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Epidemiological, clinical, and histopathological aspects of mammary neoplasms in dogs from Rio Branco, Acre, Brazil

Aspectos epidemiológicos, clínicos e histopatológicos das neoplasias mamárias em cães do município Rio Branco, Acre, Brasil

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Highlights

Mammary neoplasms.
94% malignant with no correlation between epidemiological data.

Abstract

Mammary neoplasms are very common in the clinical routine of small animals and have an enormous histological diversity and complexity. Most of these neoplasms are classified as malignant and originate from metastases, considered the main cause of death. This study aimed to present and correlate epidemiological data with the malignancy of histological types of mammary neoplasms in dogs in the municipality of Rio Branco, Acre, Brazil. The analyzed samples were collected in collaborating hospitals and veterinary clinics, the data acquired through a questionnaire filled in by them, the clinical staging carried out through the TNM system, and the histological classification according to the Consensus regarding the Diagnosis, Prognosis, and Treatment of Canine and Feline Mammary Tumors. The fixed material was evaluated macroscopically and then subjected to classical histological processing. Histological preparations were observed under optical microscopy. The data were organized statistically using Pearson's chi-square test ($p<0.05$). Of the 71 samples obtained from dogs, 50 consisted of mammary neoplasms, 18 skin neoplasms, and three non-tumor lesions. Eleven breeds of dogs were identified. Among them, 48 were females and two males with an average age of 10 years and most patients intact (90%) and the minority used progestogens (16%). Of the mammary neoplasms, 94% had a malignant

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profile, with carcinoma in a mixed tumor being the most frequent (60%). Statistically, no correlation was observed between breed, sex, age, reproductive status, use of progestogens, mammary location, or tumor size with malignancy. In general, dogs can be affected by malignant mammary neoplasms, regardless of the mentioned variables, reinforcing the importance of clinical staging and early diagnosis for the best prognosis within the small animal clinic.
Key words: Histological classification. Clinical staging. Canine mammary tumor.

Resumo

As neoplasias mamárias são muito frequentes na rotina clínica de pequenos animais e possuem uma enorme diversidade e complexidade histológica. A maior parte dessas neoplasias é classificada como malignas e originam às metástases, sendo consideradas a principal causa mortis. Este estudo objetivou apresentar e correlacionar os dados epidemiológicos com a malignidade dos tipos histológicos das neoplasias mamárias em cães dentro do município de Rio Branco, Acre. As amostras analisadas foram coletadas em hospitais e clínicas veterinárias colaboradoras, os dados adquiridos através de questionário preenchido pelos mesmos, o estadiamento clínico realizado através do sistema TNM e a classificação histológica segundo o Consenso de Diagnóstico, Prognóstico e Tratamento de Neoplasias Mamárias Caninas. O material fixado foi avaliado macroscopicamente e, em seguida, submetido ao processamento histológico clássico. As preparações histológicas foram observadas sob microscopia óptica. Os dados obtidos foram organizados na forma estatística utilizando o Teste de Qui-quadrado de Pearson ($p<0,05$). Das 71 amostras obtidas em cães, 50 foram neoplasias mamárias, 18 foram tumores de pele e três eram lesões não tumorais. Foram identificadas 11 raças caninas, 48 eram fêmeas e dois machos, com idade média de 10 anos, onde a maioria dos pacientes eram inteiros (90%) e a minoria fazia uso de progestágenos (16%). Das neoplasias mamárias, 94% apresentaram o perfil maligno, sendo o carcinoma em tumor misto o mais frequente (60%). Estatisticamente não houve correlação entre raça, sexo, idade, estado reprodutivo, uso de progestágenos, localização das mamas ou tamanho tumoral com a malignidade. A espécie canina de modo geral pode ser acometida de neoplasia mamária maligna, independente das variáveis citadas, reforçando a importância do estadiamento clínico e diagnóstico precoce para o melhor prognóstico dentro da clínica de pequenos animais.
Palavras-chave: Classificação histológica. Estadiamento clínico. Tumor de mama canino.

