Canine papillomatosis: A retrospective study of 24 cases (2001-2011) and immunohistochemical characterization[1]

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ABSTRACT.- Bianchi M.V., Casagrande R.A., Watanabe T.T.N., Wouters A.T.B., Wouters F., Boos G.S., Menegat M.B. & Driemeier D. 2012. **Canine papillomatosis: A retrospective study of 24 cases (2001-2011) and immunohistochemical characterization.** *Pesquisa Veterinária Brasileira 32(7):653-657.* Setor de Patologia Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: davetpat@ufrgs.br

A retrospective study of 24 cases of papillomas in dogs was performed from January 2001 to March 2011. Additionally, immunohistochemistry (IHC) was used to characterize and evaluate the samples. We found that disease was observed more in mixed breed dogs, ages ranging from 6 months to 10 years (mean 3.1 years), and there was no gender predilection. The main lesion sites were the skin (75%), lips (16.7%), and eyelids (8.3%). Upon histological evaluation, we observed papillary exophytic proliferation of squamous epithelium and papillary endophytic proliferation (inverted) in 87.5% and 12.5% of cases, respectively. The tumors were characterized by spinous layer hyperplasia (87.5%) with koilocytes (70.8%) and intranuclear pale basophilic inclusions bodies (8.3%), prominent granular layer with large amounts of keratohyalin granules (95.8%), and hyperkeratosis in the stratum corneum (100%). Positive immunostaining for *Papillomavirus* was found in 83.3% of cases, which were distributed between the granular layer and the stratum corneum. These findings indicate the following: that papillomas in dogs are caused by *Papillomavirus*, the viral cytopathic effect induces epithelial lesions, viral particles are found inside the cell nuclei, and inclusions bodies are rare.

INDEX TERMS: Papilloma, papillomatosis, skin disease, immunohistochemistry, dog.

RESUMO.- [**Papilomatose em cães: estudo retrospectivo de 24 casos (2001-2011) e caracterização imuno-histoquímica.**] Foi realizado um estudo retrospectivo de 24 casos de papilomas em cães diagnosticados no período de janeiro 2001 a março de 2011, bem como a sua caracterização imuno-histoquímica (IHQ). Cães sem raça definida foram os mais afetados, a idade média foi de 3,1 anos, com variação de 6 meses a 10 anos e não houve predileção sexual. Quanto à localização das lesões, 75,0% estavam na pele, 16,7% no lábio e 8,3% em pálpebra. Na avaliação histológica havia proliferação papilar exofítica do epitélio escamoso em 87,5% e papilar endofítica (invertido) em 12,5%. O tumor era caracterizado por hiperplasia do estrato espinhoso (87,5%) com coilócitos (70,8%) e inclusões intranucleares basofílicas pálidas (8,3%); o estrato granular estava proeminente com grande quantidade de grânulos de queratohialina (95,8%); e havia hiperqueratose do estrato córneo (100%). Na avaliação IHQ para *Papillomavirus* houve marcação nos estratos granuloso e córneo em 83,3%. Estes achados indicam que os papilomas em cães são causados por *Papillomavirus*, as lesões epiteliais são decorrentes do efeito citopático viral, as partículas virais estão no núcleo das células e corpúsculos de inclusão são raros.

TERMOS DE INDEXAÇÃO: Papiloma, papillomatose, doença de pele, imuno-histoquímica, cão.

INTRODUCTION

Papillomas are induced by *Papillomavirus*, a double-stranded, non-enveloped DNA virus that is usually species-specific and has a strong tropism for cutaneous squamous

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or mucosal epithelium (Gross et al. 2005). Papillomas are common in dogs and can cause single or multiple epithelial lesions at different skin sites, including mucous membranes and the mucocutaneous junction of the oral cavity and conjunctiva (Nicholls & Stanley 1999). Currently, there are six recognized syndromes related to canine papilloma: oral papillomatosis, cutaneous, inverted cutaneous, multiple pigmented cutaneous, multiple pigmented plaques, and cushions multiple papillomas (Scott et al. 2001). The pathogenesis of papillomatosis is characterized by a hyperplastic reaction of the epithelium with an increased production of keratin and virus replication (Gross et al. 2005).

