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“AVALIAÇÃO HISTOPATOLÓGICA DE AFECÇÕES PALPEBRAIS SELECIONADAS EM CÃES: INVESTIGANDO A INTERSEÇÃO DA DERMATOPATOLOGIA COM A OFTALMOLOGIA VETERINÁRIA”

CURITIBA – 2017

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*"*AVALIAÇÃO HISTOPATOLÓGICA DE AFECÇÕES PALPEBRAIS SELECIONADAS EM CÃES: INVESTIGANDO A INTERSEÇÃO DA DERMATOPATOLOGIA COM A OFTALMOLOGIA VETERINÁRIA*"*

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PARECER

A Comissão Examinadora da Defesa da Tese intitulada **"HISTOPATHOLOGICAL EVALUATION OF SELECTED PALPEBRAL DISEASES IN DOGS: INVESTIGATION OF THE INTERSECTION BETWEEN VETERINARY DERMATOPATHOLOGY AND OPTHALMOLOGY"** apresentada pela Doutoranda **JULIANA WERNER** declara ante os méritos demonstrados pela Candidata, e de acordo com o Art. 79 da Resolução nº 65/09–CEPE/UFPR, que considerou a candidata APTA para receber o Título de Doutor em Ciências Veterinárias, na Área de Concentração em Ciências Veterinárias.

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RESUMO

Os estudos, investigações e publicações sobre as alterações clínicas e histopatológicas das doenças que afetam as pálpebras de cães são muito escassos. Isso provavelmente se deve ao fato de as pálpebras serem estruturas que pertencem à área de atuação comum a duas especialidades médicas: a dermatologia e a oftalmologia, não se encaixando completamente em nenhuma delas. Tanto os dermatologistas não se sentem confortáveis em tratar de uma área que pertence ao olho; quanto os oftalmologistas, hesitam em abordar uma região tão ligada à dermatologia. Certamente o diagnóstico e consequente tratamento exitoso das afecções palpebrais exige conhecimentos sólidos de cada umas dessas especialidades. Assim sendo, estudos mais aprofundados e específicos das alterações patológicas palpebrais e que incluam conhecimentos e experiências das duas especialidades têm grande relevância no desenvolvimento da clínica veterinária de pequenos animais. Além disso, é importante que aqueles responsáveis pelo diagnóstico histopatológicos das alterações palpebrais, os patologistas, sejam apresentados a essa área de intersecção de especialidades e, uma vez habituados e conhecedores dela, tornem-se aliados dos oftalmologistas e dermatologistas no diagnóstico das afecções palpebrais.

A presente tese de doutorado está dividida em dois capítulos. O primeiro capítulo trata-se de um estudo retrospectivo de 290 casos de adenomas e epiteliomas da glândula tarsal em cães. Nele determina-se a frequência destas neoplasias palpebrais em dois laboratórios de Patologia Veterinária que recebem amostras de todo o Brasil. Além disso, discutem-se os aspectos histopatológicos dessas duas neoplasias e propõe-se uma nova forma de diferenciação entre elas. Este estudo deu origem a um artigo publicado no periódico *Brazilian Journal of Veterinary Pathology*.

O segundo capítulo avalia os achados histopatológicos e imuno-histoquímicos das lesões despigmentantes das pálpebras de cães. O estudo sugere que a despigmentação palpebral acompanhada do padrão histopatológico de dermatite de interface é uma manifestação localizada do lúpus eritematoso discoide. Além disso, o demonstra que o lúpus eritematoso discoide localizado pode ser restrito às pálpebras de cães e propõe o termo blefarite lupoide para essa manifestação. O estudo também avaliou a expressão da proteína p53 nas pálpebras despigmentadas com dermatite de interface. A correlação entre a expressão da proteína p53 com a presença de dermatite de interface em pálpebras despigmentadas pode ajudar no entendimento dos mecanismos das dermatopatias que levam à despigmentação, resultando em diagnósticos e prognósticos mais precisos. Esse artigo será publicado em periódico direcionado a clínicos veterinários dermatologistas ou oftalmologistas.

Palavras-chave: pálpebra, adenoma, epitelioma, dermatite de interface, blefarite lupoide, p53.

ABSTRACT

Studies, investigations and publications about clinical and histopathological alterations of diseases affecting the eyelids in dogs are very scarce. This is probably because the eyelids are structures belonging to an area common to two medical fields: dermatology and ophthalmology, and do not totally fit into neither. Dermatologists usually do not feel confident to treat an area belonging to the eye; and ophthalmologists hesitate to approach an area so connected to dermatology. Certainly, the diagnosis and consequent successful treatment of palpebral conditions demand solid knowledge from each of these specialties. Therefore, it is clear the need of deeper and more specific studies of palpebral diseases including knowledge and experience from both specialties. Besides, it is important that the pathologists themselves should be more familiar with this intersection area of specialties and make the connection between ophthalmologists and dermatologists in the diagnosis of eyelid diseases.

The present doctorate thesis was divided into two chapters. The first one is about a retrospective study of 290 cases of adenomas and epitheliomas of the tarsal gland in dogs. In this chapter, the frequency of these eyelid neoplasias was determined in two Veterinary Pathology laboratories that receive samples from all over Brazil. Besides, the histopathological aspects of both neoplasias have been discussed and a new form of differentiation between them has been proposed. This study gave origin to an article published in the *Brazilian Journal of Veterinary Pathology*.

The second chapter evaluates the histopathological and immuno-histochemical findings on the depigmenting lesions in eyelids of dogs. The study suggests that depigmented eyelids with interface dermatitis are, in fact, a clinical manifestation of localized discoid lupus erythematosus. Besides, the study demonstrates that localized discoid lupus erythematosus can be restricted to the eyelids of dogs and proposes the term lupoid blepharitis for those cases. The expression of p53 protein in depigmented eyelids with interface dermatitis also was investigated. The results of this study may help to better understanding of the mechanisms behind the disorders that lead to depigmentation, resulting in more precise diagnosis and prognosis. The manuscript will be published in a periodical with the focus directed to veterinary clinicians specialized in dermatology or ophthalmology.

Keywords: eyelids, adenoma, epithelioma, interface dermatitis, lupoid blepharitis, p53.

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LISTA DE ABREVIATURAS

USA – United States of America.

H&E – Hematoxylin and eosin.

RS – Rio Grande do Sul.

SC – Santa Catarina.

PR – Paraná.

AM – Amazonas.

PA – Pará.

BA – Bahia.

CE – Ceará.

GO – Goiás.

MA – Maranhão.

MS – Mato Grosso do Sul.

MG – Minas Gerais.

PB – Paraíba.

PE – Pernambuco.

RJ – Rio de Janeiro.

RN – Rio Grande do Norte.

SP – São Paulo.

SE – Sergipe.

Ad – Adenomas.

Ep – Epitheliomas.

UFPR – Universidade Federal do Paraná.

UV – Ultraviolet.

UVI – Ultraviolet index.

WHO – World Health Organization.

DCPAH – Diagnostic Center of Population and Animal Health.

MSU – Michigan State University.

DLE – Discoid lupus erythematosus.

LE – Lupus erythematosus.

CLE – Cutaneous lupus erythematosus.

ACLE – Acute cutaneous lupus erythematosus.

SCLE – Sub-acute cutaneous lupus erythematosus.

CCLE – Chronic cutaneous lupus erythematosus.

MCLE – Mucocutaneous lupus erythematosus.

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1. CAPÍTULO – 1

CANINE TARSAL GLAND EPITHELIOMAS AND ADENOMAS: A RETROSPECTIVE STUDY OF 290 CASES IN BRAZIL

ABSTRACT

Objectives: To determine the frequency and to better characterize the histopathological features of tarsal adenomas and epitheliomas in dogs.

Materials and Method: A total of 290 cases of tarsal gland adenomas and epitheliomas from canine patients were histopathologically analyzed. Tissue samples were examined in two veterinary pathology laboratories in Curitiba, Brazil.

Results: Tarsal epitheliomas were significantly more frequent than tarsal adenomas (77.9% and 20.7%, respectively). In four cases (1.4%), the lesion contained both types of neoplasms. A total of 39 breeds were represented and the most common breed was Cocker spaniel (12.4%). The upper eyelids were most commonly affected (65.2%), males and females were equally represented and there did not appear to be a predilection for either side of the face. The pigmented subtype was the most common form of epithelioma (59.7%). The subtype inflamed was highly represented (20.0%) in adenomas.

Discussion and conclusions: On the contrary of American publications, in Brazil tarsal epithelioma is significantly more frequent than tarsal adenoma in canine eyelids. Tarsal tumors are more likely to develop in the upper eyelids. Mitotic figures are more common in epitheliomas than in adenomas. Inflammation is more common in tarsal adenomas than in epitheliomas and pigmented cells are more common in tarsal epitheliomas than in adenomas. Some of these features have not been reported before in the veterinary ophthalmology and veterinary pathology literature. This study contributes to the knowledge of canine eyelid tumors.

Key words: adenoma, epithelioma, histopathology, tarsal gland, dog.

