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L1 Gene based Molecular Characterization of Bovine Papillomavirus Type 1 (BPV1) Isolated from Cutaneous Warts of Cattle, Maharashtra

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ABSTRACT

Background: Bovine papillomatosis (BP) is distributed worldwide among cattle but is relatively less common in buffaloes. Prevalence of this disease is established in buffaloes from India and Italy caused by bovine papilloma virus (BPV). The current study aimed to study the detection of bovine papilloma virus from cutaneous warts by polymerase chain reaction and confirmed by nucleotide sequencing and phylogenetic analysis.

Methods: In this study a total of 10 wart samples from affected cattle were aseptically collected. Grossly, most of cutaneous warts were of variable sizes, rough or coarse in texture, grayish/blackish/flesh colored, irregular in shape (dome or button) or resembling cauliflower-like masses and elevated from skin surface by broad base. Molecular characterization of viral DNA from wart samples and partial gene sequencing, its analysis were carried out by using bioinformatics tools to establish the phylogenetic relationship.

Result: In our investigation it revealed that out of total 10 cutaneous wart tissue samples, only six cattle were positive for BPV-1 while all the samples were found negative for BPV-2. The phylogenetic analysis of BPV-1gene of papilloma virus of cattle showed 100% homology with already reported sequence from china. The BPV-1 partial sequences generated in present study revealed close homology as well as with the earlier published BPV-1 sequences on NCBI.

Key words: Bovine Papillomatosis, BPV1, BPV-2, Cutaneous Warts, PCR, Phylogenetic analysis.

INTRODUCTION

Bovine Papillomatosis (BP) is contagious disease in the animals in which they naturally occur. It is caused by the bovine papilloma virus (BPV) and is characterized by warts that occur in cutaneous and mucosal form. Cutaneous papillomatosis (warts) in bovine is a contagious hyperplasia or benign neoplasm caused by BPV. BPV have specific tropism for squamous epithelial cells and full viral replication, including synthesis of DNA, capsid proteins and assembly of virions, occur only in the more terminally differentiated squamous epithelial cells.

Among various bovine diseases, cutaneous papillomatosis or warts are regarded as either hyperplasia or infectious neoplastic disease caused by bovine papilloma virus. Papilloma viruses is DNA virus are strictly species specific with least serological cross reactivity among capsid protein in papilloma virus of different species. The only known case of cross species infection is with BPV-1 and BPV-2, which infect animals such as cattle, horse and donkey (Singh *et al*., 2009). There are ten different types of bovine papilloma virus BPV-1 to BPV-10 producing papilloma and fibropapilloma (Ogawa *et al.,* 2004).

Only two bovine papilloma viruses are type 1 and type 2 are reported from India (Singh *et al.,* 2009). Cattle types show some site predilection or site specificity producing distinct type of papillomas grossly as well as microscopically. The virus may produce papilloma of udder/teat (BPV-1, 5 and 6), papillomas on cutaneous body parts *viz*. head, neck, shoulder and ventral abdomen (BPV-1, 2 and 3) and papilloma of alimentary tract (BPV-4). The aim of the present study was to detect BPV types prevalent in the Indian cattle in and around Shirwal, Satara region of Western Maharashtra.

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MATERIALS AND METHODS

Samples collection

A total of 10 wart samples from affected cattle were aseptically collected shown in Table 1. Grossly, most of

Table 1: Details of samples collected for the detection of BPV1.

Breed	Location	History	Tissue samples
HF cross	Jawali, Dist. Satara	Few warts were seen on head, neck, face and muzzle areas.	Wart sample
Cross bred	Tal. Bhor Dist. Satara	Warts on neck	Wart sample
HF cross	Neera, Dist. Satara	Warts on neck	Wart sample
HF cross	Jawali, Dist. Satara	Warts on udder and teat	Wart sample
HF cross	Khed Shivapur Dist. Satara	Warts on skin	Wart sample
HF cross	Aadarki Dist. Satara	Warts on udder and teat	Wart sample
HF cross	Satara	Warts on face	Wart sample
HF cross	Satara	Warts on face	Wart sample
HF cross	Tal. Bhor Dist. Satara	Warts on udder, teat and skin	Wart sample
HF cross	Satara	Warts on udder and teat	Wart samples

cutaneous warts were of variable sizes, rough or coarse in texture, grayish/blackish/flesh colored, irregular in shape (dome or button) or resembling cauliflower-like masses and elevated from skin surface by broad base. The wart sam-ples were stored at -20°C until used in further studies.

