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# Genomic characterisation of the feline sarcoid-associated papillomavirus and proposed classification as *Bos taurus* papillomavirus type 14

John S. Munday [a,*], Neroli Thomson [a], Magda Dunowska [a], Cameron G. Knight [b], Rebecca E. Laurie [c], Simon Hills [a]

[a] College of Science, Massey University, Palmerston North, New Zealand
[b] Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Canada
[c] Otago Genomics and Bioinformatics Facility, Otago University, Dunedin, New Zealand

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ABSTRACT

Feline sarcoids are rare mesenchymal neoplasms of domestic and exotic cats. Previous studies have consistently detected short DNA sequences from a papillomavirus (PV), designated feline sarcoid-associated papillomavirus (FeSarPV), in these neoplasms. The FeSarPV sequence has never been detected in any non-sarcoid sample from cats but has been amplified from the skin of cattle suggesting that feline sarcoids are caused by cross-species infection by a bovine papillomavirus (BPV). The aim of the present study was to determine the genome of the PV that contains the FeSarPV sequence. Using the circular nature of PV DNA, four specifically designed 'outward facing' primers were used to amplify two approximately 4,000 bp DNA segments from a feline sarcoid. The two PCR products were sequenced using next generation sequencing and the full genome of the PV, consisting 7,966 bp, was assembled and analysed. Phylogenetic analysis revealed the PV was closely related to the species 4 delta BPVs-1, -2, and -13, but distantly related to any carnivoran PV genus. These results are consistent with feline sarcoids being caused by a BPV type and we propose a classification of BPV-14 for this novel PV. Initial analysis suggests that, like other delta BPVs, the BPV-14 E5 protein could cause mesenchymal proliferation by binding to the platelet derived growth factor beta receptor. Interestingly BPV-14 has not been detected in any equine sarcoid suggesting that BPV-14 has a host range that is limited to bovids and felids.



## 1. Introduction

Papillomaviruses (PVs) are small circular double-stranded DNA viruses. As PVs can influence cell growth and differentiation, some are important causes of neoplasia. Papillomaviruses are classified into genera based on the sequence of the highly conserved *ORF L1* (Bernard et al., 2010). While the overwhelming majority of PVs only infect epithelium and are highly host specific (Bernard et al., 2010; Joh et al., 2011), the bovine papillomaviruses (BPVs) of the *Deltapapillomavirus* genus have the ability to infect both epithelial and mesenchymal cells and to infect multiple species (Munday, 2014).

Compared to most species PV-induced disease is currently thought to be rare in cats. Diseases that are recognised to be caused by PVs in domestic cats include

* Corresponding author at: Pathobiology, IVABS, Massey University, Palmerston North, New Zealand. Tel.: +64 6 356 9099; fax: +64 6 350 5714.
*E-mail address:* j.munday@massey.ac.nz (J.S. Munday).

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oral papillomas due to *Felis catus* (Fca) PV-1 (Munday et al., 2015), feline cutaneous viral plaques and Bowenoid in situ carcinomas due to infection with FcaPV-2 and FcaPV-3 (Lange et al., 2009; Munday et al., 2013, 2008), and feline sarcoids. Feline sarcoids are rare mesenchymal neoplasms that most commonly develop around the face and digits of younger domestic cats (Schulman et al., 2001), but have also been reported in mountain and African lions (Orbell et al., 2011; Schulman et al., 2003). While metastasis has not been reported, these neoplasms are progressive and can result in euthanasia due to recurrence following surgical excision. The same PV DNA sequence, designated the feline sarcoid-associated PV sequence (FeSarPV), has been consistently detected in multiple studies of sarcoids from domestic cats and exotic felids (Munday et al., 2010; Orbell et al., 2011; Teifke et al., 2003). As the FeSarPV sequence only comprises a short segment of the PV L1 gene, classification of this PV has not been possible. However, as FeSarPV cannot be amplified from any non-sarcoid feline sample (Munday et al., 2010) but can be amplified from samples of normal skin and fibropapillomas of cattle (da Silva et al., 2012; Munday and Knight, 2010) it appears likely that, similar to equine sarcoids, feline sarcoids are due to cross-species infection by a BPV type (Orbell et al., 2011; Schulman et al., 2001). The aim of the present study was to amplify the entire genome of the PV that contains the FeSarPV sequence. Classification and phylogenetic analysis of this PV could then be used to support a bovine definitive host.