1.1 INTRODUCTION

The eyelids of dogs can be affected by a variety of neoplasms that are typically minimally invasive and respond well to simple surgical excision.[1,2] The two most commonly diagnosed eyelid neoplasms in dogs are the tarsal adenomas and epitheliomas,[3] also known as tarsal gland adenomas and epitheliomas (Figure 1.1). The tarsal glands (*glandulae tarsales*) are modified sebaceous glands of the eyelids responsible for producing the lipid phase of the tear film,[4] and neoplastic transformation of these glands can affect ocular health. Disorders such as hordeolum or chalazion can secondarily occur if there is obstruction and/or inflammation of the ducts of affected tarsal glands; lagophthalmos, if eyelid closure is impaired; and keratitis or less commonly ulcers also may develop as a direct result of the tumor rubbing the corneal surface. Removal of these tumors is indicated[5,6] to avoid such potential complications and to allow analysis and histopathological diagnosis of the tumor.[7]

In the past, tarsal gland tumors and cutaneous sebaceous tumors were essentially studied together.[8] Sebaceous adenomas and sebaceous epitheliomas of the skin are benign tumors, but sebaceous epitheliomas can be locally aggressive and, in some cases, recur after surgical excision.[8] Even though tarsal gland tumors are histologically similar to cutaneous sebaceous tumors,[9] most authors currently consider tarsal tumors separately from its cutaneous counterparts, mainly because all reported instances of metastases from epitheliomas have been from cutaneous sebaceous epitheliomas[8] and not from canine tarsal epitheliomas.[2,9]

Macroscopically, tarsal adenomas and epitheliomas of the eyelid are very similar and are firm lobular nodules extending from the eyelid margin, rarely exceeding 5 mm in diameter,[8] with or without pigmentation, inflammation and/or ulceration of the overlying skin. Microscopically, both tumor types have nodules of epithelial cells extending to the dermis. Tarsal gland adenomas consist of lobules of grouped meibocytes (sebocytes) with abundant vacuolated cytoplasm, showing gradual and orderly maturation with a prevalence of completely “lipidized” (mature) cells. In tarsal adenomas, it is common to observe degeneration of mature meibocytes (meibum formation) in the center of the neoplastic lobes due to the holocrine nature of the gland.[8] Tarsal epitheliomas, however, consist of lobules

composed of sheets and anastomosing trabeculae of predominantly smaller and more tightly packed reserve cells with random foci of orderly sebaceous maturation.[4,7] Either type of tumor can be endophytic, exophytic, or both. Ulceration, inflammation and pigmentation can be observed histologically in both types of tumors, but pigmentation is considered more common in tarsal epitheliomas than in tarsal adenomas.[4,7] The scientific literature reports that in dogs' eyelids, the prevalence of tarsal adenomas is higher than that of epitheliomas in the United States of America and South America (USA).[2,11,12,13,14,15,16] Some authors even consider tarsal epitheliomas uncommon or rare.[12] However, in two veterinary pathology services in Brazil the reverse was observed with eyelid tarsal gland epitheliomas being much more common than adenomas - a finding reported in a previous study of only 40 cases.[17]

In order to confirm the higher frequency of tarsal epitheliomas than adenomas in the eyelids of dogs in Brazil and to better establish the standard histopathological features of these neoplasms, a larger survey was conducted combining data from two veterinary pathology laboratories over a longer period of time and including a significantly larger number of cases.

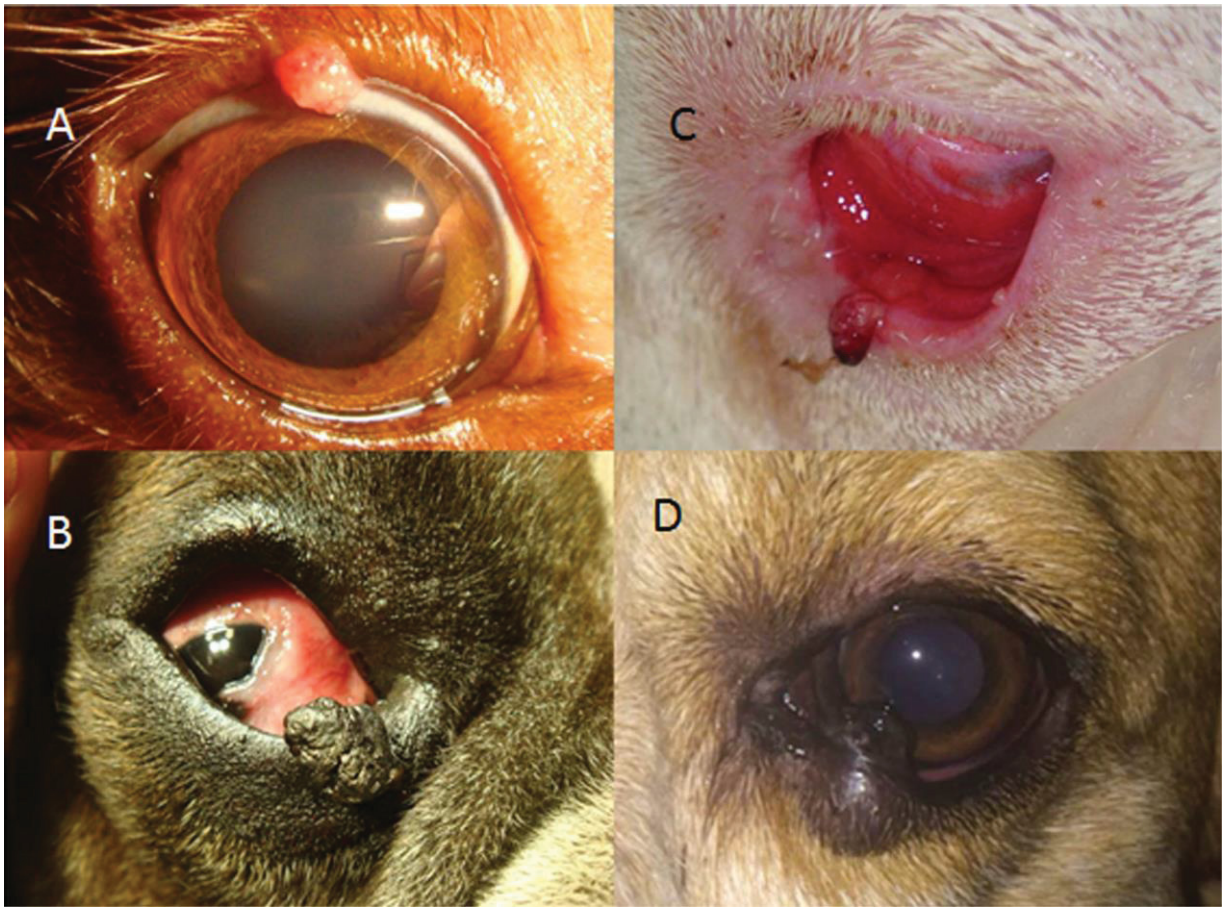

Figure 1.1 – Representative clinical photos of adenomas and epitheliomas of the tarsal gland. **A.** An eight-year old female poodle with a small (3 mm diameter), exophytic and unpigmented tarsal adenoma in the right superior eyelid. **B.** A six-year-old male boxer with a pigmented and relatively large tarsal adenoma (6 mm in diameter) in the right lower eyelid. **C.** An eight-year old Great Dane with a small (2 mm diameter), exophytic and focally ulcerated tarsal epithelioma. **D.** A seven-year old mixed-breed dog with a large (8 mm diameter) and pigmented tarsal epithelioma. Note that the clinical appearance alone is not a good parameter to differentiate between these two types of tarsal gland tumors.

1.2 MATERIAL AND METHODS

Records of 290 cases of eyelid tarsal gland adenomas and epitheliomas in dogs diagnosed in samples submitted to two different Brazilian veterinary pathology laboratories offering ocular pathology services were compiled and analyzed. One of these centers is a private laboratory (Laboratory Werner & Werner, Curitiba-PR); and the other is linked to the Federal University of Paraná (Comparative Ophthalmology Laboratory, Curitiba-PR). Samples were submitted between 2008 and 2015 from all over Brazil, and included male and female dogs of many different breeds and ages.

