



# Novel canine papillomavirus type 18 found in pigmented plaques

Christian E. Lange<sup>a,\*</sup>, Alos Diallo<sup>b</sup>, Christine Zewe<sup>c</sup>, Lluís Ferrer<sup>c</sup>

<sup>a</sup> Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA

<sup>b</sup> Immunology Division, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA

<sup>c</sup> Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts, USA



## ARTICLE INFO

### Article history:

Received 19 February 2016

Received in revised form

29 August 2016

Accepted 30 August 2016

Available online 31 August 2016

A variety of neoplasias of the skin and mucous membranes in humans and animals can be ascribed to papillomavirus infections. Some of these papillomavirus induced neoplasias can undergo malignant transformation, while most remain benign. Papillomavirus infections are often even asymptomatic. Therefore the understanding of these viruses is important for appropriate prevention, diagnostic and therapeutic approaches. The entire genomic sequence of a new canine papillomavirus was determined from DNA detected in pigmented plaques in a pug. The novel canine papillomavirus type 18 (CPV18) falls into the genus *Chipapillomavirus* whose members have all been isolated from dogs with pigmented plaques, some of them also with squamous cell carcinomas. A small partial sequence of a 2009 isolate from a dog in California is identical with CPV18.

Papillomaviruses (PVs) are non-enveloped, double-stranded DNA viruses with a circular genome of about 8,000 base pairs (bp). They are generally host species-specific with some exceptions. Numerous of the known PVs are associated with benign and malignant neoplasias of the skin and mucous membranes in humans and animals, but there is evidence, that asymptomatic infections are more common [1,9,14]. More than 200 human and 140 animal PVs have been characterized, illustrating broad genetic diversity (<http://pave.niaid.nih.gov/>) [2,6,9]. The identification and study of new animal PVs are important because new PVs are potential pathogens in veterinary medicine, some animal PVs may serve as models for human disease and tools for basic biology, and the analysis of animal PVs helps provide insights into PV evolution. Canine PVs (CPVs) have been found associated with classical exophytic papillomas such as the common canine oral papillomatosis, with endophytic papillomas, with pigmented plaques and in rare

cases with squamous cell carcinomas (Table 1) [13]. Pigmented plaques are most often seen in pugs where CPV4 seems to be the most prevalent type involved [17,18,22,23,26]. Overall seventeen complete genomes and several partial sequences of different CPVs have been communicated. The different CPVs belong to either of three different papillomavirus genera *Lambda*, *Tau* or *Chi* [2,5,11,15–23,25,26,28–31]. These three genera are not monophyletic and hence only distantly related. The aim of this study was the analysis and description of a new potentially pathogenic CPV.

A 3 year old, female spayed pug was presented to Tufts Cummings Veterinary Medical Center due to the development of multiple pigmented plaques on the medioventral abdomen and inner thigh (Fig. 1). The dog was the only pet in the household but had frequent contact with other dogs. After thorough physical examination two 8 mm punch biopsies for histopathological analysis and a cytobrush sample for microbiological assessment were taken from the lesions. The skin biopsies were fixed in 4% buffered formalin. After embedding in paraffin, 4 µm sections were stained with haematoxylin and eosin (HE) for histopathological examination. The cytobrush was moistened with sterile 0.9% NaCl solution, rubbed for thirty seconds on the affected area and stored in an 1.5 ml Eppendorf tube at –20 °C until DNA extraction.

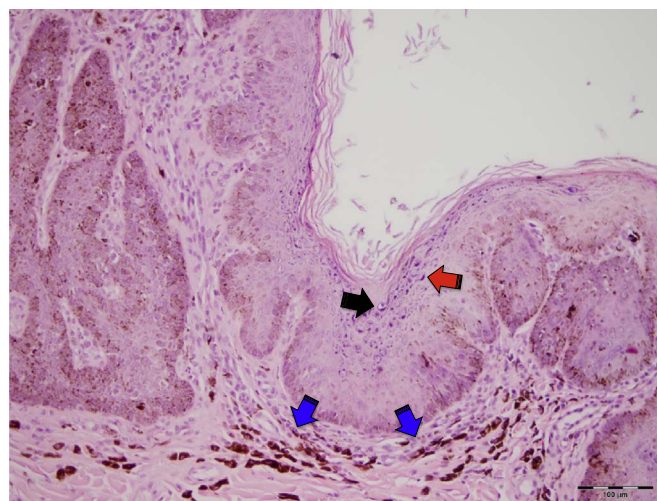
Total DNA was isolated from the cytobrush sample using a Qiagen DNeasy Blood and Tissue kit. The extract was tested for the presence of host and papillomavirus DNA by PCR using the primer combinations dogGAPDHf/dogGAPDHr, canPVf/FAP64 and CP4/CP5 [10,12,14]. Circular DNA was amplified from the total DNA extract by rolling circle amplification (RCA) using a TempliPhi Amplification kit. The RCA product was digested and entirely cloned into the *SpeI* site of a pBluescript II KS+ vector [17]. The sequences of the pure and uncloned RCA product and the genomic clones were determined independently, and the primary sequences assembled in DNA Star (DNASTAR, Inc.). Comparison of

\* Corresponding author.

E-mail address: [clange\\_virology@gmx.de](mailto:clange_virology@gmx.de) (C.E. Lange).

