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Original article

Genetic evaluation of bovine papillomavirus types detected in equine sarcoids in Poland

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Abstract

Background: Equine sarcoids are the most common neoplasms in horses. Bovine papillomavirus type 1 (BPV-1) is the main viral type identified in equine sarcoids in Europe.

Objective: The aim of the present study was to genetically evaluate BPV types based on DNA analyses of the CDS of the L1 gene. The presence of BPV DNA was confirmed by Degenerate Oligonucleotide-Primed Polymerase Chain Reaction (DOP PCR) with FAP59/FAP64 consensus primers.

Results: The DNA was detected in 21/40 (52.5%) of clinically diagnosed sarcoids. More than half of 14 isolates (66.7%) shared 100% homology with BPV-1 *Deltapapillomavirus* 4 isolate 09 asi UK (Acc. No. MF384289) and 99% nucleotide identity with BPV-1 isolate EqSarc1 (Acc. No. JX678969). A comparison with BPV-1 isolate EqSarc1 revealed one silent mutation in C5827T which did not change the amino acid codon. The remaining 6 isolates (28.6%) shared 100% nucleotide identity with the BPV-1 (Acc. No. X02346) “wild type” isolate, and 1 isolate (4.8%) demonstrated 99% nucleotide identity with BPV-2 (Acc. No. M20219).

Conclusions: Variants of BPV-1 isolate EqSarc1 (Acc. No. JX678969) constitute the most prevalent type of BPV-1 in Polish horses.

Key words: bovine papillomavirus, equine sarcoid, disease ecology, FAP59/FAP64

Introduction

Neoplastic diseases pose a growing problem in both humans and animals worldwide. Selected neoplasms have an infectious etiology. An example can be cervical cancer that evolves mainly as infection caused by human papillomavirus types 16 and 18 (HPV-16 and 18). Papillomaviruses (PVs) are generally small, non-enveloped,

double-stranded DNA viruses with circular genomes which infect all species in the world and are usually species-specific. They belong to the *Papillomaviridae* family which comprises 29 genera, from *Alphapapillomavirus* to *Dyoyiotapapillomavirus*, with several species, types, subtypes and variants (Bernard et al. 2010). Classification is based on genomic DNA homology, especially in the L1 highly conserved open reading frame

Table 1. Results of clinical, histopathological and molecular examinations.

Isolate No.	Clinical diagnosis	Histopathological lesions Tissue type	Results of molecular BPV identification (nucleotide identity %)	Total No.
14b 20c, 22b, 23, 24b, 27b	Fibroblastic sarcoid Fibroblastic sarcoid	Hyperkeratosis of epidermis Fibroblastic sarcoid	BPV-1 "wild" Acc. No. X02346 (100%)	6
5, 6, 8, 9, 10, 12, 19, 21, 25a, 25b, 25c	Fibroblastic sarcoid	Fibroblastic sarcoid	BPV-1	
13a	Fibroblastic sarcoid	Old granulation tissue	Deltapapillomavirus 4	14
7a	Sarcoid	Scar	isolate 09 asi UK	
26b	Sarcoid	Epidermal cyst	Acc. No. MF384289 (100%)	
16	Sarcoid	Old granulation tissue	BPV 2 Acc. No. M20219 (99%)	1

(ORF) which is a structural protein gene (Bernard et al. 2010).