## 2. Materials and methods

### 2.1. Initial case summary and sample collection

A young adult domestic short-haired cat was observed to have a 5 mm diameter cone-shaped mass growing dorsally from the bridge of the nose close to the junction with the nasal planum. The cat was one of 20 cats that lived in a dairy barn in New York State, USA. An intralesional injection of cisplatin was given, but the mass grew rapidly over the next few weeks (Fig. 1) and was surgically excised. Histology revealed a proliferation of dermal mesenchymal cells covered by hyperplastic epithelium and a diagnosis of feline sarcoid was made. DNA extracted from an unfixed portion of the excised mass was used for the majority of the techniques reported herein. Sarcoid regrowth was observed shortly after surgery and 5% imiquimod cream was applied to the mass every two days for two weeks. The sarcoid continued to grow slowly and an additional surgery was performed 12 months later to debulk the mass. Neoplasm recurrence was observed shortly after this surgery and euthanasia was performed 2 months later when the sarcoid began to interfere with eating.

### 2.2. PCR and DNA sequencing

DNA was extracted from the unfixed feline sarcoid using a DNeasy tissue kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions.

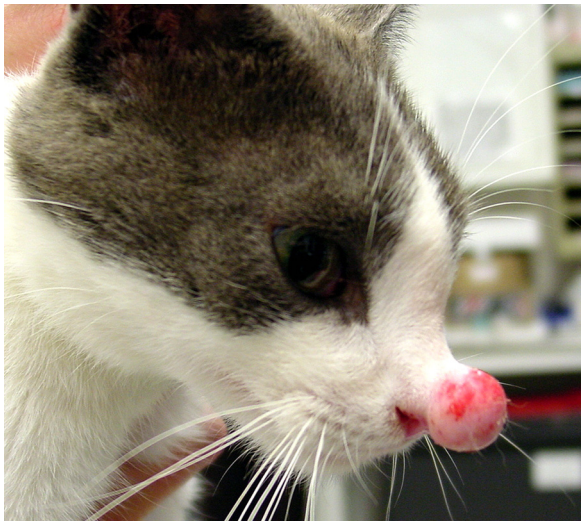

**Fig. 1.** Feline sarcoid visible as a protruding nasal mass. Photo courtesy of Dr. William Miller, Cornell University College of Veterinary Medicine, Ithaca, New York.

Amplification of PV sequences from the extracted DNA was done using the FAP59/65 and MY09/11 consensus primers and the jmpSA primers that had been specifically designed to amplify the FeSarPV sequence (Munday et al., 2010).

To amplify the full genomic sequence of the novel virus, four 'outward facing' primers were designed using the known 195 bp FeSarPV *L1* sequence and a previously reported 176 bp section of *E1* (Schulman et al., 2001). The entire circular genome was then amplified in two large sections that extended from *L1* to *E1* (BPV14F1 5′-GGAACAAACCTCACAATCAC and BPV14R1 5′-CCTCCAGCAAGCATGGGTAG, 4,000 bp) and *E1* to *L1* (BPV14F2 5′-CCTCAGTATTAGAGAACTGG and BPV14R2 5′-TCTAGGGGGCTGTGTGCTAT, 3,700 bp). Amplification was done using a GoTaq long range PCR kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions with the DNA extracted from the feline sarcoid as a template. An Illumina sequencing library was prepared from the resulting PCR products 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 $2 \times 150$ 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). Two areas of ambiguity within the NGS assembly were confirmed by sequencing the products of specific primers that were designed to amplify approximately 500 bp sections of the *L1* (BPV14L1F 5′-CCTCCAACACCTGTGTCTAAGG and BPV14L1R 5′-ATCAGTTACACAAGGACGTGC) and *E2* (BPV15E2F 5′-CGCTGGACTTGGAACGAGG and BPV14E2R 5′-GATACGCATTTAGAAGGGAAG) genes using DNA extracted from the feline sarcoid as a template.