VETERINARY CYTOPATHOLOGY: A RETROSPECTIVE STUDY

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ABSTRACT

Neoplasms are cell growths with no biological function, of multifactorial etiology, which affect all animal species. However, studies on the incidence of neoplasms in dogs in Belém do Pará through cytological diagnosis are scarce. The cytopathological evaluation comprises the study of cells that identifies the cytomorphological category, evaluates cellularity, nuclear and cytoplasmic characteristics, making it possible to classify whether the neoplasm is benign or malignant, in addition to also allowing the identification of non-neoplastic processes, such as inflammation and infections. In view of this, the objective of the research is to evaluate the occurrence of neoplasms in dogs treated at HOVET-UFRA through cytopathological examination. Retrospectively, medical records from January to December 2023 were included, surveying data on gender, age, and Pap smears. A total of 229 animals were evaluated for cytological examination, with females being the most prevalent with 64.19%, while males corresponded to 35.81%. The highest occurrence of lesions was in the reproductive system with 47.48%, followed by the integumentary system with 40.58%. Of the 377 cases of lesions analyzed, 212 (56.23%) had neoplastic diagnoses, with a prevalence of epithelial neoplasms 122 (57.55%) and round cell neoplasms 55 (25.94%). Females had a higher incidence of neoplastics, with 112 cases (69.57%), being the most common disease. Mammary neoplasms in females were the most prevalent, with mammary adenoma/carcinoma being the most frequent neoplasm with 35.40%, followed by benign epithelial neoplasia with 17.39%, in round cell neoplasms mast cell mast cell was the most common with 9.32% of all cases. In males, round cell neoplasms were the most frequent, with 15.69% for TVT cases and 13.73% for mast cell cases. The results point to the importance of the cytological examination at HOVET-UFRA, not only to guide treatments, but also to collect data on the most prevalent lesions, essential for disease control measures. Thus, the research contributes to the understanding and management of neoplasms in dogs in the region.

Keywords: Cytopathological diagnosis. Injuries. Neoplasms. Dogs.

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INTRODUCTION

Several specialties in veterinary medicine have been growing over time due to the recognition and discoveries of various diseases that affect animals, including cancer. Oncology is one of the specialties that has been gaining space in the clinical routine, due to the important prevalence of animals affected by neoplasms (DALECK & DE NARDI 2016). In a study carried out by Comin 2023, it was identified that of the 1669 lesions studied in dogs, 916 (53.9%) were neoplastic, mostly affecting elderly patients over 9 years of age (57.7%). It is known that the increase in the longevity of animals, the adoption of greater care by tutors and the advancement in studies and practice in Veterinary Medicine are one of the explanations for the increase in the prevalence of neoplasm diagnosis (SOUZA 2005).

Neoplasm is a term that means "new growth", that is, it is the formation of new tissues that are the result of genetic and epigenetic mutations in cells, thus enabling irreversible changes in the mechanisms of growth, differentiation and cell death (ZUCCARI et al., 2016). When there is a presentation of malignant potential causing tissue invasion, DNA (deoxyribonucleic acid) damage, and eventually metastasis, the most commonly used term for the condition is cancer (ZUCCARI et al., 2016; HERON & ANDERSON, 2016).

The causes of DNA damage that promote the onset of cancer are multifactorial, and include exposure, usually chronic, to chemical, physical, and biological agents, such as viruses, hormones, genotoxic or non-genotoxic chemicals, and radiation; however, they can also be inheritable, due to DNA replication and error repair processes, which are more often observed with advancing age (MEUTEN, 2016; TORRE et al., 2015).

Clinical changes in animals are variable due to the large number of existing neoplasms, and may be caused by the direct or indirect action of the growth in question. There may be an increase in volume at the affected site, wounds that do not resolve with treatment, anorexia and weight loss, exercise intolerance, persistent claudication, dyspnea, difficulty urinating or defecating, and secretions or bleeding anywhere in the body (COMIN, 2023).

The diagnosis of neoplasms is based on a thorough anamnesis and physical evaluation, in addition to tests such as cytopathology, histopathology, imaging tests such as radiography and ultrasound, and other laboratory tests. Cytopathology comprises the study of cells that identifies the cytomorphological category, evaluates cellularity, nuclear and cytoplasmic characteristics, making it possible to classify whether the neoplasm is benign or malignant, in addition to also allowing the identification of non-neoplastic processes, such as inflammation and infections (MELLO et al., 2022). Therefore, given that there is a lack of

studies on the occurrence of neoplastic processes from a cytological approach in dogs in the region of Belém do Pará, it is justifiable to carry out in-depth investigations on this topic.

LITERATURE REVIEW

CYTOPATHOLOGY IN VETERINARY MEDICINE

Veterinary cytology has experienced significant growth over the past 15 years, in part due to growing awareness among veterinarians about the advantages of the exam and its practicality. At the same time, owners are increasingly aware of the importance of a thorough diagnostic investigation to determine appropriate treatment options. As the use of cytology expands, challenging diagnostic cases become more frequent and more clinically useful responses are needed (AYELE, MOHAMMED & YIMER 2016). Microscopic evaluation of cells for diagnostic purposes is an old procedure, almost as old as microscopy itself. In human medicine, the presence of tumor cells in patients' sputum was reported in the mid-nineteenth century, but it was only after the mid-twentieth century that cytology became widely used for diagnostic purposes (NAYLOR 2000).

In veterinary medicine, cytology has a shorter history, but it has become widely disseminated in recent decades, driven by the speed of results and especially by the importance of making a diagnosis that is sometimes definitive and avoiding the use of more invasive and expensive surgical biopsies as in histopathological examination. However, there are two significant differences between veterinary and human cytology. The acquisition of material and the general interpretation of cytological samples are the responsibility of veterinarians alone and are not limited to pathologists or specialized technicians as is the case with human medicine (SHARKEY et al., 2020).

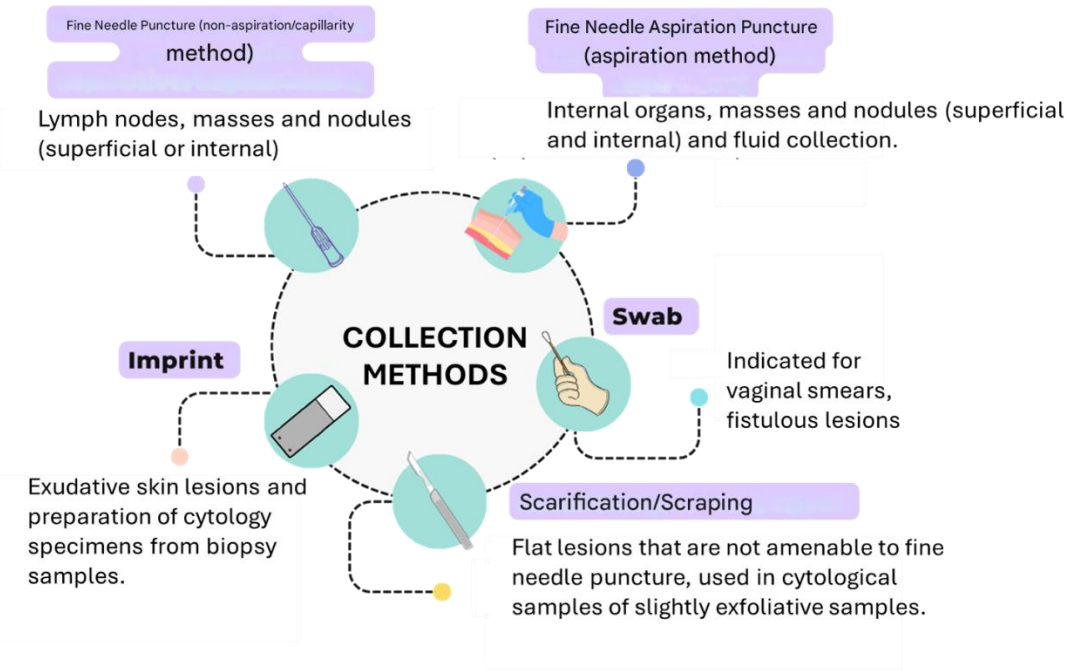
Cytological examination of aspirates, impressions, and other specimens is an important diagnostic tool in veterinary practice. Sample collection is usually quick and relatively non-invasive and the examination typically does not require any special equipment beyond what would normally be found in a veterinary clinic, with the result that a diagnosis can often be made in-house through a qualified professional (CHRISTOPHER et al., 2008).

Although there are limitations regarding sensitivity, specificity, and predictive values of positive and negative outcomes, cytologic testing is considered the preferred diagnostic modality for many types of inflammatory, neoplastic, and other lesions. It is an important tool for screening and revealing the diagnosis of lesions with suspected neoplastic (TECILLA et al., 2019; KU et al., 2016).

