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PAPILLOMA VIRUS INFECTION IN DOGS: A REVIEW

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DEDICATION

This thesis is especially dedicated to

My loving parents

Teh Chon Howi and Soh Kwee Hiang

My supportive supervisors

Dr. Ong Siew Mei

Dr. Norfitriah Mohamed Sohaimi

And

DVM 2022 Course mates

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JANGKITAN PAPILLOMAVIRUS PADA ANJING: ULASAN

Oleh

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2021

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Papillomavirus menjangkiti pelbagai spesies dan biasanya menghasilkan lesi epidermis dan mukosa jinak yang bersifat sementara. Jangkitan papillomavirus anjing (CPV) biasanya dikaitkan dengan papilloma mulut, papilloma kulit dan plak berpigmen. Terdapat bukti yang semakin meningkat bahawa jenis CPV tertentu dikaitkan dengan lesi neoplastik seperti karsinoma sel skuamosa (SCC) dan karsinoma sel basal (BCC), walau bagaimanapun, perkaitan CPV dengan neoplasma dan mekanisme asas bagi menggalakkan tumourigenesis masih kabur. Dalam kajian ini, kitaran hayat dan patogenesis CPV, termasuk penyakit dan kemungkinan mekanisme onkogenik yang terlibat telah dikaji semula. Diagnosis, rawatan dan kaedah pencegahan yang mungkin juga dibincangkan secara ringkas dalam kajian ini.

Kata Kunci: Anjing; Papillomavirus; Transformasi malignan; Ulasan

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

PAPILLOMAVIRUS INFECTION IN DOGS: A REVIEW

by

Teh Zheng Chen

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Supervisor: Dr. Ong Siew Mei

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Papillomaviruses infect various host species and commonly produce benign epidermal and mucosal lesions that are transient. Canine papillomavirus (CPV) infection in dogs is usually associated with oral papillomas, cutaneous papillomas and pigmented plaques. There are increasing evidences that certain CPV types are associated with neoplastic lesions such as squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), however, the association of CPV with neoplasms and the underlying mechanisms of promoting tumourigenesis are still vague. In this review, the life-cycle and pathogenesis of CPVs, including the diseases and possible oncogenic mechanisms involved are reviewed. The diagnosis, treatment and possible prevention methods are also briefly discussed.

Keywords: Canine; Papillomavirus; Malignant transformation; Review

1.0 INTRODUCTION

Papillomaviruses are small, double-stranded circular DNA viruses with their DNA containing about 8000 base pairs enclosed in a 50- to 55- nm non-enveloped icosahedral capsid (Lange *et al.*, 2013; Munday & Thomson, 2021). Papillomaviruses mainly infect the epithelium, targeting keratinocytes and mucous membranes inducing proliferative diseases. The genome consists of five or six early (E) genes, namely E1, E2, E4, E5, E6, and E7 as well as two late (L) genes, namely L1 and L2. The expression of these genes is vital for the complete life-cycle of the virus (Lange & Favrot, 2011). Papillomaviruses have been found to infect a wide range of host species with a majority of the established papillomavirus types being in humans. In humans, the first papillomavirus to be fully sequenced is in 1982 although the viral cause of warts was first confirmed in 1907. To date, there are about 220 types of human papillomavirus (HPV) listed as Reference Genomes for the human papillomaviruses database in the Papillomavirus Episteme (data accessed on 27 Sept 2021 at https://pave.niaid.nih.gov/). The rapid discovery and establishment of HPV types compared to the non-human species are due to the fact that some HPV types are proven to play a role in tumourigenesis with the first evidence of papillomavirus demonstration from cervical cancer (Munday *et al.*, 2017). As of now, some other examples of HPV-associated disease include high-risk HPV-5 and HPV-8 that cause the development of epidermodysplasia verruciformis (EV) as well as high-risk HPV-16 and HPV-18 that cause cervical cancer (Munger *et al.*, 2004). Other non-human species that can be infected by papillomaviruses include mammals, birds, and reptiles. To date, papillomavirus infections in amphibians is yet to be reported (Rector & Van Ranst,

2013). These infections are strictly species-specific with the exception of the bovine papillomaviruses (BPV) such as BPV-1 and BPV-2 which could infect horses causing equine sarcoids and experimentally infect rodents, while BPV-14 has been associated with feline sarcoids in cats (Lange & Favrot, 2011; Munday *et al.*, 2017).