Samples were fixed in 10% neutral buffered formalin for 2 to 7 days. After macroscopic examination, which included the measurement of the largest axis of the tumor, the samples were trimmed and, the fragments were embedded in paraffin and 5 µm sections were stained with hematoxylin and eosin (H&E). The neoplasms were classified as tarsal gland epitheliomas or adenomas according to standard histological diagnostic criteria.[8] By definition, reserve cells have scant, eosinophilic cytoplasm and slightly hyperchromatic, ovoid nuclei with inconspicuous nucleoli. Mature sebocytes have moderate, clear cytoplasm with numerous, delicate lipid vacuoles with more centrally-located nuclei with irregular margins and small nucleoli. All the samples were reviewed by the same pathologist. Other factors were considered such as tumor growth patterns, differentiation and cell maturation patterns and forms (presence of reserve cells, mature cells and lipidized cells); cell organization patterns (presence of trabeculae or lobules); number of mitotic figures per 10 high-power fields (400x); and other changes such as the presence of skin ulceration, inflammatory reaction or pigmentation. When reserve and mature cells were present in equal proportions a diagnosis of tarsal epithelioma was made. In order words: this was the cut-off point, less mature cells than that makes it an epithelioma and more than that an adenoma. Mitotic figures were evaluated and expressed as the mean of 10 high power fields analyzed (Olympus BX-45 diagnostic microscope, high power field diameter of 0.52 mm). Ordinal grade cut-offs for mean mitotic figure counts were then created: Absent (Grade 0); Mean 0-0.9 (Grade 1); Mean 1-1.9 (Grade 2): Mean 2-3 (Grade 3). When inflammation or pigmentation was present, the tumors were further divided into “inflammatory” or “pigmented” subtypes. When inflammation and pigmentation were not prominent features, the neoplasm was considered “classic”. The typical type of inflammation present was lipogranulomatous. A diagnosis of tarsal adenoma and epithelioma in the same sample was made in samples in which different lobules within a tumor showed a predominance of basaloid reserve cells whereas in other lobules differentiated cells predominated.

Results were tabulated and statistically analyzed using the chi-square test. Data were deemed significant when *P* values were less than 0.05.

1.3 RESULTS

The number of cases (221/290 cases, or 76.2%) from the official geopolitical region of Southern Brazil, which comprises the States of Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR), was significantly higher (P= 0.0001) than the numbers from the rest of the country combined (69/290 cases, or 23.8%). Figure 2 provides the number of cases of eyelid tarsal gland adenomas and epitheliomas according to geographical location within Brazil.

The diagnosis of tarsal epitheliomas was significantly more common not only in Southern Brazil – PR+SC+RS (175/221) as well as in the rest of the country (50/64) P≤ 0.006 (Figure 1.2).

The diagnosis of adenomas and epitheliomas together corresponded to 36.4% (290/797) of the laboratory´s canine ocular pathology caseload. In 177 cases the affected side of the face was reported, 92/177 (52.0%) occurred on the left side (10 adenomas and 82 epitheliomas) and 85/177 (48.0%) occurred on the right side (19 adenomas and 66 epitheliomas). There were 143 (49.3%) submissions from males and 142 (49.0%) from females. In five cases the sex of the patient was not reported. There were no significant differences between reported sexes or the side of the face affected, either for epitheliomas or for adenomas (P > 0.05).

Of the 178 cases where the affected eyelid was informed, 116 (65.2%) were removed from the upper eyelid, and 62 (34.8%) from the lower eyelid. This difference was statistically significant (P = 0.0002).

The mean largest axis of the adenomas was 6.3 ± 1.9 mm and of the epitheliomas was 6.7 ± 3.3 mm. Adenomas and epitheliomas had the same median and mode, 6 and 5 mm, respectively. There were no significant differences between the mean of the largest axis of the two tumors (P = 0.60).

The average age of the animals in this study was 9.4 years, median and mode of 10 years; with minimum age of 3 years and maximum age of 16 years. The most commonly affected breeds were: Cocker spaniel (35 cases, 12.1%); Poodle (32 cases, 11.0%); Labrador retriever (28 cases, 9.7%); and mixed breed dogs (38 cases, 13.1%). In nine cases, the breed was not reported, and the remaining 148

cases were distributed among 36 other different breeds. Epitheliomas were diagnosed in 33 (94.3%) of the samples from Cocker spaniels, in 28 (82.1%) of the samples from Labrador retrievers and in 25 (78.1%) of the samples from Poodles.

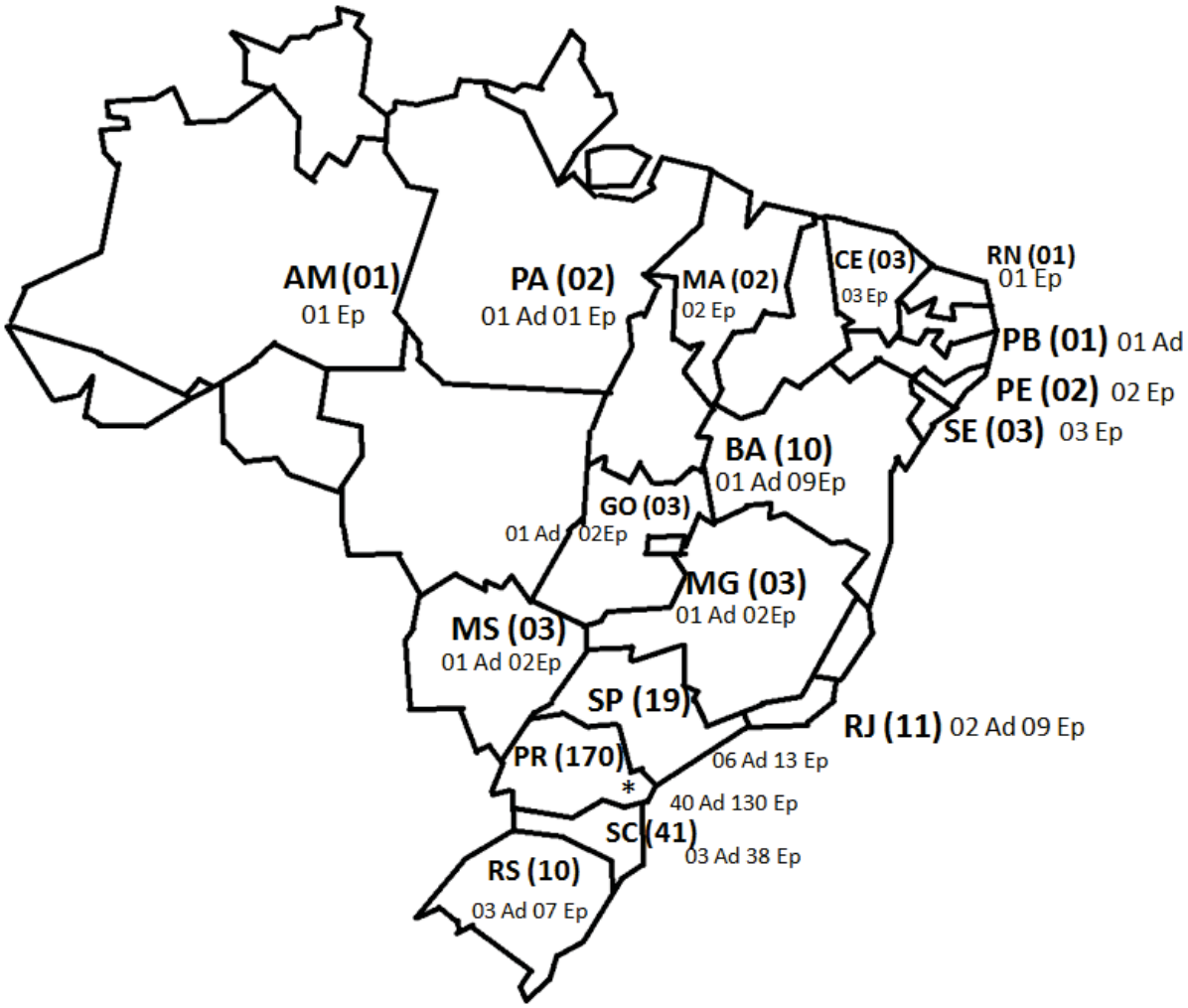

Figure 1.2 – Distribution of the 285 cases of eyelid tarsal gland adenomas and epitheliomas in dogs submitted from 2008 to 2015 according to the state of origin within Brazil. The total number of cases was 290; however, in one case there was no information about the geographic origin. AM- Amazonas; PA- Pará; BA- Bahia; CE- Ceará; GO- Goiás; MA- Maranhão; MS- Mato Grosso do Sul; MG- Minas Gerais; PB- Paraíba; PR- Paraná; PE- Pernambuco; RJ- Rio De Janeiro; RN- Rio Grande Do Norte; SC- Santa Catarina; SP- São Paulo; SE- Sergipe. The numbers in brackets represent the total number of cases from that state, followed by the numbers of adenomas (Ad) and epitheliomas (Ep). In the South, in the state of Paraná (PR), the city of Curitiba where the two veterinary pathology laboratories are located, is indicated (*).

The descriptive statistical analysis for the histological characteristics found in the survey is presented in Table 1.1. The tarsal epitheliomas (n = 226) were present at significantly higher numbers (P = 0.00001) than the tarsal adenomas (n = 60). The classic subtype (without inflammation or pigmentation) was the most common form of tarsal adenoma (P = 0.002). When inflammation was recognized, it was significantly more common in adenomas than in epitheliomas (P = 0.04). The pigmented epithelioma (Figure 1.3c) was significantly more common than any other type of epithelioma (P < 0.05), followed by the classic type (no inflammation or pigmentation). Pigmented and inflamed epitheliomas were significantly less common (p < 0.05). Pigmented epitheliomas were significantly more common than pigmented adenomas (P = 0.004).