COLLECTION METHODS IN CYTOLOGY

The evaluation of cutaneous and subcutaneous lesions, as they are easy to approach and have no contraindications during collection, is one of the reasons that makes the use of cytology an important indication, which makes it a very useful clinical tool. Cytological samples can be collected by various methods, depending on the type and location of the lesion and the patient's temperament (BAJWA, 2017) (Figure 1).

Figure 1- Materials and methods of cytological collection.



Source: Adapted Cowell and Tyler`s (2020).

Cytology or fine-needle aspiration puncture (FNAC-FNA) is the collection technique that consists of the use of the application of negative pressure with a syringe coupled to a hypodermic needle. Another option is the capillary or fine-needle puncture (FAP) technique, which does not use the attached syringe when the needle is introduced into the tissue. These techniques were first described in human medicine in 1986, but have been a commonly recognized sampling method in veterinary medicine for many years (SHARKEY et al 2020; AYELE, MOHAMMED & YIMER, 2017).

These methods are indicated in samples of proliferative formations or masses, subcutaneous glandular organs (lymph nodes, mammary or salivary gland). Specimens are generally considered sensitive, with good agreement between cytology and histopathology results for many types of lesions. Necrotic, infected, or ulcerated areas should be avoided, as they often produce samples that may not reflect the underlying process (COWELL et al, 2008).

Samples collected by capillarity are usually the most common method to be used in veterinary routine and may be better indicated in regions where blood contamination occurs, such as highly vascularized areas, such as the spleen, hemorrhagic lesions of the skin and subcutaneous tissue. Lymph nodes are often aspirated using this technique, as lymphoid cells are sensitive and prone to rupture (SHARKEY et al., 2020).

Scarification is another collection method used when it is not possible to perform FAP or FNA, such as in flat and dry skin lesions. In scarification, the sample is collected by rubbing the instrument with a blunt end on the lesion (scalpel blade), after which, the collected material is transferred to a glass slide (PELETEIRO et al., 2011).

The swab is used in areas that are sensitive such as the ear canal, vaginal cytology, tracts or draining sinuses, interdigital tissue and dry lesions that contain crusted surface. If the area to be scrubbed is dry, the ideal is to moisten the material with sterile saline solution. After smearing the injured area, the material contained in the swab is arranged on a glass slide and the smear is allowed to air dry (PAGNONCELLI, 2011).

The printing technique can be used to collect directly from skin ulcers or exudative lesions with the "direct imprint", or from masses that were surgically removed and from tissues at necropsy using the "decal technique". Ulcers should be printed, cleaned, and reprinted. To collect tissue impression samples obtained in surgery or necropsy, the material to be printed must first be cut in half to have a fresh surface. The fresh surface is then wiped (e.g., paper towel or surgical gauze) to remove as much blood and tissue fluid as possible, after which it is pressed under a clean glass slide (AYELE, MOHAMMED & YIMER, 2017).

The limitations of the printing technique show that it is only possible to collect cells that are on the surface of the lesion, therefore, in some situations they may not be representative of the underlying pathology, there are cases in which there is little or no cellularity, and only bacterial or blood contamination may occur (DUNN, 2014).

The clinical lesion pattern determines the optimal collection technique to be applied at that time. It is important to emphasize that prior knowledge about the materials and methods of cytological applicability is crucial to ensure the adequacy of the sample and the quality of the test. In addition, adequate professional training is essential for an accurate diagnosis of the conditions (CASSALI, 2017).

CANCER IN DOGS

Since the middle of the Industrial Revolution period, dogs have been genetically selected to have specific physical characteristics and functions, however, over time this continuous reproduction has made a diverse range of canine breeds favorable, some

descendant and with increased risk for certain diseases, including cancer (OSTRANDER, 2005). It is estimated that approximately one in four dogs will, at some point in their lives, develop cancer and almost 50% of dogs over 10 years old will be affected by the disease.

Cancer is the most common cause of death in dogs, affecting approximately four million dogs each year (GARDNER, FENGER & LONDON 2016). According to Gardner et al. (2016), the first study on cancer in dogs was done in the 1960s in California, where there was an attempt to identify all tumors diagnosed in animals residing in the municipalities of Contra Costa and Alameda over a period of three years. Another survey on the main causes of mortality in the region of Sweden was carried out with 43,172 dogs in the period from 1995 to 2000, and showed that, of the 62% of deaths reported with diagnosis, oncological diseases (18%) and trauma (17%) had the highest mortality rates (BONNETT et., 2005).

In Brazil, studies are still scarce on the exact incidence of cancer in dogs, since not all pets get medical care or even a definitive diagnosis of the disease. Generally, these studies are retrospective in nature and limited to specific patient populations. In the region of Santa Maria, in Rio Grande do Sul, a study cataloged 1,533 records of lesions, and in these animals, it was reported that 55.4% of dogs aged 10 years or older were affected by neoplasms, 31% by non-neoplastic lesions, while 13.6% were non-representative samples. In this, neoplasia is one of the main causes of death for the species (COMIN 2023).

In addition, the canine model has become a comparative study tool for cancer research in humans through genetic mapping, as they are naturally susceptible to most neoplasms found in humans. This stems from the fact that several dog breeds consistently have a predisposition to certain cancers, making dog breed models a unique opportunity to map genetic variants associated with neoplastic diseases (ADEYANJU et al., 2023).

It is important to note that tumors in dogs have histological, genetic, molecular, and clinical similarities, in addition to which, due to their accelerated evolution compared to tumors in humans, they provide a faster view of disease progression and treatment results (SCHWALBACK et al., 2023).

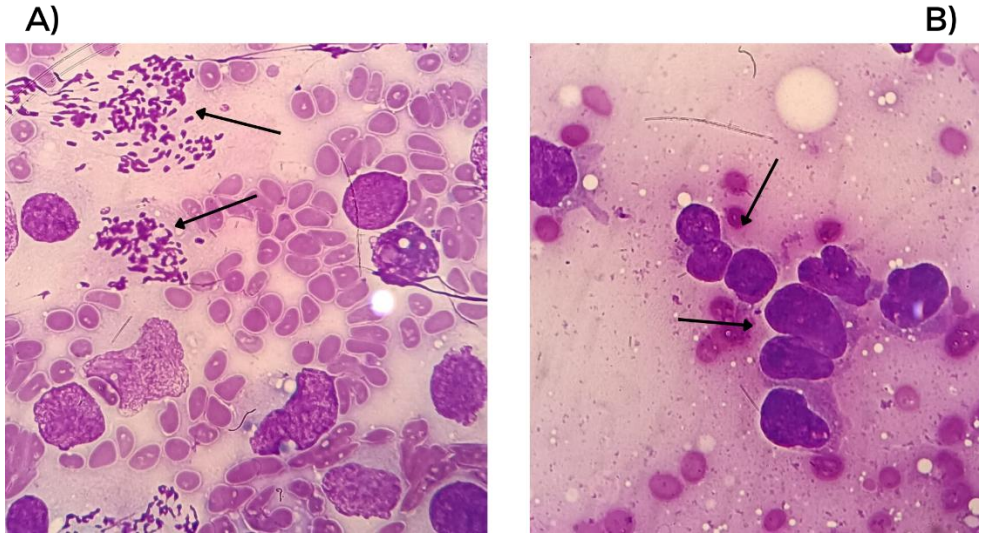
TYPES OF NEOPLASMS

In cytology, the diagnosis of neoplasms is based on the identification of monomorphic cell populations with no inflammation, since inflammation usually stimulates reactive cells, such as mesenchymal cells and some epithelial cells, which can often resemble neoplastic cells (RASKIN, MEYER & BOES 2021). It is worth mentioning that neoplastic processes may also present infiltrating inflammatory processes in their composition, such as in squamous cell carcinoma, plasmacytoma, mast cell tumor, TVT, and more detailed

evaluations are necessary to determine the type of neoplasm (CASSALI, 2017). The cytomorphology of cell characteristics is important to classify the origin, and can be epithelial, mesenchymal, round and melanocytic cells (RASKIN, MEYER & BOES 2021).