In dogs, the discovery of papillomavirus infection started with the appearance of transmissible warts in 1898 and was confirmed a viral aetiology only in 1959. The first canine papillomavirus (CPV) was fully sequenced in 1994 (Nicholls & Stanley, 1999; Munday *et al.*, 2017). Up to now, 23 types of CPV have been fully sequenced and further allocated into three different genera namely the *Lambdapapillomavirus*, *Chipapillomavirus*, and *Taupapillomavirus*. CPV-1 and 6 are classified as *Lambdapapillomavirus*; CPV-2, 7, 13, 17, 19, 21, 22, and 23 are classified as *Taupapillomavirus*; while CPV-3, 4, 5, 8, 9, 10, 11, 12, 14, 15, 16 18, and 20 are classified as *Chipapillomavirus* (Alves *et al.*, 2020). The classification of papillomavirus into the different types and genera is based on the highly conserved L1 gene. When two papillomaviruses have more than 10% difference to the L1 nucleotide sequence, they will be classified into different types; and when there is more than 40% difference to the L1 nucleotide sequence, they will be classified as different genera (Bernard *et al.*, 2010). Papillomavirus infection in dogs is usually sub-clinical and the lesions produced by the virus are able to regress spontaneously without treatment within 1 to 3 months. However, one study showed that the regression time can take up to 3 months in some dogs (5/16) (Sancak *et al.*, 2014). Immunocompetent and clinically healthy dogs may harbour papillomaviruses on their skin and oral cavity, acting as a potential reservoir for the virus

(Lange *et al.*, 2011). Unlike in humans, the role of CPV infection progressing to malignant lesions is still not elucidated. However, it has been shown that dogs with X-linked severe combined immunodeficiency (X-SCID) that underwent bone marrow transplant developed severe papillomavirus infection which later progressed into metastatic squamous cell carcinomas (Goldschmidt *et al.*, 2006). This finding supports the importance of immune response in the regression of papillomavirus infection and also supports the possibility of CPV acting as a co-factor in the development of cancer. To have a better understanding of CPV infection, this review will briefly outline the pathogenesis, clinical presentation, diagnosis as well as the treatment and prevention in dogs.

2.0 PATHOGENESIS

The transmission of papillomavirus to susceptible dogs can occur by direct cutaneous or mucosal contact with papillomavirus-induced lesions on infected dogs or contact with environmental fomites that can harbour the virus. Therefore, CPV is highly contagious and a concentrated population of animal places such as animal shelters or dog boarding centers has a high risk of papillomavirus outbreak. Besides, clinically healthy dogs may also carry the virus and act as a source of infection to susceptible dogs as the virus is able to remain latent and inactive at the superficial skin layers for months and possibly years before eliciting clinical diseases (Lange *et al.*, 2011; Rosa, 2016). Examples of lesions induced by papillomavirus infection in dogs include viral plaques, endophytic papillomas, and oral papillomas (Munday & Kiupel, 2009). In order to establish an infection, the papillomavirus on the superficial skin layers or the fomites must infect the basal epidermis layer thus requiring injury sites or micro-abrasion of the skin. The cellular tropism of papillomavirus towards basal cells is because these cells have high cellular division and differentiation ability that is crucial for the complete life-cycle and persistent infection of the virus (Lange & Favrot, 2011; Lane *et al.*, 2017).

In humans, many studies have been done on the life cycle of the high-risk HPVs has been extensively researched to determine their mechanism of oncogenesis. The expression of E5, E6, and E7 genes in high-risk HPVs have been found to act as oncoproteins that play the main role in the malignant transformation of lesions. The E5 oncoproteins protect the cell from apoptosis by inhibiting death receptor-mediated

apoptosis and endoplasmic reticulum stress-induced apoptosis whereas E6 oncoproteins prevent apoptosis by targeting the p53 tumour suppressor gene and also by inducing other p53-independent transforming activities. Meanwhile, E7 oncoproteins target retinoblastoma tumour suppressor protein (pRb) and are necessary for viral pathogenesis and cellular transformation (Jiang & Yue, 2013). In addition, the integration of the viral genome into the host cell genome is a key event that increases the stability of E6 and E7 mRNA expression, providing a selective growth advantage to the cells and therefore increasing the chance of malignant progression. There are also other mechanisms, pathways, and oncoprotein interactions that have been associated with tumourigenesis in high-risk HPVs such as induction of telomerase activity or activation of the platelet-derived growth factor β receptor (DiMaio & Mattoon, 2001; Munger *et al.*, 2004). On the other hand, E6 and E7 protein expressions in low-risk HPVs have significantly lower cellular transforming ability and do not induce genomic instability but are still critical for the viral life-cycle (Munger *et al.*, 2004). Unlike in HPVs, some CPVs lack the E5 genes and the role of E5 genes in the life-cycle of CPVs is not fully understood.

The general life-cycle of papillomavirus involves several phases namely, entry and uncoating of the virions to infect the basal epidermal cells, viral genome maintenance, proliferative phase, genome amplification, and finally virus synthesis. After successfully infecting the basal epidermal cells, the incubation period of CPV is thought to be at least 4 weeks. During that period, the viral DNA replicates and undergoes the genome maintenance phase where the expression of E1 and E2 proteins at low levels occur. This phase aims to maintain the viral DNA as a low copy number episome of about 10 to 200

copies per basal cell in order to facilitate the accurate segregation of genomes during cell replication in the next phase and also to ensure persistent infection of basal cells. E6 and E7 proteins are also expressed at low levels however their functions in this phase are still uncertain (Doorbar, 2005; Lange & Favrot, 2011; Syrjänen, 2018).