Extraction of viral DNA and detection by polymerase chain reaction and sequencing

DNA was extracted from wart tissue samples using the Genomic DNA Mini Kit (Qiagen) according to the manufacturer's protocol. PCR was performed to detect the presence of BPV serotype 1 and 2. Primers were used for BPV-1(Forward: 5′-gga gcg cct gct aac tat agg a-3′; Reverse: 5′-atc tgt tgt ttg ggt ggt gac-3′) of 301 bp and BPV-2 (Forward: 5′-gttata cca ccc aaa gaa gac cct-3′; Reverse; 5′-ctg gtt gcaaca gct ctc ttt ctc-3′) of 165 bp as described earlier (Leishangthem 2008). The reaction was set up as follows: 2 µl Template DNA, 10 µl 5× PCR buffer, 3 µl $MgCl_2$, 1 µl dNTPs, 1 µl Taq polymerase, 2 µl Forward Primer, 2 µl Reverse Primer, 29 µl Nuclease free water to make volume of 50 µl. All these ingredients were mix properly by vortexing. The PCR reaction was done as per following: Initial Denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, Annealing at 50°C for 2 min, extension at 72°C for 2 min and final extension at 72°C for 10 min. 5 µl of PCR product was then mixed with 3 µl of bromophenol blue (6×) and PCR product was run via gel electrophoresis using 1.5% agarose gel and visualized by using UV trans illuminator (Syngene G box, UK). The obtained PCR product was purified and sequenced for the confirmation of PCR product.

Gene sequencing and phylogenetic analysis

The PCR products were directly sequenced from both the end by Cellbiosis Pvt. Ltd for the confirmation of the BPV-1 and phylogenetic analysis. The obtained sequences from the field sample were subjected to manually analyses and BLAST analysis to find out the sequence similarity. The obtain sequence further aligned with reference sequences by CLUSTAL W pairwise and multiple alignment method. The nucleotide sequences of BPV-1 gene fragment of

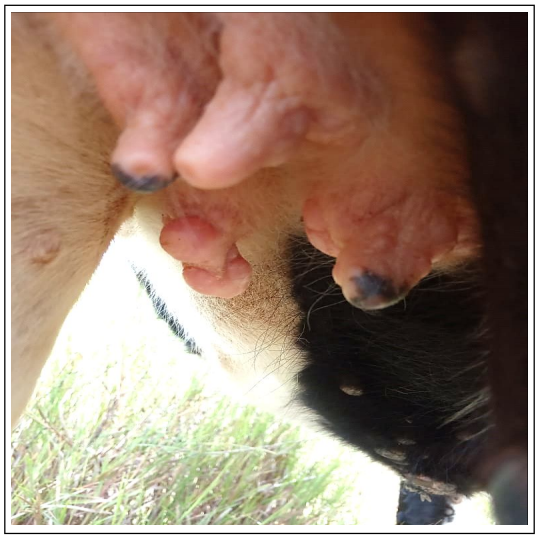
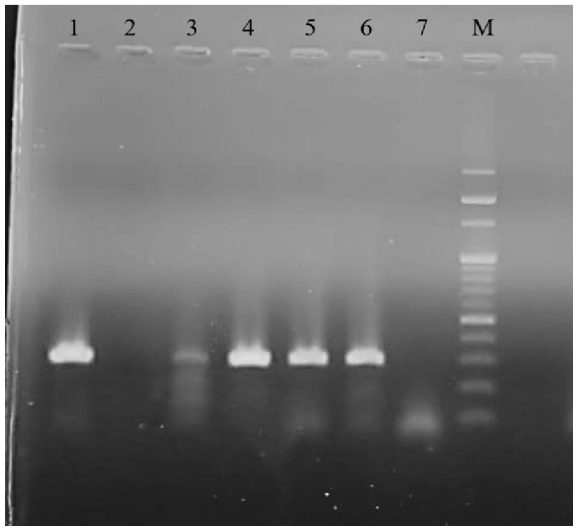


Fig 1: Cauliflower like growth on udder and teat.



Lane M-Molecular weight marker (100 bp ladder); Lane: 1-Positive control; Lane 3,4,5 and 6=Bovine positive wart samples; Lane: 2, 7=Negative.

Fig 2: Amplification of BPV1 (301 bp) of wart samples.