Table 1.1 – Prevalence of canine eyelid tarsal epithelioma, tarsal adenoma or both tumors in the same sample at two different laboratories (Werner & Werner Laboratory and Comparative Ophthalmology Laboratory at UFPR) in Brazil, during a seven-year period (2008 to 2015).

Neoplasia	Histopathological subtypes	Number of cases	Subtype within all samples	Subtype within adenomas/epitheliomas (%)
Adenoma	Classic	37	12.8	61.7
	Inflamed	12	4.1	20.00
	Pigmented	8	2.8	13.3
	Pigmented and Inflamed	3	1.03	5.00
	TOTAL ADENOMAS	60	20.7	100.00
Epithelioma	Classic	53	18.3	23.4
	Inflamed	21	7.2	9.3
	Pigmented	135	46.5	59.7
	Pigmented and Inflamed	17	5.9	7.5
	TOTAL EPITHELIOMAS	226	77.9	100.00
	ADENOMAS + EPITHELIOMAS	286	98.6	100.00
Adenoma and Epithelioma in the same sample		4	1.4	100.00
	TOTAL	290	100.00	100.00

The classic subtype (without inflammation or pigmentation) (Figure 1.3a) was the most common form of tarsal adenoma (P = 0.002). Nevertheless, the inflamed type (Figure 1.3b) occurred significantly more commonly in adenomas than in epitheliomas (P = 0.04).

Concerning the mitotic index analysis, no atypical mitoses were observed in any of the adenomas or epitheliomas. In 57 cases of the 60 adenomas evaluated, no mitotic figures were found (Grade 0). In the three remaining samples, a Grade 1 mitotic index was found. From the 226 epitheliomas analyzed, 32, 162, 21 and 14 tumors had Grade 0, 1, 2, or 3, respectively. Thus, a significantly higher number of adenomas showed no mitotic figures compared to epitheliomas (P = 0.0001). In the same manner, the presence of 1 or more mitotic figures per high power field (classified here as tumors of Grades 1-3) was significantly more common in epitheliomas than in adenomas (P = 0.0001). Regarding the tumor samples containing both adenomas and epitheliomas, one was Grade 0, two were classified as Grade 1, and one as Grade 2.

1.4 DISCUSSION

The large number of samples submitted from southern states of the country most likely reflects the fact that the laboratory is located in this area and generally receive more cases from geographically closer locations.

The significantly higher incidence of tarsal gland neoplasia (epitheliomas and/or adenomas) on the upper (rather than lower) eyelid reported in this study is consistent with literature reports and is probably due to a higher density of tarsal glands in the upper eyelid.[18]

Dogs affected by tarsal gland neoplasia were middle-aged to old as reflected by the mean age in our study and in previous studies. This result confirms data from the literature in which a typical age of 9 – 10 years is reported.[1,5,6,7,12,17,19]

The breeds most affected by these eyelid tumors are Poodles and Cocker spaniels.[6,10] This finding was confirmed in the present study, where these two breeds were the most prevalent, accounting for 22.7% of all diagnosed cases. However, it is possible that this higher prevalence merely reflects a higher prevalence of certain breeds in the general dog population in a particular region. Considering the reference population from the laboratory, the diagnosis of tarsal epithelioma is indeed over-represented Shih-tzus and Lhasa apsos since it is not a common breed listed in the laboratory´s caseload of ocular lesions. There is no convincing data in the literature

of a sex predisposition for eyelid tumors and in this study; we report a similar prevalence between the sexes.

Inflammation is significantly more common in adenomas than in epitheliomas. The inflammatory reaction is caused by the presence of free keratosebaceous material (sebum or meibum) in the dermis/substantia (Figure 1.3b). Tarsal adenomas have more sebaceous differentiated cells (which can release lipids) and hence sebum accumulates in glands and ducts, frequently causing distention and rupture. The release of sebum into the dermis incites a severe foreign body inflammatory reaction (lipogranuloma).

The pigmented subtype was the most common form of epithelioma (Figure 1.3c), and epitheliomas were more likely than adenomas to be pigmented. It is clear that epitheliomas have a significantly greater population of basal cells, which defines this tumor type. It is known that melanocytes are distributed throughout the reserve cells of an epithelium, including the epidermis and the foliculosebaceous units, pigment in epitheliomas is not located just in melanocytes but also within the basaloid reserve cells themselves (and melanophages)[8] and these facts may explain the greater numbers of epitheliomas being pigmented.

In this study, the results relating to age and breed of affected animals and the location of tarsal adenomas and epitheliomas are similar to those reported in the literature, including in the USA. However, there is a notable difference from those studies from the USA in that the number of tarsal gland epitheliomas was much higher than adenomas in our study. According to the literature, tarsal gland adenomas are the most prevalent neoplasia in dogs' eyelids.[2,11,12,14,15,16] However, in the present investigation as well as in the previous pilot study from the same group,[17] tarsal gland epitheliomas were significantly more prevalent than the tarsal gland adenomas (77.9% and 20.7%, respectively). In order to possibly explain the difference between the data obtained here and the data published in the literature, the authors hypothesize two scenarios: 1) the amount of ultraviolet (UV) radiation received in the individuals from different populations investigated and 2) the different criteria used by pathologists in the histopathological classification of the tarsal tumors, both of which are discussed below.

It is evident that hyperplasia and neoplasia are not always easily distinguishable. Even though the pathogenesis of tarsal gland hyperplasia is not well understood and its origin as either a reactive or neoplastic process is not well established, it is at least known that ultraviolet radiation is a cofactor (among others) for the development of sebaceous gland hyperplasia of the skin.[20] These studies are from the medical literature (human medicine), there are no similar studies in small animals to reflect the effect of UV light on the development of tarsal gland tumors in dogs. The country of Brazil is located in the tropical and subtropical areas of the southern hemisphere, where solar radiation is intense and ozone concentrations are naturally lower.[21] The ultraviolet radiation index (UVI) indicates the amount of UV radiation at any given time and ranges from low (1 – 2 UVI) to extreme (11+ UVI) with the higher values being more dangerous. UVIs observed in Brazil are high (between 5 and 11 or higher), reaching the highest UVI scales reported by the World Health Organization (WHO).[21] The Brazilian UVIs are similar to the Australian ones, being much higher than those observed in countries of the northern hemisphere,[21,22] since the Earth's orbit brings countries in the southern hemisphere closer to the sun the southern summer than countries in the northern hemisphere during northern summer.[22] Therefore, the authors believe that it would be interesting to investigate the prevalence of both types of canine eyelid neoplasms in other countries that experience high levels of UVIs.

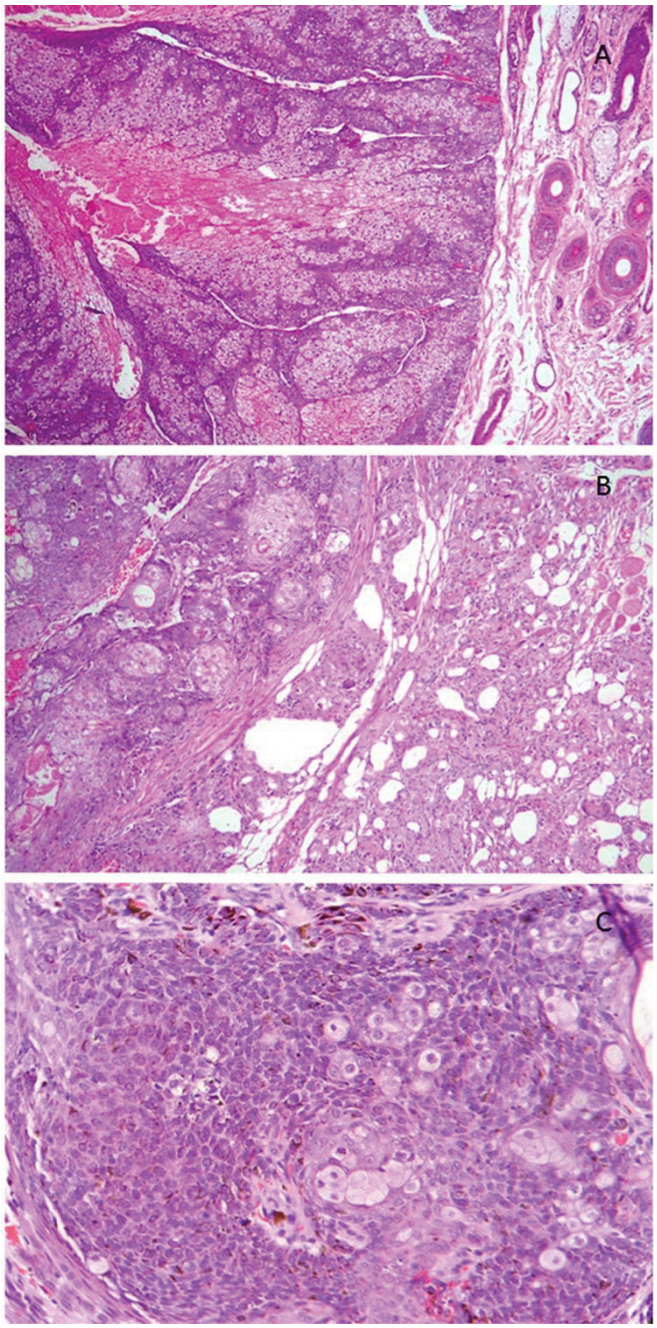

Figure 1.3 – Photomicrographs of representative cases tarsal tumors reported here. **A.** Sebaceous adenoma from the tarsal gland of the eyelid of a dog. A well demarcated neoplastic mass composed mostly of well-differentiated lipidized (clear) cells forming lobules expands the dermis of the eyelid. (H&E, 100x). **B.** Inflamed sebaceous adenoma from the tarsal gland of the eyelid of a dog. An accumulation of numerous macrophages surrounding clear spaces (lipid lakes) lies adjacent to the adenoma (lipogranulomatous inflammation). (H&E, 100x). **C.** Pigmented tarsal epithelioma in a dog. Note that the majority of cells are smaller, more tightly packed reserve cells, many of which are pigmented, and smaller numbers of well-differentiated sebocytes/meibocytes with abundant vacuolated cytoplasm (H&E, 200x).