In normal epithelium, as the basal cells undergo cell division and slowly migrate superficially, terminal differentiation of the cells would occur to form cornified keratinocytes and induce lipid secretion which together forms the physical barrier on skin layers. In papillomavirus-infected basal cells, the proliferative phase induces expression of E6 and E7 proteins causing stimulation of cell cycle progression and interference with the apoptosis of terminal cells (Doorbar, 2005). Consequently, non-scheduled replication of the infected cells occurs causing disruption to normal epithelial structural appearance such as hyperkeratosis and acanthosis leading to lesion formation (Lange & Favrot, 2011). Subsequently, genome amplification and virus synthesis occur as the papillomavirus life cycle approaches the late phase. Genome amplification begins in the proliferative compartments which are mainly the granular layers of epithelium and occurs with the additional expression of E4 and E5 gene proteins (Lazarczyk *et al.*, 2009). In this phase, expression of the E4 protein is crucial as it contributes to efficient genome amplification in which the precise mechanism is yet to be clearly reported (Doorbar, 2013). The aim of this phase is to produce infectious virions to be released and infect other hosts. After completion of genome amplification, virus synthesis begins with the expression of the L2 protein which is then followed by the L1 protein (Lazarczyk *et al.*, 2009). L2 and L1 are also known as the minor coat protein and major coat protein respectively. This phase aims

to assemble virion materials and facilitate packaging to be released to the environment (Lange *et al.*, 2013). As papillomaviruses are not lytic viruses, they are released to the environment through the death of keratinised squamous cells (Lange & Favrot, 2011). Additionally, Yajid *et al.* suggested that expression of the E4 gene can cause disruption of keratin organisation, facilitating virus release. Table 1 summarizes the phases of papillomavirus life-cycle while Table 2 shows the functions of CPV genes.

Papillomavirus infection in immunocompetent dogs if left untreated usually resolves spontaneously within a time range of 1 month to 12 months (Williams *et al.*, 2021). Infections were cleared by cell-mediated immunity and usually start late as the target cells are superficial, terminally differentiating cells that locate remotely from the systemic immune routes (Kuntsi-Vaattovaara *et al.*, 2003). Humoral immunity acts as a secondary immune response that protects the host from reinfection by the same type of papillomavirus (Sancak *et al.*, 2014).

In dogs, there are limited studies on the oncogenic mechanisms of CPV because the occurrence of CPV-associated neoplasia such as squamous cell carcinoma (SCC), is rare. Cutaneous SCCs, representing 5% of skin tumours in dogs, are most commonly found at the ventrum and are always associated with sunlight or ultraviolet (UV) light exposure. Oral SCCs are the second most common canine oral neoplasms however there is no exact cause for the occurrence of oral SCCs (Munday *et al.*, 2015; Sabattini *et al.*, 2016; Waropastrakul *et al.*, 2012). Nevertheless, the increasing reports and studies on malignant transformation of CPV-associated benign lesions have raised speculations that

certain CPV types can be a co-factor or even have a direct causal relationship with the development of malignant lesions (Munday *et al.*, 2016; Reis *et al.*, 2019). Alves et al. reported the identification of E5, E6, and E7 oncoprotein genes within the genome of CPV-16 suggesting that CPV-16, similar to high-risk HPVs, may have a high potential of tumourigenesis. A recent study showed that the E6 protein of CPV-2 does not degrade p53 tumour suppressor protein (Quinlan *et al.*, 2021) while another study demonstrated that E2 protein of CPV-9 might play a role in malignant transformation (Chang *et al.*, 2020b). Current evidences suggest that the oncogenic mechanisms vary among various CPV types and do not resemble those of high-risk HPVs. Therefore, further investigation on the oncogenic mechanisms of different CPV types is necessary to unravel the role of CPV in tumourigenesis.

Table 1. Summary of papillomavirus life-cycle

Life-cycle phase	Epithelium layer	Host-virus interaction	Gene expressions
Infection	Basal	Virus gains access to basal cells due to microabrasion	-
Genome maintenance	Basal and suprabasal	Viral DNA maintain as low number episome	E1 and E2
Proliferative	Suprabasal	Non-scheduled cellular proliferation and differentiation	E6 and E7
Genome amplification	Granular	Production of infectious virions	All E genes
Virus synthesis	Squamous	Virion assembly and packaging	L1 and L2
Virus release	Keratinised squamous	Virus release to environment by cellular death	E4

The information was collected from previous publications (Regalado Ibarra et al., 2018; Doorbar, 2005; Lazarczyk *et al.*, 2009)