**Table 1**  
List of carnivore papillomaviruses and associated lesions.

Virus	Genus	Host	Associated lesions reported	Genebank ID
CPV1	Lambda	Dog	Oral papillomatosis, endophytic papillomas, exophytic papillomas, squamous cell carcinoma	D55633
CPV6	Lambda	Dog	Endophytic papillomas	FJ492744
CcrPV1	Lambda	Spotted Hyena	Oral papillomatosis	HQ585856
FcaPV1	Lambda	Cat	Exophytic papillomas	AY057109
EIPV1	Lambda	Sea Otter	Oral papillomatosis	KJ410351
LrPV1	Lambda	Bobcat	Oral papillomatosis	AY904722
PcPV1	Lambda	Puma	Oral papillomatosis	AY904723
PIPV1	Lambda	Raccoon	Exophytic papillomas	AY763115
PIpPV1	Lambda	Asiatic Lion	Oral papillomatosis	AY904724
UuPV1	Lambda	Snow Leopard	Oral papillomatosis	DQ180494
CPV2	Tau	Dog	Endophytic papillomas, exophytic papillomas, squamous cell carcinoma	AY722648
CPV7	Tau	Dog	Exophytic papillomas, squamous cell carcinoma	FJ492742
CPV13	Tau	Dog	Oral papillomatosis	JX141478
CPV17	Tau	Dog	Oral squamous cell carcinoma	KT272399
FcaPV3	Tau	Cat	Bowenoid in situ carcinoma	JX972168
FcaPV4	Tau	Cat	Gingivitis	KF147892
MpPV1	Tau	European Polecat	No lesion	KF006988
CPV3	Chi	Dog	Pigmented plaques, squamous cell carcinoma	DQ295066
CPV4	Chi	Dog	Pigmented plaques	EF584537
CPV5	Chi	Dog	Pigmented plaques	FJ492743
CPV8	Chi	Dog	Pigmented plaques	HQ262436
CPV9	Chi	Dog	Pigmented plaques	JF800656
CPV10	Chi	Dog	Pigmented plaques	JF800657
CPV11	Chi	Dog	Pigmented plaques	JF800658
CPV12	Chi	Dog	Pigmented plaques	JQ754321
CPV14	Chi	Dog	Pigmented plaques	JQ701802
CPV15	Chi	Dog	No details reported	JX899359
CPV16	Chi	Dog	Pigmented plaques, squamous cell carcinoma	KP099966
CPV18	Chi	Dog	Pigmented plaques	KT326919
UmPV1	Omega	Polar Bear	Oral papillomatosis	EF536349
FcaPV2	Dyotheta	Cat	Bowenoid in situ carcinoma	EU796884
VvPV1	Treiseta	Fox	No lesion	KF857586
ZcPV1	Dyonu	Sea lion	Exophytic papillomas	HQ293213



**Fig. 2.** Histologic section of one of the pigmented plaques. Epidermal hyperplasia, with melanosis, large keratohyalin granules (red arrow), as well as a few single scattered clear cells reminiscent of koilocytes (black arrow). The superficial dermis presented a mild inflammatory infiltrate and pigmentary incontinence (blue arrows).

(ORFs) of the obtained sequence using the Needleman-Wunsch algorithm. A phylogenetic tree for 80 PVs including the sequences of the CPVs as well as representatives of all thus far characterized PV genera was calculated (Supplement 1). For that purpose the predicted amino acid sequences of E1, E2, L2 and L1 were translated and aligned by using MAFFT before being back-translated to DNA sequences [17]. GBLOCK (version 0.91b) was used to remove regions of poor similarity and the resulting shortened sequences were combined into a single multiple sequence alignment by concatenating the sequences from each virus [3]. The optimal model of DNA evolution was evaluated for best fit of the dataset using MODELTEST (version 1.4.4); using the default settings [4,8]. Bayesian phylogeny of the E1-E2-L2-L1 concatenate was inferred using MRBAYES (version 3.2); Markov Chain Monte Carlo with GTR substitution matrix, variable gamma rates, invariant sites, two runs, four chains of 10 000 000 generations [26]. FcPV1 was defined as outgroup, to root the tree. Trees were sampled after every 1000 steps during the process to monitor phylogenetic convergence. The average standard deviation of split frequencies was below 0.0053 (MrBayes recommended final average < 0.01). This was performed on the Orchestra High Performance Compute Cluster at Harvard Medical School. The first 10% of the trees were discarded and the remaining ones combined using TreeAnnotator (version 1.8.2; <http://beast.bio.ed.ac.uk>) and displayed with FIG-TREE (1.4.2; <http://tree.bio.ed.ac.uk/>) [24,25]. The code to run MrBayes as well as the files produced during this analysis are stored on GitHub: [https://github.com/alosdiallo/Phylogeny\\_model\\_papillomavirus](https://github.com/alosdiallo/Phylogeny_model_papillomavirus).

The histological examination revealed severe papillated epidermal hyperplasia, with melanosis, large clumped keratohyalin granules as well as a few single scattered clear cells reminiscent of koilocytes. The superficial dermis presented a mild inflammatory infiltrate and pigmentary incontinence (Fig. 2). The lesions were classified as canine viral pigmented plaques without signs of malignant transformation.

No differences between RCA product and clone were found upon comparison of the sequencing results. The circular sequence of the novel isolate consists of 7810 base pairs, which falls into the typical size range of Chi-PVs and has a GC content of 51% (Supplement 2) [11]. It contains the PV typical early (E1, E2, E4, E6, E7) and late (L1, L2) ORFs as well as non-coding region one between L1 and E6 (NCR1, 565 bp) and a second one between E2 and L2 (NCR2,



**Fig. 1.** Three year old female pug with numerous coalescing pigmented plaques and exophytic nodules (from 0.5 cm to 3 cm) on the medioventral abdomen and inner thighs.

RCA sequence and clone sequence ensured correctness and completeness especially around the *Spel* cloning site. Pairwise sequence alignments were performed with the open reading frames

Download English Version:

<https://daneshyari.com/en/article/4372302>

Download Persian Version:

<https://daneshyari.com/article/4372302>

[Daneshyari.com](https://daneshyari.com)