Introduction

Mammary gland neoplasms are among the most common diseases in dogs in the small animal clinical routine (Feliciano et al., 2012; Cassali et al, 2017). They are ideal and accepted models for the study of tumor biology due to the similarity of histopathological and behavioral presentation of tumors that affect humans (D. C. Martins & Ferreira, 2003).

The study of mammary neoplasms in female dogs has been growing when compared to other affections due to their high rates, with great importance in veterinary medicine. In this sense, efforts have been made for the adoption of criteria to standardize the diagnosis, the understanding of the behavior and evolution of mammary neoplasms, and the definition of prognostic and predictive factors (Cassali et al., 2014).

Epidemiological studies on neoplasms have revealed important data, such as occurrence, age, and histological classification, allowing the planning and adoption of prevention and treatment measures for that species, and encouraging further research on the subject (Daleck & Nardi, 2016).

Among many characteristics, these neoplasms can appear as firm and well-demarcated nodules, mobile or fixed, presenting cutaneous and/or muscular involvement, reaching large dimensions, followed by ulcerations, and occurring in more than one mammary gland (Cogliati, 2015).

Clinical staging is essential for selecting and evaluating treatment and should be specific according to the TNM Classification of Malignant Tumors (Owen, 1980).

According to Ribeiro et al. (2009), approximately 50% of mammary neoplasms in female dogs are classified as malignant, and metastases are considered the main cause of death. The incidence of tumors in male dogs is low, representing less than 1% of mammary neoplasms (Bearss et al., 2012; Han et al., 2016) and their expression of hormone receptors is considered a prognostic factor (Misdorp et al., 1999; Carvalho et al., 2011).

Currently, the Consensus regarding the Diagnosis, Prognosis, and Treatment of Canine and Feline Mammary Tumors, proposed by Cassali et al. (2020), contemplates a more comprehensive classification, adopting new subtypes not yet described by Misdorp et al. (1999).

Material and Methods

This scientific research is in accordance with the rules issued by the National Council for the Control of Animal Experimentation (CONCEA) and approved by the Ethics Committee on the Use of Animals (CEUA-UFAC), registered with process number 23107.01654/2019-52 and protocol 30/2019.

Seventy-one dogs were collected, and non-tumoral lesions and tumors of origin other than the mammary gland were adopted as an exclusion method. The aspects associated with the mammary tumor were analyzed by applying a questionnaire to the guardians of the 50 patients affected by lesions located in the mammary chain to recognize factors of the agent related to the patient, age, breed, sex, animal size, reproductive status, use of hormones, and clinical history based on the date that the guardians noticed the onset of tumor appearance until the moment of diagnosis in order to carry out the epidemiological study.

Clinical staging of dogs with neoformations in the mammary chain was performed according to the TNM system, evaluating the extent of tumor involvement, as well as tumor size, including cytological evaluation of lymph nodes and chest X-ray in three projections. Some samples were collected during necropsies, which made clinical staging unfeasible. Some samples were collected during necropsies and made clinical staging unfeasible.

The samples came from the Dr. Mário Alves Ribeiro Teaching Veterinary Clinic at

the Federal University of Acre and private veterinary clinics in the municipality of Rio Branco, Acre, Brazil, being received from September 2019 to April 2021. The samples were fixed in 10% formaldehyde for better conservation and sent to the Laboratory of Pathology and Support for Silvestre Life at UFAC for histopathological examination. The slides produced were duplicated and sent to the Laboratory of Comparative Pathology at ICB/UFMG to confirm the tumor histological classification.

All samples were macroscopically photographed, weighed, measured, and also processed following the standard protocol. The fragments were cleaved and then processed using the Histotécnico Mod 808 apparatus, according to routine techniques for embedding in paraffin blocks. The 5-µm histological sections were obtained using a microtome and stained using Hematoxylin-Eosin techniques. The reading of the material was carried out in optical light microscopy, following the criteria: 10x to evaluate the cellularity and staining quality, 20x for the characteristics of cell types, and 40x magnification for individual morphological analysis of cells (nuclear and cytoplasmic characteristics) to establish a definitive diagnosis.