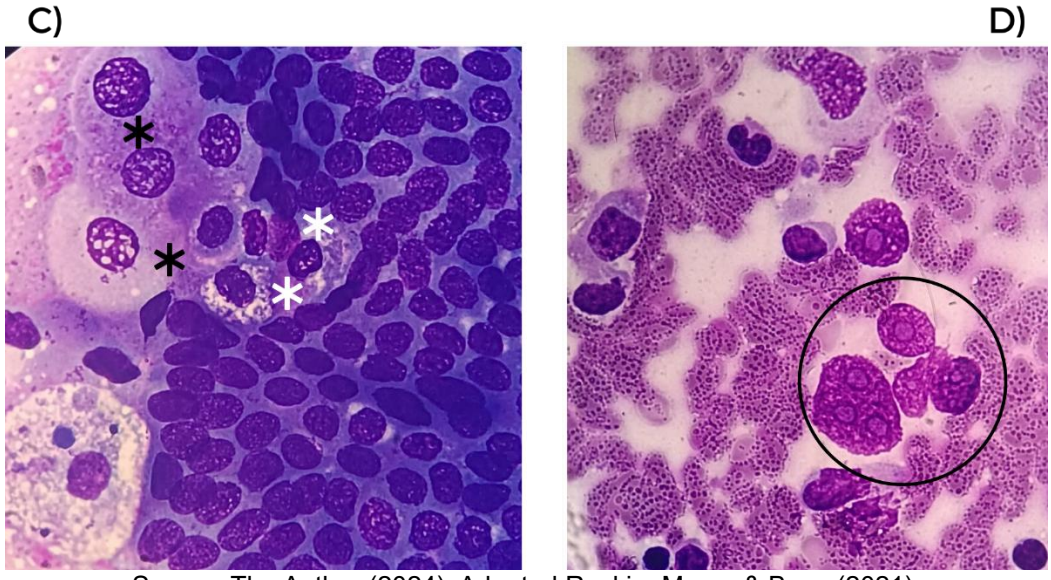
In addition, it is possible to assist in the differentiation of benign and malignant neoplasms through the pattern of cells such as size, nucleus/cytoplasm ratio and other cellular aspects. In malignant cells, the criteria for malignancy are more evident, such as cellular and nuclear pleomorphism, variation in the nucleus/cytoplasm ratio, variation in nucleoli, multinucleation, atypical mitosis figures, among others (COWELL et al., 2008). The most commonly evaluated malignancy criteria in cytology are presented in the following figures.

Figure 2- (A): Atypical mitosis figures- chromosomal fragments with irregular length, isolated or wide (arrows). (B): Pleomorphism - variation in the size, shape, or state of maturation of cells and their nuclei (arrows). (Panopticon, Fast-magnification, 100x).



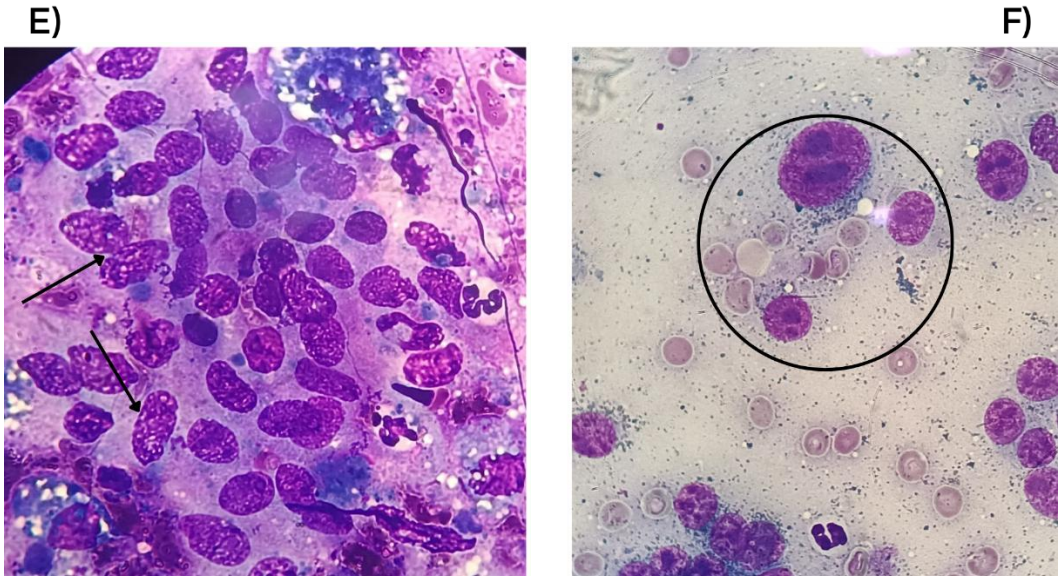
Source: The Author (2024). Adapted Raskin, Meyer & Boes (2021).

Figure 3- (C): Nucleus-cytoplasm ratio variation - high or variable cytoplasmic nuclear ratio between cells of similar origin (asterisks). (D): Anisochariosis - variation in nuclear size between cells of the same origin (circle). (Panopticon-fast 100x magnification).



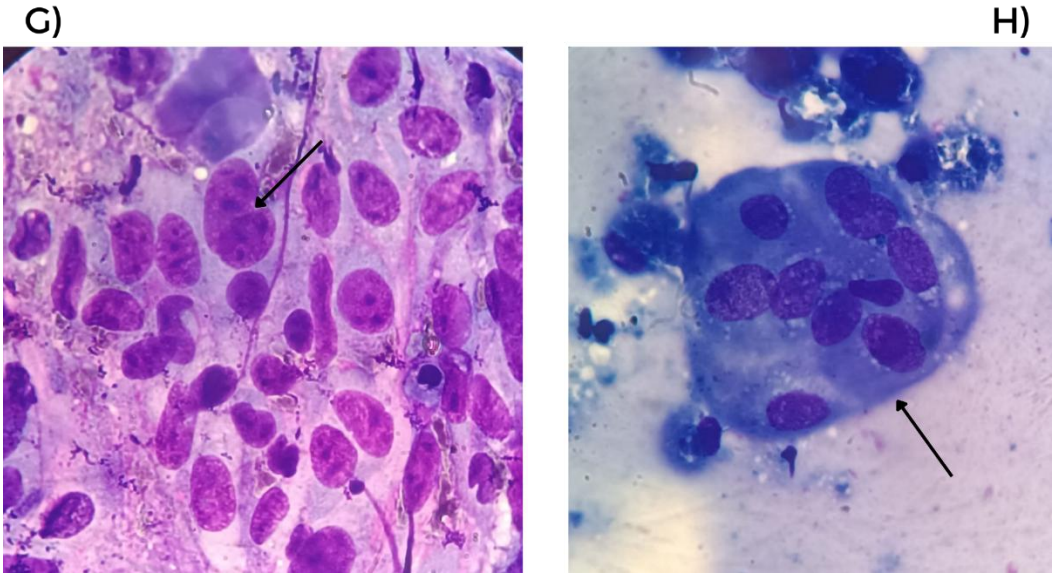
Source: The Author (2024). Adapted Raskin, Meyer & Boes (2021).

Figure 4- (E): Coarse chromatin - chromatin with a clustered (coarse) aspect of its nucleus (arrows). (F): Anisonucleosis - variation in the size of the nucleolus that can be multiple and of variable shape (circle). (Panopticon-fast 100x magnification).



Source: The Author (2024). Adapted Raskin, Meyer & Boes (2021).

Figure 5- (G): Nuclear molding - abnormal nuclear form related to the rapid growth of cells, where there is tight cell spacing even though there is no inhibition (arrow). (H): Multinucleations - two or more nuclei occupying the same cell (arrow). (Panopticon-fast 100x magnification).



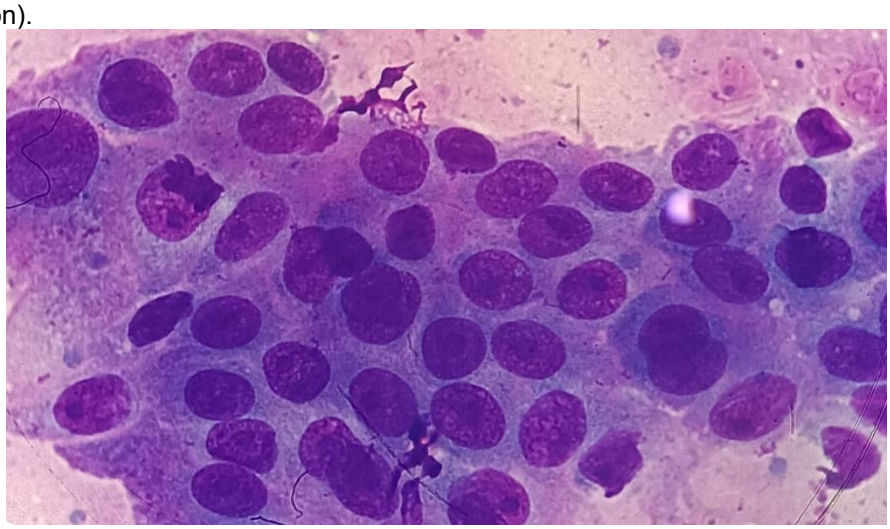
Source: The Author (2024). Adapted Raskin, Meyer & Boes (2021).

In addition to the classification into malignant and benign, cytological evaluation can, in many cases, allow the identification of the tissue that originated it, which may be of epithelial, mesenchymal, round or melanocytic cell origin.

