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*Short communication*

# **Phylogenetic analysis of bovine papillomavirus E5 detected in equine sarcoids in Poland**

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## **Abstract**

The aim of the study was to analyse a part of the sequence of the E5 gene of bovine papillomaviruses (BPV) associated with equine sarcoids in Polish horses. Samples of 40 skin lesions obtained from 29 horses were collected for molecular examination. The PCR amplicons of BPV DNA were detected in 38 specimens. After phylogenetic analysis 37 specimens were recognized as BPV-1 and one as BPV-2. Phylogenetic analysis has allowed the classification of the amplicons into two phylogenetic groups (A1,) and four separate isolates (2, 10, 16, 17).

**Key words:** BPV, equine sarcoids, E5, phylogenetic analysis

## **Introduction**

Papillomaviruses (PV) constitute a group of viruses associated with benign and malignant lesions of cutaneous and mucosal epithelia. Bovine PV (BPV) are usually species-specific and, even in experimental conditions, do not infect other species, except BPV-1 which is accepted to be an aetiological agent of equine sarcoids (Chambers et al. 2003). Our previous retrospective data showed the presence of E5 gene European variants of BPV-1 also in Poland (Szczerba-Turek et al. 2009). The aim of the study was to analyse a part of the sequence of the E5 gene of BPV associated with equine sarcoids in Polish horses and to compare the results with previous retrospective and literature data.

## **Materials and Methods**

The study was carried out using 40 tissue samples of skin lesions from 29 horses, clinically diagnosed as sarcoids. The purpose of PCR was to amplify the fragment of the E5 gene of BPV-1 and/or the fragment of the E5, E25 genes of BPV-2. PCR was carried out using primer sets E<sub>5</sub>L<sub>2</sub>up/E<sub>5</sub>L<sub>2</sub>lo published by Teifke et al. (1994). Phylogenetic analysis was conducted using the freeware Computational Evolutionary Biology package MEGA4 (Tamura et al. 2007).

## **Results and Discussion**

Histopathological examination confirmed sarcoids in 27 (67.5%) of all skin lesions. The fibroblastic sar-

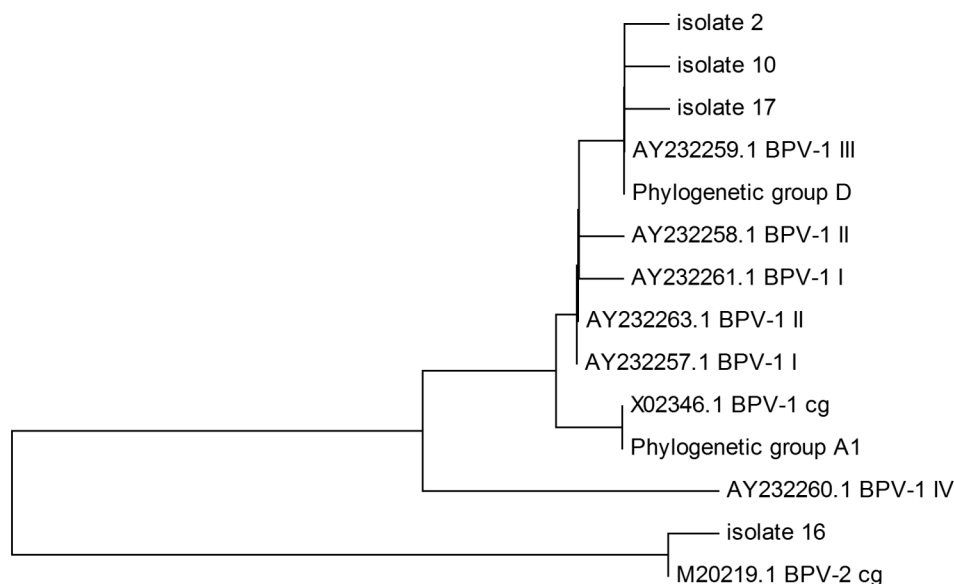


Fig. 1. Evolutionary relationships of 14 taxa of the part of E5 gene of BPV-1 and E5, E25 genes of BPV-2.

Table 1. Nucleotide changes of isolates 2, 10, 17 examined in position 3760-4003 in comparison with BPV-1 genome.

Isolate no.	Point mutation – transition Position in BPV-1 Acc. No. X02346	Point mutation – transversion Position in BPV-1 Acc. No. X02346
2	C3854T, T3858C, A3938G	G3921T
10	C3854T, A3938G	G3921T, C3930G
17	C3854T, A3938G	T3920G, G3921T

coid was diagnosed in 26 (96.3%) and verrucous sarcoid only in 1 (3.7%) skin lesions. After molecular examination the amplicons of BPV DNA were found in 38 (95%) skin lesions. Phylogenetic analysis of nucleotide sequence of all isolates revealed that 37 of them were recognized as BPV-1 and one as BPV-2. BPV-1 isolates were classified into two phylogenetic groups: A1 (8 isolates) and D (26 isolates). Four isolates were separated – 2, 10, 16, 17. Evolutionary relationships of 14 taxa of the partial E5 BPV-1 or E5, E25 BPV2 genes are shown in Fig. 1. The mutations in nucleotide sequences of E5 ORF BPV-1 from 2, 10, 17 isolates are shown in Table 1. Sequence variant from isolate 16 (BPV-2) contains one point mutation T3856A, in comparison with the nucleotide sequence of BPV-2 (Acc. No. M20219). The results presented in this paper support our earlier observations (Szczerba-Turek et al. 2009, 2010) that in equine sarcoids in Poland the European variants of BPV-1 E5 ORF described by Chambers et al. (2003) were observed.

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### References

- Chambers G, Ellsmore VA, O'Brien PM, Reid SW, Love S, Campo MS, Nasir L (2003) Sequence variants of bovine papillomavirus E5 detected in equine sarcoids. *Virus Res* 96: 141-145.
- Szczerba-Turek A, Siemionek J, Szweda W, Banczerz-Kisiel A, Raś A, Rotkiewicz T (2009) Sequence variants of E5 ORFs bovine papillomavirus detected in formalin-fixed and paraffin -embedded tissues obtained from equine sarcoids. *Bull Vet Inst Pulawy* 53: 563-567.
- Szczerba-Turek A, Siemionek J, Wąsowicz K, Szweda W, Raś A, Platt-Samoraj A (2010) Partial sequence analysis of the L1 gene of bovine papillomavirus type 1 detected by PCR with MY09/MY11 primers in equine sarcoids in Poland. *Pol J Vet Sci* 13: 241-246.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24: 1596-1599.
- Teifke JP, Hardt M, Weiss E (1994) Detection of bovine papillomavirus DNA in formalin-fixed and paraffin-embedded equine sarcoids by polymerase chain reaction and non-radioactive *in situ* hybridization. *Eur J Vet Pathol* 1: 5-10.