L1 gene based Molecular Characterization of Bovine papillomavirus type 1 (BPV1) isolated from cutaneous warts of cattle, Maharashtra (India)

U. M. Tumlam (uma_tumlam@yahoo.com)
Krantisinh Nana Patil College of Veterinary Science
D. N. Desai
Navsari Agricultural University
M. M. Pawade
Krantisinh Nana Patil College of Veterinary Science
P. P. Mhase
Krantisinh Nana Patil College of Veterinary Science
D. M. Muglikar
Krantisinh Nana Patil College of Veterinary Science

Research Article

Keywords: Bovine Papillomatosis, Cutaneous Warts, BPV1, BPV-2, PCR, Phylogenetic analysis

Posted Date: June 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1641649/v1

Abstract

Bovine Papillomatosis (BP) is a contagious disease of the animals in which it naturally occurs. It is caused by the bovine papillomavirus (BPV) and is characterized by warts that occur in cutaneous and mucosal forms. Cattle are affected by six different types of papillomaviruses. BPV-1 and – 2 infections can cause warts on the skin, teats, udders, penis, and tumors in the urinary bladder. In the present study, total of 10 wart samples from cattle were studied. PCR was performed to detect the presence of BPV and its type. Further, the PCR amplified product was sent for the sequencing for the confirmation and phylogeny analysis. The result revealed that the cutaneous warts of six cattle were positive for BPV-1 while all the samples were found negative for BPV-2. BPV-1 was detected in cutaneous warts by a PCR and was confirmed by nucleotide sequencing and phylogenetic analysis. In conclusion, the BPV type prevalent in the cutaneous warts of Indian cattle is the BPV-1 type.

Introduction

Bovine Papillomatosis (BP) is a contagious disease in the animals in which it naturally occurs. It is caused by the bovine papillomavirus (BPV) and is characterized by warts that occur in cutaneous and mucosal form. Cutaneous papillomatosis (warts) in cattle is a contagious hyperplasia or benign neoplasm caused by BPV. BPV only infects squamous epithelial cells, and full viral replication, including the production of DNA, capsid proteins, and the assembly of virions, can only happen in squamous epithelial cells that have reached their final stage of differentiation (Campo, 2006).

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

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

Material And Methods

Samples collection

A total of 10 wart samples from affected cattle were aseptically collected. The Majority of cutaneous warts were of variable sizes, rough or coarse in texture, grayish or flesh-colored, irregular in shape (dome or button), or resembling cauliflower-like masses, grossly and elevated from the skin surface by a broad base. The wart samples were stored at –20 °C until used in further research. The details of the samples are presented in Table 1.

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 et al., 2008). The reaction was set up as follows: 2 µl Template DNA, 10 µl 5X 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 a volume of 50 µl. All these ingredients were mix properly by vortexing. The PCR reaction was done as per the following: Initial denaturation at 94 °C for 5 minutes, followed by 30 cycles of 1 minute at 94 °C, 2 minutes annealing at 50 °C, 2 minutes extension at 72 °C, and 10 minutes final extension at 72 °C.5 µl of PCR product was then mixed with 3 µl of bromophenol blue (6X) and the PCR product was run via gel electrophoresis using 1.5% agarose gel and visualized by using a UV trans illuminator (Syngene G box, UK). The obtained PCR product was purified and sequenced for the confirmation of the PCR product.

Gene Sequencing and Phylogenetic Analysis

The PCR products were directly sequenced from both ends by Cellbiosis Pvt. Ltd. for the confirmation of the BPV-1 and phylogenetic analysis. The obtained sequences from the field sample were subjected to manual analysis and BLAST analysis to find out the sequence similarity. The obtained sequence is further aligned with reference sequences by the CLUSTAL W pairwise and multiple alignment method. The nucleotide sequences of the BPV-1 gene fragment of papillomavirus were aligned using MEGA 6.0 software and a phylogeny was constructed.

Results And Discussion

A total of 10 wart samples from cattle were collected. The Majority of cutaneous warts were of variable sizes, rough or coarse in texture, grayish or flesh-colored, irregular in shape (dome or button), or resembling cauliflower-like masses, grossly and elevated from the skin surface by a broad base (Fig. 1). PCR was performed to detect the presence of BPV-1 and BPV-2 serotypes of bovine papillomavirus. It revealed that out of a total of 10 cutaneous wart tissue samples, only six cattle were positive for BPV-1 while all the samples were found negative for BPV-2 (Fig. 2). A similar finding was observed by Jangir 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 cutaneous warts of cattle (Pangty et al. 2010; Kumar et al.

