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## Cellular profile of the skin and ocular surface in healthy shih-tzu dogs

### Perfil citológico da pele e superfície ocular de cães da raça shih-tzu saudáveis

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### ABSTRACT

Cytology involves analyzing individual cells and is crucial for diagnosing skin and ocular surface diseases. The skin acts as a barrier against various threats and communicates with the immune system via receptors. The conjunctiva also provides protection to the eyes. This study aimed to describe the cytological profiles of healthy Shih-Tzu dogs' skin and ocular surfaces, assessing how age impacts these profiles. Materials for cytological analysis were collected using a nylon microbrush and evaluated with the Schirmer test. Results showed lymphocytes were predominant in ocular cytology (RE - 47%, LE - 41.93%), while polymorphonuclear cells dominated in nasal fold (62%) and palmar pad cytologies (100%). Superficial cells were prevalent in all regions (RE 83.11%, LE 90.52%, palmar pads 100%, nasal fold 96.91%). *Malassezia* spp was detected in all areas. This research underscores the cellular variations across different anatomical sites and provides insights into normal cytological patterns in these dogs.

**Keywords:** Canids; Cytopathology; Dermatology; Morphology; Ocular health

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### RESUMO

A citologia envolve a análise de células individuais e é crucial para diagnosticar doenças da pele e da superfície ocular. A pele atua como uma barreira contra várias ameaças e se comunica com o sistema imunológico por meio de receptores. A conjuntiva também fornece proteção aos olhos. Este estudo teve como objetivo descrever os perfis citológicos da pele e das superfícies oculares saudáveis de cães Shih-Tzu, avaliando como a idade impacta esses perfis. Os materiais para análise citológica foram coletados usando um microbrush de nylon e avaliados com o teste de Schirmer. Os resultados mostraram que os linfócitos predominaram na citologia ocular (OD - 47%, OE - 41,93%), enquanto as células polimorfonucleares dominaram nas citologias da dobra nasal (62%) e das almofadas palmares (100%). Células superficiais foram prevalentes em todas as regiões (OD 83,11%, OE 90,52%, almofadas palmares 100%, dobra nasal 96,91%). *Malassezia* spp foi detectada em todas as áreas. Esta pesquisa destaca as variações celulares em diferentes locais anatômicos e fornece insights sobre os padrões citológicos normais nesses cães.

**Palavras-chave:** Canídeos; Citopatologia; Dermatologia; Morfologia; Saúde ocular.

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### INTRODUCTION

Corneal conjunctival impression cytology is an important non-invasive test that aids in the diagnosis and staging of various diseases, with the most significant being ocular surface alterations due to lacrimal film deficiency (Putz, 2017).

The skin has defense mechanisms consisting of two basic processes represented by physical and chemical barriers, where receptors are present to capture information about the pathogen and relay it to the innate immune system. Consequently, leukocytes and mast cells are recruited for peptide production (Afshar and Gallo, 2013).

The conjunctival region is composed of a moist mucous membrane covering the anterior portion of the eyeball adjacent to the limbus, the inner surface of the eyelids, and the third eyelid. Its epithelium is stratified cuboidal, and its lamina propria is made up of loose connective tissue (Junqueira and Carneiro, 2023).

Ocular impression cytology is considered a valid diagnostic tool in ophthalmology. Collection with a spatula is not recommended as it produces low-quality samples containing few intact cells, while the mini-brush method is considered the best method, causing no discomfort and yielding a high-quality sample with a sufficient quantity of cells for analysis (Kovalcuka, Sarpio, and Nikolajenko, 2023).

Bron et al. (2017) reported that in humans, conjunctival goblet cells play an important role in mucin production, which, upon contact with water, forms a mucous layer that constitutes the innermost part of the lacrimal film. This film serves as a functional interface between the eye and the external environment and contributes significantly to the adaptive response of the ocular surface immune system to dry eye.

