BOVINE PAPILLOMAVIRUS TYPE 2 IS HARBOURDED IN CATTLE CUTANEOUS WARTS

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Abstract

Papillomaviruses (PV) are epitheliotropic double-stranded DNA viruses, known to infect animals. Up to date, more than 280 different papillomavirus types have been described in human and animals. In cattle, up to 29 genotypes of bovine papillomaviruses have been described and classified based on the nucleotide sequence identity of L1 open reading frame. The aim of this study was to detect de bovine papillomavirus type 2 in cutaneous warts. Twenty six (n=26) cutaneous lesions were collected from a cattle slaughterhouse, located in Iasi County, North-Eastern Romania. The viral DNA was extracted using PureLink™ Genomic DNA Mini Kit, following the manufacture's instruction. Detection of papillomavirus DNA was confirmed by PCR, using degenerated primers - FAP59/64 and type specific primers for BPV-2 L2 gene. PCR products were electrophoresed on 1.5% agarose gel stained with SybrSafe and each band was visualized with UV transillumination. When FAP 59/64 primers were used, PV DNA was detected in 11 (42.3%) out of 26 samples, while in 15 (57.69%) samples no PV DNA was identified. The fragment length was consisting in 478 base pairs from L1 gene. A fragment of 164 base pairs corresponding to BPV-2 L2 gene was amplified in 24 (92.2%) samples, while 2 samples were negative (7.69%). Two samples were proved to be negative when tested with both primers pairs. These results are in accordance with previous reported results. The use of type specific primers represent a useful tool in bovine papillomavirus detection.

Key words: BPV, warts, PCR

Papillomaviruses (PV) are epitheliotropic double-stranded DNA viruses able to infect human and animals. These viruses are associated with benign and malignant epithelial lesions. Up to date, more than 280 different PV types have been described in human and animals, while in cattle, up to 29 bovine papillomavirus (BPV) types have been identified and fully characterized (Yamashita-Kawanishi N., Haga T., 2020).

The bovine papillomavirus genome is divided into three different regions: early, late, and noncoding long control region (LCR). The early control region is comprising 50% of the viral genome and encodes seven early proteins: E1, E2, E3, E4, E5, E6, and E7. The late control region occupies 40% of the viral genome and encodes two late proteins or the L1 and L2 capsid proteins and LCR, which comprises 10% of the genome, with 850 bp (Zheng Z.M. and Baker C.C., 2006; Araldi R.P. *et al*, 2013). The viral oncoproteins encoded by BPV are known to be involved in several pathways of the cell transformation (Boracchiello G., Roperto, F., 2008; Lunardi *et al*, 2013).

According to criteria proposed by the International Committee on the Taxonomy of Viruses (ICTV), PVs are classified based on the nucleotide sequence identity of the major capsid protein, L1 ORF: one PV isolate is admitted as a new type if the complete genome has been cloned and the DNA sequence of L1 differs by more than 10% from the closest known PV type. Differences between 2% and 10% define a subtype and less than 2% are proposed for a variant (de Villiers E.-M. *et al*, 2004; VanDoorslaer *et al*, 2018).

According to the papillomavirus episteme (PaVE) (Bianchi R.M. *et al*, 2020), there are currently 29 fully characterized BPVs types that are classified into five genera, namely: the *Deltapapillomavirus* genus with BPV types -1, -2, -13, and -14; *Epsilonpapillomavirus* genus with BPV types -5 and -8; *Dyoxipapillomavirus* genus with BPV type -7; *Dyokappapilomavirus* genus with BPV types -16, -18 and -22 and *Xipapillomavirus* genus with BPV types -3, -4, -6, -9, -10, -11, -12, -15, -17, -20, -23, -24, -28, -29; BPV -19, BPV -21 and BPV -27 remain unclassified (Yamashita-Kawanishi N. *et al*, 2020a; Yamashita-Kawanishi N. *et al*, 2020b; Daudt *et al*, 2018). Improvement in genomic science technologies and molecular biology, such

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as viral detection and sequencing led to identify new types of PVs (Yamashita-Kawanishi N. *et al*, 2020a).