2013a), buffalo (Singh and Somvanshi 2010; Kumar et al. 2013b; Kumar et al. 2013c) and yak (Bam et al. 2012). BPV-1 was detected in three cases of teat warts with a rice grain-like morphological appearance (Kumar 2013b). Earlier, BPV was detected in cutaneous warts, blood, and various tissue fluids of bovine papilloma affected animals (Freitas et al., 2003; Leishangthem et al., 2008).

BPV-1 was detected from cutaneous warts by PCR and was confirmed by nucleotide sequencing and phylogenetic analysis. 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 the BPV-1gene of papilloma virus of cattle showed 100% homology with the already reported sequence from China. The BPV–1 partial sequenced in present study revealed close homology as well as distinct difference with the earlier published BPV–1 sequences on NCBI. Our study is in agreement with Alcigir et al. (2016) who found that the L1 genotype has association with the development of cutaneous papillomatosis as papilloma or fibropapilloma on the surface. 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 Hamad et al., (2017) compared the alignment of different sequences and the alignment comparison showed high identity of 100% among the Iraqi isolates KY662042-1, KY662043-1, and KY662040-1 with BPV-1 L1 partial cds, as well as small variety with isolates KY662041-1, 99%.

Conclusions

Papillomatosis in cattle and buffaloes is a less known disease, but it is a separate infectious ailment and require more attention. In further future studies to better understand the *in vivo* carcinogenesis of papillomavirus, Moreover, the correlation between farm animal papillomavirus and small animals should be determined. A specific treatment regime should be planned for the number 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 a need to conduct more molecular studies to diagnose if there are other types of the disease in the same area of study and other geographical areas of Maharashtra state.

Declarations

Acknowledgements

The authors are grateful to the Associate Dean, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara (Maharashtra) for providing necessary facilities to conduct the present study.

Author Contribution

U.M.T design and conducted the study. U.M.T, D.N.D performed the study and wrote the first draft. U.M.T, D.N.D, and D.M.M wrote the manuscript and edited. M.M.P, P.P.M, help in experiment and proof reading

work. All authors reviewed the manuscript.

Funding

No special funding was received.

Conflict of interest

The authors declare no conflict of interests.

Animal ethics

No experiment was conducted on animals.

References

1. Alcigir M E, Atalay Vural S, 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(9), 549–557. https://doi.org/10.21521/mw.5562
2. Bam J, Kumar P, Leishangthem G D, Saikia A, Somvanshi R (2012) Spontaneous cutaneous papillomatosis in yaks and detection and quantification of bovine papillomavirus-1 and – 2. Transboundary and Emerging Diseases, doi:10.1111/j.1865-1682.2012.01361.x
3. Freitas A C d, Carvalho C d, Brunner O, Birgel-Junior E H, Dellalibera A M M P, Benesi F J, Gregory L, Beçak W, Santos R d C S d. (2003) Viral DNA sequences in peripheral blood and vertical transmission of the virus: A discussion about BPV-1. Braz. J. Microbiol. 34, 76–78. [CrossRef]
4. Jangir B L, Singh V K, Saikumar G, Pawde A M, Churamani C P, Somvanshi R (2013) Preliminary Pathological Studies on Equine Skin Lesions with Specific Reference to Equine Sarcoid and Detection of Bovine papillomaviruses 1 and 2. Adv. Anim. Vet. Sci.1 (6): 197–201
5. Kumar P, Jangir B L, Saikumar G, Somvanshi R. (2013a) Co-infection of bovine papillo-mavirus type-1 and – 10 in teat warts of dairy cattle. Vet Med; 58: 605–608
6. Kumar P, Nagarajan N, Kumar D, Bind R B, Somvanshi R (2013b) Detection and quantification of bovine papillomaviruses (BPVs) in cutaneous warts of cattle and buffaloes. Advances in Animal and Veterinary Science 1, 53–58.
7. Kumar P, Nagarajan N, Saikumar G, Arya R S, Somvanshi R (2013c) Detection of bovine papillomaviruses (BPVs) in war-like lesions of upper gastrointestinal tract of cattle and buffaloes. Transboundary and Emerging Diseases, doi:10.1111/tbed.12127
8. Leishangthem G D, Somvanshi R, Lauren DR (2008) Pathological studies on bovine papilloma virus-fern interaction in hamsters. Ind J Exp Biol 46: 100–107.
9. Pangty K, Singh S, Saikumar G, Goswami R and Somvanshi R (2010) Detection of BPV–1 and – 2 and quantification of BPV–1 by real–time PCR in cutaneous warts in cattle and buffaloes.

