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Cell proliferation and apoptosis in canine oral papillomatosis

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Abstract

This study was aimed at the evaluation of cell proliferation, *p53* level and apoptotic index by immunohistochemical methods in canine oral papillomatosis. The study material comprised of tumor tissue samples taken from six dogs being admitted to the Pathology Department of Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye. Choice of immunohistochemical staining was avidin-biotin peroxidase method. Cases of canine oral papillomatosis, determined to have been caused by canine papillomavirus-1, were found to have a rather high cell proliferation index. Furthermore, all cases were immunohistochemically demonstrated to carry a mutant *p53* gene. Despite the mutation of *p53* gene, the shift in the *Bax*/*Bcl-2* ratio of dogs diagnosed with tumor was in favor of the pro-apoptotic *Bax* gene. The apoptotic mechanism was determined to occur through both the caspase-dependent and caspase-independent pathways. While the lesions occupied the entire oral cavity in some cases, histopathologically, malignant transformation was not detected in any of the six cases.

Introduction

Canine papillomaviruses (CPVs) are double-stranded DNA viruses with a viral genome contained in a non-enveloped capsid.[1] The CPVs are classified under three subgenera and 20 types as follows: *Lambdapapillomavirus* (types 1 and 6), *Taupapillomavirus* (types 2, 7, 13, 17 and 19) and *Chipapillomavirus* (types 3, 4, 5, 8, 9, 10, 11, 12, 14, 15, 16 and 20).[2,3] The long control region of the CPV genome, known to control viral replication and transcription, is comprised of a late region and an early region, the latter of which includes six open reading frames (*E1*, *E2*, *E4*, *E5*, *E6* and *E7*).[1] The *E6* is an oncoprotein, targeting basic cellular proteins to induce cell immortality and proliferation.[4]

The CPVs are major etiological agents associated with various skin lesions in dogs.[5] Clinical problems caused by CPVs include pigmented viral plaques, oral papillomatosis, cutaneous exophytic papillomas, cutaneous endophytic papillomas, digital papillomatosis, genital papillomas and although rarely, *in situ* or invasive squamous cell carcinomas (SCCs).[6,7]

Generally, CPV warts (verrucae) do not require any treatment and tend to regress spontaneously within 3 to 12 months.[8] The CPV frequently affects dogs with a weakened or underdeveloped immunity and may cause rapidly growing lesions tending to expand.[9] The CPV is transmitted by direct contact and easily spread among dogs.[3] In dogs, tumor prevalence is not correlated with breed or gender.[10,11] The CPV mostly affects dogs younger than four years of age. Oral papillomatosis is characterized by high morbidity and low mortality. The prognosis is quite good and recovered dogs are immune for the rest of their life.[12]

Autoimmune therapy, laser therapy, surgery, cryotherapy, photodynamic therapy and chemotherapeutic agents such as vincristine are used for the treatment of canine oral papillomatosis.[2,12]

The present study was aimed at the evaluation of cell proliferation including proliferating cell nuclear antigen (PCNA), *p53* level and apoptotic index (*Bax*, *Bcl-2*, *Caspase-3*) and apoptosis inducing factor (AIF) through the immunohistochemical methods in canine oral papillomatosis.

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Materials and Methods

Animals. The study material comprised of tumor tissue samples taken from six dogs (four males and two females, mixed breed, three of which died due to the secondary bacterial infection or distemper disease and the other three of which underwent biopsy), being admitted to the Pathology Department of Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye, between the years 2013 - 2021 for routine histopathological examination. This study was approved by the Local Ethics Board for Animal Experiments of Kafkas University, Kars, Türkiye (Decision Number: KAU-HADYEK-2022/129, Date: 29.08.2022).

Nucleic acid extraction from paraffin-embedded tissue blocks. The extraction procedure was performed as described by Pikor *et al*.[13] The extracts were stored at –20.00 ˚C until further processing.

Molecular analysis. Polymerase chain reaction (PCR) targeting *E6* gene was used for the investigation of CPV, and primer pairs were used as described by Teifke *et al*.[14] The expected amplicon size was 350 bp. The PCR products were visualized in a transilluminator after electrophoresis in 1.00% agarose gel containing Safe-Red DNA stain (SafeView™, Richmond, Canada).

Phylogenetic analysis. The amplicons were submitted to a commercial company (BM Yazilim Danis. ve Lab. Sis. Ltd. Sti, Ankara, Türkiye) for Sanger sequencing. Sequence alignment was performed using Bioedit (version 7.0; Ibis Therapeutics, Carlsbad, USA).[15] Sequence similarities were compared with the GenBank nucleotide sequence database using the Basic Length Alignment Search Tool Software of the National Center for Biotechnology Information (NCBI).[16] Phylogenetic analyses of the gene sequences were performed using MEGA Software (version 7.0; Biodesign Institute, Tempe, USA).[17] Neighbor-joining method was selected and sequence differences were calculated with the Kimura two-parameter model; while, the confidence level was assessed by bootstrapping using 1,000 replicates.

Histopathological examination. The biopsy samples taken from the dogs were fixed in 10.00% buffered formaldehyde solution. After routine tissue processing, 5.00 µm-thick serial sections were cut from the paraffin blocks. The sections were stained with hematoxylin and eosin to determine histopathological changes. At least two pathologists reviewed the slides (presence of hyper-keratosis, acanthosis, rete ridge, keratohyaline granules, koilocytes and inclusion bodies) under an upright light microscope (Bx53; Olympus, Tokyo, Japan), and all detected lesions were imaged.