The use of PCR assays with degenerated primers, followed by sequencing has allowed the identification of several PV types in human and other animal hosts (Forslund O. *et al*, 1999; Antonsson A., Hansson B. G., 2002). The PCR primer FAP set was designed from two relatively conserved regions found in the L1 gene and has been shown to amplify PVs DNA from both papillomas and healthy tissue of many animal species, including BPVs in bovines (Carvalho R.F. *et al*, 2013). The aim of this study was to detect de bovine papillomavirus type 2 in cutaneous warts by employing degenerated primers FAP59/64 and type specific BPV-2 primers.

MATERIAL AND METHOD

The bovines with cutaneous warts were slaughtered in one slaughterhouse located in Iași County, North-Eastern Romania, from August 2020 to January 2021. Fragments of skin presenting warts were collected from 26 bovines and each sample was fixed in 10% neutral buffered formalin, routinely processed for histopathology and stained with hematoxylin and eosin (H&E).

DNAs from 26 paraffin embedded bovine cutaneous tumour samples were recovered using the PureLink™ Genomic DNA Mini Kit, following the manufacture's instruction.

Partial amplification of the PV L1 gene was performed with the forward primer FAP59 and the reverse primer FAP64 (Table 1).

amplification, it was used the protocol recommended by Araldi *et al*, 2013, as follows: 5 minutes at 95°C, followed by 35 cycles of 1 minute and 30 seconds at 98°C, 2 minutes at 52°C, and 1 minute and 30 seconds at 72°C and a final extension step of 5 minutes at 72°C. A positive control consisting in a BPV-2 positive sample was used (Bocaneti F. *el al*, 2015). PCR products were analyzed by electrophoresis in 1.5 % agarose gels stained with SybrSafe (Invitrogen) and visualized under UV light.

RESULTS AND DISCUSSIONS

Diagnosis of cutaneous warts or papillomatosis was performed by evaluating each sample. Microscopically, all samples showed specific features of BPV infections: epithelial and dermal hyperplasia, hyperkeratosis and koilocytosis.

Conventional PCR using the degenerated FAP59/FAP64 primer pair generated the expected 478 bp L1 gene fragment (*figure 1*) in 11 (42.3%) of the 26 warts samples, while in 15 (57.69%) no papillomavirus DNA was amplified (*figure 2*).

A fragment of 164 base pairs corresponding to BPV-2 L2 gene (*figure 3*) was amplified in 24 (92.2%) samples, while 2 (7.69%) samples were negative (*figure 4*). Two samples were proved to be negative when tested with both primers pairs.

Primers used for PCR amplification Table 1

Primers	Region	Sequence	Reference
FAP 59/64	L1	5" TAACWGTIGGICAYCCWTATT 3" and 5 "CCWATATCWVHCATITCICCATC 3"	Forslund O. *et al,* 1999
BPV-2	L2	5" GTTATACCACCCAAAGAAGACCCT 3" 5" CTGGTTGCAACAGCTCTCTTTCTC 3"	Araldi R.P. *et al,* 2013

For BPV-2 detection, L2 gene was targeted, resulting in a 164 amplicon. The PCR were performed with a previously described protocol (Ogawa O. *et al*, 2004, Araldi R.P. *et. al*, 2013). Briefly, 5 µL of extracted DNA was mixed with [1×] PCR mix buffer Platinum II Hot-Start Green, 20 µmol of each primer, (Invitrogen, Platinum II Hot-Start Green PCR Master Mix) in a total volume of 20 µL adjusted with water, nuclease free. For PV amplification, after an initial incubation at 95 °C for 5 min, the reaction conditions consisted of 40 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, and extension at 75 °C for 1 min. For BPV-2

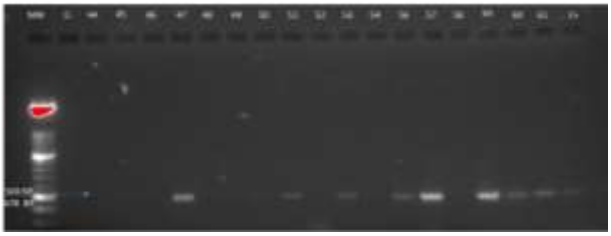

Figure 1 PCR results showing a specific band of 478 bp amplified with FAP59/64 primers. MW – 100 bp; C- negative control; C+ positive control; 44-61 bovine cutaneous warts

