



# Genomic characterisation of canine papillomavirus type 17, a possible rare cause of canine oral squamous cell carcinoma



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## ARTICLE INFO

### Article history:

Received 4 August 2015

Received in revised form 9 November 2015

Accepted 14 November 2015

### Keywords:

Dog

Papillomavirus

Oral

Taupapillomavirus

Squamous cell carcinoma

Oncogenesis

## ABSTRACT

Squamous cell carcinomas (SCCs) are the second most common cancer of the canine oral cavity resulting in significant morbidity and mortality. Recently a dog with multiple oral SCCs that contained a novel papillomavirus (PV) was reported. The aim of the present study was to determine the genome of this novel PV. To do this a short section of PV DNA was amplified from an oral SCC and 'back-to-back' primers were designed. Due to the circular nature of PV DNA, these primers were then used to amplify the remainder of the genome by inverse PCR. The PCR product was sequenced using next generation sequencing and the full genome of the PV, consisting of 8007 bp, was assembled and analysed. As this is the seventeenth PV identified from the domestic dog, the novel PV was designated *Canis familiaris* papillomavirus (CPV) type 17. Similar to other CPV types, the putative coding regions of CPV-17 were predicted to produce 5 early and 2 late proteins. Phylogenetic analysis of *ORF L1* revealed greater than 70% similarity to CPV-2 and CPV-7 and we propose that CPV-17 also be classified as a *Taupapillomavirus* 1. While it appears CPV-17 is only rarely present in canine oral SCCs, evidence suggests that this PV could influence the development of oral SCCs in this species.

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## 1. Introduction

Papillomaviruses (PVs) are small circular double-stranded DNA viruses. As part of their normal life cycle, PVs produce proteins that influence cell growth and differentiation. The majority of PV infections do not cause clinical lesions; however, infection by some PV types can alter normal host cell regulation resulting in self-resolving hyperplastic papillomas (warts) or neoplasia (Munday, 2014). Papillomaviruses are classified into genera based on the sequence of the highly conserved *ORF L1* (Bernard et al., 2010). The overwhelming majority of PVs only infect epithelium and infections are typically restricted to a single location within a specific host (Bernard et al., 2010; Joh et al., 2011).

Sixteen PVs from 3 different genera are currently recognised to infect domestic dogs with *Canis familiaris* papillomavirus (CPV) types 1 and 6 classified as lambdaPVs, CPV-2, -7, and -13 within the *Taupapillomavirus* genus, and the remaining 11 PV types all classified as chiPVs (Luff et al., 2015; Rector and Van Ranst, 2013). Of the 16 currently recognised CPV types, only CPV-1 and CPV-13 have been detected within the oral cavity (Lange et al.,

2012; Rector and Van Ranst, 2013). Diseases caused by PVs in dogs include oral papillomas caused by CPV-1, cutaneous papillomas associated with CPV-2 and -6, and cutaneous pigmented plaques associated with the canine chiPVs (Lange and Favrot, 2011; Rector and Van Ranst, 2013). Less frequently PVs have also been associated with the development of cutaneous squamous cell carcinomas (SCCs) in dogs (Munday and Kiupel, 2010).

Oral cancer is common in dogs with SCCs being the second most common neoplasm at this site (Head et al., 2002). While no cause of canine oral SCCs has been identified thus far, up to 25% of human oral SCCs are thought to be caused by PV infection (Fakhry et al., 2008). Recently, a case of a dog that developed multiple oral SCCs that contained a novel PV was reported (Munday et al., 2015b). Evidence suggested that the PV could have influenced the development of the oral SCCs in this dog and the aim of the present study was to amplify the entire genome of this novel potentially oncogenic PV.

## 2. Materials and methods

### 2.1. Initial case summary and sample definition

A 7-year-old intact male Labrador dog presented at a veterinary clinic due to excessive salivation. Examination of the oral cavity

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revealed a 1.5 cm diameter exophytic mass and 7 smaller raised, pale, roughened plaques within the right buccal gingiva. Radiology revealed osteolysis of the underlying maxilla and histology was used to confirm a diagnosis of multiple SCC and in situ carcinomas. A sample of gingiva that contained a raised pale plaque was surgically excised with half of the plaque fixed in formalin for histological evaluation and the other half frozen for DNA extraction.

Histological examination of the plaque revealed a proliferation of neoplastic epithelial cells that contained PV cytopathic changes that were characterised by increased blue–grey cytoplasm and swollen eosinophilic nuclei. Immunohistochemistry to detect p16<sup>CDKN2A</sup> protein (p16) was performed as previously described (Munday et al., 2011) and revealed intense intranuclear and intracytoplasmic immunostaining within the neoplastic cells. These tests confirmed that the DNA used for sequencing was from an oral SCC that contained histological evidence of PV infection.