Neoplasms of epithelial origin present equivalent cytomorphology, and the main characteristic among them is the cell arrangement in groups, consisting of a variable number of cohesive cells across the cytoplasmic membrane. In addition, they are easy to obtain high to moderate exfoliation during collection. The cytomorphological aspects of epithelial cells are rounded to polygonal in shape, with a distinct margin cytoplasm and a predominantly round to oval nucleus (MARTÍNEZ, 2022; CIAN & MONTI, 2019).

Epithelial cells are part of lining tissues, glandular or parenchymal tissues. Neoplasms of these profiles include squamous cell carcinoma (SCC), sebaceous epithelioma, mammary adenomas, and perianal gland neoplasia (CIAN & MONTI, 2019) (Figure 6).

Figure 6- Epithelial cell cytology of a breast tumor presenting a cohesive group arrangement. (Panopticon-fast 100x magnification).

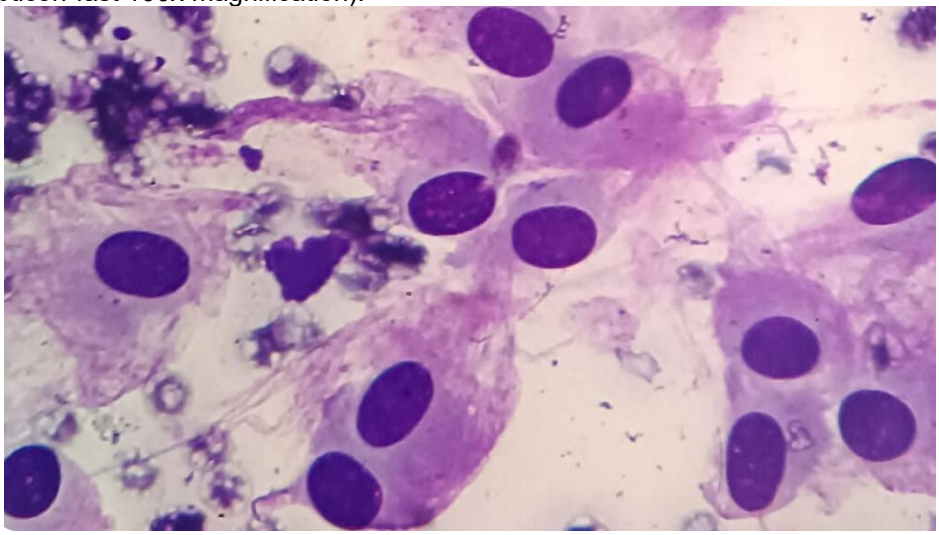


Source: The author (2024)

Mesenchymal cell neoplasms exfoliate little to many cells with a spindle-shaped profile or stellate shape, they are individualized with indistinct and clear cytoplasm, that is, without cytoplasmic delimitation and can also be in large aggregates, it is common to find multinucleation and presence of eosinophilic extracellular matrix, neoplasms of this category originate in the dermis and subcutaneous tissue and can be classified based on the type of cell from which they arise. (CIAN & MONTI, 2019)

In connective tissues, the common cells that appear are fibrocytes and fibroblasts, neoplasms of this type of tissue include fibroma, fibrosarcoma, myxoma, and myxosarcoma. Cells made up of vessels such as endothelial and perivascular cells include hemangioma/hemangiosarcoma and perivascular wall tumors, in adipose tissue (adipocytes) constitute lipoma and liposarcoma (CIAN & MONTI, 2019) (Figure 7).

Figure 7- Cytology of mesenchymal cell neoplasm of soft tissue sarcoma presenting stellate to spinal cells. (Panopticon-fast 100x magnification).

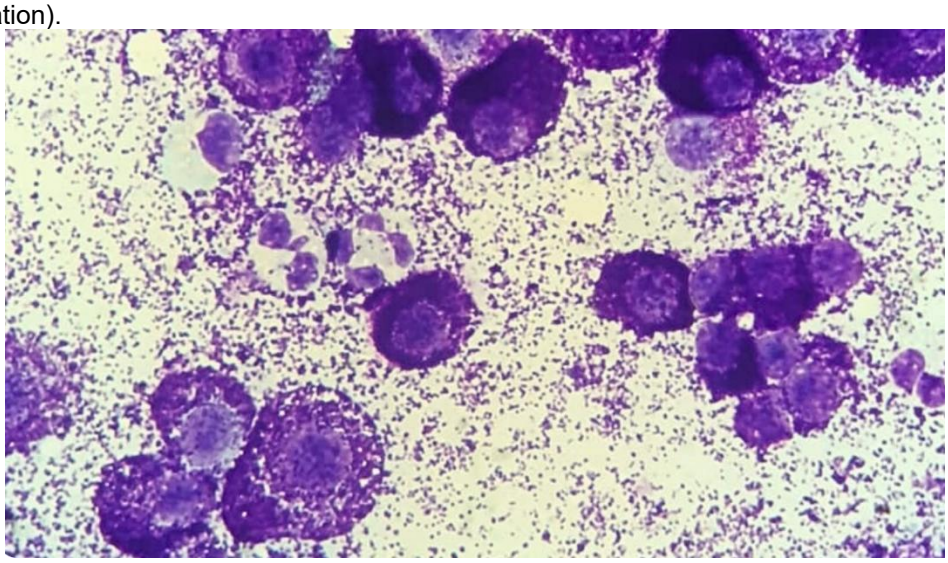


Source: The author (2024)

The group constituted by round cell neoplasia originates from different cell lines, but has historically been grouped because they have similar cytological characteristics. The aspiration of these tumors generally has high cellularity, the cells have a discrete characteristic of round to oval shape, do not group together or produce extracellular matrix (CIAN & MONTI, 2019).

When aspirates are very thick and hypercellular, they can give the impression of forming organized structures. Therefore, it is important to observe its arrangement in thinner areas of the blades. Tumors in this category include mast cell tumors (MCTs), cutaneous histiocytoma, plasmacytoma, transmissible venereal tumor (TVT), and lymphoma (COWELL et al., 2008; CIAN & MONTI, 2019) (Figure 8).

Figure 8- Round cell neoplasm cytology. Mastocytoma. These cells are characterized by having a round, individualized and discrete cell shape and several purplish-red granules by the cytoplasm. (Panopticon-fast 100x magnification).



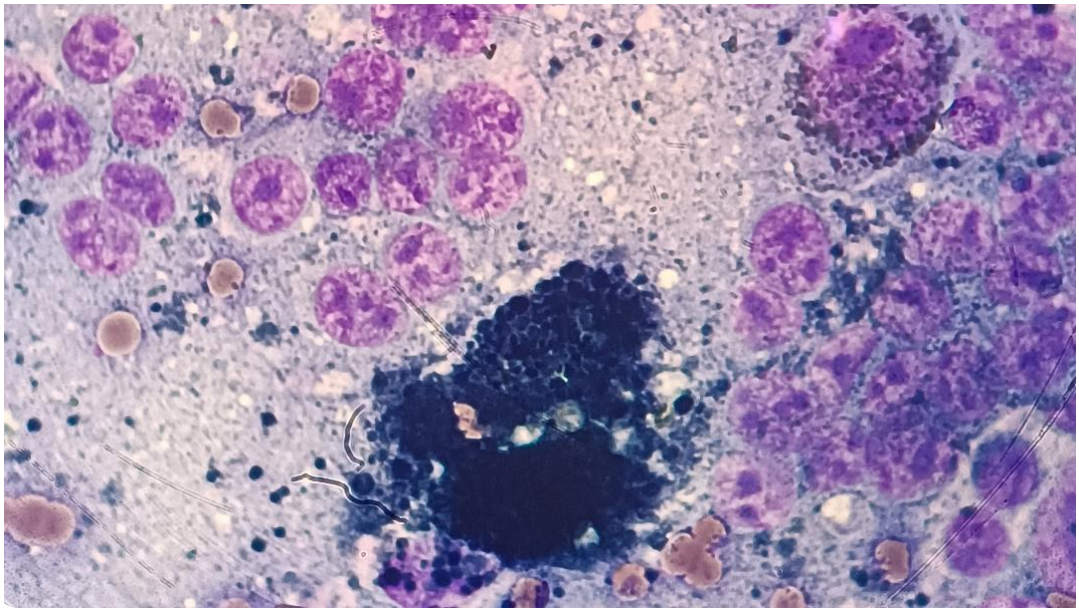
Source: The author (2024)

Melanocytic neoplasms in dogs have variable biological behavior regardless of their location, prognostic evaluation requires a combination of microscopic evaluation for nuclear atypia, mitotic count, degree of pigmentation, level of infiltration, and vascular invasion together with immunohistochemical determination, in addition, the general diagnosis of canine melanocytic neoplasms is often performed with fine-needle aspirates, however, cell morphology is variable, and may be spindle-shaped, rounded, and others with an epithelial shape, and is therefore a type of neoplasm that is difficult to classify as to its origin, but histopathology is often necessary to differentiate between benign and malignant forms (SMEDLEY et al, 2010).