Canine oral papillomatosis is a contagious disease that mainly affects young dogs and it manifests as single or multiple verrucous lesions with an average size of 1.0 cm in diameter, which can be found in the oral mucosa, eyelids, and skin (Goldschmidt & Hendrick 2002). Cutaneous inverted papillomas also affect young animals and the lesions are located in the abdomen and the inguinal region (Campbell et al. 1988).

The disease has an incubation period of 4 to 8 weeks and typically regresses after 4 to 8 weeks of evolution (Chambers & Evans 1959, Bredal et al. 1996, Nicholls et al. 2001). Dogs that successfully recover from papillomatosis become immune to subsequent infections (Chambers et al. 1960). Circulating IgG antibodies induced by spontaneous regression of canine papillomas that protect against subsequent infections have been observed (Ghim et al. 2000). However, in some cases, the lesion may progress to squamous cell carcinoma (Nicholls & Stanley 1999). Diagnoses of canine viral papillomas are determined mainly by anatomical distribution, histological characterization, immunohistochemistry, electron microscopy, immunofluorescence, and in situ hybridization (Scott et al. 2001).

The goal of this study was to review papilloma cases in dogs submitted to the Laboratory of Veterinary Pathology of the Universidade Federal do Rio Grande do Sul (UFRGS), Brazil from 2001 to 2011. We aimed to describe the cases and define their histological and immunohistochemistry characteristics.

MATERIALS AND MET,HODS

The canine biopsies archives from the Laboratory of Veterinary Pathology, UFRGS, Brazil, from January 2001 to March 2011, were reviewed. All papilloma cases were analyzed and the paraffin blocks were recut and stained with hematoxylin and eosin (HE).

For immunohistochemical analysis, the monoclonal papillomavirus antibody (clone K1H8, DakoCytomation) was used. The slides were deparaffinized, rehydrated, and treated with 10% hydrogen peroxide in methanol solution. The antigens were retrieved by boiling the sections in citrate buffer (pH 6) for 40 min at 96°C in a water-bath. Sections were incubated overnight at 4°C with primary antibody (1:100 dilution). Amplification signal was achieved by using biotinylated secondary antibody, followed by labeled streptavidin-biotin-peroxidase complex, and both reagents were obtained from the LSAB Universal kit (DakoCytomation). The reaction was revealed with 3-amino-9-etilcarbazol (AEC, K3469, DakoCytomation) chromogenic substrate. Slides were counterstained with Mayer's hematoxylin and coverslipped with aqueous medium (S1964, DakoCytomation) for microscopic examination. Bovine papilloma was included as a positive control for this procedure. The tissue samples were categorized as negative (-), discrete reaction intensity (+), moderate (++), and accentuated (+++).

RESULTS

Characterization of the cases

From January 2001 to March 2011, 24 canine papilloma cases were diagnosed (Table 1). The percentage of mixed breed dogs was overrepresented at 29.3% (7/24) and 12.5% (3/24) were unreported breeds. Each of the following breeds comprises 8.4% of the population (2/24): Beagle, English Bulldog, Labrador, and Pitbull. Moreover, German Shepherd, Golden Retriever, Lhasa Apso, Pinscher, Poodle, and Pug breeds each comprised 4.1% (1/24). Of the dogs in the study, females comprised 54.2% (13/24), males comprised 41.7% (10/24), and 4.1% (1/24) did not report gender. Ages ranged from 6 months to 10 years (average 3.1 years), with 54.2% (13/24) that were less than 3 years; 20.8% (5/24) that were more than 3 years; and 25% of undetermined age.

