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

A. Szczerba-Turek¹, J. Siemionek¹, A. Bancerz-Kisiel¹, A. Raś², W. Szweda¹

¹ Department of Epizootiology
² Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 13, 10-718 Olsztyn, Poland

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 E5L2up/E5L2lo 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.

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Point mutation – transition</th>
<th>Point mutation – transversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Position in BPV-1 Acc. No. X02346</td>
<td>Position in BPV-1 Acc. No. X02346</td>
</tr>
<tr>
<td>2</td>
<td>C3854T, T3858C, A3938G</td>
<td>G3921T</td>
</tr>
<tr>
<td>10</td>
<td>C3854T, A3938G</td>
<td>G3921T, C3930G</td>
</tr>
<tr>
<td>17</td>
<td>C3854T, A3938G</td>
<td>T3920G, G3921T</td>
</tr>
</tbody>
</table>

Sarcoid was diagnosed in 26 (96.3%) and verrucose sarco-
d only in 1 (3.7%) skin lesions. After molecular ex-
amination the amplicons of BPV DNA were found in 38 (95%) skin lesions. Phylogenetic analysis of nu-
cleotide 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 com-
pared with the nucleotide sequence of BPV-2 (Acc. No. M20219). The results presented in this paper sup-
port our earlier observations (Szczereba-Turek et al. 2009, 2010) that in equine sarcoids in Poland the Euro-
pean variants of BPV-1 E5 ORF described by Chambers et al. (2003) were observed.

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References

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