Being common in dogs and occurring mainly on the skin and oral cavity, melanocytic neoplasms comprise 30-40% of all canine oral neoplasms (DOBSON et al., 2002). Although

the diagnosis of pigmented melanocytic neoplasms is simple, amelanotic forms can pose a diagnostic challenge (SMEDLEY et al., 2010). (Figure 9).

Figure 9- Cytology of a melanoma in a dog's digital region. Several greenish-black pigments are observed by the cytoplasm, evident nucleoli, and marked anisocytosis and anisocariosis. (Panopticon, Fast-magnification, 100x).



Source: The Author (2024)

OBJECTIVES

GENERAL OBJECTIVE

- To evaluate the occurrence of neoplasms in dogs treated at the Mário Dias Texeira Veterinary Hospital (HOVET-UFRA) through cytopathological examination from January to December 2023.

SPECIFIC OBJECTIVES

- To classify according to cytomorphological characteristics the neoplastic, non-neoplastic, inflammatory and non-conclusive processes.
- To identify the main neoplastic origins that affect dogs as a function of sex, age and organ system affected.

MATERIAL AND METHODS

SAMPLE SELECTION

Retrospectively, medical records of cytopathological tests of dogs from the routine of the Clinical Analysis Laboratory located at the Mário Dias Teixeira Veterinary Hospital (HOVET) of the Federal Rural University of the Amazon (UFRA) from January to December

2023 were selected. Information was collected about the sex, age of the animals and the cytopathological classification. Incomplete forms were excluded from the study.

The selected patients were segregated into females and males, regardless of whether they were castrated or not, and regardless of breed. Regarding the age group, the classification was made into three categories according to Fighera et al. (2008), in puppies (less than 1 year of age), adults (from 1 to 8 years of age) and elderly (9 years of age or older). Neoplastic lesions were classified according to their origin according to Raskin et al. (2021) into neoplasms of epithelial, mesenchymal, round cell, melanocytic and affected organ system (Integumentary System, Hemolymphatic System, Reproductive System, Urinary System, Eyes or Ears, and Oral Cavity/Gastrointestinal Tract and associated structures). Samples with scarce cellularity, showing blood contamination or inconclusive diagnosis were classified as non-diagnostic. Animals that exhibited more than one lesion were all included, as there were lesions with different diagnoses. Thus, the number of alterations may be greater than the number of patients included, since each medical record reviewed will correspond to a single dog.

COLLECTION, PROCESSING OF SAMPLES AND ANALYSIS OF DATA

Biological samples were collected by resident veterinarians of clinical pathology at HOVET-UFRA during the hospital routine according to the most suitable methodology for each case: Fine Needle Aspiration Puncture (FNA) and/or Fine Needle Puncture (FNP), scarification or direct/indirect imprint.

The material was collected mainly with 25 mm x 0.7 mm (22G) and/or 25 x 0.80 mm (21G) gauge needles connected to a 5 mL syringe and deposited on a 26 x 76 matte-tip glass slide for microscopy. For lesions where it was not possible to use the FAP or FNA technique, disposable carbon steel scalpel blades (Descarpack®) or sterile swab were used. The slides were stained with Romanowsky-type dye (Panopticon fast®. Newprov, Brazil). The samples were analyzed by optical microscopy using Olympus ® CX21/CX40 microscopes using 10x, 20x, 40x and 100x objectives.

The statistical data were evaluated through a descriptive analysis, where the frequency and percentage of the use of the Pap smear test in the veterinary practice of the animals treated was determined. The results were tabulated and analyzed using Microsoft Excel.

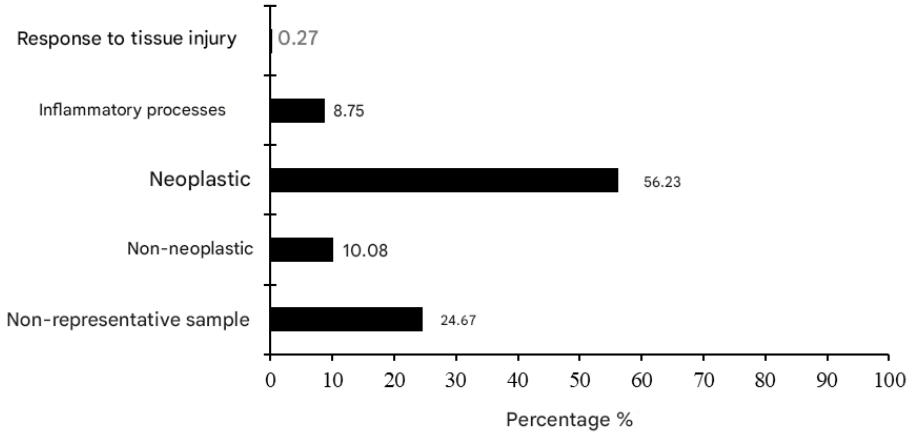
RESULTS AND DISCUSSION

During the period analyzed, a significant number of cytological tests were conducted, highlighting the feasibility and efficacy of this technique for diagnosis in clinical practice. Therefore, based on the survey of data regarding cytological routines in 2023, 262 reports were evaluated, of which 33 medical records contained incomplete information and were thus excluded from the study.

The results revealed a total of 377 lesions identified in 229 reports of the animals evaluated. Of the 377 lesion samples analyzed, suggestive cytological diagnoses were established for 212 (56.23%) neoplastic cases, 38 (10.08%) non-neoplastic cases, 33 (8.75%) cases of inflammatory processes, and only one lesion related to the response to tissue injury (0.27%) (Figure 10). As in humans, the occurrence of neoplasia is common in pets, possibly due to increased life expectancy. In addition, advances in the availability and quality of clinical diagnostic methods have also contributed to broader case detection (CASSALI 2017). Non-neoplastic processes are characterized by the presence of mature epithelium in a cutaneous mass, usually indicating a benign condition. These findings are common in keratin-containing cysts, glandular hyperplasias, or increased collagen deposition. Examples of these types of lesions include infundibular cysts, apocrine cysts, sebaceous nodular hyperplasia, hamartomas, among others. (RASKIN et al, 2021).

Non-representative samples were those in which it was not possible to make an adequate diagnosis. These samples were considered non-diagnostic for clinical suspicion due to the presence of blood contamination, scarce cellularity or sampling error, which totaled 93 cases (24.67%). It is relevant to mention that, in certain cases, such as in poorly exfoliative lesions, common in mesenchymal neoplasms, and in cases of blood contamination and inflammatory process, frequent in breast neoplasms, complementation with other tests, such as histopathological examination, immunohistochemistry, among others, as indicated by Peters et al. (2016), Oliveira et al. (2020) and Silva et al. (2020), become necessary, thus increasing the reliability of the technique and enhancing its therapeutic efficacy. In addition, bleeding can occur in inflamed and ulcerated tissues due to tissue neovascularization, as well as by the sample collection method. For example, there may be aspiration of surrounding fat or non-target structures, such as the salivary gland when trying to aspirate the mandibular lymph node, among others (ALBANESE, 2018). It is worth noting that, regardless of the cytological evaluation strategy, obtaining good samples and adequate training in cytopathology are essential to obtain clinically relevant results, thus increasing diagnostic sensitivity and specificity (CASSALI, GOBBI & SCHMITT, 2007).

Figure 10- Cytological diagnosis of lesions in dogs treated at HOVET-UFRA from January to December 2023.



Source: The author (2024)

Data related to sex, the highest occurrence of visits for cytological examination were in females 64.19% (147). Regarding the age of the animals, the results obtained showed a prevalence of elderly dogs with 61.14% (140) of animals attended. The propensity for lesions in females was 70.82% (267) while in males it was 29.18% (110) (Table 1). Corroborating this disposition, Comin (2023) observed that of the 902 dogs in his study, 621 (68.8%) corresponded to females and 520 (57.7%) were geriatric animals. It is worth noting that for Silva et al. (2020), in cytological tests, There is no correlation between the sexual predisposition of dogs and the development of diseases diagnosed by cytology, since the lesions can be caused by inflammatory, infectious or neoplastic processes.

Table 1- . Data evaluated regarding the sex, distribution of lesions and age of the animals treated at HOVET-UFRA from January to December 2023.