Immunohistochemical examination. The 5.00 µm-thick serial sections, being cut from the paraffin blocks of the biopsy samples, were stained with the avidin-biotin-peroxidase complex technique using commercial anti-bodies against PCNA: Pre-treatment using microwave oven (Santa Cruz Biotechnology, Dallas, USA), Dilution: 1:100 and Incubation conditions: Overnight at 4.00 ˚C; *p53*: Pre-treatment: Microwave oven (ABclonal Technology, Woburn, USA), Dilution: 1:100 and Incubation conditions: Overnight at 4.00 ˚C; *Bax*: Pre-treatment using the microwave oven (Santa Cruz Biotechnology), Dilution: 1:100 and Incubation conditions: Overnight at 4.00 ˚C; *Bcl-2*: Pre-treatment using the microwave oven (ABclonal Technology), Dilution: 1:100 and Incubation conditions: Overnight at 4.00 ˚C; *Caspase-3* : Pre-treatment using the microwave oven (ABclonal Technology), Dilution: 1:100 and Incubation conditions: Overnight at 4.00 ˚C and AIF: Pre-treatment using the microwave oven (ABclonal Technology), Dilution: 1:100 and Incubation conditions: Overnight at 4.00 ˚C according to the manufacturer's instructions. All immunostainings were performed using a Histostain Immunohistochemistry Kit (Thermo Fisher Scientific, Waltham, USA). Amino-ethylcarbazole (AEC; Thermo Fisher Scientific) was used as a chromogen and incubated for 15 min. The slides were washed with distilled water for 5 min, stained with Mayer's hematoxylin, and mounted with the AEC mounting solution. The mounted preparations were examined under the light microscope (Olympus Bx53) and the tissue sections were imaged with the Cell ^P Software (version 3.4; Olympus Soft Imaging Solutions GmbH, Münster, Germany). The detailed analysis of the images was made with the Image J Software (version 1.51j8; National Institutes of Health, Bethesda, USA). The immunohisto-chemical staining results were analyzed with a scoring system based on the number of positive tumor cells in the regions, being determined to display the strongest staining when assessed for immunopositive reactions. In each tumor tissue, three different areas were evaluated at 400 × magnification. The scoring was performed as follows: (-): Absence of immunoreactivity; (+): Weak immuno-reactivity, 1.00 - 10.00% positivity; (++): Moderate immunoreactivity, 11.00 – 59.00% positivity and (+++): Strong immunoreactivity, ≥ 60.00% positivity.[18]

Statistical analysis. The number of cells stained positive for PCNA, *p53*, *Bax*, *Bcl-2*, *Caspase-3* and AIF was recorded separately for each case with oral papilloma (three different sites at 400×). The mean values of the positive cell scores of these three different regions were calculated using the Excel (version 2002; Microsoft Corporation, Redmond, USA).

Results

Molecular results. Amplicons of the correct size were detected in all of the six samples. Only three samples could be sequenced and the sequences were deposited in GenBank under the accession No. of OP321268, OP321267 and OP321269.

After sequencing, samples were classified as *Lambdapapillomavirus 2* according to the phylogenetic tree constructed with the reference strains (Fig. 1). According to the NCBI Papillomavirus Episteme, CPV-1 is classified as *Lambdapapillomavirus 2*.

Macroscopic results. The macroscopic examination of the tumors demonstrated multiple cauliflower-like pale masses of varying size (average diameter: 1.83 cm) and hard consistency in the oral region (lips, palate, gingiva, buccal mucosa, tongue, etc.; Fig. 2).

Microscopic results. The microscopic examination of the tumor masses revealed hyperkeratosis, acanthosis and fingerlike extensions of the proliferated squamous epithelium, protruding from the epidermis into the dermis (rete ridges). The rete ridges were supported by a fibrovascular layer. The stratum spinosum was observed to be thickened and contained a large number of degenerated cells. Koilocytes, being characterized by a dark, peripherally situated and wrinkled nucleus being surrounded by a clear cytoplasm, were abundant. Epidermal cells contained numerous large and small keratohyalin granules in the cytoplasm. The keratinocytes contained intra-nuclear inclusion bodies. Furthermore, there were scarcely mitotic features. Inflammatory cells infiltration, necrosis and hemorrhage were negligible (Fig. 3).

Immunohistochemical results. Immunopositivity scores and staining patterns are presented in Table 1. All papilloma cases showed PCNA-positive staining. No statistically significant difference was detected between the cases regarding staining intensity. The PCNA immunoreactivity was largely localized to the cells in the basal layer. These cells exhibited very strong nuclear immunoreactivity. In addition to the cells of the basal layer, cells of the spinosum and granular layers also showed intra-nuclear PCNA expression.