The histological classification and grading were carried out based on the Consensus regarding the Diagnosis, Prognosis, and Treatment of Canine and Feline Mammary Tumors, with the final diagnosis being based on histopathological findings, allowing the evaluation of histomorphology, nuclear pleomorphism, mitotic index, and its differentiation in degrees: (grade 1) well differentiated, (grade II) moderately differentiated, and (grade III) poorly differentiated (Elston & Ellis, 1991; Cassali et al., 2020).

The data were tabulated associating the epidemiological and histopathological factors of the mamma neoplasms found. Subsequently, they were subjected to the Pearson Chi-square Test, using the R Software to describe the variables and know the degree of independence between the mentioned factors, better predicting the result of one of them when the result of the other is known. Values were considered statistically significant when $p<0.05$. Importantly, the results were also distributed in percentage frequencies.

Results and Discussion

This study evaluated clinical and epidemiological data according to sex, age, breed pattern, reproductive status, use of progestogens, and location and tumor measurement correlated to the malignancy of mammary tumors in dogs (Table 1).

The frequency of mammary gland tumors in dogs is not significantly related to sex and tumor malignancy ($p=0.7154$). For the universe sampled in this study, females and males with malignant tumors are in greater numbers, representing 96% (45/48) and 100% (2/2), respectively. However, it was not statistically preponderant for tumor malignancy occurrence.

Very similar averages of the age of female dogs with benign and malignant tumors were observed (9.66 and 10.21 years old, respectively). A higher frequency was observed in elderly animals when

we correlated age with the occurrence of malignant and benign neoplasms. Statistically, the malignancy of the neoplasm is not correlated with the age group of the animal (p = 0.475). These results can be attributed to the small sample in a short period of observation.

Regarding the breed pattern, the animals affected by mammary tumors were classified as a mixed breed, with 48% (24/50), and pure breeds, with 52% (26/50), divided into Pinscher, with 12% (6/50), Dachshund 10% (5/50), German Shepherd 6% (3/50), Pitbull 4% (2/50), Poodle 4% (2/50), English Cocker Spaniel 4% (2/50), Yorkshire 4% (2/50), Boxer 4% (2/50), Rottweiler 2% (1/50), and Shih-Tzu 2% (1/50). There was no relationship between breeds and the presence of malignancy.

Despite the statistical data indicating no correlation between intact animals and malignant tumors (p=0.1647), intact animals showed a malignancy rate proportional to the rate of castrated animals, that is, 80% (4/5), agreeing with the statistics and not being preponderant this correlation. Moreover, we observed that 94% (45/50) of the animals that presented mammary tumors at the time of diagnosis were not castrated.

A total of 5% (5/50) of the studied females used progestogen-based contraceptives but no statistical correlation (p=0.5214) was observed between the use of progestogens and the occurrence of malignant tumors although all of them developed malignant mammary neoplasms.

Considering the location of tumors along the mammary chain, the inguinal mammae were more affected and the thoracic mammae less, 16% (12/50) with involvement of more than one mammary gland, with 19% (14/50), 29% (22/50), and 36% (27/50) of tumors being found in the thoracic, abdominal, and inguinal mammary glands, respectively. The inguinal glands were the most affected by malignant tumors although no significant relationship was observed between the factors malignancy and location of mammae. These findings do not support the hypothesis that there is a correlation even with a higher occurrence in abdominal and inguinal areas.

Tumor size showed that only 12% (6/50) were T1, 24% (12/50) were T2, and 64% (32/50) were T3. A prevalence of malignant tumors was observed in T3 (62%, 31/50), but no significant correlation was found between the two variables and tumor size is not a preponderant factor (p = 0.4075).

Tumor size (T) was evaluated within the clinical staging, in which T3 occurred in 64% (32/50), thorax X-ray with the presence of metastasis (M1) in 72.72% (8/11), and lymph nodes with the presence of metastasis (N1) in 28.57% (2/7). Deaths occurred in 32% (16/50), with adenoma occurring in 33.33% (1/3) and benign mixed tumors in 66.66% (2/3) (Figure 1).