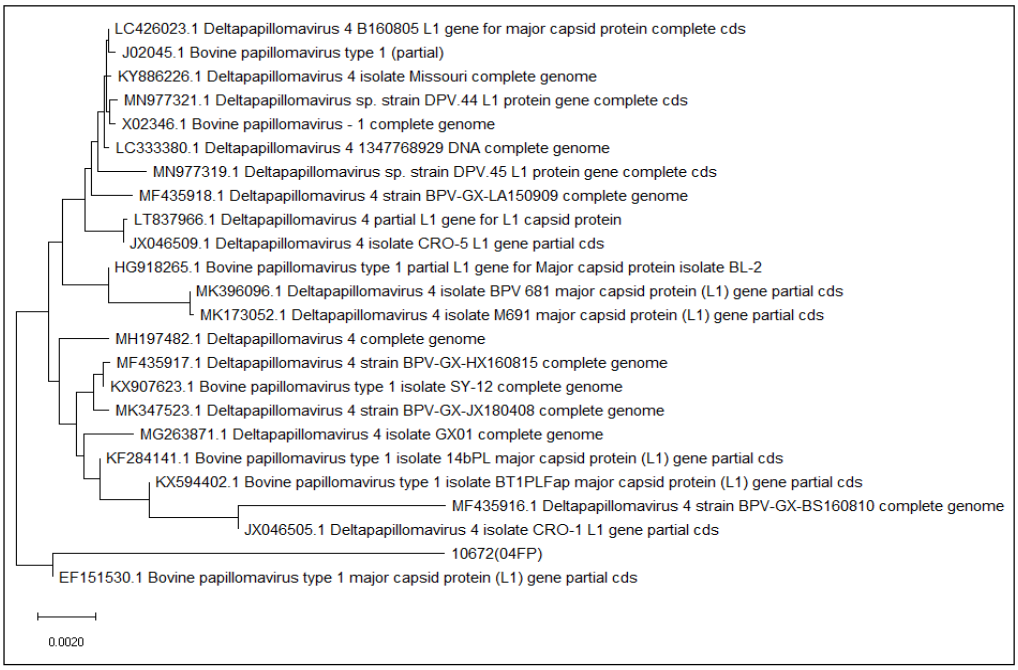


Fig 3: Phylogenetic analysis of the sequence of positive PCR products of wart samples (10672(04FP)).

papillomavirus were aligned using in MEGA 6.0 software and phylogeny was constructed.

RESULTS AND DISCUSSION

A total of 10 wart samples from cattle were collected. Grossly, most of the cutaneous warts were of variable sizes, rough or coarse in texture, grayish/blackish/flesh colored, irregular in shape, cauliflower-like masses and elevated from skin surface. PCR was performed to detect the presence of BPV-1 and BPV-2 serotype of bovine papilloma virus. It revealed that out of total 10 cutaneous wart tissue samples, only six cattle were positive for BPV-1 while all the samples were found negative for BPV-2 (Fig 1). Similar finding was observed by Jangir *et al*. (2013), Kumar *et al*. (2013) where they reported overall, 68.75% (11/16) and 56.25% (9/16) positivity for BPV-1 and BPV-2, respectively. BPV-1 and BPV-2 in the cutane-ous warts of cattle (Pangty *et al*. 2010; Kumar *et al.,* 2013a and Kumar *et al.,* 2013b), buffalo (Singh and Somvanshi 2010; Kumar *et al.,* 2013a) and yak (Bam *et al*., 2012). BPV-1 was detected in the three cases of teat warts with rice grain-like morphological appearance. Earlier, BPV was detected from cutaneous wart, blood and various tissue fluids of bovine papilloma affected animals (Freitas *et al*., 2003); (Leishangthem, 2008)

BPV-1 was detected from cutaneous warts by PCR and was confirmed by nucleotide sequencing and phylogenetic analysis as shown in (Fig 2). On phylogenetic analysis of the sequence of PCR products (Acession Nos: 10672(04FP), having closed 98% and 100% homology was seen with the genome sequences (Ac. No:JX046505) and (Ac. No:KX594402) of L1 major gene protein of Deltapapilloma virus BPV-1 from china (Fig 3) In the present study, the phylogenetic analysis of BPV-1gene of papilloma virus of cattle showed 100% homology with already reported sequence from china. The BPV-1 partial sequences generated in present study revealed close homology as well as with the earlier published BPV-1 sequences on NCBI. Our study are in agreement of Alcigir *et al.* (2016) who found that L1 genotype has been found in association with the development of cutaneous papillomatosis as papilloma or fibropapilloma. This genotype (BPV-1) has been found in association with the development of cutaneous papillomatosis manifesting as papilloma or fibropapilloma. Khalefa *et al.* (2016) and Hamed *et al.* (2017) compared the alignment of different sequences and alignment comparison showed high identity 100% among the Iraqi isolates Accesion No.KY662042-1, KY662043-1 and KY662040-1 with BPV-1 L1 partial cds where as small variety with isolates KY662041-1 99%.

CONCLUSION

Papillomatosis in cattle and buffaloes is a little known disease, but it is a separate infectious ailment and deserves more attention. In further future study for better understand *in vivo* carcinogenesis of papillomavirus. Moreover, the correlation between farm animal papillomavirus and small animals should be determined. Specific treatment regime should be planned as the numbers of papillomatosis in the different animal species**.** In addition, the present study showed that skin BPV-1 infection was present in around Satara district of Maharashtra and there is need of conducting more molecular studies will be required to diagnose if there are other types of the disease in the same area of study and other geographical areas of Maharashtra state.

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Conflict of interest

There is no conflict of interests.

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