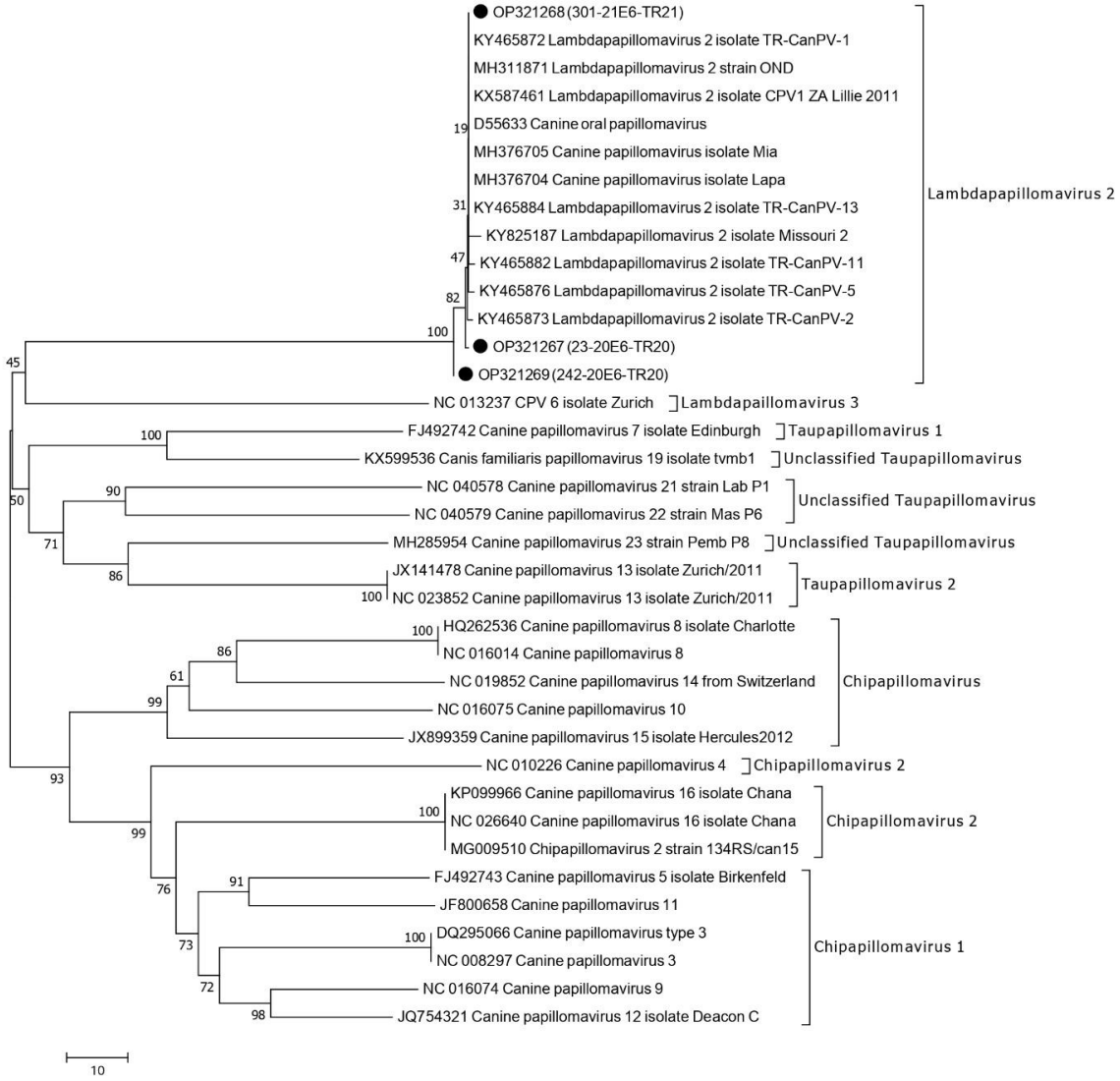

Fig. 1. Phylogenetic tree according to the *E6* primer pairs.

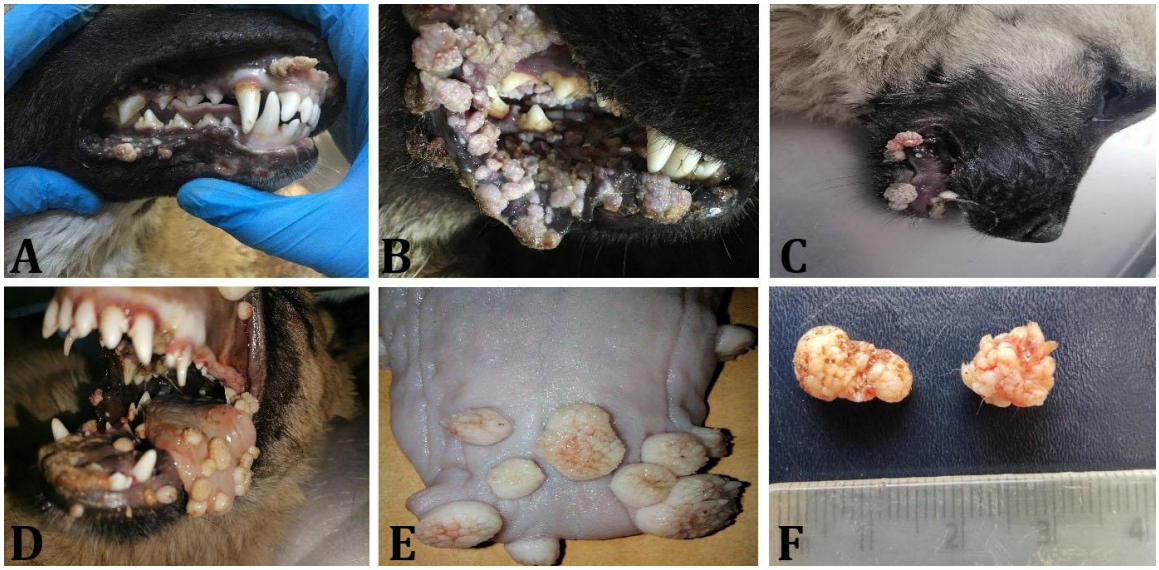

Fig. 2. Cauliflower-like tumor masses on **A)** lips and gingiva, **B)** lips, gingiva, palate, buccal mucosa and tongue, **C)** lips, **D)** lips, gingiva, palate, buccal mucosa and tongue, and **E)** tongue. **F)** Macroscopic view of surgically removed warts is also showcased.