Skin cytology allows for histological characterization of tissues, and its results provide important information about the patient's dermatological condition, helping to understand the behavior of skin-resident microbiota in dogs and consequently aiding clinicians in reaching a diagnosis (Gasparetto et al., 2013).

*Malassezia* spp is a widely distributed fungus on our planet, and new species are expected to be discovered. Although some *Malassezia* spp species are adapted to specific hosts, many are shared between animals and humans. There are reports of zoonotic transmission, especially for *M. pachydermatis*. *Malassezia* spp species are associated with skin diseases in companion animals, production animals, birds, exotic animals, as well as in humans (Hobi et al., 2022).

Therefore, the objective of this study was to propose a cytological profile of the skin and ocular surface of healthy Shih-Tzu dogs, as well as to investigate whether there are age-related changes in these profiles.

## **MATERIALS AND METHODS**

Approved by the Committee on Ethics in Animal Experimentation (CEUA) under protocol No. 01/2023.

The inclusion criteria for animals in the experiment were based on the absence of ocular and cutaneous lesions, determined through clinical evaluations of the skin and ocular surface. Additionally, normal results on the Schirmer test were considered, with values within the range established by Martin (2010) between 15 and 25mm/min, and the absence of corneal ulceration, determined by fluorescein utilization.

Samples were collected by the same professional from 45 healthy Shih-Tzu dogs, distributed into three groups of 15 animals each, categorized by age. Group 1 (G1) consisted of animals aged 1 to 4 years, Group 2 (G2) included animals aged 5 to 8 years, and Group 3 (G3) encompassed animals aged 9 to 12 years. Before sample collection, a Schirmer test was conducted to quantify tear production and exclude cases of dry eye that could interfere with cytology. The fluorescein test was performed to exclude corneal ulceration.

The Schirmer test involved folding the end of a measuring strip, which was then inserted into the lower conjunctival sac, near the medial canthus of the eye. After one minute, the strip was removed, and the wetted portion was measured in millimeters.

The fluorescein test consisted of applying a drop of dye to each eye. After a few seconds, excess dye was washed out with saline solution and cotton. The presence or absence of corneal ulceration was observed, with animals having ulcers being excluded from the experiment.

For cytology, samples were collected from the conjunctival epithelium, nasal fold, and interdigital region of the palmar pads using a fine nylon microbrush, pre-moistened with sterile injection water, with 10 rotational movements performed in each region. Subsequently, smears were prepared on microscope slides. Approximately 30 minutes after collection, the slides were stained using the Rapid Panoptic staining method and examined under a light microscope at magnifications of 10x, 40x, and 100x with immersion oil, by a single examiner.

To standardize slide reading, a differential cell count method was employed until reaching 100 cells. Separate counts were performed for leukocytes and desquamative cells by region (conjunctiva, nasal fold, and palmar pad region). The presence or absence of *Malassezia* spp was also noted in the cytology samples analyzed.

Statistical correlation between groups was evaluated using the median due to the absence of normal data distribution. The Mann-Whitney U test was employed at a significance level of 5% ( $p < 0.05$ ) using the R statistical software (R Core Team, 2022).

## RESULTS AND DISCUSSION

The methodology employed to evaluate the cytological profile of the conjunctiva, nasal fold, and interdigital region of the palmar pads allowed for satisfactory identification of cells on the slides.

In conjunctival cytology, lymphocytes were present in 73% of the right eye slides, predominantly in 42.3% of samples, and in 60% of the left eye slides, predominantly in 42.3% of samples. Polymorphonuclear cells were present in 57.7% of the right eye slides, predominantly in 26.7% of samples, and in 44.4% of the left eye slides, predominantly in 24.4% of samples, as evidenced in Table 1.

In cytologies of the palmar pad region, there was an absolute predominance of polymorphonuclear cells, with no other types of leukocytes found. For the nasal fold region, lymphocytes were predominant in 17.7% of cytologies, and polymorphonuclear cells showed predominance in 31.2%.