Regarding the tumor sites, 75% (18/24) involved the skin, including the inguinal region. Both the thoracic member and foreskin comprised 8.3% of tumor sites (2/24). Comprising 4.2% of tumor sites (1/24) are: the face, interdigit, flank, and areas close to the nipple. Also, the skin distribution was not specified in 33.3% of the medical records (8/24). Less common sites included the lip and eyelid at 16.7% (4/24) and 8.3% (2/24), respectively.

Microscopic features

During histological evaluations, we observed papillary exophytic proliferation of squamous epithelium and papillary endophytic proliferation (inverted) in 87.5% (21/24) (Fig.1A) and 12.5% of cases (3/24), respectively. Theses tumors were characterized by spinous layer

Table 1. Characterization of the dogs affected by papillomatosis

Case	Sex	Age	Breed	Site
1	F	-	Pinscher	Skin[a]
2	M	7 months	Mixed breed	Prepuce
3	F	4.5 years	Pitbull	Close to the nipple
4	M	1.6 years	German Shepherd	Inguinal region
5	M	10 years	Pug	Prepuce[b]
6	F	1 year	Poodle	Inner surface of upper lip
7	M	-	Labrador	Skin[a]
8	NI	-	-	Skin[a]
9	F	-	Mixed breed	Thoracic member and thoracic ventral[b]
10	F	9 years	Beagle	Lip
11	F	2.5 years	Beagle	Skin[a]
12	M	1 year	Pitbull	Interdigit[b]
13	M	8 years	Lhasa Apso	Right flank
14	F	9 months	Mixed Breed	Left lower eyelid[b]
15	M	3 years	-	Eyelid
16	F	1.7 years	Labrador	Inguinal region
17	F	5 years	-	Lip
18	M	2 years	Mixed breed	Skin[a]
19	F	6 months	English Bulldog	Face
20	M	8 months	English Bulldog	Skin[a]
21	M	3 years	Golden Retriever	Lip
22	F	-	Mixed breed	Skin[a]
23	F	-	Mixed breed	Skin[a]
24	F	1.5 years	Mixed breed	Left thoracic member

F = female, M = male, - = unreported; [a] Site unreported, [b] multiple lesion.

hyperplasia and the normal eosinophilic cytoplasm was replaced by pale basophilic cytoplasm in 87.5% of cases (21/24) (Fig.1A). Also, intracytoplasmic eosinophilic material (aggregates of keratin) was observed in 37.5% of cases (9/24). The nuclei of these cells were round or oval with dispersed and condensed chromatin in 95.8% (23/24) and 4.2% of cases (1/24), respectively. Additionally, 1-2 and 1-3 evident nucleolus were observed in 91.7% (22/24) and 8.3% of cases (2/24), respectively. The granular layer was prominent, and the keratohyalin granules were large, round, or irregular in 95.8% of cases (23/24) (Fig.1A). The stratum corneum showed parakeratotic hyperkeratosis and orthokeratotic hyperkeratosis in 70.8% (17/24) and in 29.2% of cases (7/24), respectively (Fig.1A). Koilocytes (enlarged keratinocytes with eccentric pyknotic nuclei surrounded by a clear halo) were observed in the spinous and granular layer and their "ghost" cells in the stratum corneum in 70.8% (17/24) and 54.2% of cases (13/24), respectively (Fig.1B). Intranuclear basophilic inclusions that completely filled the nucleus or had distinct margination of the nuclear chromatin surrounded by a clear halo in 8.3% of cases (2/24) (Fig.1C) were observed in the spinous layer.

The papillomas were supported by small (75.0%; 18/24), moderate (20.8%; 5/24) and severe (4.2%; 1/24) amount of dermal fibrovascular stroma. In addition, 45.8% (11/24) of the papillomas had prominent and dilated capillaries (Fig.1B). Intracytoplasmatic melanin pigment was observed in 45.9% of cases (11/24), with a small amount in rare cells (25%; 6/24); moderate amount in some cells (16.7%; 4/24), and severe amount in all cells (4.2%; 1/24).