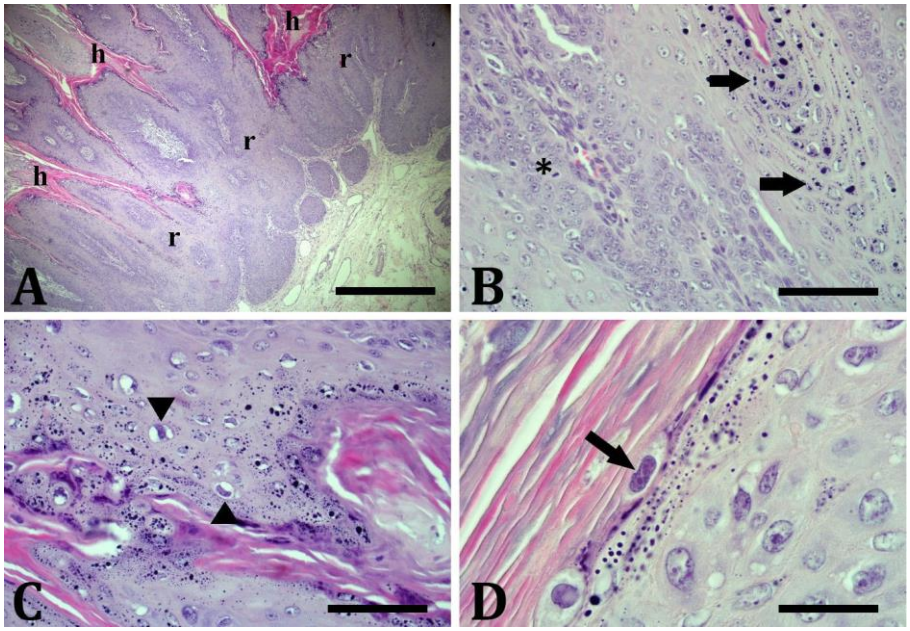

Fig. 3. Histopathological examination of the biopsy samples taken from dogs with canine oral papillomatosis. **A)** Lip: Hyperkeratosis (h) and rete ridges (r); **B)** Buccal mucosa: Intra-cytoplasmic keratohyalin granules (thick arrows) and mitotic feature (asterisk); **C)** Gingiva: Koilocytes (arrowheads); **D)** Palate: Intra-nuclear inclusion bodies in keratinocytes (arrow). Hematoxylin and eosin staining.

The *P53* immunopositive staining was observed in the nucleus of the basal and supra-basal cells. Staining intensity was stronger particularly in the cells peripheral to the rete ridges. All of the cases were positive for *p53* immunoreactivity. However, the intensity of staining ranged from weak to moderate. The *Bax* immunoreactivity was concentrated particularly in basal region. However, immunoreactions were also present in the spinosum and granular layers. Immunoreactions were localized to the cytoplasm of tumor cells and were rather strong. Degenerated keratinocytes were determined to be positive for *Bax* immunoreactivity. Similar to the results obtained for *Bax* immunoreactivity, *Bcl-2* immunopositive staining was detected in the cytoplasm of the cells lining in the basal layer. But, the intensity of *Bcl-2* immunopositive staining was much weaker than *Bax* immunopositivity (Fig. 4). *Caspase-3* and AIF immunopositive stainings were observed in the cells of the basal, spinosum and granular layers. The immunopositive reactions were intra-cytoplasmic. The AIF expression was mostly granular. No statistically significant difference was detected between the cases regarding both apoptosis indicators.

Table 1. Immunopositivity scores and staining patterns.

Case	PCNA	*p53*	*Bax*	*Bcl-2*	*Caspase-3*	AIF	PCR (*E6*)	Type
1	+++ IN	+ IN	++ IC	+ IC	+++ IC	+++ IC	Positive	-
2	+++ IN	+++ IN	+++ IC	++ IC	+ IC	+++ IC	Positive	Canine papillomavirus-1
3	+++ IN	+ IN	+ IC	+ IC	+++ IC	+++ IC	Positive	-
4	+++ IN	+ IN	++ IC	+ IC	+++ IC	+++ IC	Positive	-
5	+++ IN	++ IN	++ IC	+ IC	+++ IC	+++ IC	Positive	Canine papillomavirus-1
6	+++ IN	+ IN	+++ IC	+ IC	+++ IC	+ IC	Positive	Canine papillomavirus-1

AIF: Apoptosis inducing factor; IC: Intra- cytoplasmic; IN: Intra- nuclear; PCNA: Proliferating cell nuclear antigen; PCR: Polymerase chain reaction.