Macrophages did not show predominance in any conjunctival cytology but were present in 15.5% of the right eye cytologies and 11.11% of the left eye cytologies. They were not found in palmar pad samples and showed predominance in 2.2% of nasal fold samples.

Regarding the results of desquamative cell counts from cytologies, superficial cells were predominant in all regions. Goblet cells were observed in only two cytologies (4.4%) of the conjunctival region of the right eye and were not predominant in any cytology.

For the investigation of *Malassezia* spp, it was observed that they were present in 4.4% of ocular cytologies, 2.22% of palmar pad cytologies, and 20% of nasal fold cytologies, as evidenced in Table 5.

**Table 1-** Percentage Distribution indicating predominance, between Leukocytes and Desquamative Cells found in Conjunctival Cytology by skin and conjunctival exfoliation in healthy Shih-Tzu dogs.

<b>Cytology</b>				
<b>Leukocytes</b>	<b>RE%</b>	<b>LE%</b>	<b>Palmar Pads%</b>	<b>Nasal Fold%</b>
Lymphocytes	42,3	42,3	0	17,7
Polymorphonuclear Cells	26,7	24,4	100	31,2
Macrophages	0	0	0	2,2
Uniform Distribution	31	33,3	0	48,8
<b>Desquamative Cells</b>				
Superficial	84,5	95,6	100	97,8
Intermediate	11,1	2,2	0	2,2
Goblet Cells	0	0	0	0
Homogeneous distribution	4,4	2,2	0	0

Descriptive Statistics. RE: right eye; LE: left eye

Using the same collection method, Kovalcuka, Sarpio, and Nikolajenko (2023) reported that this type of material collection is safe, causing minimal discomfort to the patient and not requiring chemical restraint for its execution. In the present study, it was observed that there were no lesions or irritation on the conjunctiva or skin of the animals with the use of the fine nylon microbrush.

Kovalcuka, Sarpio, and Nikolajenko (2023) emphasized that impression cytology using the mini-brush collection method is a viable technique that produces a high-quality sample with a good monolayer of cells. Perazzi et al. (2017) stated that cytological brushes with nylon bristles, used for collecting conjunctival cytology samples, form uniform monolayers, resulting in good quality with satisfactory cellular yield for microscopic evaluation of slides.

The Rapid Panoptic staining method was effective in staining the investigated cell types. Borges et al. (2012) affirmed that the Papanicolaou staining technique, in humans, provides the best accuracy in cell identification; however, the method employed in this

study is the most widespread in Veterinary Medicine due to being faster, easier, and less costly.

The relevant literature on conjunctival cytology does not standardize the cell types for the region or quantify them, but it reports the identification of leukocytes, such as neutrophils, eosinophils, and monocytes, in small quantities in cytologies of clinically healthy dogs (Bolzan et al., 2005; Venâncio et al., 2012).

Bolzan et al. (2005), in their research, performed impression cytologies using asymmetrical Millipore strips on the conjunctiva and reported the visualization of superficial desquamative cells in 33% of slides from clinically healthy dogs. The present study, using a different collection method, observed the presence of this cell type in 95.55% OD and 97.7% OE of conjunctival cytologies, with them being predominant in 84.5% OD and 95.6% OE of slides (Table 1).

This study corroborates the findings of Azevedo et al. (2009), who identified that the most common cells in exfoliative conjunctival cytology of healthy dogs were desquamative cells from superficial and intermediate layers, with 50% of slides showing predominance of intermediate desquamative cells and the other 50% showing predominance of superficial desquamative cells.

Borges et al. (2012), using an abrasion method with cytological brush on the conjunctiva of 13 healthy dogs, despite visualizing all types of desquamative cells, obtained predominance of intermediate cells (87.06% and 73.10%); in the present study, the result was a predominance of superficial desquamative cells (Table 1). It is worth noting that according to the collection method employed in both studies, the applied force may interfere with the depth of the layers reached.