Table 2. Genes of CPV and their viral protein function

Genes	Protein function
E1	Mediates episomal viral DNA replication as low number copies
E2	Regulates episomal viral DNA replication and facilitates segregation of viral genome during basal cell division
E4	For efficient genome amplification and facilitate virion release from keratinised squamous epithelial cells
E5	Viral genome amplification; Involves in malignant transformation in high-risk PVs
E6	Targets several host-cell proteins (e.g. inhibits apoptosis by degradation of p53, induces malignant transformation in high-risk PVs)
E7	Stimulates cell cycle progression by binding pRb; induces malignant transformation together with E6 in high-risk PVs
L1	Major coat protein; allow assembly of infectious particles
L2	Minor coat protein; enhance packaging of virions

The information was collected from previous publications (Doorbar, 2005; Doorbar, 2013; Lazarczyk *et al.*, 2009; Syrjänen, 2018; Yajid et al., 2017)

3.0 CANINE PAPILLOMAVIRUS-ASSOCIATED BENIGN LESIONS

CPV infection causes various clinical manifestations in dogs. These include oral and cutaneous papillomas, and pigmented viral plaques (Munday *et al.*, 2017). Table 3 summarizes the clinical symptoms associated with the different CPVs. Healthy dogs can be asymptomatically infected by papillomavirus and only develop visible lesions when they are affected by various risk factors which promote papillomavirus replication. The risk factors include genetic predisposition, acquired immunosuppression, or exposure to external stimuli like UV light or chemicals (Lange *et al.*, 2013).

3.1 Canine Oral Papillomas

Oral papillomas are characterised by benign exophytic growth in the oral mucosa that has a cauliflower-like appearance and is occasionally fringed- or nodular-shaped. The lesions can be observed within the oral cavity, which may involve the tongue or oesophagus, on the lips, and mucocutaneous junction (Lange & Favrot, 2011). CPV-1, which was formerly referred to as canine oral papillomavirus (COPV), is the most prevalent and common type of CPV in causing oral papillomas, especially in juvenile dogs (Munday *et al.*, 2017; Reis *et al.*, 2019). In some instances, CPV-1 can infect haired skin causing exophytic cutaneous lesions (Alcântara *et al.*, 2014; Reis *et al.*, 2019) and inverted papillomas (Lange *et al.*, 2009). Other than CPV-1, other types of CPV including CPV-2, 13, 17, and 19 have also been detected in canine oral papillomatous lesions (Lange *et al.*, 2012; Munday *et al.*, 2016; Tisza *et al.*, 2016). Most evidences indicated that CPV-2 has higher occurrence and stronger association with endophytic papillomas

(Lange *et al.*, 2019; Lange & Favrot, 2011). Symptomatic CPV infection of canine oral papillomatosis usually regresses spontaneously with the aid of cell-mediated immunity particularly the lymphocytes (Porcellato *et al.*, 2014). However, it is possible for the infection to persist and progress to oral SCC (Regalado Ibarra *et al.*, 2018).

3.2 Canine Cutaneous Papillomas

Cutaneous papillomas can be histologically subdivided into exophytic and endophytic papillomas. Exophytic papillomas, also known as warts, are characterised by hyperplastic reaction of the epithelium with increased production of keratin causing protrusion of the folded epidermis above the skin surface (Bianchi *et al.*, 2012). Endophytic papillomas, also known as inverted papillomas, are characterised by downward growth of the skin where papillary extensions of the epidermis project into the dermis resulting in raised and smooth nodules with a keratin-filled central pore (Lange & Favrot, 2011). Cutaneous papillomas can be presented as single or multiple nodules with lesions commonly found at the ventral abdomen, inguinal region, and footpads (Orlandi *et al.*, 2021). Cutaneous papillomas are often associated with CPV-2, 6, and 7 and are commonly seen in young dogs (Munday *et al.*, 2017). The lesions frequently contain papillomavirus DNA (96%) and L1 protein (92%) with CPV-2 dominating in the endophytic papillomas (Lange *et al.*, 2019). Similar to oral papillomas, cutaneous papillomas undergo spontaneous regression without medical intervention in most healthy dogs and may also develop into SCCs in immunosuppressed dogs. (Goldschmidt *et al.*, 2006; Lange & Favrot, 2011)

3.3 Canine Pigmented Plaques

Pigmented plaques are characterised by dark, hyperkeratotic lesions that can be flat or slightly raised and usually appear as clusters. The size of lesions ranges from 1 to 10 mm in diameter and are commonly found at the ventral and medial aspects of the limbs, axilla, and abdominal region (Lange & Favrot, 2011). Canine pigmented plaques are so far exclusively associated with the *Chipapillomavirus* types, namely CPV-3, 4, 5, 8, 9, 12, 15, 16, 18, and 20. Although the role of breed predilection as a risk factor in the development of canine pigmented plaques is undefined, Pug dogs are the most common breed presented with pigmented plaques associated with CPV-4 and -18 (Lange *et al.*, 2016; Yu *et al.*, 2019). Nevertheless, any dog breed can be infected and develop canine pigmented plaques. Generally, other than cosmetic implications, pigmented plaques do not cause harmful effects to the health of dogs and can spontaneously regress without intervention.