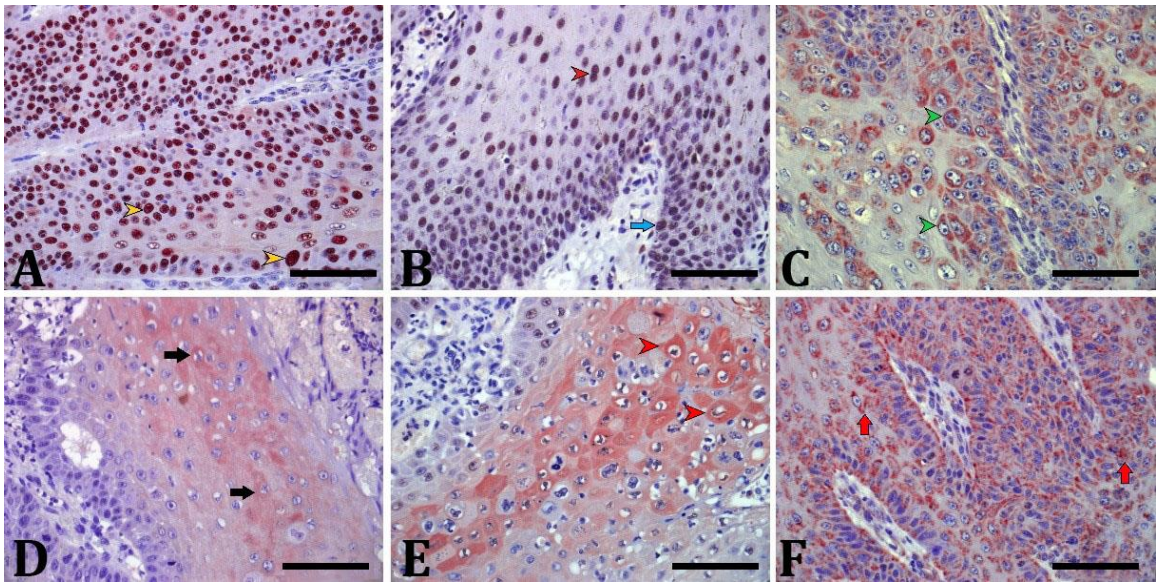

Fig. 4. Immunohistochemical examination of the biopsy samples taken from dogs with canine oral papillomatosis. **A)** Tongue: Severe intra-nuclear proliferating cell nuclear antigen-positive staining in tumor cells (yellow arrowheads); **B)** Lip: Moderate *p53* immunoreactivity in the nuclei of basal (blue arrow) and supra-basal (red arrowhead) cells; **C)** Buccal mucosa: Intra-cytoplasmic and severe *Bax* expressions in epidermal cells (green arrowheads); **D)** Gingiva: Very weak *Bcl-2*-positive staining in the cytoplasm of tumor cells (black arrows); **E)** Tongue: Severe and intra-cytoplasmic *Caspase-3* immunoreactivity in the epidermis (red arrows); **F)** Lip: Granular apoptosis inducing factor expressions in the cytoplasm of neoplastic cells (red arrows). Immunohistochemical staining.

Discussion

Papillomatous growths mostly display a typical cauliflower-like exophytic appearance on the lips and in the mouth.[9] However, they may also present a fringed or nodular appearance, and occur in the oral mucosa, including the lips and mucocutaneous junctions.[4,19] Although rarely, the tongue and esophagus may also be affected.[6] In agreement with former reports,[2,10,12] the present study revealed cauliflower-like tumor masses localized to the lips, palate, gingiva and buccal mucosa. Only two out of the six cases included in this study had papillomatous lesions on the tongue.

The CPV infections most frequently occur in dogs with a weakened or underdeveloped immunity.[9] Mostly dogs younger than four years of age are affected.[12] In agreement with literature data,[9,12] in the present study, three out of the six dogs with oral papillomatosis died due to secondary bacterial infection or canine distemper disease. All of the cases were below the age of four years.

Various methods including histopathology, PCR, immunohistochemistry, *in situ* hybridization and electron microscopy are used for the papillomatosis diagnosis.[2,19] Almost all reported cases of widespread canine oral papillomatosis are associated with CPV-1.[7] In this study, in the cases histopathologically diagnosed as papillomatosis, the *Lambdapapillomavirus 2* strains were identified, which were previously reported in CPV cases in Türkiye.[11]

The PCNA is a 36-kDa non-histonic nuclear peptide required for DNA replication.[20] Compared to mitotically quiescent cells, proliferating cells show a significantly increased expression of PCNA. Expression rapidly increases at the late phase of G1, peaks during the S phase, and decreases to an undetectable level during the G2 and M phases of the cell cycle. For this reason, the rate of PCNA synthesis is considered to be directly associated with the proliferation rate of cells.[21] Being one of the major prognostic factors for cancer diagnosis, PCNA is considered as a determinant of tumor aggressiveness and malignancy as well as cancer progression.[22,23] In

compliance with previous studies,[21,23,24] in the present study, PCNA-positive intra-nuclear reactions were mostly determined in cells lining the basal layer. The proliferation rate of neoplastic cells was rather high. Nonetheless, the cases investigated did not show any statistically significant difference regarding cell proliferation.