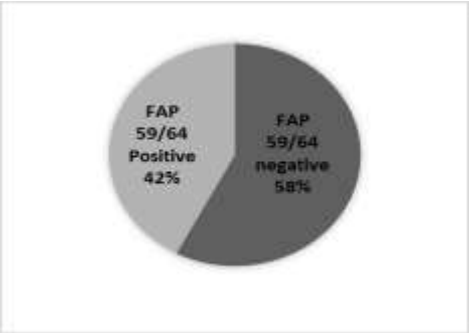

Figure 2 Result of PCR amplification using FAP59/64 degenerated primers

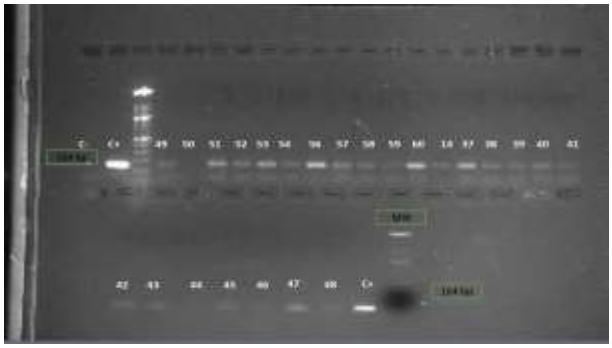

Figure 3 PCR showing a specific band of 164 bp amplified with BPV-2 L2 specific primers. MW – 100 bp; C-negative control; C+ positive control; 37-61 bovine cutaneous warts

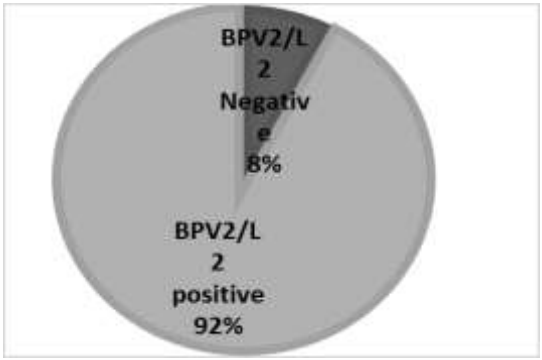

Figure 4 Result of PCR amplification using BPV-2 L2 specific primers

With respect to papillomavirus identification, these results are in accordance with previous reported results (Forslund O. *et al*, 1999; Silva M.A. *et al*, 2013). PCR assays using the FAP59/FAP64 degenerated primers to amplify partial fragments of the PV L1 gene, followed by use of specific BPV primers is an important tool that is widely used to determine the presence of different BPV types in cattle herds from different countries. Indeed, two sets of primers (FAP59/FAP64 and MY09/MY11), which were originally designed from two conserved regions of the HPV L1 gene, are widely used for identifying PVs in both humans and a wide range of animals (Manos M. *et al*, 1989; Forslund O. *et al*, 1999; Antonsson A., Hansson B.G., 2002; Ogawa T. *et al*, 2004). However, studies are showing that FAP59/64 consensus primers have a lower level of sensitivity than BPV type-specific primers (Araldi R.P. *et al*, 2013).

It is well know that both FAP and BPV type-specific primer sets are able to amplify a wide range of BPV types in cutaneous lesions, as well in blood and semen samples. However, BPV type-specific primers were more sensitive than consensus primers (Araldi R.P. *et al*, 2013). On the other hand, the consensus primers are a very suitable way of detecting new BPV types and subtypes. Therefore, the choice of the PCR primer system plays an important role in epidemiological analysis of bovine papillomavirus distribution in cattle (Araldi R.P. *et al*, 2013).

In our study, the positivity rate was for BPV-2 was 92%.This is in accordance with the results obtained by other authors who demonstrated that BPV-2 is the main BPV circulating in Moldova area, North-Eastern Romania (Balcos L. *et al*, 2008; Silva M. *et al*, 2013, Bocaneti F. *et al*, 2016).

CONCLUSIONS

The BPV type-specific primers are a sensitive method to detect papillomavirus DNA in cutaneous samples than the consensus primers.

In this study, only two sample were negative when tested with both pair of primers. However, since the rate of positivity when using degenerated primers is low, we believe that two sample is possible to harbor other type of BPV.

ACKNOWLEGMENTS

This work was supported by a grant of the Ministery of Research, Innovation and Digitalization, CNCS/CCCDI_UEFISCDI, project number 50/2020 within PNDI III.

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