Although tarsal adenomas and tarsal epitheliomas are clinically similar, histopathologically they are quite distinct. In classic tarsal adenomas, most proliferating cells are mature and are completely "lipidized" i.e. the cells exhibit features of mature meibocytes and are organized into defined lobes (Figure 1.3a). On the other hand, in tarsal epitheliomas, the majority of cells typically resemble the basaloid reserve cells at the periphery of normal tarsal glands with only individual cells or clusters of cells showing sebaceous maturation. In epitheliomas, there is no formation of distinct sebaceous lobules, and mitotic figures are more common in epitheliomas than in adenomas.[6] It is important to bear in mind that the distinction between adenomas and epitheliomas is highly subjective, thus it depends on the pathologist. Objective standards to differentiate tarsal adenomas and epitheliomas are yet to be established. As an “unwritten rule”, epitheliomas should have a substantial number of basal cells. For skin tumors, however, some authors use a threshold of up to 90% of the tumor cells being of the basal type to deserve the diagnosis of tarsal epithelioma.[8,9,23]. Nevertheless, in samples seen at veterinary histopathology laboratories, a significant percentage of tarsal gland eyelid neoplasms show similar proportions of reserve and mature cells. In these cases, the authors believe that a diagnosis of tarsal epithelioma should be made. It is possible that in such cases other pathologists opt for the diagnosis of tarsal adenoma. However, it is important to remember that in the skin low-grade epitheliomatous sebaceous carcinoma is histologically similar to sebaceous epithelioma and consequently tarsal epithelioma,[8] making it difficult to differentiate between them at times. In these cases, it is possible that some pathologists opt for the diagnosis of low-grade epitheliomatous sebaceous carcinoma. The authors believe that the diagnosis of low-grade epitheliomatous tarsal carcinoma should only be made in the presence of obvious signs of malignancy, in particular the presence of infiltrative margins and/or invasion of the underlying subcutis. Nevertheless, it is impossible to examine margins in many eyelid tumors since they are submitted in several fragments, so for those samples, they would be unable to be diagnosed as either benign or malignant.

As observed here, mitoses may be quite frequent in tarsal epitheliomas. However, other features of malignancy are usually not observed.[9] This is an important feature to be remembered for the diagnosis of this type of tumor and it is worth mentioning

that some tumors that should be diagnosed as tarsal epitheliomas have been published as carcinomas based on the number of mitoses.[9,24,25]

The histopathological classification of sebaceous tumors is still far from being well established in both animals and people. In people, some authors suggest classifying the sebaceous tumors into six categories: 1) sebaceoma, 2) trichoblastoma with sebaceous differentiation, 3) apocrine poroma with sebaceous differentiation, 4) low grade sebaceous carcinoma, 5) sebaceous carcinoma, and 6) basal carcinoma with sebaceous differentiation.[18] In veterinary dermatopathology the term "sebaceoma" has not been adopted. Moreover, human low-grade sebaceous carcinoma is very similar to what veterinary pathologists define as sebaceous epithelioma.[8] It is worth mentioning that eyelid tumors in general are quite rare in humans, especially sebaceous gland carcinoma, representing 1–5.5% of all the eyelid malignancies.[26]

This difficulty in establishing histopathological parameters and standards supports the hypothesis that the benign sebaceous and tarsal tumors, and those of low-grade malignancy, in reality, may be part of a continuum.[8] In other words, they are different morphological manifestations of the same neoplastic process from the same source and with the same causes. In this investigation, the diagnosis of four cases of simultaneously occurring tarsal adenoma and epithelioma in the same sample (Table 1.1) reinforces this possibility. We recognize, however, as a matter of semantics, some authors do not consider different lobules with basaloid reserve cells and lobules with differentiated cells predominate, necessarily as different tumor. Thus, a plausible hypothesis is that tarsal gland adenoma, epithelioma and low-grade epitheliomatous carcinoma reflect a spectrum of histopathological patterns of the same neoplasia. Furthermore, that spectrum can vary from tumor to tumor and even within the same tumor, depending on the portion analyzed histopathologically. One factor that could help prove this hypothesis would be better understanding of the progression and evolution of these neoplasms. Nevertheless, such data are lacking in the literature and, for this study, there was no information as to how long tumors had been present before excision and histopathological diagnosis.

1.5 CONCLUSION

This study concludes that the frequency of tarsal epithelioma is significantly greater than tarsal adenoma in canine eyelids in Brazil. Breeds in which epitheliomas are overrepresented are Labradors, Poodles, Cocker spaniels, Shih tzus and Lhasa apso. Tarsal tumors are more likely to develop in the upper eyelids. Mitotic figures are more common in epitheliomas than in adenomas. Inflammation is more common in tarsal adenomas than in epitheliomas and pigmented cells are more common in tarsal epitheliomas than in adenomas. Some of these features have not been reported before in the veterinary ophthalmology and veterinary pathology literature. This study contributes to the knowledge of canine eyelid tumors.

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2. CAPÍTULO – 2

DEPIGMENTING INTERFACE BLEPHARITIS (LUPOID BLEPHARITIS) AND p53 IMMUNOEXPRESSION IN DOGS

ABSTRACT

Objectives: To confirm that depigmented eyelids with interface dermatitis is a clinical manifestation of localized discoid lupus erythematosus; to demonstrate that localized discoid lupus erythematosus can be restricted to the eyelids of dogs; and to investigate the expression of p53 protein in depigmented eyelids with interface dermatitis.

Material and methods: From 86 samples of eyelid biopsies, 11 cases of depigmentation with histopathological pattern of interface dermatitis were selected. The cases were histopathologically diagnosed as cell-poor or lymphocyte-rich interface dermatitis. The samples were submitted to immunochemistry evaluation for p53 protein expression.

Results: Four of the 11 samples (36.36%) had depigmentation exclusively in the eyelids. Seven samples (63.63%) were from dogs presenting depigmented eyelids associated with additional depigmentation in other facial areas. In five of these dogs (71,43%), the additional depigmentation was located in nasal planum; one sample (14,29%) was from a dog with additional depigmentation in nasal planum and lip, and one sample (14,29%) came from a dog with additional depigmentation in scrotum In the histopathological exam, eight of the samples (72.73%) showed lymphocyte-rich (lichenoid) interface dermatitis, and three (27.27%) showed cell-poor interface dermatitis. Protein p53 was detected in eight of the nine samples (88.89%) evaluated using immunohistochemistry, with results that varied from three to 55 positive nuclei per 5-grid unit.

Discussion and conclusions: Depigmented autoimmune skin diseases can affect the eyelids only. When the histopathological examination reveals interface dermatitis, the most adequate term for the diagnosis is “lupoid blepharitis”. Lupoid blepharitis was more common in female dogs. The expression of p53 in lupoid blepharitis can occur in the absence of histopathological neoplastic or pre-neoplastic disease. Independently of the cause or association of causes, actinic or autoimmune, depigmented eyelids with interface dermatitis express p53 protein, which suggests loss of regulation of cell proliferation and, therefore, higher risk to neoplastic transformation.

Keywords: eyelids, depigmentation, blepharitis, interface dermatitis, lupoid blepharitis, p53, immunohistochemistry, discoid lupus erythematosus.

2.1 INTRODUCTION

The eyelids are a mucocutaneous structure and the most external and visible adnexa of the eye. Diseases that affect the skin of other areas of the body also can affect the cutaneous component of the eyelids. However, the eyelids have some particularities that demand special attention. Most importantly, it is a mucocutaneous junction and marks the boundary between two different anatomical structures. Besides, they share aspects of interests of two veterinary specialties, dermatology and ophthalmology. Maybe because of that, they are partially neglected in most dermatology investigations, ophthalmology texts, and even more in dermato-histopathology publications. As a consequence, many diseases of the eyelids need further clarification and standardization, especially in veterinary medicine.