4.0 CANINE PAPILLOMAVIRUS-ASSOCIATED NEOPLASMS

Reports on the association of CPV with neoplasms usually begin with persistent benign lesions, which over time undergo malignant transformation. Recently, numerous reports suggested the possible association of CPV with squamous cell carcinoma (SCC) (Alves *et al.*, 2020; Goldschmidt *et al.*, 2006; Luff *et al.*, 2016; Munday *et al.*, 2016; Thaiwong *et al.*, 2018). However, unlike in humans, evidence on the causal relationship is yet to be established.

4.1 Oral Squamous Cell Carcinoma

For many years, researchers have hypothesised that CPV-1 may contribute to the malignant transformation of oral papillomas into oral SCCs. From an earlier research, CPV-1 deoxyribonucleic acid (DNA) was detected in 3 out of 29 oral SCCs (Teifke, 1998) while another research successfully amplified CPV-1 from 1 out of 9 CPV-positive SCCs with the remaining CPV-positive SCCs concluded to be associated with novel CPV types (Zaugg *et al.*, 2005). Besides, there is also a well-documented case report where a CPV-1 induced oral papilloma developed into oral SCC, suggesting that CPV-1 might play a significant role in the emergence of the oral SCC (Regalado Ibarra *et al.*, 2018).

Thaiwong *et al.* suggested the possibility of this "low-risk" CPV-1 to increase its oncogenic properties due to influences from various co-factors. Interestingly, another recent research detected a putative novel CPV-1 variant with a mutation associated with a change in protein structure. This raised the hypothesis that new variants of CPV-1 might be closely associated with canine oral SCCs (Reis *et al.*, 2019). Despite these emerging

evidences, some reported that the detection rate of CPV-1 in oral SCCs is low and concluded that CPV-1 is not a significant cause of canine OSCCs (Munday et al., 2015; Porcellato et al., 2014).

Besides CPV-1, numerous reports have suggested the role of other CPV types in the development of SCC (Chang et al., 2019; Munday *et al.*, 2016). Thus, more conclusive and thorough research on the association of CPV types with oral SCCs is needed to have a better understanding on the association and pathogenesis of CPVs in oral SCC development.

4.2 Cutaneous Squamous Cell Carcinoma

The progression of cutaneous papillomas to SCCs is possible, especially in immunosuppressed dogs which were reported in a study where a group of dogs with canine X-linked severe combined immunodeficiency (X-SCID) that underwent bone marrow transplant developed metastatic SCC from CPV-2 induced inverted papillomas (Goldschmidt *et al.*, 2006). Nevertheless, evidence of cutaneous papilloma progressing to SCCs in naturally infected dogs have not been reported and documented yet.

Similar to other CPV-associated benign lesions, pigmented plaques can also undergo malignant transformation (Munday *et al.*, 2017) and progress into different SCC types, which include in situ, invasive and metastatic SCC (Alves *et al.*, 2020). CPV-9, 12, 15, and 16 have been detected from different SCC lesions in dogs with CPV-16 being the most frequently detected type (Chang *et al.*, 2020a; Luff *et al.*, 2016). Recent research on CPV-16 has successfully recovered the full genome of CPV-16 by high throughput

sequencing and identified E5, E6, and E7 oncoprotein genes. Interestingly, CPV-16 viral genome integration into four locations of the host genome was also identified and was the first discovery of integration of a CPV genome into the host genome. These findings raised suspicions that CPV-16 may be a potential high-risk CPV type that is strongly associated with the development of SCCs (Alves *et al.*, 2020; Luff *et al.*, 2019). Nevertheless, the direct causal association of CPV types with oncogenesis, especially CPV-16, still requires further investigation on the transforming abilities of the oncogenes to precisely determine their roles in inducing oncogenesis. Besides, the roles of other risk factors should also be considered when studying this causal relationship.

4.3 Basal Cell Carcinoma

BCCs are malignant cutaneous neoplasms and papillomavirus-associated BCCs were previously reported in cats but not in dogs (Munday *et al.*, 2017). However, an association of CPV with BCC was first reported in a study where CPV-18 was detected in a basal cell tumour (Yu *et al.*, 2019). Another case report identified CPV-3 from invasive BCC and trichoblastoma lesions in an English pointer dog with multiple skin lesions (Orbell *et al.*, 2020). These studies have raised speculations that CPV may be associated with neoplastic proliferation of epidermal basal cells. Hence, further investigation on the involvement of CPV in BCC development, especially CPV-3 and CPV-18 is warranted.

Table 3. Summary of lesions associated with the different CPVs.