Regarding lymphoplasmocytary infiltration, 16.7% (4/24) and 12.5% (3/24) were classified as focal mild or moderate, respectively. Infiltration of lymphocytes, plasma cells and neutrophils were classified as focal mild (16.7%; 4/24), focal moderate (8.3%; 2/24), or locally extensive and severe (4.2%; 1/24). Epithelial erosion with lymphocytes, plasma cells and neutrophils was locally extensive in 4.2% of cases (1/24).

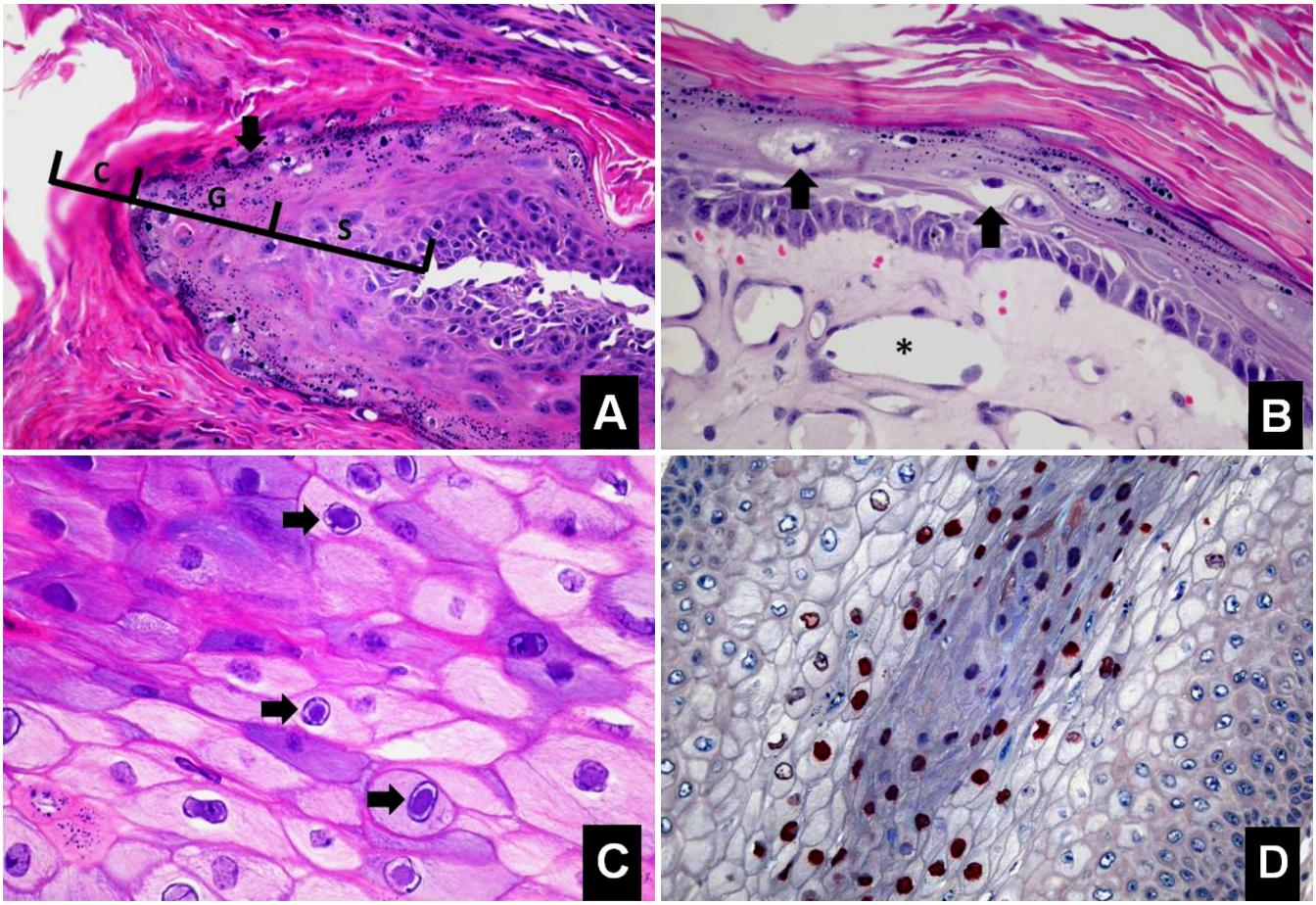

Fig.1. Papillomatosis in dogs. (**A**) Papillary exophytic proliferation of squamous epithelium with spinous layer hyperplasia (S) and the normal eosinophilic cytoplasm was replaced by pale basophilic; the granular layer was prominent (G) and the keratohyalin granules were large, round, or irregular (arrow); and the stratum corneum (C) with parakeratotic hyperkeratosis. HE, obj.10x. (**B**) The spinous and granular layer with koilocytes that are enlarged keratinocytes with eccentric pyknotic nuclei surrounded by a clear halo (arrows). The papilloma was supported by moderate amount of dermal fibrovascular stroma with prominent and dilated capillaries (*). HE, obj.10x. (**C**) The spinous layer with intranuclear basophilic inclusions that completely filled the nucleus or with distinct margination of the nuclear chromatin surrounded by a clear halo (arrows). HE, obj.40x. (**D**) Immunostaining for *Papillomavirus* in the epithelial cells nuclei of the granular layer. Streptavidin-biotin-peroxidase, obj.20x.

Table 2. Immunohistochemical characterization of papillomatosis in dogs

Nº case	Diagnostic	Immunohistochemical for *Papillomavirus*	
		Distribution	Intensity
1	Inverted papilloma	Granular layer	+
2	Inverted papilloma	Stratum corneum	+
3	Papilloma	-	-
4	Inverted papilloma	Stratum corneum	++
5	Papilloma	-	-
6	Papilloma	Granular layer and stratum corneum	++
7	Papilloma	Granular layer and stratum cor neum	$+^{G}$ $+++^{C}$
8	Papilloma	Granular layer and stratum corneum	++
9	Papilloma	Granular layer and stratum corneum	+++
