

## Genomic Characterisation of Equine Sarcoid-derived Bovine Papillomavirus Type 1 and 2 Using Nanopore-Based Sequencing

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**Abstract :** Bovine papillomavirus (BPV) types 1 and 2 play a central role in the etiology of the most common neoplasm in horses, the equine sarcoid. The unknown mechanism behind the unique variety in a clinical presentation on the one hand and the host-dependent clinical outcome of BPV-1 infection, on the other hand, indicate the involvement of additional factors. Earlier studies have reported the potential functional significance of intratypic sequence variants, along with the existence of sarcoid-sourced BPV variants. Therefore, intratypic sequence variation seems to be an important emerging viral factor. This study aimed to give a broad insight in sarcoid-sourced BPV variation and explore its potential association with disease presentation. In order to do this, a nanopore sequencing approach was successfully optimized for screening a wide spectrum of clinical samples. Specimens of each tumour were initially screened for BPV-1/-2 by quantitative real-time PCR. A custom-designed primer set was used on BPV-positive samples to amplify the complete viral genome in two multiplex PCR reactions, resulting in a set of overlapping amplicons. For phylogenetic analysis, separate alignments were made of all available complete genome sequences for BPV-1/-2. The resulting alignments were used to infer Bayesian phylogenetic trees. We found substantial genetic variation among sarcoid-derived BPV-1, although this variation could not be linked to disease severity. Several of the BPV-1 genomes had multiple major deletions. Remarkably, the majority of the cluster within the region coding for late viral genes. Together with the extensiveness (up to 603 nucleotides) of the described deletions, this suggests an altered function of L1/L2 in disease pathogenesis. By generating a significant amount of complete-length BPV genomes, we succeeded in introducing next-generation sequencing into veterinary research focusing on the equine sarcoid, thus facilitating the first report of both nanopore-based sequencing of complete sarcoid-sourced BPV-1/-2 and the simultaneous nanopore sequencing of multiple complete genomes originating from a single clinical sample.

**Keywords :** Bovine papillomavirus, equine sarcoid, horse, nanopore sequencing, phylogenetic analysis

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