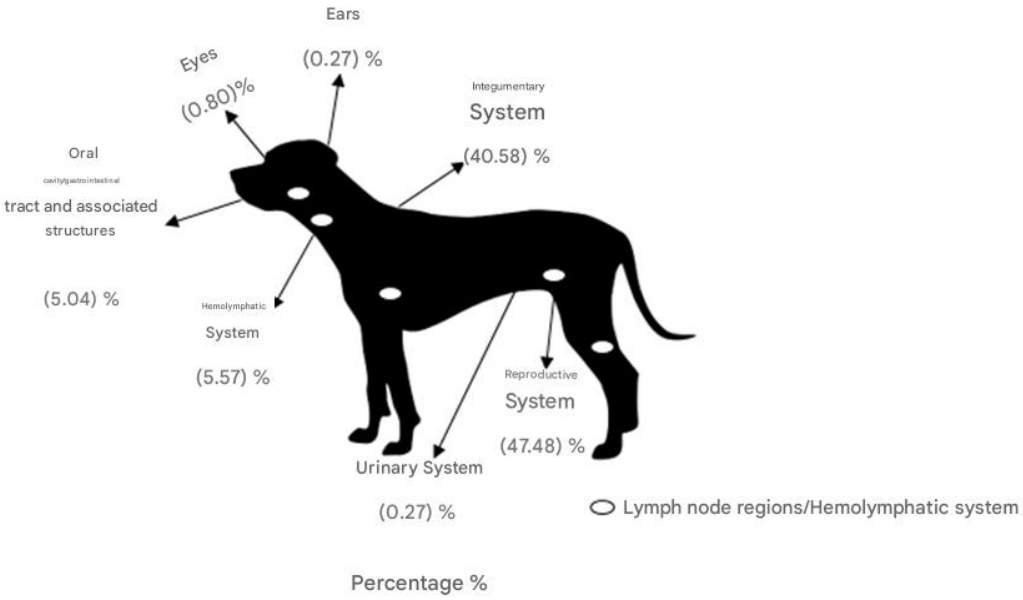
Animals by sex	Frequency	%
Female	147	64,19
Male	82	35,81
Total	229	100
Number of injuries by sex	Frequency	%
Female	267	70,82
Male	110	29,18
Total	377	100
Classification by age group	Frequency	%
Puppies < 1 year	2	0,87
Adults (1 to 8 years)	87	37,99
Seniors (9 years and older)	140	61,14
Total	229	100

Source: The author (2024).

The distribution of lesions in dogs by organic system is shown in Figure 11. The results showed that the highest occurrences of lesions occurred in the reproductive system with 47.48% (179), integumentary system 40.58% (153) and in third place in the hemolymphatic system with 5.57% (21) of the cases. Comin (2023) in his study of

cytopathological screening of lesions found that the most recurrent conditions in both dogs and cats occurred in the integumentary, reproductive and hemolymphatic systems, respectively. It is believed that the higher number of lesions in the reproductive system of animals is due to the low awareness of owners about the importance of elective and early sterilization. The teaching hospital is located on the outskirts of Terra Firme, in Belém do Pará, where most residents who use veterinary services are low-income and often unaware of the concept of responsible guardianship and the necessary care for their animals, so that animals that are not sterilized are more likely to develop pathological diseases in the reproductive system, therefore, sterilization becomes necessary and is considered a public policy instrument of a national nature aimed at the protection of companion animals (SANTANA & OLIVEIRA, 2020).

Figure 11- Distribution of lesions in dogs by organic system treated at HOVET-UFRA from January to December 2023.

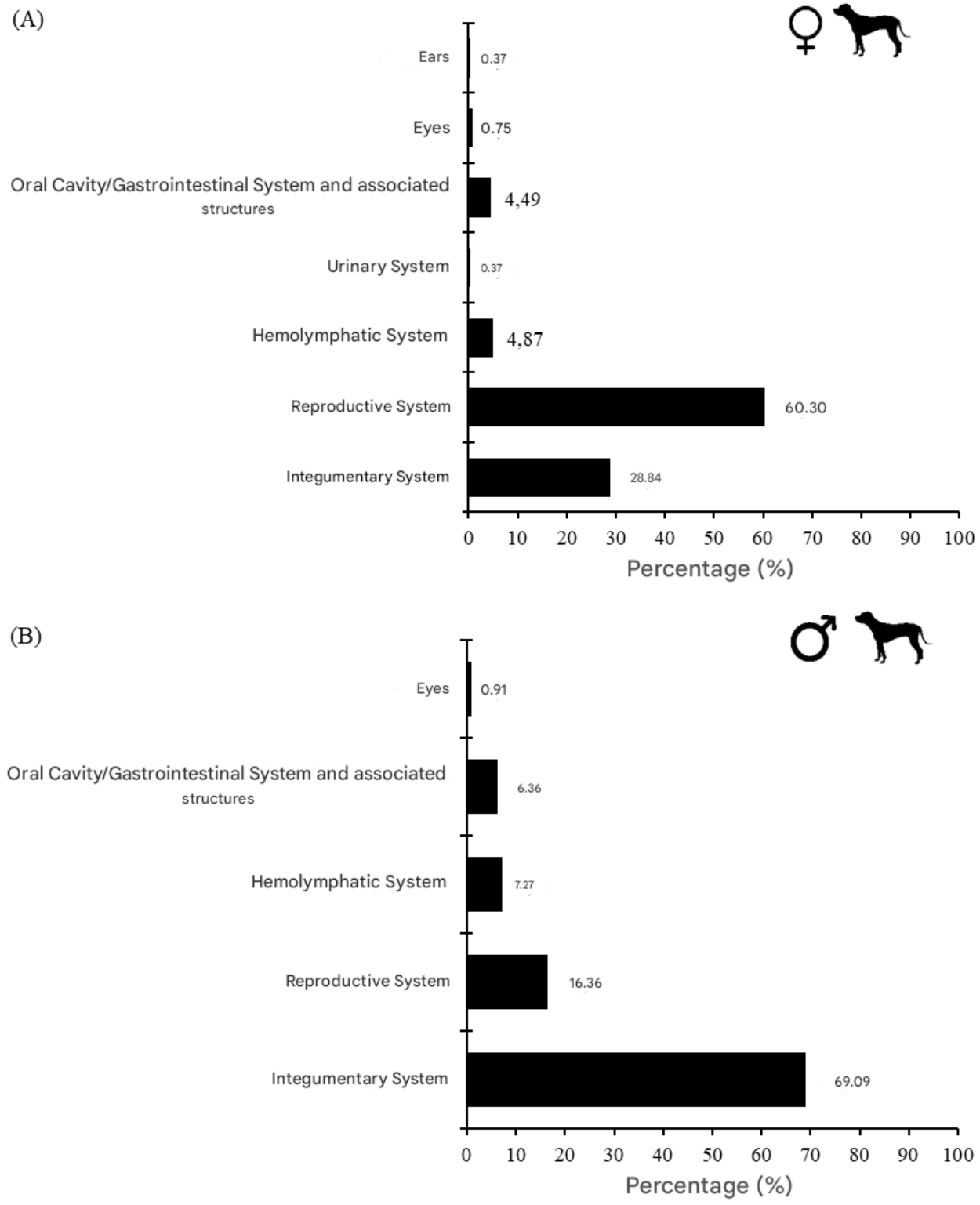


Source: The author (2024).

For the distribution of lesions according to sex, it was found that both sexes showed divergent values regarding the affected organ system, and in females the highest prevalence of lesions observed were in the reproductive system: 60.30% (161) (Figure 12). In females, lesions in the reproductive system tend to be more common, and the main cytological disorders found include: inflammatory processes, mammary cysts, hyperplasia and mammary neoplasms, conditions that are usually related to animals that are not castrated, females in pseudocyesis, and the indiscriminate use of exogenous hormones (RASKIN, MEYER & BOES, 2022). In males, the lesions were more distributed in the integumentary system 69.09% (76), regarding the results obtained on the incidence of skin

tumors in dogs carried out by Subapriya and collaborators (2021), of the 160 dogs included in the study, it was shown that cases of skin tumors were more incident in males with 68.13% of the cases, while in females it was 31.88%.

Figure 12- Distribution of lesions by sex and organic system of dogs treated at HOVET-UFRA from January to December 2023.



Source: The author (2024).

The distribution of neoplastic processes according to the affected organ system is shown in Table 2. It is possible to observe that neoplasms in the reproductive system were the most prevalent, representing 115 cases (54.25%) of the occurrences. Next, neoplasms in the integumentary system totaled 74 cases (34.91%), followed by the hemolymphatic system with 14 cases (6.60%). Neoplasms in the oral cavity, gastrointestinal system and associated structures accounted for 7 cases (3.30%), while cases in the urinary system and ears were less frequent, with only one case each (0.47% for both). The predominant occurrences of neoplasms in the reproductive system include breast tumors, uterine tumors, vaginal tumors, and testicular tumors (KUDNIG & SÉGUIN, 2022). A retrospective study on reproductive disorders in female dogs conducted by Beaudu-Lange and collaborators (2021), show that the main cause of death from reproductive disorders is breast tumors. Testicular neoplasms are more frequent in elderly dogs and those with cryptorchidism, with Leydig cell tumors, seminomas, and Sertoli cell tumors being the most prevalent types (ARGENTA et al., 2018). In addition, canine transmissible venereal tumors are also common in dogs, which affects the reproductive system of both females and males (ARAUJO et al., 2016).

