Quantitative PCR High-Resolution Melting (qPCR-HRM) curve analysis, a new approach to rapid detection and differentiation of bovine papillomavirus detected in equine sarcoids

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Abstract

The aim of the study was to evaluate a novel diagnostic scheme which combines quantitative PCR and High-Resolution Melting (qPCR-HRM) curve analysis for rapid differentiation based on E5 partial CDS of bovine papillomavirus type 1 or 2 (BPV-1 or BPV-2), and to perform a phylogenetic analysis of the complete CDS of the E5 gene of BPV detected in equine sarcoids. Samples of 38 skin lesions obtained from 27 horses were collected for molecular examinations. All lesions were clinically diagnosed as sarcoids, but results of histopathological examinations did not always corroborate the clinical diagnosis. Although all the samples were positive for the presence of BPV DNA, after qPCR-HRM analysis 6 (16%) specimens were recognized as BPV-1 “wild”, 24 (63%) as BPV-1 “European” and 8 (21%) as a “variant” of BPV E5 ORF partial CDS. Phylogenetic analysis based on nucleotide sequences of E2 ORF partial CDS and E5 ORF complete CDS was conducted on 7 specimens, whose sequences were published in GenBank and recognized as: 2PL (Accession Number – Acc. No. KC684939) – “variant” BPV-1, 7aPL (Acc. No. KC684940) – “European” BPV-1, 10PL (Acc. No. KC693480) – “variant” BPV-1, 16PL (Acc. No. KC693484) – “variant” BPV-2, 17PL (Acc. No. KC693481) – “variant” BPV-1, 20aPL (Acc. No. KC693482) – “European” BPV-1 and 20cPL (Acc. No. KC693483) – “wild” BPV-1. Amino acid (aa) sequences of E5 ORF complete CDS were also analyzed. The E5 variant of aa sequences found in isolate 10PL (protein identification – ID: AGM 20700) is a novel variant of E5 ORF complete CDS of BPV-1 detected in equine sarcoid in Poland.

Key words: BPV-1, BPV-2, equine sarcoids, E5 gene, PCR HRM, phylogenetic analysis

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