To date, 193 human papillomavirus (HPV) types have been identified, but only 24 bovine papillomaviruses (BPVs) have been detected (<http://pave.niaid.nih.gov>). Bovine papillomaviruses are grouped into four genera: *Deltapapillomavirus* – BPV-1, 2, 13 and 14, *Epsilon-papillomavirus* – BPV-5 and 8, *Xipapillomavirus* – BPV-3, 4, 6, 9, 10, 11, 12, 15, 17, 20, 23 and 24, *Dyokapapillomavirus* – 16, 18 and 22, *Dyoxipapillomavirus* – BPV-7. The remaining BPVs (19 and 21) have not been classified. The *Deltapapillomavirus* is the most interesting genus which breaks the species-specific barrier: BPV-1, 2 and BPV-13 infect horses, and BPV-1 and 2 are the main causative agents of equine sarcoids (Lunardi et al. 2013a,b). BPV-14 has been recently detected in feline sarcoids (Munday et al. 2015). Several years ago, two new BPVs, BPV-13 (Acc. No. JQ798171) and BPV-1 isolate EqSarC1 (Acc. No. JX678969), were detected in equine sarcoids (Lunardi et al. 2013a,b, Wilson et al. 2013). Equine sarcoids are the most common skin tumour in horses. Recently, PCR assays using degenerate primers that amplify partial fragments of the L1 gene of BPV-13 have been used to identify numerous putative new PV types in both humans and cattle from diverse geographical regions (Lunardi et al. 2013a). Initially, the primer pairs FAP59/FAP64 and MY09/MY11 were applied to detect 11 putative new BPV types in skin warts from the teats and in healthy skin of cattle in Japan and Sweden (Antonsson and Hansson 2002, Ogawa et al. 2004).

The aim of the present study was to genetically evaluate the BPV types associated with equine sarcoids in Polish horses.

Material and Methods

An ethical approval was not required as this study was performed retrospectively. Samples of DNA from

equine sarcoids were obtained from previous studies, performed in adherence to the Local Ethics Committee of the University of Warmia and Mazury in Olsztyn No. 49/N, 19.12.2006.

Experimental material

Forty skin lesions clinically diagnosed as sarcoids were collected from 29 horses. Histopathological examinations were carried out in the Department of Pathological Anatomy at the Faculty of Veterinary Medicine of the University of Warmia and Mazury in Olsztyn, Poland.

Degenerate Oligonucleotide-Primed Polymerase Chain Reaction (DOP-PCR) analysis

Total DNA was isolated using the Genomic Mini kit (A&A Biotechnology, Poland) in accordance with the manufacturer's instructions. Relative purity and quality were determined spectrophotometrically (BioPhotometer, Eppendorf). PCR assays were performed with the HotStarTaq Plus PCR kit (Qiagen, Germany) according to the manufacturer's instructions using FAP59/FAP64 primers described by Furslund et al. (1999). Cloned BPV-1 was the positive control, and H₂O served as the negative control. The amplicons were purified using the Clean-up purification kit (A&A Biotechnology, Poland) according to the manufacturer's recommendations. Purified amplicons were independently sequenced (Genomed S.A., Poland) in both directions.

Data analysis

Sequence data from the specimens were compared to the nucleotide sequence of the previously identified BPVs in the BLASTN v. 2.2.18 program (Altschul et al. 1997). A multiple sequence alignment was carried out