Hashitomo (2013) emphasize that macrophages are considered first-line cells in immune defense, highlighting their multifunctionality as components of the mononuclear phagocytic system. Venâncio et al. (2012) assert that the presence of inflammatory cells such as leukocytes, in reduced quantities, in animals without ocular conditions is not significant and should be disregarded.

Polymorphonuclear leukocytes are typically numerous in the conjunctiva of healthy animals and, when present, are migrating through the epithelium towards the conjunctival sac to combat infectious agents (McGavin and Zachary, 2018).

Borges et al. (2012), in their study using a cytological brush for collection, presented a percentage of 0.12% of goblet cells in conjunctival cytology of 13 healthy dogs. In the present study, the percentage was 0.022% (2/90) of conjunctival cytologies.

Lima et al. (2014) did not find goblet cells in exfoliative cytologies using an interdental brush on the palpebral conjunctiva of 10 healthy dogs, and Venâncio et al. (2012) described the absence of these cells in the palpebral conjunctiva of 50 healthy cats. Morais (2016), in a study with 38 Gir breed cattle, reported a percentage of 0.34% of goblet cells in conjunctival cytology.

**Table 2-** Comparison of medians of leukocytes and desquamative cells found in ocular exfoliative cytologies among groups of healthy Shih-Tzu dogs of different age ranges.

Parameters	Median			p-value
	G1	G2	G3	
Lymphocytes	0,00 <sup>C</sup>	75,00 <sup>A</sup>	17,00 <sup>B</sup>	0,0169
Polymorphonuclear cells	0,00 <sup>C</sup>	1,50 <sup>B</sup>	49,00 <sup>A</sup>	0,0487
Macrophages	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,2339
Superficial	99,00 <sup>A</sup>	91,00 <sup>A</sup>	96,00 <sup>A</sup>	0,1152
Intermediate	1,00 <sup>B</sup>	9,00 <sup>A</sup>	4,00 <sup>AB</sup>	0,0464
Goblet cells	0,00	0,00	0,00	0,2005

Different uppercase letters on the same line represent statistical differences between groups by the Mann-Whitney U test at a 5% level of significance ( $p < 0.05$ ).

In the comparison between groups according to age range, statistically significant differences were observed in the percentages of lymphocytes (Figure 3) ( $p=0.0169$ ), polymorphonuclear cells ( $p=0.0487$ ), and intermediate desquamative cells ( $p=0.0464$ ), as evidenced in Table 2. Notably, animals in groups G2 and G3 demonstrated higher percentages of polymorphonuclear cells and intermediate desquamative cells compared to group G1. These results highlight distinct cytological responses among the investigated age groups.

According to Raskin (2003), when there is a predominance of neutrophils in cytological samples, they should be classified as inflammatory; however, this was not the case in the study, as despite a higher percentage of polymorphonuclear cells in these groups, they were not predominant.



In the cytological analysis by palm pad exfoliation, as evidenced in Table 3, there was a predominance of superficial desquamative cells (Figure 4) and polymorphonuclear leukocytes (Figure 1), with no difference ( $p>0.05$ ) between the groups.

**Table 3-** Comparison of medians of leukocytes and desquamative cells found in cutaneous cytologies by exfoliation of the palmar pad region among groups of healthy Shih-Tzu dogs of different age ranges.

Parameters	Median			p-value
	G1	G2	G3	
Lymphocytes	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	1,0000
Polymorphonuclear cells	100,00 <sup>A</sup>	100,00 <sup>A</sup>	100,00 <sup>A</sup>	0,2703
Macrophages	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	1,0000
Superficial	100,00 <sup>A</sup>	100,00 <sup>A</sup>	100,00 <sup>A</sup>	1,0000
Intermediate	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	1,000
Goblet cells	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	1.000

Equal uppercase letters on the same line indicate no difference according to the Mann-Whitney U test at a 5% level of significance ( $p < 0.05$ ).