The eyelids are immunologically active structures[1], and as such, inflammation is a very common event. The causes of blepharitis are similar to and as varied as the causes of dermatitis in general.[2] Inflammation of these structures may occur via different processes and, in some of them, the result is depigmentation. There is a diversity of skin diseases capable to promote inflammation with depigmentation of mucocutaneous junctions mostly due to damage to the epidermal basal layer and destruction of keratinocytes and melanocytes.[2,3] It is not known if the already established dermato-histopathological patterns for the canine skin in general are totally applicable to the eyelids.

The main histopathologic pattern that accompanies depigmentating skin and mucosal diseases is “interface dermatitis”. Histologic features of this histological pattern consist of mild vacuolar change in the basal cell layer of the epidermis with some individually apoptotic basal keratinocytes. The superficial dermis shows inflammatory lichenoid lymphoplasmacytic infiltrate with pigmentary incontinence.[4] Mild interface and/ or superficial perivascular inflammation without a lichenoid band may be observed. Chronically inflamed interface dermatitis lesions with prominent plasma cell infiltrates often lack obvious basal cell damage and are more purely lichenoid.[3]

Depigmenting blepharitis, besides discomfort, pain, pruritus and decrease in ocular surface health, expose the eyelids to ultraviolet rays due to loss of the melanin

pigment. Overexposure to ultraviolet (UV) radiation has harmful effects that go from a simple sunburn, hyperpigmentation or keratosis to skin tumors.[5]

The tumor suppressor p53 protein is activated by various types of DNA damage and cellular stress and plays a central role in the suppression of development of cancer. The p53 protein is now known to induce apoptosis, cell cycle arrest, senescence, and DNA repair. Its purpose appears to be removal of DNA-damaged cells that might otherwise become mutated.[6] Consequently, p53 protein is considered the genomic guardian.[7]

The expression of p53 suggests gene mutation. It has been shown that mutant p53 accumulates in the nucleus of cells probably due to its increased half-life. Furthermore, expansion of p53 in the epidermis correlates with sun exposure and sun UV damage, even in the absence of premalignant changes.[8] In addition, there is evidence indicating that the dysfunction of p53 is associated with the development of autoimmune diseases.[9] The expression of p53 is frequently demonstrated by immunohistochemistry in neoplastic, immune-mediated and inflammatory diseases studies in human beings.[7,10] Expression of p53 also has been reported in canine melanomas, and in mammary, eyelid and corneal tumors.[11, 12,13,14] However, studies on the expression and mutations of p53 in the depigmentating interface blepharitis of dogs are nonexistent.

The purpose of the present study was to confirm that depigmented eyelids with interface dermatitis is a clinical manifestation of a localized form of discoid lupus erythematosus and to demonstrate that localized discoid lupus erythematosus can be restricted to the eyelids of dogs. Besides, the study aimed to relate the expression of p53 protein in depigmented eyelids with interface dermatitis and without histopathological evidence of neoplastic or pre-neoplastic disease. The correlation between interface dermatitis and immunoexpression of p53 in depigmented diseases of the eyelids may help better understanding of the mechanisms behind the disorders that lead to skin and eyelid depigmentation, resulting in more precise diagnosis and prognosis.

2.2 MATERIAL AND METHODS

Eighty-six samples of canine eyelid margin biopsies of non-nodular lesions, received from 2010 to 2015, were submitted to histopathological evaluation in a private veterinary pathology laboratory (Werner & Werner, Curitiba, PR, Brazil). The surgically removed specimens were fixed with 10% neutral formalin immersion from two to seven days, dehydrated through a graded series of alcohol, washed with xylene and embedded in paraffin wax. Sections of 5-µm-thick were serially cut of each of those samples was stained with hematoxylin and eosin (H&E). Eleven out of the 86 samples met the criteria of inclusion in this study,

The criteria for inclusion in the study were the presence of depigmentation of the eyelid at the physical examination and a histopathological pattern of interface dermatitis on the histopathological diagnosis. Accordingly to the density of the superficial dermal inflammatory infiltrate, the interface dermatitis was classified as "cell-poor"[3] or "lymphocyte-rich".[15]

The eleven selected samples were submitted to immunochemistry evaluation for the p53 protein expression at the Diagnostic Center for Population and Animal Health (DCPAH) at Michigan State University (MSU). Immunohistochemical staining for p53, was performed. Deparaffinization, antigen retrieval, immunohistochemical staining, and counterstaining were performed using the Benchmark Automated Immunostainer (Ventana Medical Systems Inc, Tucson, AZ) and the Bond Max AutomatedStaining System (Vision Bio Systems, Norwell, MA). The Benchmark System was used to stain slides with a rabbit polyclonal anti-p53 antibody (Signet Laboratories, Dedham, MA) at a dilution of 1:100. A biotinylated anti-mouse and anti-rabbit secondary antibody conjugated with alkaline phosphatase-streptavidin (enhanced alkaline phosphatase red detection kit, Ventana Medical Systems Inc.) was applied to visualize the bound primary antibody. Sections were counterstained with hematoxylin, followed with a bluing reagent to change the hue of hematoxylin to blue color (Bluing Reagent, Ventana Medical Systems Inc.). Immunostaining quantification of p53 protein expression was determined by manually counting the number of positive epidermal keratinocytes nuclei within the area of 1.0 mm^2 optical-grid reticle at 400x using a standard light microscope and a cell counter. Five grid areas within the areas of highest labeling were counted. The p53 data was

processed to generate mean, standard deviation and median using Graphpad Prism® 5.0 (California, United States of America) for Windows.

2.3 RESULTS

From the 86 samples examined, 11 (11/86 or 12.79%) had clinically detectable depigmentation and a histopathological pattern of interface dermatitis. Eight of the dogs were females (8/11 or 72.72%) and three (3/11 or 27.27%) were males, ranging in age from one to 11 years of age. The mean age was 5.5 years. One case was from a Rottweiler, one from a Lhasa Apso, and one from a Pinscher, and eight from mixed-breed dogs (Table 2.1).

Seven cases (7/11 or 63.63%) came from dogs which had depigmented eyelids associated with additional depigmentation in other facial areas (Table 2.1). Five of those (5/7 or 71.43%) the additional depigmentation was located in nasal planum (Figure 2.1); one sample (1/7 or 14.29%) came from a dog with depigmentation also in scrotum, and one sample (1/7 or 14.29%) from a dog with additional depigmentation in nasal planum and lip. A total of four samples (4/11 or 36.36%) had depigmentation exclusively in the eyelids (Figure 2.2).

Table 2.1. – Clinical features of the 11 samples of depigmented eyelids of dogs with interface dermatitis.

Samples	Breed	Age (years)	Sex	Sites of additional depigmented lesions
1	Mix breed	11	Female	-
2	Mix breed	2	Female	Nasal planum
3	Mix breed	6	Female	Nasal planum and labial
4	Rottweiler	7	Female	Nasal planum
5	Pinscher	1	Female	-
6	Lhasa apso	4	Male	Scrotal
7	Mix breed	10	Female	Nasal planum
8	Mix breed	3	Female	Nasal planum
9	Mix breed	2	Female	-
10	Mix breed	-	Male	-
11	Mix breed	6	Male	Nasal planum

Figure 2.1 – Clinical aspect of a dog, included in this study, presenting depigmented areas in the lower eyelid (left) and in the nasal planum (right), concomitantly. Note the stitches used for suturing the biopsy site in the nasal planum (right).

Figure 2.2 – Clinical aspect of a dog, included in this study, presenting depigmented area exclusively in the lower eyelid (lupoid blepharitis).

All of the eleven samples showed interface dermatitis. Eight of them (8/11 or 72.73%) showed lymphocyte-rich interface dermatitis (Figure 2.3), and three (3/11 or 27.27%) showed cell-poor interface dermatitis (Figure 2.4). Pigmentary incontinence was observed in nine samples (9/11 or 81.82%); seven of these (7/9 or 77.78%) were associated to lymphocyte-rich interface dermatitis, and two (2/9 or 22.22%) to cell-poor interface dermatitis. The data is summarized in Table 2.2.