The *p53* regulates cell apoptosis, ageing and growth arrest as well as DNA integrity and repair.[25] In response to ultra-violet (UV)-B radiation-induced DNA damage, the *p53* gene is up-regulated such that either the end of the cell cycle is induced to allow DNA repair or the apoptotic mechanisms are initiated in the event of extensive damage.[26] The *p53* has multiple functional roles in the regulation of the cell cycle, and its being devoid of a suppressor mechanism contributes to the development of SCC and ameloblastoma in humans and bovine papillomavirus-induced tumors in animals.[27] The oncogenicity of human *E6* is associated with aversion from cell proliferation and apoptosis related to the proteasome-mediated degradation of *p53*. However, the oncogenic mechanism of CPV-2 *E6* in dogs is considered not to be associated with the capability of *E6* to degrade the well-defined *p53*. Quinlan *et al*.[26] reported that CPV-2 *E6*-expressing keratinocytes, which were exposed to UV radiation, did not down-regulate *p53* levels or prevent the expressions of *p21*, *Bcl-2* antagonist/killer (*Bak*) and *Bax*. These results suggest that undefined oncogenic mechanisms other than *p53* could be involved in the malignancy of *E6* in dogs.[1] Our study demonstrated the presence of a mutant *p53* gene in CPV-1 *E6*-positive papillomas, being different from findings previously reported for CPV-2 *E6*-positive tumors.[26]

Hassanien *et al*.[28] reported that in cases of bovine fibropapilloma, *p53*-positive reactions were of both intra-nuclear and intra-cytoplasmic localizations and observed in cells of the stratum corneum, and granulosum, spinosum and basal layers. In a similar study by Al-Salihi *et al*.[27], in samples taken from bovine papillomas and fibro-papillomas, intra-cytoplasmic and peri-nuclear *p53* stainings were observed primarily in the cells of the stratum corneum, as well as the basal and para-basal layers. In their investigation on cases of bovine cutaneous papilloma, Bocaneti *et al*.[25] determined that intra-cytoplasmic and peri-nuclear *p53* immunoreactivity was localized to the cells of the basal and para-basal layers at a rate of 75.00%. Also, Thaiwong *et al*.[29] demonstrated intra-nuclear positive reactions for *p53* in canine papillomas caused by CPV-1, and determined a potential malignant transformation at a rate of 11.00%. On the other hand, Teifke *et al*.[14] reported no expression of *p53* in papilloma samples taken from the tongue, gingiva, larynx, lips, abdomen, penis and toes. These researchers determined that *p53* was intra-nuclearly expressed in the epithelial cells of cutaneous papilloma samples taken from the forehead of only two dogs. In a study by Mayr *et al*.[30] on *exon 8*, which is considered as a conservative region of *p53*, mutation was detected in a canine papilloma of the oral mucosa. In the present study, all cases of canine oral papillomatosis were positive for mutant *p53* expression, and the positive reactions were mostly localized to the cells of the basal layer. Intra-nuclear stainings predominated over intra-cytoplasmic stainings.

The *Bcl-2* family regulates apoptosis, being described as a programmed cell death or cell suicide. Besides pro-apototic factors such as *Bax* and *Bak*, *Bcl-2* shows an anti-apoptotic effect.[25] Stimulants, including DNA damage, hypoxia and expression of certain oncoproteins, activate the *p53*-dependent apoptotic pathway. Once activated, *p53* suppresses anti-apototic factors such as *Bcl-2* and activates pro-apoptotic factors like *Bax* and thereby, paves the way to apotosis.[31] In the present study, it was determined that, in the tumor samples, the *Bax*/*Bcl-2* ratio had increased in favor of *Bax*. Accordingly, the increase of *Bax* and decrease of *Bcl-2* in cases with *p53* mutation suggest the presence of cell cycle regulators other than *p53* that may affect apoptosis.[31] An increase in favor of the anti-apoptotic *Bcl-2* in the *Bcl-2*/*Bax* ratio has been reported to be associated with cancer progression, neoplastic transformation and metastasis in various canine tumors, and a high *Bcl-2*/*Bax* ratio has been determined to indicate resistance against apoptosis.[32-34] On the other hand, data obtained in the present study pointed out to a pro-apoptotic tendency in canine oral papillomatosis. The oncoprotein *E6* disrupts the biological activity of many different types of cells in the body. One of the main biological functions of *E6* is the inhibition of apoptotic cell death.[1,11] Furthermore, the *E6* oncogen of the human papillomavirus has been reported to block apoptosis either through the inactivation of *p53* or independently from *p53*.[35] In the present study, the shift in the *Bax*/*Bcl-2* ratio of the tumor samples demonstrated that apoptosis had been triggered rather than having been inhibited in the CPV-1 *E6*-positive canine oral papillomas.