Genus	Type	Associated lesions	References
Lamda	CPV-1	Oral papilloma, oral SCC, exophytic papilloma, endophytic papilloma	Regalado Ibarra *et al.*, 2018; Reis *et al.*, 2019; Lange *et al.*, 2009,
	CPV-6	Endophytic papilloma	Regalado Ibarra *et al., 2018;* Munday *et al.*, 2017
Tau	CPV-2	Exophytic papilloma, endophytic papilloma, metastatic SCC, invasive SCC, oral papilloma	Goldschmidt *et al.*, 2006; Lange *et al.*, 2009; Lange & Favrot, 2011; Lange *et al.*, 2019; Tisza *et al.*, 2016
	CPV-7	Exophytic papilloma, in situ SCC	Lange & Favrot, 2011
	CPV-13	Oral papilloma	Lange *et al.*, 2012
	CPV-17	Oral papilloma, oral SCC	Munday *et al.*, 2016
	CPV-19	Oral papilloma	Tisza *et al.*, 2016
	CPV-21	Not known	Altan *et al.*, 2019
	CPV-22	Not known	Altan *et al.*, 2019
	CPV-23	Not known	Altan *et al.*, 2019
Chi	CPV-3	Pigmented plaques, in situ SCC, invasive SCC, invasive basal cell carcinoma, trichoblastoma	Orbell *et al.*, 2020; Lange & Favrot, 2011
	CPV-4	Pigmented plaques	Munday *et al.*, 2017
	CPV-5	Pigmented plaques	Munday *et al.*, 2017
	CPV-8	Pigmented plaques	Munday *et al.*, 2017
	CPV-9	Pigmented plaques, cutaneous SCC	Chang *et al.*, 2020a
	CPV-10	Pigmented plaques	Munday *et al.*, 2017
	CPV-11	Pigmented plaques	Munday *et al.*, 2017
	CPV-12	Pigmented plaques, metastatic SCC, invasive SCC	Luff *et al.*, 2016
	CPV-14	Pigmented plaques	Munday *et al.*, 2017
	CPV-15	Pigmented plaques, verrucous SCC	Chang *et al.*, 2020a
	CPV-16	Pigmented plaques, metastatic SCC, in situ SCC, invasive SCC	Alves *et al.*, 2020; Chang *et al.*, 2020a; Luff *et al.*, 2016
	CPV-18	Pigmented plaques, basal cell tumour	Yu *et al.,* 2019
	CPV-20	Pigmented plaques	Regalado Ibarra *et al.*, 2018

5.0 DIAGNOSIS

Clinical diagnosis of CPV-associated diseases can be easily conducted based on signalment of the patient which are either young or immunosuppressed dogs, and gross appearance of the lesion. However, in order to obtain a definitive diagnosis, histopathology, immunohistochemistry (IHC) and molecular techniques such as polymerase chain reaction (PCR) testing are required (Lange & Favrot, 2011).

Histopathology is used to observe papillomavirus-induced cell abnormalities caused by viral replication in the lesions. Generally, viral replication that causes greater epithelial proliferative lesions is more likely to contain papillomavirus-induced cell changes. On the contrary, lesions with fair epithelial proliferation especially pigmented plaques, contain lesser viral replication as well as papillomavirus-induced cell changes. Typical cellular features of a papilloma lesion include enlarged keratinocytes with a shrunken nucleus surrounded by clear cytoplasmic halo (koilocytes), parakeratosis, increased amount of grey or blue fibrillary material in the cytoplasm, intracytoplasmic inclusion bodies, enlarged vesicular nuclei or clumped keratohyalin granules in the granular skin layers (Munday *et al.*, 2017). Occasionally, ballooning degeneration and intranuclear inclusion bodies can be observed (Orlandi *et al.*, 2021). Commonly observed features of pigmented plaques include acanthosis, clumped keratohyalin granules and hyperkeratosis with hyperpigmentation while koilocytes and inclusion bodies are rarely observed (Munday *et al.*, 2017; Yu *et al.*, 2019). For neoplastic lesions, viral replication

is usually scarce and histological features of papillomavirus-induced cell abnormalities are usually absent (Munday & Thomson, 2021).

IHC allows the detection and localization of CPV-associated proteins in the cells. This provides strong evidence that a lesion was caused by CPV infection. Two IHC methods are commonly used by researchers to investigate a CPV aetiology of the lesion. Firstly, IHC can be used to detect the L1 viral protein in the lesions. However, L1 protein is only expressed late in the replication cycle and can only be detected in lesions containing actively replicating CPV (Munday *et al.*, 2017). In addition, viral replication is scant in papillomavirus-induced neoplasms. Thus, results for immunostaining of L1 protein in neoplastic lesions are usually negative (Munday & Thomson, 2021).