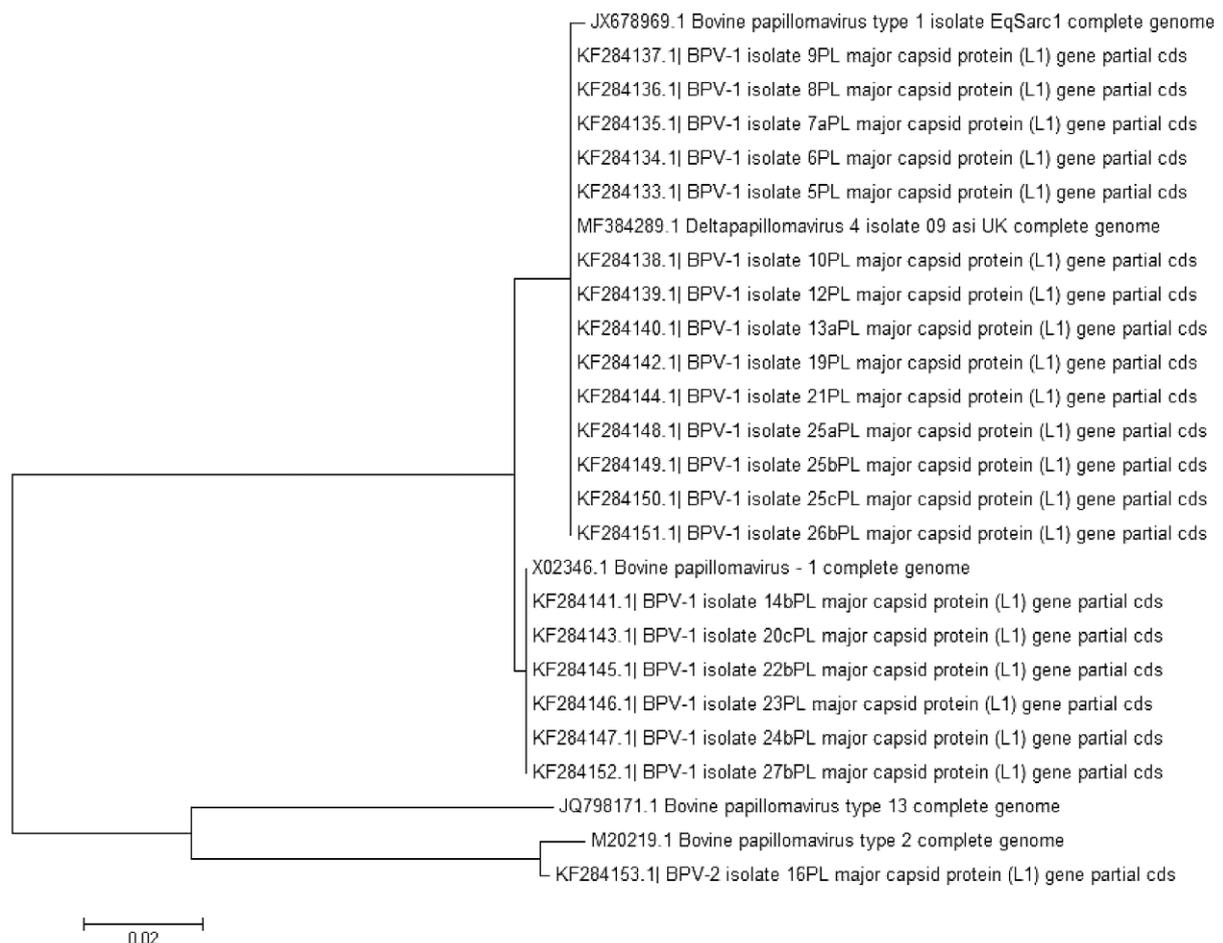


Fig. 1. A phylogenetic tree created with the neighbour-joining method based on a 445 base pair (bp) fragment of partial nucleotide sequences of the open reading frame (ORF) of the late L1 gene of bovine papillomaviruses isolated from equine sarcoids in Poland. The analysis involved 26 nucleotide sequences. The sequences used in the analysis have the following GenBank accession numbers: BPV-1 *Deltapapillomavirus* 4 isolate 09 asi UK – MF384289, BPV-1 isolate EqSarc1 – JX678969, BPV-1 “wild type” – X02346, BPV-13 – JQ798171, BPV-2 – M20219, BPV-1 isolates from Poland – KF284133-KF284152, BPV-2 isolates from Poland – KF284153.

using ClustalW (Larkin et al. 2007) algorithms incorporated in the MEGA v. 5.2.1. Computational Evolutionary Biology freeware package (Tamura et al. 2011). Evolutionary history was inferred using the neighbour-joining method with a total branch length of 0.25617348. Nucleotide and amino acid (aa) sequences were determined using BioEdit v.7.2.0 software. The sequences described in this paper have the following GenBank Acc. No.: KF284133-KF284153.