10	Papilloma	-	-
11	Papilloma	Stratum corneum	+
12	Papilloma	Granular layer and stratum corneum	++
13	Papilloma	Granular layer	+++
14	Papilloma	Granular layer and stratum corneum	++
15	Papilloma	Granular layer and stratum corneum	+
16	Papilloma	Granular layer and stratum corneum	+++
17	Papilloma	Granular layer and stratum corneum	+++
18	Papilloma	-	-
19	Papilloma	Granular layer and stratum corneum	++
20	Papilloma	Granular and spinous layer, and stratum corneum	+++
21	Papilloma	Granular layer and stratum corneum	+
22	Papilloma	Granular layer and stratum corneum	+
23	Papilloma	Granular layer and stratum corneum	$+^{G}$ $+++^{C}$
24	Papilloma	Granular layer and stratum corneum	$+++^{G}$ $+^{C}$

- Negative, + discrete, ++ moderate, +++ accentuated; ^{G} granular layer, ^{C} stratum corneum.

Immunohistochemical characterization

The IHC results are reported in Table 2. Immunostaining for *Papillomavirus* in the epithelial cells nuclei was observed in 83.3% of cases (20/24) and with accentuated intensity (+++) in 40% (8/20), moderate (++) in 30% (6/20), and discrete (+) in 30% of cases (6/20). Staining occurred in the granular layer and stratum corneum (70%; 14/20), stratum corneum (15%; 3/20), granular layer (10%; 2/20), and in the granular layer, spinous layer, and stratum corneum (5%; 1/20) (Fig.1D).

DISCUSSION

Papillomas are often diagnosed as exophytic (Scott et al. 2001). Cutaneous inverted papilloma is a rare endophytic variant in dogs (Campbell et al. 1988). Exophytic viral papilloma occurs most commonly on the face, ears, and extremities, but it may also occur in the mucocutaneous junctions (Gross et al. 2005). In this study, lips, inguinal region, thoracic member, prepuce, and eyelid were the most commonly affected sites; however, it is not possible to determine the precise site prevalence because this information was not specified in 33.3% of the clinical records. Endophytic viral papillomas are common in the abdominal and inguinal region (Campbell et al. 1988).