Table 2- Distribution of neoplastic processes by organic system of dogs treated at HOVET-UFRA from January to December 2023.

NEOPLASMS BY ORGANIC SYSTEM		%
Reproductive System	115	54,25
Integumentary System	74	34,9
Hemolymphatic System	14	6,60
Oral Cavity/gastrointestinal system and associated structures	7	3,30
Urinary System	1	0,47
Ears	1	0,47
Total	212	100

Source: The author (2024)

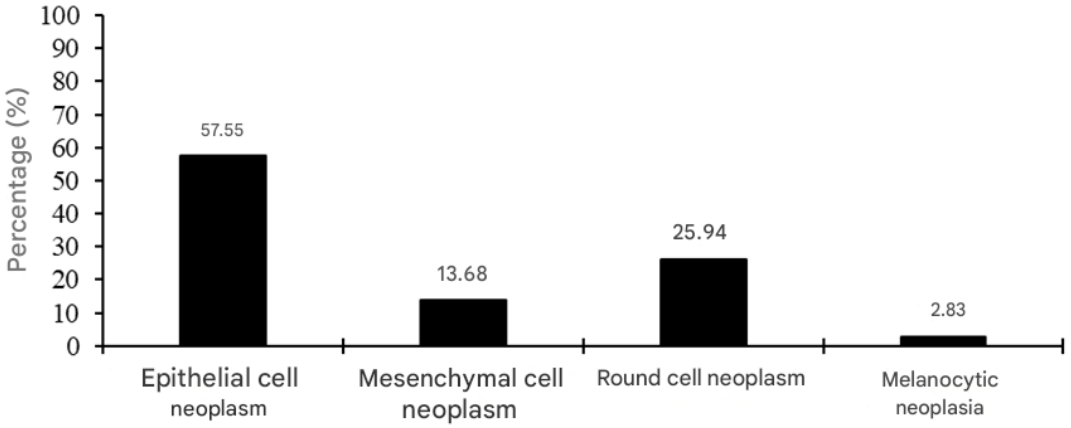
Regarding the occurrence of the main neoplastic origins, the prevalence of neoplasms of epithelial origin was observed 122 (57.55%), as can be seen in Figure 13. These results are in line with those obtained by Subapraya et al. (2020) where the incidence of neoplastic skin tumors in dogs was evaluated. In this study, it was shown that of the 160 cases, 80 were contained by epithelial neoplasms.

Epithelial neoplasms are common in dogs and cats, and can occur on the skin or in various organs. As with most neoplasms, those of epithelial origin can present benign or malignant biological behavior depending on the degree of differentiation and potential aggressiveness of the cells that compose them (O'BRIEN et al, 2000). They can be classified as benign when the cells are well differentiated and resemble the tissue of origin (ZACHARY, 2018). Benign tumors have little or no tissue invasion without metastasis,

responding well to chemotherapy treatments and patients generally have a good prognosis, examples of these neoplasms include breast adenomas, basal cell tumors, trichoblastoma, among others (MEUTEN, 2016).

At another time, malignant epithelial neoplasms are usually undifferentiated, and, in certain cases, the determination of their origin is based on other diagnostic methods, such as immunohistochemistry (IHC), histopathology and polymerase chain reaction (PCR). Therefore, due to their high presentation of infiltration and metastasis, animals when not treated often have an unfavorable prognosis, examples of these neoplastic types are composed of mammary carcinomas, squamous cell carcinomas, among others (MEUTEN, 2016).

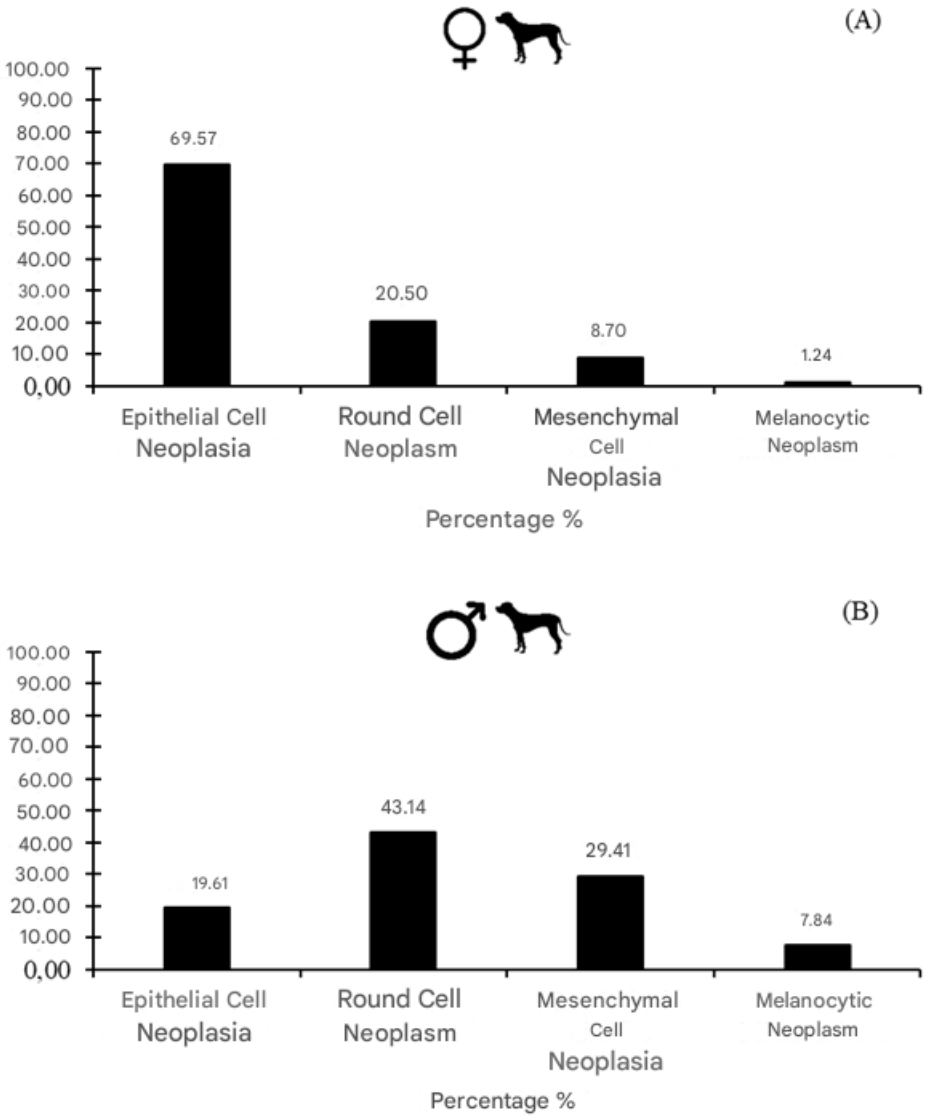
Figure 13- Cytological classification as to the origin of neoplastic lesions in dogs treated at HOVET-UFRA from January to December 2023.



Source: The author (2024).

The prevalence of gender-related neoplastic types is shown in Figure 14. Females had higher incidences of cases of neoplastic processes with a predominance of neoplasms of epithelial origin (69.57%) (112), followed by round cell neoplasms (20.50%) (33). In males, the predominant neoplasms were round cell neoplasms (43.14%) (22), followed by mesenchymal cells (29.41%) (15).

Figure 14- Prevalence of sex-related neoplastic types of dogs treated at HOVET-UFRA from January to December 2023.



Source: The author (2024).

In female dogs, the most frequent types of epithelial tumors are those that affect the mammary chain, this occurs due to the prolonged and intense exposure of the breast tissue to sex hormones, such as estrogen, prolactin and progesterone, which are identified as risk factors for breast cancer (CASSALI, 2017). Therefore, the association of different groups of neoplasms with specific epidemiological characteristics emphasizes the importance of diagnostic approaches and personalized treatment based on the type of neoplasm (SANTOS et al., 2020).

Regarding the cytological diagnosis of neoplastic processes in female dogs in the reproductive system, mammary epithelial cell neoplasms, in general, were the most important cases diagnosed, with mammary adenoma/carcinoma being the most frequent neoplasm with 35.40% (27/161), followed by benign epithelial neoplasms 17.39% (28/161).

Mastocytoma and lymphoma were the most common types of round cell neoplasia, with results of 9.32% (15/161) and 6.83% (11/161), respectively (Table 3).