The results indicate uniformity in the cytological cutaneous characteristics obtained by exfoliation of the palmar pad region in healthy Shih-Tzu dogs, regardless of the age groups investigated. According to Cowell (2009), desquamated epithelial cells are commonly present in cutaneous cytology using swabs/scrapings, and superficial cells are found in the vast majority of skin samples collected by scraping, considered an expected finding in healthy dogs or those with dermatopathies.

With cytological analysis by exfoliation of the nasal fold, as evidenced in Table 4, there was a predominance of superficial desquamated cells (Figure 4) and polymorphonuclear leukocytes (Figure 1), with no statistical significance ( $p>0.05$ ) observed between the groups.

**Table 4-** Comparison of medians of leukocytes and desquamated cells found in cutaneous cytologies by exfoliation of the nasal fold region among groups of healthy Shih-Tzu dogs of different age ranges.

Parameters	Median			p-value
	G1	G2	G3	
Lymphocytes	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0.9383
Polymorphonuclear cells	40,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0.7590
Macrophages	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0.2752
Superficial	100,00 <sup>A</sup>	100,00 <sup>A</sup>	100,00 <sup>A</sup>	0.7769
Intermediate	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0.7769
Goblet cells	0,00 <sup>A</sup>	0,00 <sup>A</sup>	0,00 <sup>A</sup>	1.0000

Equal uppercase letters in the same row indicate that there was no difference according to the Mann-Whitney U test at the 5% level of significance (p<0.05).

These results indicate uniformity in the cytological characteristics of the nasal fold region in healthy Shih-Tzu dogs, regardless of the age groups investigated. Cowell (2009) states that desquamated epithelial cells are present in cutaneous cytologies using swabs/scrapings, and superficial cells are found in the vast majority of skin samples collected by scraping, considered an expected finding in healthy dogs.

**Table 5-** Percentage distribution of *Malassezia* spp found in conjunctival cytologies by exfoliation of the right eye (RE) and left eye (LE), and dermatological (palmar pads and nasal fold) in healthy Shih-Tzu dogs.

	Cytology – <i>Malassezia</i> spp							
	RE	%	LE	%	Palmar Pads	%	Nasal Fold	%
Present	1	2,22	3	6,66	1	2,22	9	20
Absent	44	97,77	42	93,33	44	97,77	36	80

Descriptive\_statistics

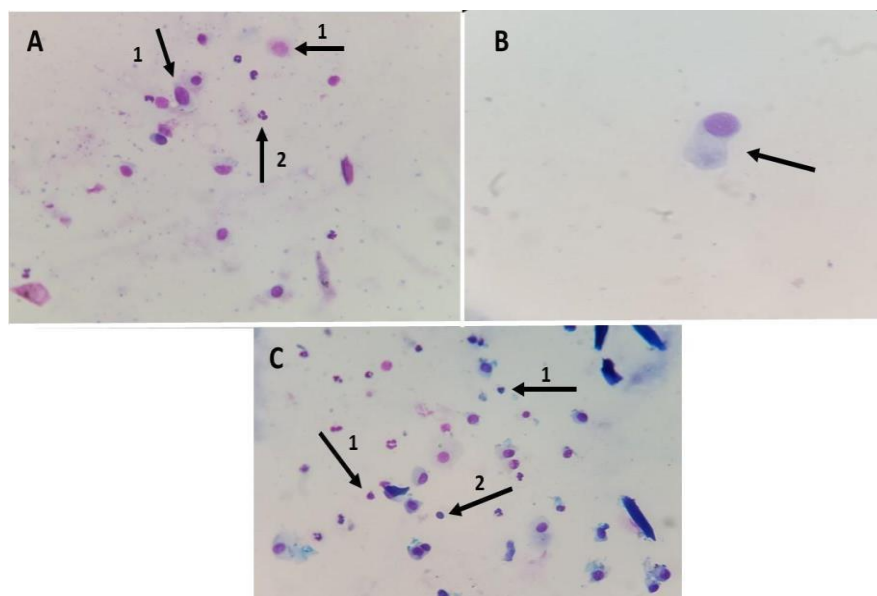
Diaz et al. (2023) reported that methods using cytological and molecular techniques showed a good correlation between sampling methods and demonstrated their value as a diagnostic tool for *Malassezia* spp in the interdigital fold of dogs with pododermatitis.