There is no known sex predilection and dogs at increased risk are the Great Dane, Irish Setter, and Beagle, while mixed breed dogs are at decreased risk (Goldschmidt & Hendrick, 2002). However, this study suggests that mixed breed dogs were affected more than pure breeds. Exophytic papillomas occur at any age but are most often seen in dogs less than 2 years of age (Gross et al. 2005), and inverted papillomas affect dogs less than 3 years of age (Campbell et al. 1988). This type of tumor is rare in older animals (Narama et al. 1992) and the higher incidence in younger animals supports the idea that older animals have developed immunity as a result of previous exposure to the virus (Chambers et al. 1960).

In this report, typical viral papilloma lesions were observed in 87.5% of cases and these lesions comprise multiple finger-like projections of thickened squamous epithelium (Gross et al. 2005). The presence of koilocytes, which were seen in 70.8% of these cases, in the spinous layer and their "ghost" cells in the stratum corneum is a sign of viral cytopathic effect, which causes a ballooning degeneration and the nucleus becomes eccentric and picnotic (Gross et al. 1992).

The papillomatosis regression in dogs is spontaneous; therefore, the prognosis is favorable (Goldschmidt & Hendrick 2002). Usually, disease regression is marked by a moderate, interface infiltrate that primarily consists of T lymphocytes (Gross et al. 2005). In this study, there were inflammatory infiltrates in 62.6% of cases, which suggests that the lesion may be regressing.

Benign lesions usually do not cause clinical problems, except when the site leads to airway obstruction or dysphagia. The treatment consists of surgical removal by excision, cryosurgery, or electrosurgery. The treatment with autogenous vaccines has no proven therapeutic effect in dogs, although some studies suggest that it may prevent disease (Sundberg et al. 1994, Scott et al. 2001). A spectrum of proliferative cutaneous lesions occurred in 12 dogs at the injection site of live canine oral papillomavirus vaccine, suggesting a viral etiology for the disease. Lesions included epidermal hyperplasia, epidermal cysts, squamous papilloma, basal cell epithelioma, and squamous cell carcinoma. Tumor sections revealed papillomavirus antigen in five of 12 masses (Bregman et al. 1987).

A severe, naturally occurring, nonregressing oral papilloma was observed in a 3.5-year-old neutered female Labrador. The papillomas proved refractory to surgical and medical treatments, including autogenous vaccination and vaccination with capsid (L1) virus-like particles. The papillomas spread to oesophageal mucosa, perioral haired skin, and remote cutaneous sites. Experimental infection of Beagle dogs with this viral isolate resulted in the uncomplicated development and regression of oral warts within the usual period. These findings support the hypothesis that

the recurrent lesions are associated with specific defects in host immunity rather than variations in viral pathogenicity (Nicholls et al. 1999).

The results of the present study confirm that *Papillomavirus* was responsible for 83.3% of papillomatous lesions observed in dogs and immunostaining was concentrated in the granular layer and the stratum corneum. The same immunostaining was seen in an outbreak of canine oral papillomavirus in Korea (Yheel et al. 2010). This antibody targets the whole virus, which is formed only in differentiated squamous epithelial cells. Thus, positive reactions tend to be limited to the skin surface and stratum corneum (Gross et al. 2005). Negative IHC cases could be explained by the lack of virus localization in the lesion.

The presence of basophilic intranuclear inclusions in cells from the spinous layer was only noted in two cases. Inclusions are less frequently observed because they are more evident when the biopsy is conducted during the initial development of the papilloma (Goldschmidt et al. 2002).