Table 1
Clinical and epidemiological data correlated to the malignancy of naturally occurring mammary tumors found in dogs in the municipality of Rio Branco, Acre, Brazil

	Sex		Age		Breed		Reproductive status		Use of progestogens		Location			Size		
	Male	Female	Young	Elderly	Mixed breed	Pure breed	Intact	Castrated	Yes	No	M1 and M2	M3 and M4	M5	Several mammae	T1	T2	T3
MT	02 (100%)	45 (94%)	08 (89%)	39 (95%)	23 (96%)	24 (92%)	45 (96%)	02 (67%)	05 (100%)	24 (92%)	13 (93%)	20 (91%)	23 (85%)	12 (100%)	05 (83%)	11 (92%)	31 (97%)
BT	0	03 (6 %)	01 (11%)	02 (5%)	01 (4%)	02 (8%)	02 (4%)	01 (33%)	0	02 (8%)	01 (7%)	02 (9%)	04 (15%)	0	01 (17%)	01 (8%)	01 (3%)
TOTAL	02 (4%)	48 (96%)	09 (18%)	41 (82%)	24 (48%)	26 (52%)	47 (94%)	03 (6%)	05 (16%)	26 (84%)	14 (19%)	22 (29%)	27 (36%)	12 (16%)	06 (12%)	12 (24%)	32 (64%)

MT: malignant tumor; BT: benign tumor; M1: cranial thoracic mamma; M2: caudal thoracic mamma; M3: cranial abdominal mamma; M4: caudal abdominal mamma; M5: inguinal mamma; T1: up to 3 cm; T2: 3 to 5 cm; T3: above 5 cm.

Of the 50 dogs with mammary neoplasms in the north region, 94% (47/50) had a malignant profile and 6% (3/50) a benign profile, results similar to those also found in the southeast and northeast regions (Cassali et al., 2017). Thus, the histological types observed in this research suggest a predominance of carcinomas, including the two males, which presented carcinoma in mixed tumor and non-invasive papillary carcinoma.

Carcinoma in a mixed tumor was the most frequent, with 64% (30/47), followed by papillary carcinoma with 7% (3/47), micropapillary carcinoma with 4% (2/47), secretory carcinoma with 2% (1/47), solid carcinoma with 2% (1/47), basaloid carcinoma with 2% (1/47), cribriform carcinoma with 4% (2/47), pleomorphic lobular carcinoma with 2% (1/47), carcinosarcoma with 2% (1/47), malignant adenomyoepithelioma with 7% (3/47), sarcoma with 2% (1/47), and osteosarcoma with 2% (1/47) (Figure 2).

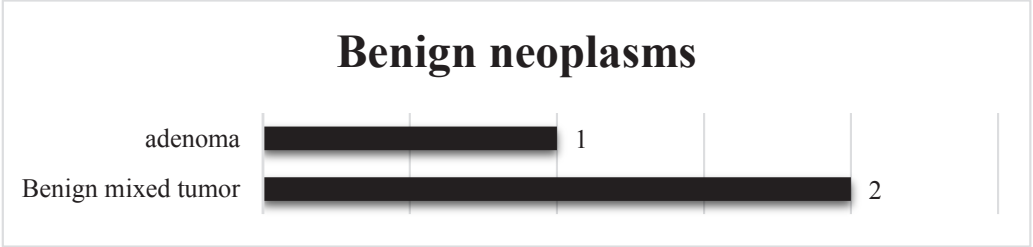

Figure 1. Histological classification of benign mammary neoplasms in dogs from the city of Rio Branco, Acre, Brazil.