Secondly, $p16^{CDKN2A}$ protein (p16) is commonly used as a surrogate marker for HPV-associated cancers. Expression of papillomavirus oncoproteins that cause degradation of pRb subsequently results in marked elevation of p16. Detection of p16 also means detection of the repercussions caused by papillomavirus proteins instead of detecting the presence of the virus itself. Therefore, immunostaining of p16 can be used on lesions that may not contain active viral replications especially neoplastic lesions (Munday & Thomson, 2021). In humans, p16 immunostaining is generally considered to accurately predict HPV aetiology of oral SCCs (Munday *et al.*, 2015). However, the sensitivity and specificity of p16 immunostaining in dogs are still unclear where a study had shown that there is no association between CPV infection and p16 immunostaining in a subset of canine SCCs (Sabattini *et al.*, 2016). The non-association of CPV infection

with p16 immunostaining could be due to other cofactors causing upregulation of p16. Additionally, oncogenes of CPV might have expressed different oncogenic mechanisms instead of degrading pRb proteins.

PCR assays are highly sensitive as they allow the detection of small quantities of CPV DNA, even in lesions without replication activities. Additionally, the use of consensus primers in PCR enables detection of novel CPV. Other than the conventional PCR, reverse transcriptase PCR can also be used to detect CPV ribonucleic acid (RNA) which is indicative of gene expression within the lesion (Munday *et al.*, 2017). However, the presence of CPV DNA or RNA within lesions does not indicate the causal relationship of the virus as CPV can also be detected on the skin of clinically healthy dogs (Lange *et al.*, 2011). Thus, PCR results must be interpreted alongside the signalment, epidemiological data, gross and histopathology findings as well as IHC results. To overcome the limitation of PCR, in situ hybridization which allows localisation of papillomavirus DNA or RNA in fixed tissues, has been used. By demonstrating the presence of viral DNA or RNA in the basal and suprabasal epidermis, better evidence supporting the role of CPV in lesion development can be achieved (Munday & Thomson, 2021).

6.0 TREATMENT

Treatment for papillomavirus infection in dogs is usually not required as the lesions normally undergo spontaneous regression, from 1 to 2 months extending up to 12 months (Munday *et al.*, 2017). For oral papillomas, issues with prehension, mastication, swallowing and infection can occur if the lesion growth is significant and located at concerning locations, affecting the quality of life (Williams *et al.*, 2021). In such cases, intervention is warranted to relieve the discomfort. To treat persistent and extensive CPV-associated papillomas, surgical excision, laser ablation or cryotherapy is often done. Although surgical excision and laser ablation are widely used, there is no published report of the efficacy rate of these treatments. Meanwhile, a study utilising cryotherapy to treat three dogs with persistent papillomas achieved permanent regression showing that cryotherapy appears to be an effective treatment modality for refractory cases (Richman *et al.*, 2017).

Numerous medical treatments to hasten the resolution of CPV-associated papillomas have been proposed, but most have not been assessed adequately and recognised treatment to date is still limited (Williams *et al.*, 2021). Azithromycin, an azalide subclass macrolide antibiotic is the most reported medical treatment for canine papillomatosis (Gould *et al.*, 2021). From a study by Yac *et al*, azithromycin suspension at a dosage of 10 mg/kg per os given every 24 h for 10 days, was found to be safe and effective where all the treated CPV-infected dogs achieved complete clinical remission in 10 to 15 days while the placebo group still had evident lesions after 50 days. However,

there was also a case showing the lack of response towards azithromycin therapy in a dog with disseminated cutaneous papillomas (Levy *et al.*, 2017). Other medical treatment options that have been reported include interferon alpha-2B, topical imiquimod cream and therapeutic vaccines such as CPV-1 autologous vaccine, recombinant COPV vaccine or recombinant CPV-2 L1 vaccine (Kuntsi-Vaattovaara *et al.*, 2003; Levy *et al.*, 2017; Levinson *et al.*, 2019). Interferon has been theorised to enhance immunological response against CPV-infected cells and was reported anecdotally as an adjunctive therapy (Levinson *et al.*, 2019). For autologous vaccine, in which a wart is removed and made into a crude vaccine to be injected into the same animal, has been reported to be effective in treating canine papillomas (Nicholls & Stanley, 1999).

Other alternative treatments have also been proposed and reported with convincing outcomes. In a study to evaluate efficacy of homeopathic drugs, namely, Sulfur 30C, Thuja 30C, Graphites 30C, and Psorinum 30C in combination against canine oral papillomas, lesions in the homeopathic group regressed between 7 to 15 days while the lesion regression in the placebo group occurred between 90 to 150 days (Raj *et al.*, 2020). This suggest that the treatment is effective against canine oral papillomas. *Tarantula cubensis* extract (Theranekron) as a homeopathic therapy was also experimented in 10 CPV-infected dogs, given subcutaneously at 2 ml per 10 kg, two times per week for 3 weeks where all dogs achieved total remission within the span of 3 to 5 weeks. However, no placebo group were used in this study and the efficacy of Theranekron in treating canine papillomatosis cannot be evaluated objectively (Icen *et al.*, 2011). Next, the application of a novel topical anti-neoplastic agent, tigilanol tiglate, which is primarily