## 2.2. PCR and DNA sequencing

DNA was extracted from the frozen half of the gingival SCC using a High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The MY09/11 consensus primers were then used to amplify PV DNA from the extracted DNA and the amplicon was sequenced as previously described (Waropastrakul et al., 2012).

Two 'back-to-back' primers (CPV17inv1 5'-GCTCGACTCTTCAA-CATGG and CPV17inv2 5'-CTACATAGAGTCACTTGCAACC) were

designed on the basis of the 450 bp PV sequence obtained by MY09/11 PCR. The whole circular viral genome was then amplified by inverse PCR using a GoTaq long range PCR kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. An Illumina sequencing library was prepared from the resulting PCR product by first shearing the DNA into 300–400 bp fragments. Illumina adapters containing sequencing primer recognition sites were then annealed to each DNA fragment using the Illumina TruSeq DNA sample preparation v2 protocol (Illumina Inc., San Diego, CA). Paired-end 2 × 150 bp sequencing of the DNA library was then performed on an Illumina MiSeq sequencer. To assemble the viral genome, around 200,000 reads were assembled into a single contiguous sequence using Geneious version 8.04 software (Drummond et al., 2010).

## 2.3. DNA and protein sequence analysis

The putative coding regions in the PV sequence were predicted using FGENESV0 (<http://linux1.softberry.com>). The characteristics of the putative viral proteins, the presence of conserved protein domains and motifs, and the presence of regulatory sequences were predicted using Geneious version R8.1.6 software including third party plugins (InterProScan).

## 2.4. Phylogenetic analysis

Complete genomes of 67 PV representative species from each of the currently recognised genera (Bernard et al., 2010) were

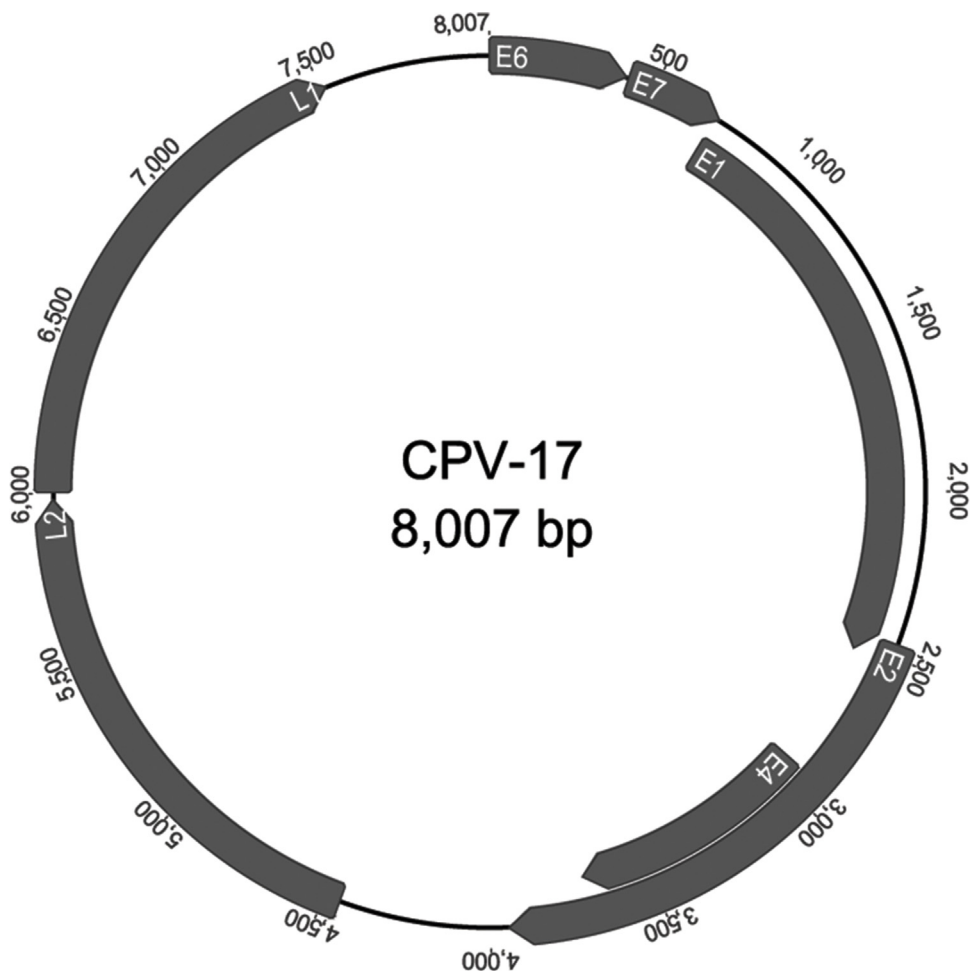


Fig. 1. Schematic genomic organisation of the proposed CPV-17.

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