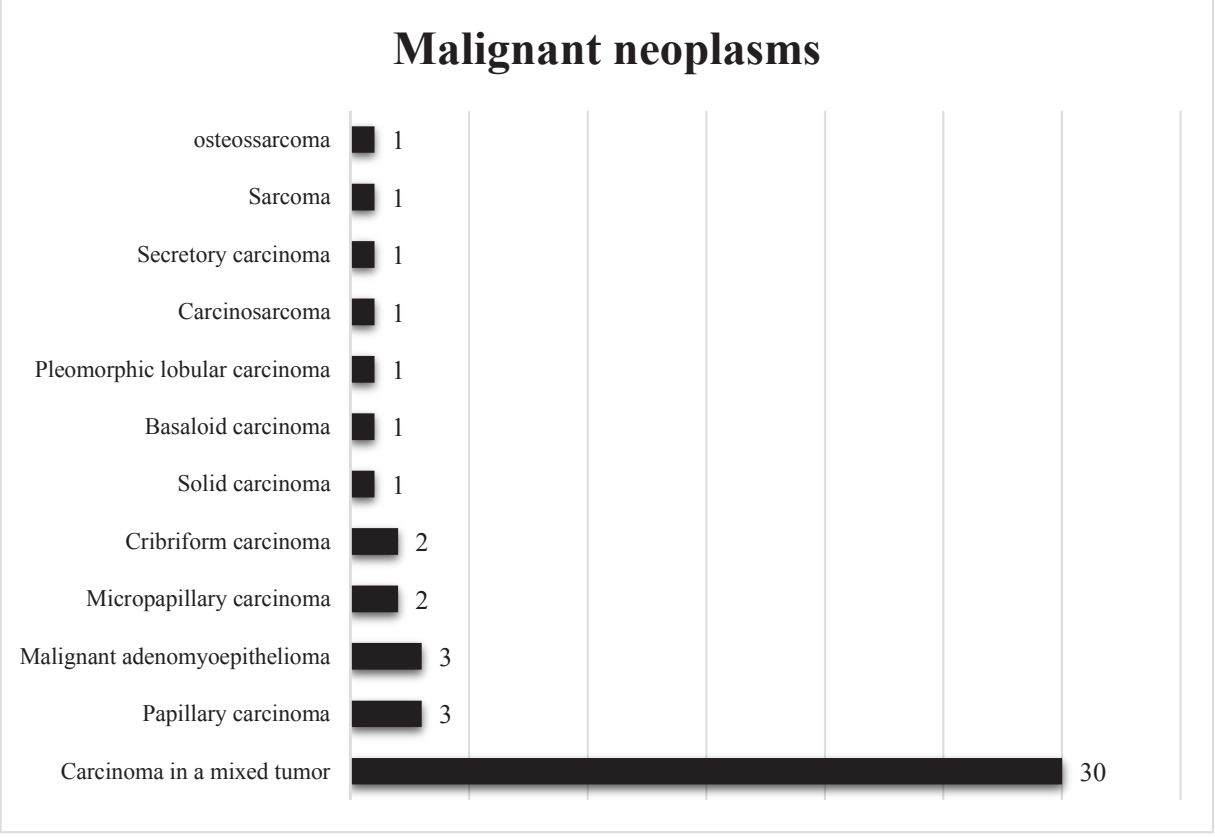

Figure 2. Histological classification of malignant mammary neoplasms in dogs from the city of Rio Branco, Acre, Brazil.

Mammary tumors in male dogs have low incidence but high malignancy. The low occurrence of neoplasms in males has also been reported in other studies (Bearss et al., 2012; Han et al., 2016). Although rare, this research demonstrated that mammary tumors could occur in male dogs and the differential diagnosis of neoformations located in the mammary chain should be considered.

Mammary tumors develop more frequently in middle-aged and elderly female dogs, with the highest occurrence in the age group between eight and 10 years old (Chang et al., 2005), corroborating with the present study, in which the average age of affected female dogs was 10 years. Some authors have reported lower averages for the occurrence of benign tumors (8.5 years) (Sorenmo et al., 2009; Oliveira et al., 2010).