approved as intratumoural treatment of non-metastatic, non-resectable canine mast cell tumors (Brown *et al.*, 2021), have been reported for treating viral pigmented plaques in a dog. The topical tigilanol tiglate appeared to be a potentially useful agent showing reduction of size and number of lesions within 9 days in the dog (Hansen *et al.*, 2017). Meanwhile, another report utilising a combination of four botanical extracts as a topical formulation to treat CPV-associated warts in a dog leads to regression of the lesions in approximately 9 days (Williams *et al.*, 2021). These studies demonstrated the safety and effectiveness of alternative therapies in hastening the regression of CPV-associated papillomas. Nevertheless, more conclusive and in-depth research or clinical trials should be conducted to have a comprehensive understanding on the potential novel treatments for persistent CPV-associated lesions.

7.0 PREVENTION

Prevention of CPV-associated diseases can be achieved by controlling the risk factors as they play a huge role in eliciting clinical diseases. Minimising the use of immunosuppressive drugs and treating immunosuppressive disorders are important to reduce the likelihood of disease occurrence. Besides, reducing contact with CPV-infected dogs is advisable. However, since healthy dogs can be a carrier for CPV and papillomaviruses can also be transmitted through fomites, transmission of CPV is possible even without direct contact with infected dogs (Munday *et al.*, 2017).

In humans, various prophylactic HPV vaccines have been developed and commercialised, from a bivalent or quadrivalent to a 9-valent vaccine which could prevent almost 90% of all cervical cancer cases (Jentschke *et al.*, 2020). The development of HPV vaccines is important because HPV is the strongest risk factor for cervical cancer with approximately 90% of all cervical cancer reported to be associated with it and also cervical cancer is the second most common type of cancer for women worldwide (Beyazit *et al.*, 2018).

At present, vaccine against CPV is not commercially available. Instead, experimental vaccines are still being developed. While vaccines are commonly used therapeutically to treat existing infection, a number of experimental studies have confirmed the efficacy of these vaccines as prophylaxis against CPV infection. Nicholls & Stanley (1999) reported that autologous vaccines prevented the development of warts when the oral mucosa of dogs was infected by scarification 2 to 3 weeks later. However,

vaccination with live COPV extract has been implicated in neoplastic development at the injection site (Bregman *et al.*, 1987; Nicholls & Stanley, 1999). Previous studies have also shown that immunisation with purified virus-like particles (VLPs) protect dogs against experimental papillomavirus infection (Suzich *et al.*, 1995; Yuan *et al.*, 2001). Recombinant VLP vaccine provided effective protection against oral papillomas and had better safety profile as it was found to be efficacious even at extremely low doses while still antigenically similar to authentic papillomavirus particles (Suzich *et al.*, 1995). Hence, development of recombinant or VLP vaccines may be safer and more efficient in preventing infection compared to the autologous vaccines. This is because recombinant vaccines use purified L1 protein as its component and does not contain viral DNA or RNA.

Despite advancements in vaccine development, a commercial vaccine to prevent CPV infection in dogs is still unavailable due to some limitations. Fundamentally, in order for a vaccine to be effective, the vaccine should be given prior to first infection by CPV. This criteria can be achieved in humans as high-risk HPVs are venereally-transmitted (Munday & Thomson, 2021). However, transmission of CPV is by direct contact and dogs can be asymptomatically infected by CPV (Lange *et al.*, 2011). Due to the transient nature of CPV infection in dogs, the development of a commercial vaccine would not be economically practical (Munday *et al.*, 2017). Although there has been evidence of CPV infection developing into more life-threatening neoplastic diseases, concrete scientific evidence is still lacking and not to mention their rare occurrence. Additionally, each VLP type confers protection for a single CPV type only and the CPV types that are strongly

associated with neoplastic transformation are still unknown. Therefore, the economic value and feasibility of CPV vaccine to prevent infection is debatable.

8.0 CONCLUSION

Most papillomavirus infection in dogs causes mild disease and often spontaneously resolves. Since the discovery of CPV, it has been speculated to be associated with oral and cutaneous neoplasms. Throughout the past decade, there are increasing reports of certain CPV types that are associated with the development of neoplasms such as SCCs and BCCs. These reports have raised concern regarding potential factors contributing to their increasing oncogenic potential and also possible recognition of certain existing CPV types as "high-risk" CPVs. Diagnosis of CPV-associated diseases can be achieved clinically alongside patient and epidemiological data, while advanced laboratory diagnoses are more commonly conducted for research purposes. Treatment for CPV-infected dogs is only indicated if the lesions are non-regressing and extensive, and prevention of CPV infection in dogs appear to be difficult and redundant. Thus, the aim of future research should focus on the oncogenic potential and mechanism of CPVs in order to facilitate the development of effective treatment and prevention strategies against CPV-induced malignant transformation.

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