4: Polymerase chain reaction (PCR) used for determination of Bovine Papillomavirus (BPV) with amplicon size 850 bp of L1 gene using BPV L1 Primers

the specificity of the amplified products. The L1 gene is known to be highly conserved among papillomaviruses and is therefore widely accepted as a reliable molecular marker for viral identification and classification (Bernard *et al*., 2010; de Villiers *et al*., 2004).

Comparative nucleotide sequence analysis revealed that the BPV sequences generated in the present study shared a high percentage of nucleotide identity with BPV L1 gene sequences deposited in the NCBI GenBank database from different geographical regions worldwide. The observed nucleotide similarity ranged within the limits commonly reported for BPV strains belonging to the same viral type, supporting the accurate molecular confirmation of BPV infection in the studied samples. Similar levels of global sequence conservation in BPV L1 gene sequences have been documented by earlier researchers (Delius *et al*., 1994; Ogawa *et al*., 2004).

Phylogenetic analysis

Bootstrap consensus analysis revealed one strongly supported clade (87%) and one moderately supported clade (51%), while remaining internal nodes showed low support due to limited nucleotide divergence among isolates. It also shows an out-group (Fig. 5).

The Maximum likelihood phylogeny revealed host-specific clustering of papillomaviruses, a hallmark of papillomavirus evolution. All study isolates grouped within the Deltapapillomavirus clade, supporting their bovine origin and consistent host restriction. Host-associated phylogenetic segregation based on L1 gene sequences has been well documented in recent papillomavirus evolutionary analyses (Willemsen and Bravo *et al*., 2019 and Van Doorslaer, 2022).

Histopathology

The histo-architectural examination of suspected papillomatous tissues were performed which revealed moderate to severe, dense finger like projections of keratin resting on stratum corneum. Extending from the hyperplastic epidermis is an unencapsulated, exophytic neoplasm composed of squamous epithelial cells that progress from a hyperplastic stratum basale to a thickened stratum spinosum (acanthosis) and stratum granulosum, forming papillary projections supported by fine fibrovascular cores and overlain by prominent orthokeratotic and parakeratotic hyperkeratosis. Within the stratum basale, neoplastic cells have variably distinct cell borders, a moderate amount of eosinophilic cytoplasm and round to elongate nuclei with finely stippled chromatin