The onset of apoptosis depends on the activation of a series of cysteine-aspartic proteases (caspases).[31] Upon the detection of cell damage, among the inactive pro-caspases, the initiator caspases (8 and 9) are activated, and these continue to activate the executioner caspases (3, 6 and 7). The activation of the executioner caspases triggers a series of events starting with the activation of endo-nucleases and continues by DNA degradation, destruction of nuclear proteins and cytoskeleton, cross-linking of proteins and expression of ligands for phagocytic cells, finally resulting in the formation of apoptotic bodies.[36,37] Two main apoptosis pathways are known including caspase-dependent and caspase-independent pathways.[38] The caspase-dependent pathway involves the formation of death-inducing signaling complex through an extrinsic death program activated by tumor necrosis factor receptors.[39] On the other hand, the intrinsic apoptotic

mechanism is activated by death signals such as cellular stress, and regulated mainly by mitochondria being independent from caspase. These stimuli affect mitochondria; so that, apoptosis-related factors such as AIF are released. The AIF is a pro-apoptotic flavoprotein, inducing apoptosis *via* caspase-independent pathway.[40] In the present study, it was demonstrated that both caspase-dependent and caspase-independent pathways were triggered in the cases of canine oral papillomatosis. Immunohistochemical examination showed that there was no significant difference between the two pathways.

Oral papillomas are considered as benign tumors. In general, they show spontaneous regression and do not transform into invasive cancer.[41] Furthermore, excessive proliferation of the epithelium may result in the malignant transformation of lesion into SCC. While there are several potential causes of oral SCC in dogs, the exact role of papillomavirus infections remains unknown.[19] The CPV-1 together with CPV-13, -17 and -19 causes lesions in the oral cavity of puppies and immunocompromised dogs.[2,3] Although some reports indicate that CPV-1 does not transform cells, recent reports suggest that CPV-1 is highly associated with oral SCC.[2,29] Several types of CPV other than CPV-1 have been determined to be associated with SCC. The CPV-2 is known to cause metastatic SCC in immunocompromised dogs. Moreover, CPV-7 has been detected in cases of canine cutaneous SCC.[1]

Cases of canine oral papillomatosis, determined to have been caused by CPV-1, were found to have a rather high cell proliferation index. Furthermore, all cases were immunohistochemically demonstrated to carry a mutant *p53* gene. Despite the mutation of *p53* gene, the shift in the *Bax*/*Bcl-2* ratio of the dogs diagnosed with tumor was in favor of the pro-apoptotic *Bax* gene. The apoptotic mechanism was determined to occur through both caspase-dependent and caspase-independent pathways. While the lesions occupied the entire oral cavity in some cases, histopathologically, malignant transformation was not detected in any of the six cases.

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Conflict of interest

The authors declare no conflicts of interest.

References

1. Cruz-Gregorio A, Aranda-Rivera AK, Pedraza-Chaverri J. Pathological similarities in the development of papillomavirus-associated cancer in humans, dogs, and cats. Animals (Basel) 2022; 12(18): 2390. doi: 10.3390/ani12182390.
2. Regalado Ibarra AM, Legendre L, Munday JS. Malignant transformation of a canine papillomavirus type 1-induced persistent oral papilloma in a 3-year-old dog. J Vet Dent 2018; 35(2): 79-95.
3. Chang CY, Chen WT, Haga T, et al. The detection and association of canine papillomavirus with benign and malignant skin lesions in dogs. Viruses 2020; 12(2): 170. doi: 10.3390/v12020170.
4. Porcellato I, Brachelente C, Guelfi G, et al. A retrospective investigation on canine papillomavirus 1 (CPV1) in oral oncogenesis reveals dogs are not a suitable animal model for high-risk HPV-induced oral cancer. PLoS One 2014; 9(11): e112833. doi: 10.1371/journal.pone.0112833.
5. Boehm TMSA, Bettenay S, von Bomhard W, et al. A case series of canine cutaneous inverted papilloma with one case showing evidence of recurrence. Vet Dermatol 2021; 32(3): e268-e274. doi: 10.1111/ vde.12961.
6. Lange CE, Favrot C. Canine papillomaviruses. Vet Clin North Am Small Anim Pract 2011; 41(6): 1183-1195.
7. Lange CE, Jennings SH, Diallo A, et al. Canine papillomavirus types 1 and 2 in classical papillomas: High abundance, different morphological associations and frequent co-infections. Vet J 2019; 250: 1-5.
8. Munday JS, Thomson NA, Luff JA. Papillomaviruses in dogs and cats. Vet J 2017; 225: 23-31.
9. Williams A, Scally G, Langland J. A topical botanical therapy for the treatment of canine papilloma virus associated oral warts: A case series. Adv Integr Med 2021; 8(2): 151-154.
10. Yhee JY, Kwon BJ, Kim JH, et al. Characterization of canine oral papillomavirus by histopathological and genetic analysis in Korea. J Vet Sci 2010; 11(1): 21-25.
11. Oğuzoğlu TÇ, Timurkan MÖ, Koç BT, et al. Comparison of genetic characteristics of canine papillomaviruses in Turkey. Infect Genet Evol 2017; 55: 372-376.
12. Raj PAA, Pavulraj S, Kumar MA, et al. Therapeutic evaluation of homeopathic treatment for canine oral papillomatosis. Vet World 2020; 13(1): 206-213.
13. Pikor LA, Enfield KS, Cameron H, et al. DNA extraction from paraffin embedded material for genetic and epigenetic analyses. J Vis Exp 2011; 49: 2763. doi: 10.3791/2763.
14. Teifke JP, Löhr CV, Shirasawa H. Detection of canine oral papillomavirus-DNA in canine oral squamous cell carcinomas and p53 overexpressing skin papillomas of the dog using the polymerase chain reaction and non-radioactive in situ hybridization. Vet Microbiol 1998; 60(2-4): 119-130.
15. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 1999; 41: 95-98.