Rosa et al. (2019) reported that *Malassezia* spp is a commensal microorganism of dogs' microbiota, causing disease when associated with other conditions, and its prevalence is related to low host immunity combined with increased temperature and humidity, creating a conducive environment for its development.

Santos et al. (2020) highlighted the presence of *Malassezia* spp in 2.6% of skin examinations from 39 dogs, and Chiurco (2016), in a study with 33 healthy dogs, observed a more pronounced positivity for *Malassezia* spp in ear canal samples compared to skin, with 72% of ear canal samples testing positive versus 12% positivity in skin samples.

Santos et al. (2009) isolated *Malassezia* spp from the conjunctival microbiota in 1.84% of dogs. In contrast to the present study, Diaz et al. (2023) concluded that *Malassezia* spp is predominant in samples from healthy animals' interdigital folds and is present post-treatment for pododermatitis.

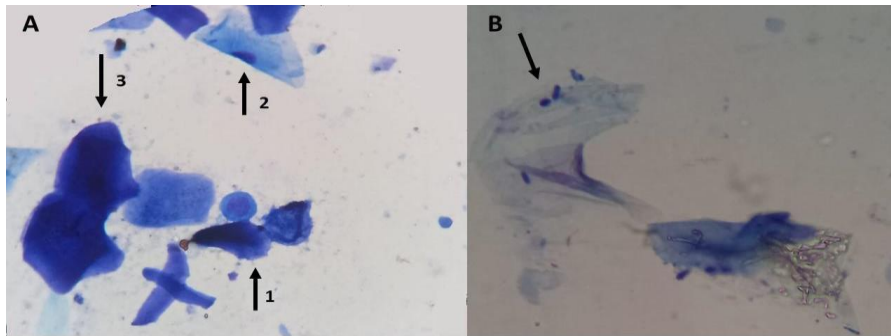
**Figure 1-** Conjunctival cytological micrograph of healthy Shih-Tzu dogs stained with panoptic dye under a 100x objective magnification.



Observe in A desquamated cells indicated by arrows 1 and polymorphonuclear leukocyte by arrow 2. Observe in B desquamated cell indicated by arrow. Observe in C lymphocytes indicated by arrow 1 and intermediate desquamated cell by arrow 2.

Source: author (2024)

**Figure 2-** Cytological micrograph of the palmar pads (A) and nasal fold (B) of healthy Shih-Tzu dogs stained with panoptic dye under a 100x objective magnification.



Observe in A, arrow 1 indicating an intermediate desquamated cell, arrow 2 superficial desquamated cell, arrow 3 anucleated superficial desquamated cell. Observe in B, arrow indicating *Malassezia spp.*

Source: author (2024)

## CONCLUSION

Based on the results obtained in this study, we can infer that cytology proved to be an effective method in characterizing the cytological profile of the skin and ocular surface in healthy Shih-Tzu dogs. When analyzing conjunctival cytology in Shih-Tzu dogs, a significant statistical predominance of lymphocytes was observed in the total leukocyte count, with notable variations among different age groups. The prominent presence of polymorphonuclear leukocytes was highlighted in the palmar pad and nasal fold regions. In the analysis of desquamated cells, a significant prevalence of superficial desquamated cells was evident in all examined areas.

Additionally, the presence of *Malassezia spp.* was identified in the analyzed samples. These findings contribute to a more comprehensive understanding of cytology as a diagnostic tool in Shih-Tzu dogs, providing valuable insights into normal cytological characteristics and potential age-related variations.

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