Results

The results of histopathological examinations are presented in the Table 1. In the present study, we analysed a 445 bp fragment in position 5732-6176 partially coding L1 ORF of BPV-1 isolate EqSarc1 (Acc. No. JX678969). BPV DNA was detected with the use of FAP primers in the skin lesions of 21 out of 40 (52.5%)

clinically diagnosed equine sarcoids. BPV nucleotide sequences were obtained in 21 of 21 (100%) specimens and deposited in GenBank under Acc. No. KF284133-KF284153. Fourteen of the 21 (66.6%) BPV DNA-positive samples (isolates 5PL, 6PL, 7aPL, 8PL, 9PL, 10PL, 12PL, 13aPL, 19PL, 21PL, 25aPL, 25bPL, 25cPL, 26bPL) shared 100% nucleotide identity with BPV-1-*Deltapapillomavirus* 4 isolate 09 asi UK (Acc. No. MF384289) from asinine (*Equus asinus*) tissue samples from the United Kingdom and 99% nucleotide identity with BPV-1 isolate EqSarc1 from equine (*Equus caballus*) tissue samples from the United Kingdom (Acc. No. JX678969) (Koch et al. 2018). Six of the 21 (28.6%) BPV DNA-positive samples (isolates 14bPL, 20cPL, 22bPL, 23PL, 24bPL, 27bPL) shared 100% nucleotide identity with the BPV-1 “wild type” isolate (Acc. No. X02346) and one of the 21 isolates (4.8%) (isolate 16PL) shared 99% nucleotide identity with

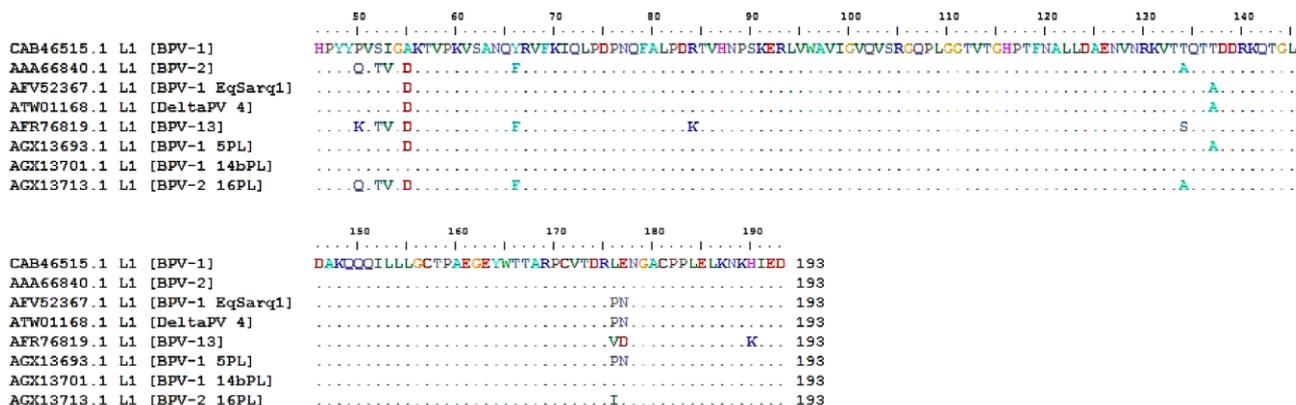


Fig. 2. A comparison of amino acid (aa) sequences in bovine papillomaviruses detected in equine sarcoids from Poland. The analysis involved 8 aa sequences. The analysed proteins have the following GenBank ID numbers: BPV-1 “wild type” L1 – CAB46515, BPV-2 L1 – AAA66840, BPV-1 isolate Eqsarc1 L1 – AFV52367, BPV-1 DeltaPV 4 isolate 09 asi UK L1 – ATW01168, BPV13 L1 – AFR76819, BPV-1 isolate 5PL L1 – AGX13693, BPV-1 isolate 14b PL L1 – AGX13701, BPV-2 isolate 16PL L1 – AGX13713.

