BOVINE PAPILLOMAVIRUS TYPE 2 DETECTION IN BLOOD OF ASYMPTOMATIC LIMOUSINE BREED CATTLE

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Abstract

Bovine Papillomaviruses (BPV) are double-stranded DNA viruses, known to infect ruminants and equines. In bovines, up to 29 genotypes of BPV have been described and classified based on the nucleotide sequence identity of L1 open reading frame. The aim of this study was to detect bovine papillomavirus type 1, 2 and 4 in the blood collected from clinically healthy Limousine cattle, with history of papillomatosis. Fourteen blood samples were collected from a cattle farm, located in Suceava County, Romania. The viral DNA was extracted using PureLink™ Genomic DNA Mini Kit, following the manufacture's instruction. Papillomavirus DNA was confirmed by PCR, using type specific primers for BPV-1 L1 gene, BPV-2 L2 gene and BPV-4 E7 gene detection. PCR products were electrophoresed on 2% agarose gel and visualized with UV transillumination. None of the blood samples tested positive for BPV-1 nor BPV-4. A fragment of 164 base pairs corresponding to BPV-2 L2 gene was amplified in 10 (92,2%) samples, while 4 samples were negative (7,8%). This is the first time when in Romania, to authors best knowledge, BPV-2 is detected in blood samples collected from cattle. Moreover, BPV-2 persists and is maintained in the bloodstream of the asymptomatically cattle, representing a potential reservoir of viral infection.

Key words: BPV-2, blood, PCR

Bovine Papillomaviruses (BPV) are double-stranded DNA viruses, known to infect ruminants and equines. In cattle, up to 29 bovine papillomavirus (BPV) types have been detected and their genome fully characterized (Yamashita-Kawanishi N., Haga T., 2020).

The bovine papillomavirus genome characterized by three different regions: early, late, and noncoding long control region (LCR). The early control region encodes seven early proteins: E1, E2, E3, E4, E5, E6, and E7. The late control region encodes two late proteins or the L1 and L2 capsid proteins and LCR 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, especially E5, are known to be involved in different pathways of the cell transformation (Boracchiello G., Roperto, F., 2008; Lunardi *et al,* 2013; Bocaneti F. *et al,* 2016).

According to criteria proposed by the International Committee on the Taxonomy of Viruses (ICTV), BPVs are classified based on nucleotide sequence identity of the major capsid protein L1 ORF and there are described 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 C. *et al*, 2018).

Papillomavirus DNA is known to be detected most commonly as episomal molecules in lesions, in lymphocytes and in precursor lesions (Campo M.S. *et al*, 1994).

BPV has been detected in non-epithelial sites such as gametes and fluids in recent years (Freitas A.C. *et al,* 2003; Lindsey C.J. *et al,* 2009; Roperto S. *et al,* 2011; Silva M.A. *et al,* 2011). Since BPV DNA has been identified in other body tissues, blood has been hypothesized as a vehicle of BPV to various body parts (Freitas A.C. *et al,* 2007; Roperto S. *et al,* 2011). BPV DNA has also been found in peripheral blood mononuclear cells of sheep, the skin of wild ruminants, and the

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placenta and blood of mares (Roperto S. *et al*, 2018; Savini F. *et al*, 2019).

Recently, BPV-2 and BPV-4 DNA were identified in embryonic tissues as well as in female reproductive tissues taken from infected cows, demonstrating viral presence in tissues other than cutaneous epithelium (Yaguiu A. *et al*, 2006, 2008).

The aim of this study was to detect de bovine papillomavirus type -1, -2 and -4 in the blood collected from clinically healthy Limousine cattle, but with history of papillomatosis in the past.

MATERIAL AND METHOD

The bovines with history of cutaneous warts in the past were reared into a farm located in Marginea, Suceava County, with a flock of 62 animals. During 2015-2016, 14 bovines: 10 cows and 4 bulls, Limousine pure breed were imported from France and adapted to the climate of Bucovina. The bovines are kept in free range system during the summer, while in the winter are kept in stable and feed with dry hay (*figure 1* and *2*).