Transbound. Emer. Dis. 57(3): 185–196.http://dx.doi.org/10.1111/j.1865-1682.2009.01096.x PMid:20113447

10. Singh V, Somvanshi R (2010) BPV-2 associated papillomatosis in Indian Water Buffaloes (Bubalus bubalis). Indian Journal of Animal Science 80, 956–960.
11. Khalefa A M. (2016) Clinical, histopathological and molecular detection of cutaneous papillomatosis associated with bovine papillomavirus types 1and 2 in cattle from Al-Qadissiyia Governorate. Kufa J Vet Med Sci. 7(1):182–191. 14. Santos EUD, Silva MAR, Pontes NE, Countinho LCA, Paiva SSL, Castro RS, Freitas AC. Detection of different bovine papillomavirus types and co-infection in bloodstream of cattle. Transbound Emerg Dis. 2016;63:103–108.
12. Hamad M, Al-Shammari A M, Odisho S M, Yaseen N Y. (2017) Molecular epidemiology of bovine papillomatosis and identification of three genotypes in central Iraq. Inter Virol. 60:156–164

Tables

Table 1. Details of samples collected for the detection of BPV

Sr. No.	Breed	Location	History	Tissue samples
1	HF cross	Jawali, Dist. Satara	Few warts were seen on head, neck, face and muzzle areas.	Wart sample
2	Cross bred	Tal. Bhor Dist. Satara	Warts on Neck	Wart sample
3	HF cross	Neera, Dist. Satara	Warts on Neck	Wart sample
4	HF cross	Jawali, Dist. Satara	Warts on Udder and teat	Wart sample
5	HF cross	Khed Shivapur Dist. Satara	Warts on Skin	Wart sample
6	HF cross	Aadarki Dist. Satara	Warts on Udder and teat	Wart sample
7	HF cross	Satara	Warts on face	Wart sample
8	HF cross	Satara	Warts on face	Wart sample
9	HF cross	Tal. Bhor Dist. Satara	Warts on Udder, teat and skin	Wart sample
10	HF cross	Satara	Warts on Udder and teat	Wart samples