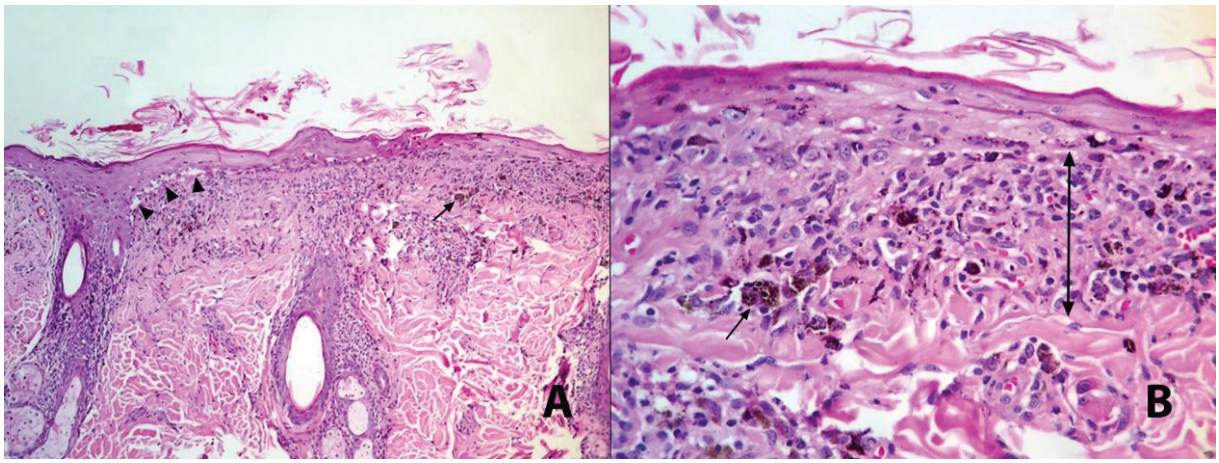

Figure 2.3 – Photomicrograph of a representative case of lymphocyte-rich interface dermatitis in depigmented eyelid of a dog. **A**: Notice the hypercellular aspect of the superficial dermis due to a band-like accumulation of inflammatory cells, and vacuolar degeneration of basal cells (arrow-heads). (H&E, 100x). **B**: Higher magnification of the left picture. Notice pigmentary incontinence (arrow – the same area is indicated in the left picture) and the obliteration of the dermal-epidermal junction by the inflammatory infiltrate (double-headed arrow). (H&E, 400x).

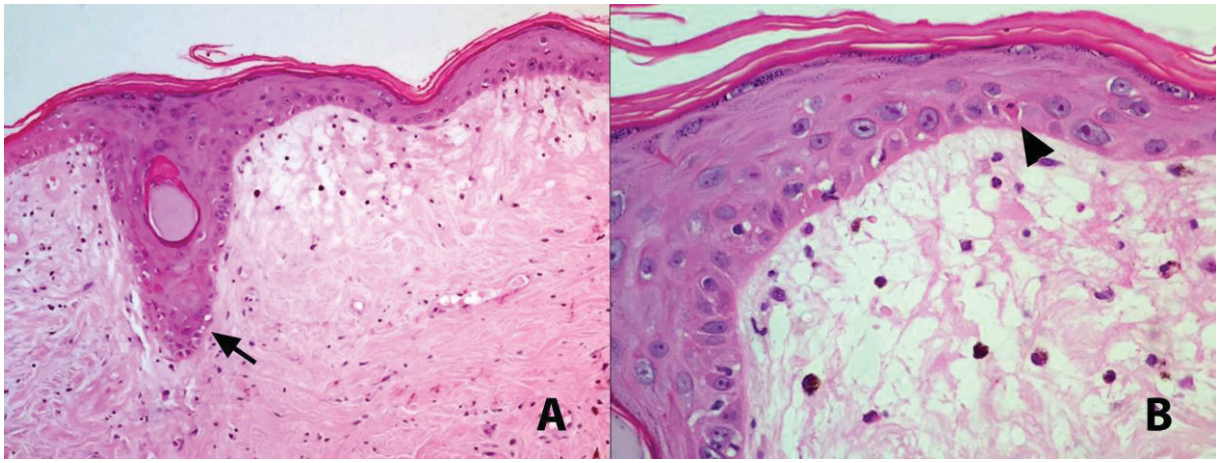

Figure 2.4 – Photomicrograph of a representative case of cell-poor interface dermatitis in depigmented eyelid of a dog. **A**: The superficial dermis is pale, with discrete inflammatory infiltrate. Notice vacuolar degeneration of the basal cells of the infundibular epidermis (arrow). (H&E, 100x). **B**: Higher magnification of the left picture. Notice hypocellular superficial dermis and apoptosis of basal cell keratinocyte (arrow-head). (H&E, 400x).

For the expression of p53, from the 11 samples submitted to the evaluation, nine were adequate; two were deemed inadequate. Protein p53 was detected (Figure 2.5) in eight of the nine samples (8/9 or 88.89%); the results varied (range) from three to 55 (mean of 15.87) nuclei per 5-grid unit; in one of the samples (1/9 or 11.11%) there was no expression of p53 (Table 2.2).

Table 2.2 – Histopathological features of the 11 samples of depigmented eyelids of dogs with interface dermatitis.

Samples	Interface dermatitis	Cell-poor	Lymphocyte-rich	Pigmentary incontinence	p53
1	+	-	+	+	5
2	+	-	+	+	-
3	+	-	+	+	-
4	+	-	+	+	19
5	+	+	-	+	55
6	+	-	+	+	5
7	+	-	+	+	12
8	+	+	-	-	20
9	+	-	+	+	3
10	+	+	-	-	8
11	+	-	+	+	0

* Present (+); Absent (-); and Protein p53 (number of positive epidermal keratinocytes nuclei in 5 grids using a high power objective - 400x).

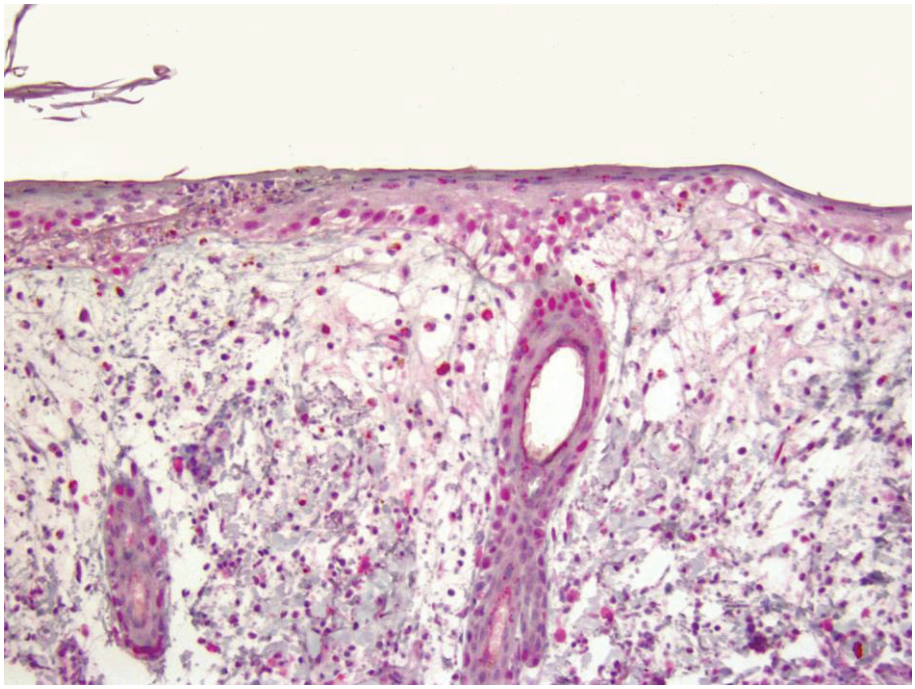

Figure 2.5 – Representative case of depigmented eyelid of a dog. Positive immunohistochemical staining of p53 expression. Keratinocytes bearing the antibody-antigen complexes are stained in red (100x).

2.4 DISCUSSION

Interface dermatitis is a well-known dermato-histopathological pattern of depigmented skin diseases. For descriptive clarity, the authors define interface dermatitis as superficial dermal inflammation with inflammatory infiltrate oriented towards and that can obscure the dermal-epidermal junction, and more importantly, with the presence of epidermal basal cell damage characterized by vacuolar degeneration of basal cells and apoptosis of basal cell keratinocytes.[2,3,4] This report proves, that just like in the skin of other regions, lymphocyte-rich interface dermatitis or cell-poor interface dermatitis can occur in depigmented eyelids.

The main depigmenting skin diseases with interface dermatitis that can affect the eyelids are discoid lupus erythematosus, systemic lupus erythematosus, mucocutaneous lupus erythematosus, ischemic dermatopathy/canine dermatomyositis, solar dermatitis, and drug eruption.[3,15,16,17] Despite having the same general histopathological pattern, they can be differentiated by some subtle histopathological features correlated to the history and clinical presentation.

Discoid lupus erythematosus (DLE) is an important depigmenting skin disease with a well-established histopathological pattern: lichenoid interface dermatitis,[3,4] also called lymphocyte-rich interface dermatitis.[15,18] Lymphocyte-rich interface dermatitis was the main histopathological pattern present in the samples in our study, which places DLE as the principal differential diagnosis in the cases analyzed.