CONCLUSIONS

Our findings indicate that:

1) dogs with papillomatosis were young;
2) mixed breed dogs were affected the most;
3) papilloma lesions occurred mainly in the skin;
4) the exophytic form was predominant;
5) papillomas were caused by *Papillomavirus*;
6) lesions were caused by viral cytopathic effect;
7) inclusion bodies were rarely observed; and
8) intranuclear immunostaining occurred mainly in the granular layer and in the stratum corneum.

REFERENCES

Bredal W.P., Thoresen S.I., Rimstad E., Aleksandersen M. & Nafstad P.H.J. 1996. Diagnosis and clinical course of canine oral papillomavirus infection. J. Small Anim. Pract. 37:138-142.

Bregman C.L., Hirth R.S., Sundberg J.P. & Christensen E.F. 1987. Cutaneous neoplasms in dogs associated with canine oral papillomavirus vaccine. Vet. Pathol. 24:477-487.

Campbell K.L., Sundberg J.P., Goldschmidt M.H., Knupp C. & Reichmann M. E. 1988. Cutaneous inverted papillomas in dogs. Vet. Pathol. 25:67-71.

Chambers V.C. & Evans C.A. 1959. Canine oral papillomatosis. I. Virus assay and observations on the various stages of the experimental infection. Cancer Res. 19:1188-1195.

Chambers V.C., Evans C.A. & Weiser R.S. 1960. Canine oral papillomatosis. II. Immunologic aspects of the disease. Cancer Res. 20:1083-1093.

Ghim S., Newsome J., Bell J., Sundberg J.P., Schlegel R. & Jenson A.B. 2000. Spontaneously regressing oral papillomas induce systemic antibodies that neutralize canine oral Papillomavirus. Exp. Mol. Pathol. 68:147-151.

Goldschmidt M.H. & Hendrick M.J. 2002. Tumors of the skin and soft tissues, p.45-118. In: Meuten D.J. (Ed.), Tumors in Domestic Animals. 4th ed. Blackwell, Iowa.

Gross T.L., Ihrke P.J. & Walder E.J. 1992. Veterinary Dermatopathology: A macroscopic and microscopic evaluation of canine and feline skin disease. Mosby, St Louis. 520p.

Gross T.L., Ihrke P.J., Walder E.J. & Affolter V.K. 2005. Skin Diseases of the Dog and Cat: Clinical and histopathologic diagnosis. 2nd ed. Blackwell, Oxford. 932p.

Narama I., Ozaki K., Maeda H. & Ohta A. 1992. Cutaneous papilloma with viral replication in an old dog. J. Vet. Med. Sci. 54:387-389.

Nicholls P.K. & Stanley M.A. 1999. Canine papillomavirus: A centenary review. J. Comp. Pathol. 120:219-233.

Nicholls P.K., Klaunberg B.A., Moore R.A., Santos E.B., Parry N.R., Gough G.W. & Stanley M.A. 1999. Naturally occurring, nonregressing canine oral papillomavirus infection: Host immunity, virus characterization, and experimental infection. Virology 265:365-374.

Nicholls P.K., Moore P. F., Anderson D.M., Moore R.A., Parry N.R., Gough G.W. & Stanley M.A. 2001. Regression of canine oral papillomas is associated with infiltration of CD41 and CD81 lymphocytes. Virology 283:31-39.

Scott D.W., Miller D.H. & Griffin C.E. 2001. Muller and Kirk's Small Animal Dermatology. 6th ed. W.B. Saunders, Philadelphia. 1528p.

Sundberg J.P., Smith E.K., Herron A.J., Jenson A.B., Burk R.D. & Van Ranst M. 1994. Involvement of canine oral papillomavirus in generalized oral and cutaneous verrucosis in a Chinese Shar Pei dog. Vet. Pathol. 31:183-187.

Yheel J.Y., Kwon B.J., Kim J.H., Yu C.H., Im K.S., Lee S.S., Lyoo Y.S., Chang B.J. & Surl J.H. 2010. Characterization of canine oral papillomavirus by histopathological and genetic analysis in Korea. J. Vet. Sci. 11(1):21-25.