Table 3- Neoplastic cytological diagnosis of female dogs attended at HOVET-UFRA from January to December 2023.

Neoplastic Cytological Diagnosis in Females		
Neoplasm of Epithelial Cells	Frequency	%
Adenoma due to Glândula ceruminosa	1	0,62
Adenoma/Carcinoma, Mammary	57	35,40
Squamous cell carcinoma	2	1,24
Mixed type carcinoma	6	3,73
Carcinoma Urotelial	1	0,62
Epithelioma Sebáceo	2	1,24
Basal cell neoplasm	5	3,11
Neoplasia Epitelial Benigna	28	17,39
Neoplasia Epitelial Maligna	9	5,59
Perianal Gland Tumor	1	0,62
Mesenchymal Cell Neoplasm		
Epulis	3	1,86
Hemangiopericitoma	1	0,62
Lipoma	3	1,86
Liposarcoma	2	1,24
Mixoma/Mixossarcoma	2	1,24
Mesenchymal Cell Neoplasm	1	0,62
Sarcoma	1	0,62
Undifferentiated Sarcoma	1	0,62
Round Cell Neoplasia		
Lymphoma	11	6,83
Mastocytoma	15	9,32
Plasmacytoma	2	1,24
TVT	4	2,48
Melanocytic Neoplasms		
Amelanocytic tumor	2	1,24
Total	161	100,00

Source: The author (2024).

Canine mammary neoplasms are appropriate and valid experimental models for the study of cancer biology in humans, due to the similarity in epidemiological, clinical, biological and genetic characteristics (SILVA et al. 2004; KUMARAGURUPARAN et al. 2006). Studies on the main causes of breast neoplasms indicate that factors such as obesity, age, gender, nutrition, and hormonal activities are associated with the etiology of breast cancer in dogs and women (CASSALI et al, 2014, TAKALKAR et al. 2016). These factors also highlight the importance of early ovariohysterectomy (OSH) before the third estrous cycle, which has a significant positive impact on reducing the development of mammary neoplasms in, constituting an important prevention measure (FONSECA & DALECK 2000).

Based on what Cassali (2017) provides, breast tumors in cytology are classified into stages such as the evaluation of the suitability of the samples; the determination of the presence or absence of cell populations (epithelial and/or myoepithelial/mesenchymal); the

determination of malignant potential and the classification of the cytotype, when possible. Therefore, the cytological examination helps in the provisional diagnosis and serves as a screening criterion for the diagnosis of benign and malignant tumors of the mammary gland. Therefore, histopathology remains a crucial diagnostic tool for accurate tumor classification due to its ability to assess the degree of infiltration, tissue architecture, and vascular invasion (TKACZYK-WLIZŁO et al., 2023).

One of the most frequent diagnoses in canines is mast cell tumor, cytologically it is a type of neoplasm with high specificity and diagnostic sensitivity, establishing a definitive diagnosis for most cases (92-96%), as the characteristics of mast cells are widely recognizable as the presence of purplish-red granulations around the cytoplasm and extracellular (MERLO, 2022; CIAN & MONTI, 2019). Despite the high diagnostic accuracy, cytology does not evaluate the degree of tissue invasion and the degree of malignancy, therefore, histopathology is relevant to designate the prognosis of this type of tumor, to evaluate cell and nuclear morphology and mitotic count, which are important factors for the recognition of treatment protocols and evaluation of surgical margins, at another time, studies through immunohistochemical and molecular genetic analyses have also been shown to be efficient in terms of the prognosis of the disease (CIAN & MOTIN, 2019; CONRAD et al., 2023).

Data regarding neoplastic cytological diagnoses in males are presented in Table 4. In general, the most recurrent neoplasms were of round origin, with TVT being the most frequent neoplastic condition (15.69% (8/51), followed by mast cell tumors with 13.73% (7/51) of the cases. In neoplastic lesions of mesenchymal origin, lipoma was the most common 9.80% (5/51) of the conditions evaluated. There are also two cases of epithelial cell neoplasia, with perianal gland tumor being 7.84% (4/51) the most common.

Table 4- Neoplastic cytological diagnosis of male dogs treated at HOVET-UFRA from January to December 2023.

Neoplastic Cytological Diagnosis in Males		
Neoplasm of Epithelial Cells	Frequency	%
Perianal Gland Tumor	4	7,84
Sebaceous adenoma	1	1,96
Squamous Cell Carcinoma	1	1,96
Basal Cell Neoplasia	3	5,88
Interstitial Cell Tumor	1	1,96
Mesenchymal Cell Neoplasm		
Hemangiossarcoma	1	1,96
Lipoma	5	9,80
Liposarcoma	3	5,88
Myxosarcoma	2	3,92
Mesenchymal Cell Neoplasm	2	3,92
Malignant Mesenchymal Cell Neoplasm	1	1,96
Sarcoma	1	1,96

Round Cell Neoplasia		
Histiocytoma	1	1,96
Lymphoma	2	3,92
Mastocytoma	7	13,73
Seminoma	1	1,96
TVT	8	15,69
Melanocytic Neoplasms		
Melanoma	2	3,92
Amelanocytic tumor	2	3,92
Total	51	100,00

Source: The author (2024).

Round cell tumors in general have additional specific morphological characteristics that facilitate their recognition in cytology, so it is always important to be able to differentiate these types of tumors, since the biological behavior of these neoplasms and the therapeutic options differ significantly, such as cases of cutaneous histiocytoma often suffer spontaneous regression, while mast cell tumors are treated for surgical excision and may have malignant potential, in cases of TVT, chemotherapy treatments are necessary, and there may be risks of metastases in organs (CIAN & MONTI, 2019).

Transmissible Venereal Tumor (TVT) is one of the important dog neoplasms that occur most frequently in sexually active animals, it does not have a racial predisposition, predominantly affecting dogs aged 1 to 7 years and both sexes (CONTE et al, 2022). On the other hand, an epidemiological analysis of TVT in dogs carried out by Pimentel et al. (2021), observed that females were the most affected by this type of neoplasm, although the present research demonstrates a predominance in males and based on the study of the epidemiological profile of TVT carried out by Costa and collaborators (2023), it was observed that there were disagreements from several studies on the incidence rate of TVT in both males and females, Therefore, it is concluded that there is no greater predisposition to TVT in either sex. The compromised immune system plays a main role in the transmission of TVT and the main site of affection is the external genitalia, and its transmission occurs mechanically through intercourse, licking, bites or scratches, through the mucous membranes or abrasions of the skin (DAS et al., 2020).

In a retrospective study on skin tumors in dogs, epithelial neoplasms were the most common conditions in males, with hepatoid gland tumors standing out as the most frequent (MARTINS et al. 2022). In unneutered males, there is evidence that androgen receptors (ARs) are present in all normal canine hepatoid tissues. Therefore, the higher levels of androgens circulating in males may justify the higher incidence of these lesions in this genus (PISANI et al. 2006).

Lipoma is a neoplasm of mesenchymal origin, characterized as a benign tumor of adipose tissue, being common in dogs and representing approximately 9% of all skin and

subcutaneous tumors (CIAN & MONTI, 2019; RASKIN et al, 2023). Studies conducted These tumors are often associated with obesity or being overweight. Although lipomas are fatty masses that do not usually cause substantial direct problems to dogs, they can generate great anxiety in owners, especially when they are multiple or large in size (O'NEILL et al. 2018).

Although lipomas are benign neoplasms, cytological screening is a crucial tool to differentiate them from other neoplasms that may appear benign, such as mast cell tumors. It is common to find nodules with macroscopic characteristics similar to those of lipomas, but when performing the cytological examination, it is often discovered that it is a mast cell neoplasm. This neoplastic behavior underscores the importance of using cytology to distinguish between benign and malignant neoplastic processes (KOLYCH et al, 2023).

FINAL CONSIDERATIONS

The use of cytological examination at the Mário Dias Teixeira Veterinary Hospital (HOVET-UFRA), in Belém do Pará, proved to be an important diagnostic tool. The diagnostic results not only guided treatments, but also provided data on the most common lesions, which are critical for disease control in the region. In the present study, the sample of care for cytological examination in females was prevalent and presented higher neoplastic occurrences in the reproductive system linked to the mammary chain, with elderly animals being the most affected. Therefore, it is recommended that this technique be routinely integrated into veterinary clinical examination due to its ease of execution, affordability, and relevance in identifying conditions such as inflammatory, infectious, and neoplastic processes. For the neoplastic processes, which were prevalent in this study, it is suggested that additional studies be carried out on their clinical, laboratory and epidemiological characteristics in the region, due to their importance. In addition, histopathological analysis is also necessary to define a definitive diagnosis of neoplastic processes, thus validating what is observed in cytology.

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