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 E5Lup/E5Llo 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-
coid was diagnosed in 26 (96.3%) and verrucose sarco-
coid 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 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
gene 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. The results presented in this paper sup-
port our earlier observations (Szczerba-Turek et al.
2009, 2010) that in equine sarcoïds in Poland the Euro-
pean variants of BPV-1 E5 ORF described by Cham-
bars et al. (2003) were observed.

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papillomavirus DNA in formalin-fixed and paraffin-em-
bedded equine sarcoïds by polymerase chain reaction and
non-radioactive in situ hybridization. Eur J Vet Pathol
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Table 1. Nucleotide changes of isolates 2, 10, 17 examined in position 3760-4003 in comparison with BPV-1 genome.

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<tbody>
<tr>
<td>2</td>
<td>C3854T, T3858C, A3938G</td>
<td>G3921T</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>C3854T, A3938G</td>
<td>G3921T, C3930G</td>
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</tr>
<tr>
<td>17</td>
<td>C3854T, A3938G</td>
<td>T3920G, G3921T</td>
<td></td>
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Fig. 1. Evolutionary relationships of 14 taxa of the part of E5 gene of BPV-1 and E5, E25 genes of BPV-2.