In human beings, lupus erythematosus (LE) with characteristic microscopic changes of lymphocyte-rich interface dermatitis with basal keratinocyte damage, i.e. lupus-specific skin disease or cutaneous lupus erythematosus (CLE), is subdivided into acute, sub-acute, and chronic types (ACLE, SCLE, and CCLE, respectively). These classifications depend on morphologic aspects and the average duration of individual skin lesions.[19] A purely histological classification is impossible, because there is a significant superposition between the various clinical subtypes of CLE.[20] Among the variants of human CCLE, DLE represents the most common form and it is divided into a localized form, where the skin lesions are confined to the head and neck, and a generalized form, in which skin lesions also occur below the neck.[21] Mucocutaneous lesions are seen only in the context of DLE, but in less than 25% of patients with this disease. In humans, mucocutaneous lesions of DLE are most often noted either on the lips or in the oral cavity.[22] Using the principles that guide the classification of human CLE, most variants of canine CLE would fall under the category of CCLE, and this group should include DLE.[18]

Apart from nasal planum involvement, mucosal lesions are normally not seen in the classical facial-predominant DLE, in its generalized variant, or in exfoliative cutaneous lupus erythematosus. The diagnosis given to dogs with mucocutaneous erosive lesions and histopathology typical of CLE has varied for decades. However, after two German shepherd dogs with perivulvar lesions were first reported as having DLE, an author coined the diagnostic term “mucocutaneous LE” (MCLE) for the canine disease manifesting ulcerative lesions of juxtamucosal areas with microscopic pathology typical of CLE.[23] A recent study[18] reports 21 dogs with mucocutaneous erosive lesions and lupus-specific histopathology. Lesions were noticed on or around the genital (57%), anal (33%), perioral (24%), periocular (24%) or nasal skin (14%). So, it is possible to conclude that depigmented eyelids with histopathology pattern of lymphocyte-rich dermatitis like the cases of the present study fall in the category of

MCLE. Therefore, this is the first study to investigate and characterized MCLE restricted to the eyelids and we propose the term “lupoid blepharitis” for those.

The classical histological pattern of DLE is interface dermatitis accompanied by intense band-like lymphocytic infiltration that can obscure the dermal-epidermal junction. Gross TL, et al. (2005) classifies this pattern as “lichenoid interface dermatitis”. However, in this study we considered more adequate the term lymphocyte-rich infiltrate, as described by Olivry T, et al (2015). Eight samples studied here presented this pattern. The remaining three samples had a less intense inflammatory infiltrate, and for these we opted for the term “cell-poor” interface dermatitis.[3] This pattern can occur in cases of DLE, systemic lupus erythematosus, ischemic dermatitis, and solar dermatitis. The differentiation between them depends on clinical findings, history, and the presence of subtle histopathological features.

Literature suggests German shepherd dogs as being genetically predisposed to mucocutaneous lupus erythematosus.[18] However, the results in our study showed no breed predisposition for interface blepharitis. In the same way, there was no age predisposition. The veterinary literature has no information about breed, sex or age predisposition of depigmented eyelids diseases. However, Olivry, T, et al (2015) indicates that there is a higher tendency for female dogs to manifest MCLE. In our study, females were indeed affected more frequently than males (8:3).

Photosensitivity has been proven to be an important factor in the pathogenesis of lupus erythematosus in people due to the clinical evidence demonstrating a clear relationship between ultraviolet (UV) light exposure and skin lesions of LE.[24] It is important to consider that p53 protein expression increases in response to solar ultraviolet radiation (UV) exposition. Its increased expression controls apoptosis and the removal of potential cancer cells in the skin.[25,26,27] Ultraviolet radiation can cause aberrant induction of apoptosis in keratinocytes and contribute to the appearance of excessive apoptotic cells in the skin of CLE patients. In addition to UV-mediated induction of apoptosis, UV-induced direct DNA damage can activate the p53-dependent cell death pathways.[24] This corroborates our results, where p53 protein expression was observed in lesions compatible with those of MCLE and located in photo-exposed areas.

In the present study, most of the samples showed pigmentary incontinence. Although pigmentary incontinence is a sign of depigmentation of the epidermis, its presence is not pathognomonic of interface dermatitis. It can occur in other histopathologic patterns, like superficial perivascular dermatitis, and it is not always present in interface dermatitis. Besides, in interface dermatitis, pigmentary incontinence depends on the chronicity of the depigmented lesions. The more chronic the lesions, lesser are the chances of finding histopathological signs of depigmentation.

The p53 protein has a short half-life and it is not detected in immunohistochemical samples unless is has suffered a mutation, with increased expression and loss of function.[28,29] It is known that p53 plays a role in the suppression of systemic autoimmune and inflammatory diseases, maintaining immune homeostasis.[7,30,31,32,33] Its deficiency can predispose to a decrease on its autoimmunity suppression capacities. It has been shown that patients with systemic lupus erythematosus have an autoantibody to the C-terminal domain of p53, which can inhibit p53 functions.[32]

Published immunohistochemical studies of p53 protein in eyelids and third eyelids of dogs are restricted to neoplasia, such as squamous cell carcinoma, melanoma and sebaceous epithelioma.[13,25] In our study we analyzed p53 expression in 11 samples of lupoid blepharitis. Two of the samples evaluated were considered inadequate, probably because of formalin over-fixation. Eight of the nine samples evaluated (88.89%) showed immunoexpression of p53 protein. Our results demonstrate that the p53 expression is not related to neoplasia only, since none of the samples examined showed dysplasia or any signs of neoplastic transformation. Our results are similar to the literature as they demonstrate that p53 can be related to chronic inflammatory and autoimmune diseases of the eyelids, besides neoplasia.

These findings also point to the possibility that the interface dermatitis in the eyelids, besides a MCLE or lupoid pattern, has a potential of cellular mutation. Thus, the same suggestion that the p53 alterations may precede morphologic changes seen during tumor development in the esophagus[34] could well be applied to depigmentating diseases of the eyelids.

The results attained in this study demonstrate that there is a clear relation between the expression of p53 and the histopathological pattern of interface dermatitis. On the other hand, the expression of p53 in depigmented eyelids with interface dermatitis indicates that DNA damage and genetic instability are involved in this particular histopathological pattern, therefore a possibility for malignant transformation should also be considered.[35] It has been shown that squamous cell carcinoma can occur in photo-exposed areas of chronic discoid lupus erythematosus.[36]

2.5 CONCLUSION

From the data collected in this study it may be concluded that:

- Depigmented skin diseases can affect the eyelids only.
- Lupoid blepharitis seems to be the most adequate term to describe cases where skin depigmentation is observed exclusively in the eyelids and the histopathological examination reveals interface dermatitis.
- Lupoid blepharitis appears to be a localized variant of mucocutaneous lupus erythematosus.
- The expression of p53 in lupoid blepharitis can occur in the absence of histopathologically detectable neoplastic or pre-neoplastic disease.
- Independently of the cause or association of causes, actinic or immune-mediated, depigmented eyelids with interface dermatitis express p53 protein suggesting loss of regulation of cell proliferation and, therefore, higher risk to neoplastic transformation.

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Canine tarsal gland epitheliomas and adenomas: a retrospective study of 290 cases in Brazil

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Abstract

A total of 290 cases of tarsal gland adenomas and epitheliomas from canine patients were analyzed to determine the frequency and to better characterize their histopathological features. Tissue samples were examined in two veterinary pathology laboratories in Curitiba, Brazil, which receive samples from all over the country. Tarsal epitheliomas were significantly more frequent than tarsal adenomas (77.9% and 20.7%, respectively). In four cases (1.4%), the lesion contained both types of neoplasms. A total of 39 breeds were represented. The most common breeds were: English cocker spaniel (12.4%), poodle (10.3%) and Labrador retriever (9.3%). Mixed breed dogs represented 13.1% of the total. The upper eyelids were most commonly affected (65.2%), males and females were equally represented and there did not appear to be a predilection for either side of the face. Mitotic figures were most common in epitheliomas. The pigmented subtype was the most common form of epithelioma (59.7%). Whereas within the adenomas, the classic subtype (without inflammation or pigmentation) was the most common form (61.7%); and the inflamed subtype was also highly represented (20.0%).

Key words: adenoma, epithelioma, histopathology, tarsal gland, dog.

Introduction

The eyelids of dogs can be affected by a variety of neoplasms that are typically minimally invasive and respond well to simple surgical excision (1, 22). The two most commonly diagnosed eyelid neoplasms in dogs are the tarsal adenomas and epitheliomas (25), also known as tarsal gland adenomas and epitheliomas (Fig. 1). The tarsal glands (*glandulae tarsales*) are modified sebaceous glands of the eyelids responsible for producing the lipid phase of the tear film (12), and neoplastic transformation of these glands can affect ocular health. Disorders such as hordeolum or chalazion can secondarily occur if there is obstruction and/or inflammation of the ducts of affected tarsal glands; lagophthalmos, if eyelid closure is impaired; and keratitis or less commonly ulcers also may develop as a direct result of the tumor rubbing the corneal surface. Removal of these tumors is indicated (14, 17) to avoid such potential complications and to allow analysis and histopathological diagnosis of the tumor (10).

In the past tarsal gland tumors and cutaneous sebaceous tumors were essentially studied together (9). Sebaceous adenomas and sebaceous epitheliomas of the skin are benign tumors, but sebaceous epitheliomas can be locally aggressive and in some cases recur after surgical excision (9). Even though tarsal gland tumors are histologically similar to cutaneous sebaceous tumors (13), most authors currently consider tarsal tumors separately from its cutaneous counterparts, mainly because all reported instances of metastases from epitheliomas have been from cutaneous sebaceous epitheliomas (9) and not from canine tarsal epitheliomas (13, 22).

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