16. Altschul SF, Madden TL, Schäffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997; 25(17): 3389-3402.
17. Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011; 28(10): 2731-2739.
18. Beytut E. Pathological and immunohistochemical evaluation of skin and teat papillomas in cattle. Turkish J Vet Anim Sci 2017; 41(2): 204-212.
19. Öztürk Gürgen H, Egeden E, Şennazlı G. Clinicopathologic evaluation of oral squamous cell carcinoma in a young dog. Ankara Univ Vet Fak Derg 2021; 68(1): 77-81.
20. Mestrinho LA, Faísca P, Peleteiro MC, et al. PCNA and grade in 13 canine oral squamous cell carcinomas: association with prognosis. Vet Comp Oncol 2017; 15(1): 18-24.
21. Paramjeet, Jangir BL, Saharan S, et al. Patho-morphological and immunohistochemical evaluations of pancytokeratin, vimentin and proliferating cell nuclear antigen in epithelial tumours of dogs. Indian J Vet Pathol 2021; 45(2): 128-135.
22. Martano M, Restucci B, Ceccarelli DM, et al. Immunohistochemical expression of vascular endothelial growth factor in canine oral squamous cell carcinomas. Oncol Lett 2016; 11(1): 399-404.
23. Subapriya S, Pazhanivel N, Shafiuzama M, et al. Immunohistochemical diagnosis of skin tumours in dogs. Pharma Innov J 2021; 10(5): 612-619.
24. Jun D, Yi G, Na T, et al. Canine oral papillomavirus infection: clinical course, pathology, L1 gene and NCR2 gene sequencing. Isr J Vet Med 2010; 65: 111-116.
25. Bocaneti F, Altamura G, Corteggio A, et al. Expression of bcl-2 and p53 in bovine cutaneous fibropapillomas. Infect Agent Cancer 2015; 10(1): 2. doi: 10.1186/1750-9378-10-2.
26. Quinlan S, May S, Weeks R, et al. Canine papillomavirus 2 E6 does not interfere with UVB-induced upregulation of p53 and p53-regulated genes. Front Vet Sci 2021; 8: 570982. doi: 10.3389/fvets.2021.570982.
27. Al-Salihi KA, Al-Dabhawi AH, Ajeel AA, et al. Clinico-histopathological and immunohistochemical study of ruminant's cutaneous papillomavirus in Iraq. Vet Med Int 2020; 2020: 5691974. doi: 10.1155/2020/5691974.
28. Hassanien RT, Hamdy ME, Elnomrosy SM, et al. Molecular characterization and pathological identification of a novel strain of delta papillomavirus-4 (bovine papillomavirus-2) in Egypt. Vet World 2021; 14(9): 2296-2305.
29. Thaiwong T, Sledge DG, Wise AG, et al. Malignant transformation of canine oral papillomavirus (CPV1)-associated papillomas in dogs: An emerging concern? Papillomavirus Res 2018; 6: 83-89.
30. Mayr B, Schellander K, Schleger W, et al. Sequence of an exon of the canine p53 gene--mutation in a papilloma. Br Vet J 1994; 150(1): 81-84.
31. Xu X, Lai Y, Hua ZC. Apoptosis and apoptotic body: disease message and therapeutic target potentials. Biosci Rep 2019; 39(1): BSR20180992. doi: 10.1042/BSR20180992.
32. Pieper JB, Stern AW, LeClerc SM, et al. Coordinate expression of cytokeratins 7 and 14, vimentin, and Bcl-2 in canine cutaneous epithelial tumors and cysts. J Vet Diagn Invest 2015; 27(4): 497-503.
33. Madej JA, Madej JP, Dzimira S, et al. An immunohistochemical analysis of lymphocytic infiltrations in canine skin cancers. Pol J Vet Sci 2017; 20(1): 141-147.
34. Dos Anjos DS, Bueno C, Magalhães LF, et al. Electrochemotherapy induces tumor regression and decreases the proliferative index in canine cutaneous squamous cell carcinoma. Sci Rep 2019; 9(1): 15819. doi: 10.1038/s41598-019-52461-6.
35. Cai Q, Lv L, Shao Q, et al. Human papillomavirus early proteins and apoptosis. Arch Gynecol Obstet 2013; 287(3): 541-548.
36. Bongiovanni L, Romanucci M, Fant P, et al. Apoptosis and anti-apoptotic heat shock proteins in canine cutaneous infundibular keratinizing acanthomas and squamous cell carcinomas. Vet Dermatol 2008; 19(5): 271-279.
37. D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int 2019; 43(6): 582-592.
38. Pistritto G, Trisciuoglio D, Ceci C, et al. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY) 2016; 8(4): 603-619.
39. Goldar S, Khaniani MS, Derakhshan SM, et al. Molecular mechanisms of apoptosis and roles in cancer development and treatment. Asian Pac J Cancer Prev 2015; 16(6): 2129-2144.
40. Shimada M, Yamashita A, Saito M, et al. The human papillomavirus E6 protein targets apoptosis-inducing factor (AIF) for degradation. Sci Rep 2020; 10(1): 14195. doi: 10.1038/s41598-020-71134-3.
41. Gil da Costa RM, Peleteiro MC, Pires MA, et al. An update on canine, feline and bovine papillomaviruses. Transbound Emerg Dis 2017; 64(5): 1371-1379.