Figures

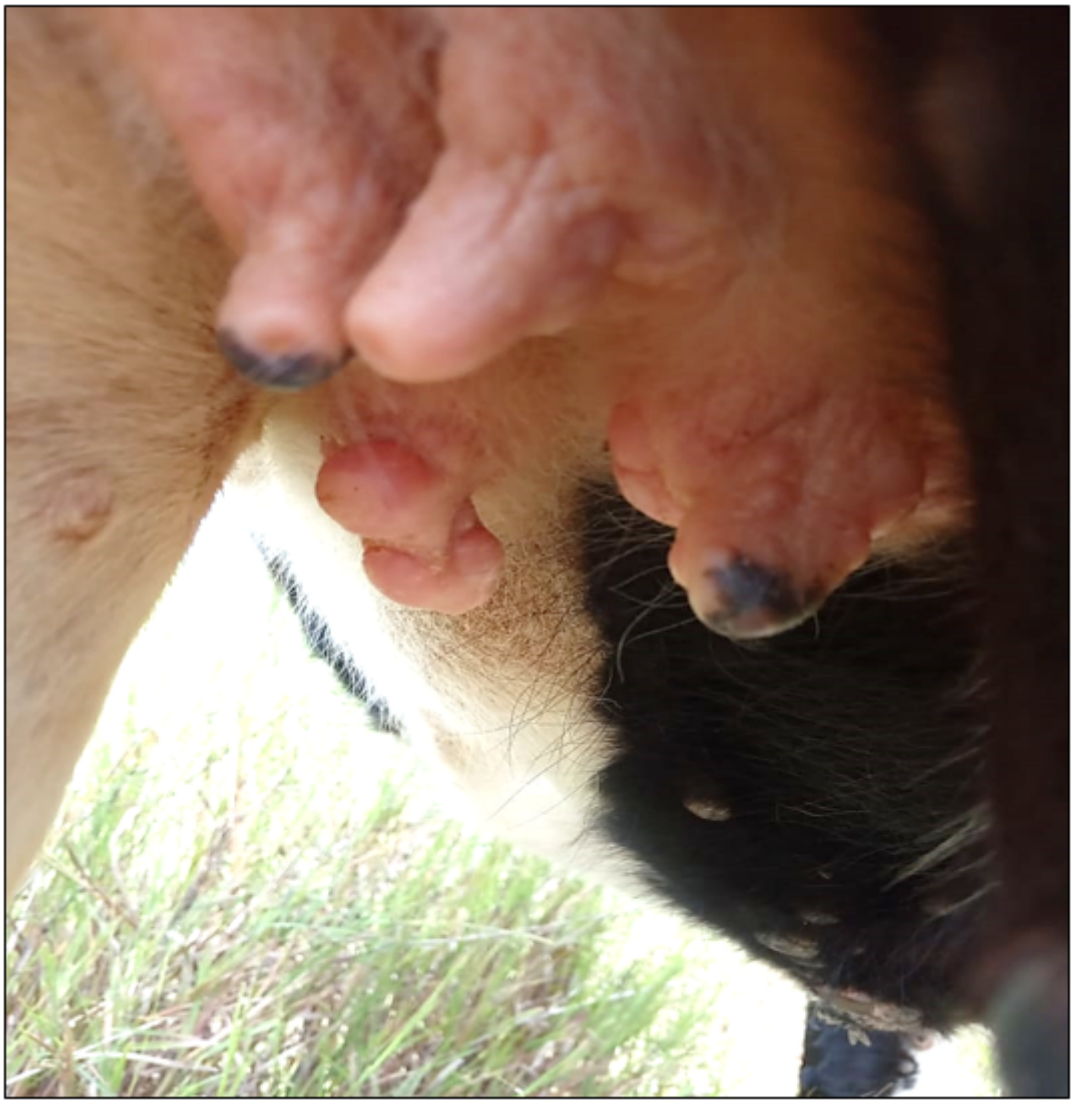

Figure 1

Cauliflower like growth on udder and teat

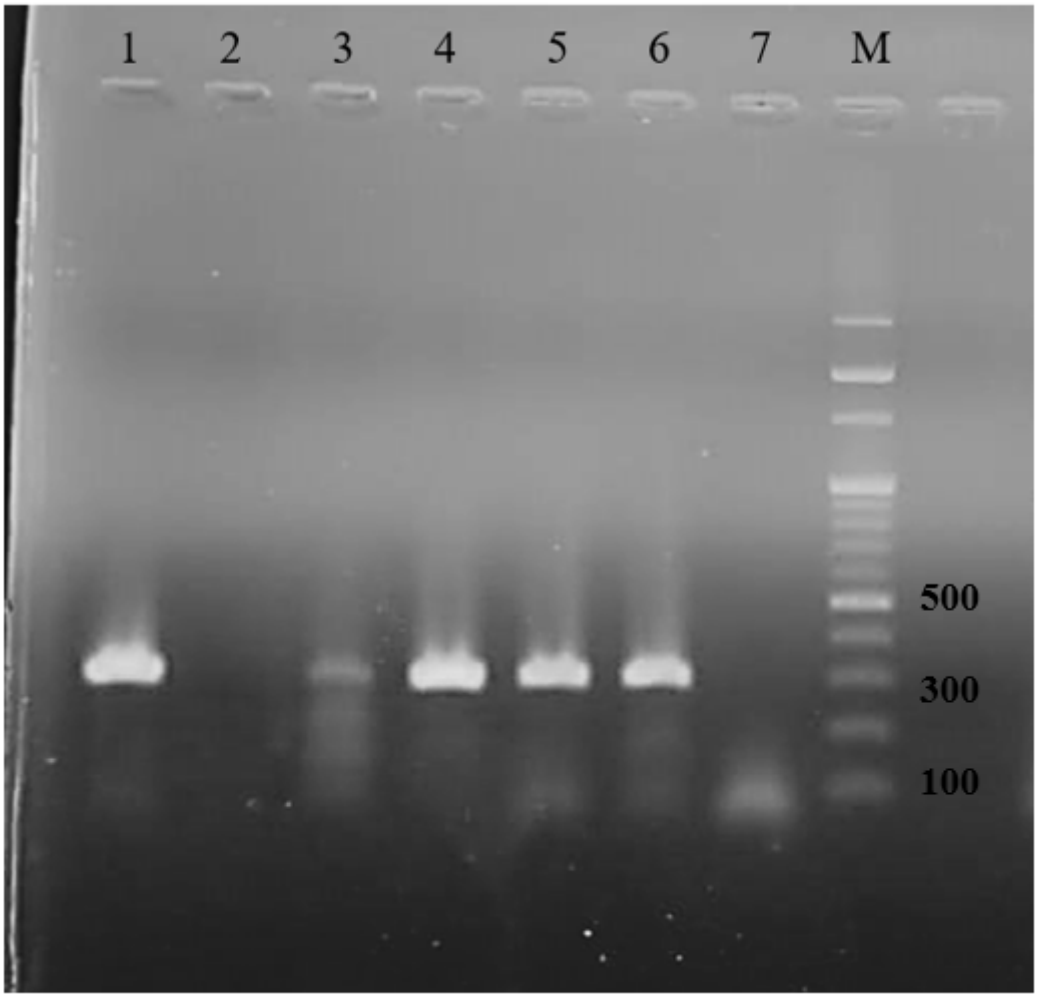

Figure 2

Amplification of BPV1 (301bp) of wart samples

Lane M-Molecular weight marker (100bp ladder)

Lane: 1,3,4,5 and 6=Bovine positive wart samples, Lane: 2, 7=Negative

Figure 3

phylogenetic analysis of the sequence of positive PCR products of wart samples (10672(04FP))