For this study, 14 blood samples were collected from animals that were known by the owner to suffer of cutaneous papillomatosis. At the moment of blood collection none of the animals showed signs of papillomatosis. From each sample, a quantity of three mL of blood was collected by coccygeal venipuncture using EDTA-containing tubes. The viral DNAs for each blood sample were recovered using the PureLink™ Genomic DNA Mini Kit, following the manufacture's instruction.

Figure 1 Limousine bull

Figure 2 Limousine cattle kept in stable

For BPV-1 detection, a fragment of 301 bp of L1 gene was amplified. For BPV-2 detection, L2 gene was targeted, resulting in a 164 amplicon, while for BPV-4 detection a fragment of 170 bp of E7 was targeted. The amplification was 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

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
BPV-1	L1	5" GGAGCGCCTGCTAACTATAGGA 3" and 5" ATCTGTTGTTTGGGTGGGTGGTGAC 3"	Araldi R.P. *et al,* 2013
BPV-4	E7	5" GCTGACCTTCCAGTCTTAAT 3" 5" CAGTTTCAATCTCCTCTTCA 3"	Araldi R.P. *et al,* 2013

Partial amplification of the PV L1 gene was intended in a first step of papillomavirus surveillance with the forward primer FAP59 and the reverse primer FAP64 (*table* 1).

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-1, BPV-2 and BPV-4 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. PCR products were analyzed by electrophoresis in 1.5 % agarose gels stained with SybrSafe (Invitrogen) and visualized under UV light.

RESULTS AND DISCUSSIONS

PCR using the degenerated FAP59/FAP64 primer pair did not generated the expected 478 bp L1 gene fragment in none of the samples.

A fragment of 164 base pairs corresponding to BPV-2 L2 gene was amplified in 10 (92.2%) samples, while 4 (7.8%) samples were negative (*figure 3*). The results for each tested blood sample by PCR is shown in table 2.

Figure 3 PCR results representing a specific band of 164 bp amplified with BPV-2 L2 primers. MW – 100 bp; C-negative control; C+ positive control; 1-14 bovine blood

Table 2

PCR results for each tested blood sample

Sample	ID	Sex	DOB	FAP 59/64	B-PV-1	BPV-2	BPV-4
1	RO502009339386	F	07/04/2019	-	-	-	-
2	RO502008553961	F	26/05/2018	-	-	-	-
3	RO506008553976	F	02/04/2018	-	-	-	-
4	RO507008587840	F	05/01/2019	-	-	-	-
5	RO509009342976	F	26/04/2019	-	-	+	-
6	RO509009339387	F	06/04/2019	-	-	+	-
7	RO500000009396	F	06/03/2019	-	-	+	-
1.	FR2278711432	F	03/04/2016	-	-	+	-
2.	RO503000009717	F	29/01/2011	-	-	+	-
3.	RO500000009396	F	06/03/2019	-	-	+	-
4.	RO506007638922	M	27/04/2017	-	-	+	-
5.	RO508006920149	F	30/06/2016	-	-	+	-
6.	RO507008587840	F	05/01/2019	-	-	+	-
7.	RO50000009721	F	29/01/2011	-	-	+	-

With respect to BPV-1 and BPV-4 detection, none of the sample showed a positive response. 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 confirm the presence of different BPV types in cattle herds from different countries.

However, in our study, we failed to detect in the first step PV DNA when using degenerated primers. Indeed, 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).

With respect to BPV-2 detection, this is the first time when in Romania, to authors best knowledge, when BPV-2 is detected in blood samples collected from cattle. Accordingly, there are studies confirming the presence of BPV-2 among cattle from Moldova, where this BPV type was detected in cutaneous warts or in urinary bladder tumours (Balcos L. *et al*, 2008; Silva M. *et al*, 2013, Bocaneti F. *et al*, 2016; Bocaneti et al., 2021, in press).

CONCLUSIONS

BPV-2 persists and is maintained in the bloodstream of the asymptomatically cattle, representing a potential reservoir of viral infection.

Knowledge of BPV diversity and epidemiology is of great importance for establishing distribution, prevention strategies and understanding the evolution of this group of oncoviruses.

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