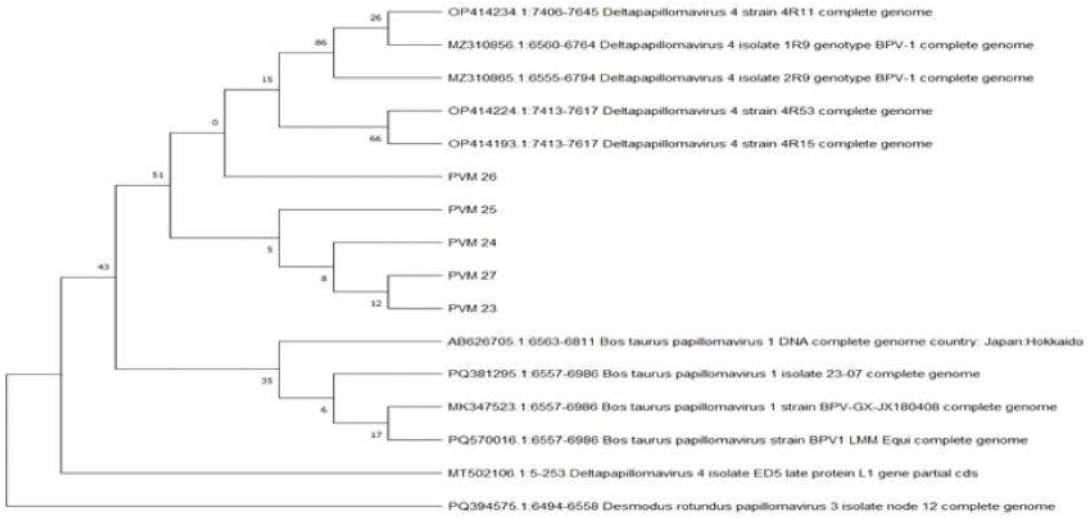

Fig. 5: Maximum likelihood phylogenetic tree of L1 gene

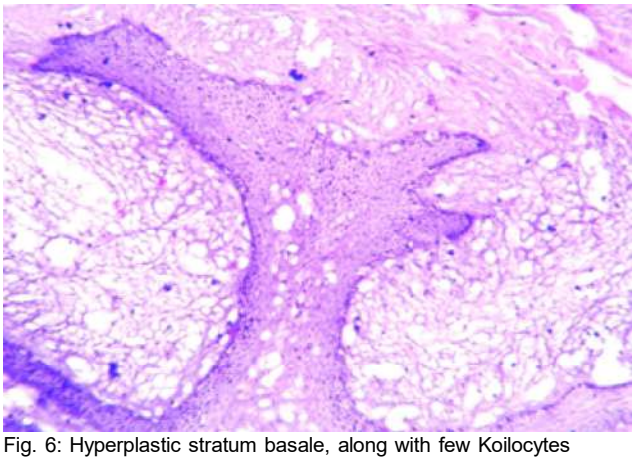

Fig. 6: Hyperplastic stratum basale, along with few Koilocytes

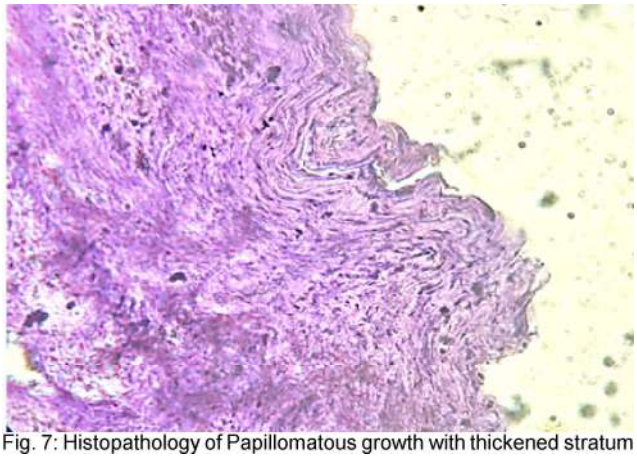

Fig. 7: Histopathology of Papillomatous growth with thickened stratum corneum characterized by thick Keratin deposits

and 1-4 distinct nucleoli. Within the stratum spinosum and granulosum, neoplastic cells are often enlarged with abundant finely granular, amphophilic cytoplasm and clear cytoplasmic vacuoles and have eccentric vesiculate or occasionally pyknotic nuclei surrounded by a clear halo and 1-3 prominent magenta nucleoli (koilocyte) (Fig. 6 and 7).

The detection rate of 15.62% observed in the present study is comparable to previous reports, suggesting that not all clinically suspected lesions harbor detectable viral DNA. The restriction of positive cases to bovine samples highlights the higher prevalence of BPV in cattle compared to other species. These observations are consistent with earlier descriptions of bovine cutaneous papillomatosis (Al-Salihi *et al*., 2020).

The higher occurrence of infection in Holstein Friesian crossbred cattle may be linked to multiple factors, including genetic predisposition, environmental stress, and intensive management conditions. These animals often exhibit reduced adaptability to local climatic environments and experience greater metabolic stress due to high production demands, which may compromise their resistance to infectious agents. In contrast, indigenous *Bos indicus* cattle are generally more resilient, demonstrating greater tolerance to ectoparasites, environmental challenges, and certain infections. This inherent robustness may indirectly limit viral transmission by reducing skin injuries and exposure to vector-borne factors (Frisch, 1998).

Age-wise distribution indicates increased susceptibility in younger animals, likely due to immature immune responses and increased exposure to mechanical trauma. These findings are in agreement with previous studies (Araldi *et al*., 2017) indicating a higher incidence of papillomatosis in young cattle.

The high nucleotide similarity with global isolates confirms the conserved nature of the BPV genome and supports previous findings regarding the low evolutionary rate of papillomaviruses. Similar levels of global sequence conservation in BPV L1 gene sequences have been documented by earlier researchers (Delius *et al*., 1994; Ogawa *et al*., 2004).

The lack of similarity with Indian isolates suggests possible regional variation or limited sequence availability. Phylogenetic clustering within Deltapapillomavirus further confirms host specificity and evolutionary stability. The findings reinforce the utility of L1 gene–based phylogenetic analysis as a robust approach for elucidating evolutionary relationships, tracking viral circulation, and understanding the molecular epidemiology of bovine papillomaviruses (Timurkan and Alcigir, 2017).

Histopathological findings revealed moderate to severe, dense finger like projections of keratin resting on Stratum corneum. Extending from the hyperplastic epidermis is an unencapsulated, exophytic neoplasm composed of squamous epithelial cells that progress from a hyperplastic stratum basale to a thickened stratum spinosum (acanthosis) and stratum granulosum, forming papillary projections supported by fine fibrovascular cores and overlain by prominent orthokeratotic and parakeratotic hyperkeratosis. These findings are in accordance with previous studies (Méndez Rodríguez *et al.,* 2021), where, they also found similar histopathological findings. These findings corroborated molecular results, reinforcing the diagnosis of papillomavirus infection.

Conclusion

The present study confirms the occurrence of bovine papillomavirus belonging to delta papillomavirus in Hosein Fresian cattle in Maharashtra. Molecular detection using PCR, followed by sequencing and phylogenetic analysis, proved to be an effective approach for virus identification and characterization. The findings highlight the need for continued molecular surveillance to understand the epidemiology and control of papillomavirus infections.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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