BPV-2 (Acc. No. M20219). The results of the partial phylogenetic analysis of the L1 gene are shown in Fig. 1. and Table 1. A comparison of 14 isolates exhibiting 100% identity with BPV-1 *Deltapapillomavirus* 4 isolate 09 asi UK (Acc. no. MF384289) with BPV-1 isolate EqSarc1 (Acc. no. JX678969) revealed one silent mutation in C5827T which did not change the amino acid codon. The results are presented in Fig. 2. The group of 14 isolates was represented by the aa sequences of BPV-1 isolate 5PL L1 (Protein id. AGX13693), the group of 6 isolates was represented by BPV-1 isolate 14b PL L1 (Protein id. AGX13701), and only one representative of the aa sequence of BPV-2 isolate 16PL L1 was identified (Protein id. AGX13713).

Discussion

In our previous study, we used MY primers to analyse a 425 bp fragment in position 6545-6969 of L1 ORF of BPV-1 isolate EqSarc1 (Acc. No. JX678969) (Szczerba-Turek et al. 2010). BPV DNA was found in 77.5% (31 samples) of skin lesions. Six isolates in the analysed fragment demonstrated 100% identity with BPV-1 (Acc. No. X02346), which is consistent with the results of the present study. Eleven isolates (phylogenetic group B – Acc. No. GQ451823) showed 100% identity with BPV-1 isolate EqSarc1 (Acc. No. JX678969), *Deltapapillomavirus* 4 isolate 04_asi_UK (Acc. No. MF384288), isolate 25_equ_CH (Acc. No. MF 384286) and isolate 18_equ_CH (Acc. No. MF384280). Thirteen isolates (phylogenetic group C – Acc. No. GQ451824) exhibited 99% nucleotide and protein identity with BPV-1 isolate EqSarc1 (Acc. No. JX678969) and 100% protein identity with BPV-1 *Deltapapillomavirus* 4 isolate 23_equ CH (Acc. No.

MF384285) from equine (*Equus caballus*) tissue samples from Switzerland and isolate 01_asi_UK (Acc. No. MF384275) from asinine (*Equus asinus*) tissue samples from the United Kingdom.

In our previous study, we also compared the 584/582 bp sequences of the partial CDS of E2 ORF and E5 ORF (Acc. No.: KC684939, KC684940, KC693480, KC693481, KC693482, KC693483, KC693484) with the complete CDS of the E5 gene in position 3673-4256 of BPV-1 isolate EqSarc1 (Acc. No. JX678969) (Szczerba-Turek et al. 2014). This analysis confirmed that variants of BPV-1 EqSarc1 (Acc. No. JX678969) constitute the most prevalent type of BPV-1 in equine sarcoids in Poland. The BPV-1 “wild type” isolate (Acc. No. X02346) was detected in 16% of the clinically diagnosed sarcoids.

In conclusion, our study provides new information about BPV-1 in equine sarcoids in Poland. Variants of BPV-1 isolate EqSarc1 (Acc. No. JX678969) constitute the most prevalent type of BPV-1 in Polish horses. Our findings can be used to develop specific immunopreventive methods and minimize losses caused by BPV infections in horses.

List of abbreviations

BPV: *bovine papillomavirus*; DNA: deoxyribonucleic acid; CDS: coding DNA sequence; DOP-PCR: Degenerate oligonucleotide-primed polymerase chain reaction; HPV: *human papillomavirus*; NCBI: National Centre for Biotechnology Information; PVs: Papillomaviruses; ORF: open reading frame; aa: amino acid; Acc. No.: accession number; bp: base pair.

Availability of data and materials

PaVE: Papilloma virus genome database: <http://pave.niaid.nih.gov>

Deltapapillomavirus 4 major capsid protein (L1) gene, partial cds – PopSet – NCBI: <https://www.ncbi.nlm.nih.gov/popset/?term=KF284133-KF284153&report=genbank>

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