According to Chang et al. (2005) and Dobson and Lascelles (2011), some breeds have a higher risk of developing mammary neoplasms, such as the Poodle, Cocker Spaniel, Yorkshire, German Shepherd, Boxer, and Dachshund, corroborating the results found in the present study. However, most studies in the literature have mentioned mixed-breed animals as those with a high incidence of mammary neoplasms (Morris et al., 2008; Nardi et al., 2009), as observed in this study, perhaps due to the fact they represent a large proportion in the studied population.

Studies have shown a higher incidence in intact animals, as well as the role of hormone levels in the occurrence of mammary tumors (Sleeckx et al., 2011; Ribas, et al., 2012; Beauvais et al., 2012), corroborating with studies that have shown that female dogs with this profile are more affected by these types of neoplasms (Feliciano et al., 2012; Kamiguchi et al., 2016).

Fonseca and Daleck (2000) stated that the use of hormones based on estrogens and progestogens at low concentrations to control heat in females is not correlated with the development of mammary neoplasms, unlike what was mentioned by Lana et al. (2007), Nardi et al. (2009), and Dias et al. (2016), who pointed out that the use of progestogens in females is directly related to the late appearance of mammary neoplasms. According to Oliveira et al. (2010), a single administration of the contraceptive can induce the occurrence of mammary hyperplasia and mammary tumors. However, this study was not sufficient to measure the relevance and correlation of heat suppressants and their effects on mammary neoplasms.

In general, the caudal abdominal and inguinal mammae are the most affected by neoplasms because they have higher tissue volume, consequent to the hormonal stimulus and because they are more subject to trauma (Fonseca & Daleck, 2000). According to Cirillo (2008), 60% of female dogs have simultaneous involvement of more than one mammary gland, with the occurrence of different histological types being common.

According to Queiroga and Lopes (2002), most malignant mammary tumors in dogs were between 3 and 5 cm in size (T2). Most of the benign tumors analyzed by these authors were smaller than 3 cm (T1). Moreover, some malignant neoplasms were larger than 5 cm (T3). The observed dimensions for benign and malignant neoplasms can be explained by the speed of tumor growth. In general, benign neoplasms tend to grow slowly over a period of months, or even years, as nodular, well-

circumscribed masses. However, malignant neoplasms vary widely in their growth rate.

Misdorp et al. (1999) and Nunes et al. (2018) stated that most neoplasms are malignant and may be associated with high mortality, and expansive tumor growth has a better prognosis than infiltrative growth. These authors considered this parameter as one of the most important prognostic factors. Tumor size is considered an intended prognostic feature. Dogs with mammary tumors larger than 5 cm (T3) have a shorter survival time compared to those at stages T1 and T2 (Ferreira et al., 2009; Nunes et al., 2018). The impact of lymph node involvement on survival time is higher when there is no lymphatic spread (Papazoglou et al., 2014). The detection of metastases has an important impact on the survival time of dogs. The overall survival time can be reduced from 331 to 236 days for dogs with primary tumors larger than 5 cm without or with the detection of metastases, respectively (Nunes et al., 2018).

Most benign mammary neoplasms in female dogs correspond to benign mixed tumors, reaching a proportion of 57.1 to 60% of cases of benign neoplasms (Queiroga & Lopes, 2002), and adenoma with 51.21% of cases (A. M. C. R. P. F. Martins et al., 2002), corroborating with this research.

According to A. M. C. R. P. F. Martins et al. (2002), Queiroga and Lopes (2002), and Nunes et al. (2018), malignant mammary neoplasms in dogs have an incidence of 25-50% of cases, and the histological classification found in this study corroborates with the findings of these authors, who found a prevalence of malignant tumors.

Histological grading is based on tissue architecture (tubular formation), cell pleomorphism, and mitosis count, always considering invasive areas for evaluation, demonstrating great prognostic value. Grade I and II carcinomas are more frequent and have a better prognosis (Peña et al., 2013), in agreement with the literature, as it is a rare neoplasm in dogs, but with an unfavorable prognosis when it presents a high histological grade (II and III) and frequent invasive areas (Cassali et al., 2017) (Figures 3 and 4).

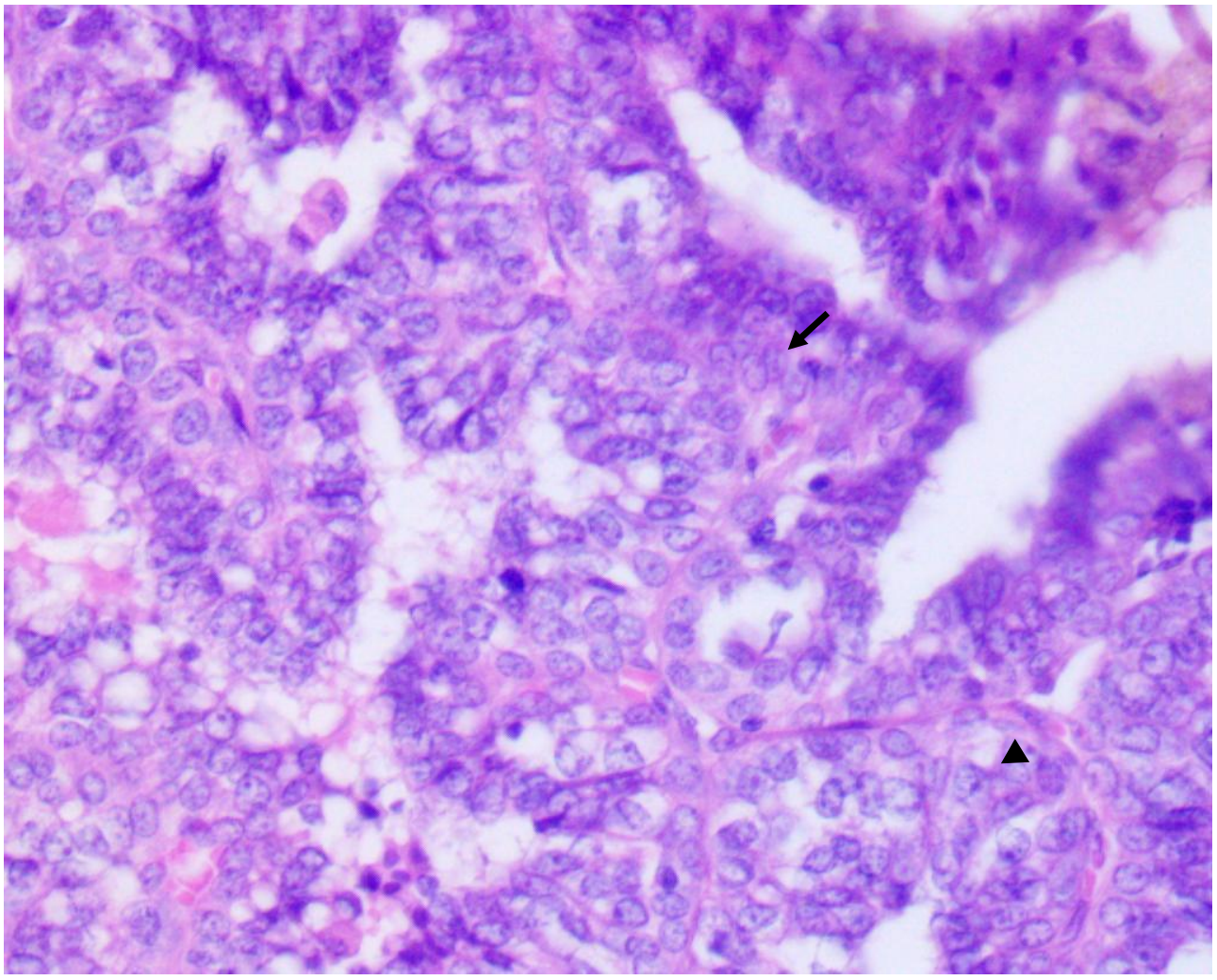

Figure 3. Optical micrograph of grade II invasive papillary carcinoma with evident fibrovascular support axis (arrowhead) and numerous cell layers (arrow) (female dog, mammary gland. HE - 40X).

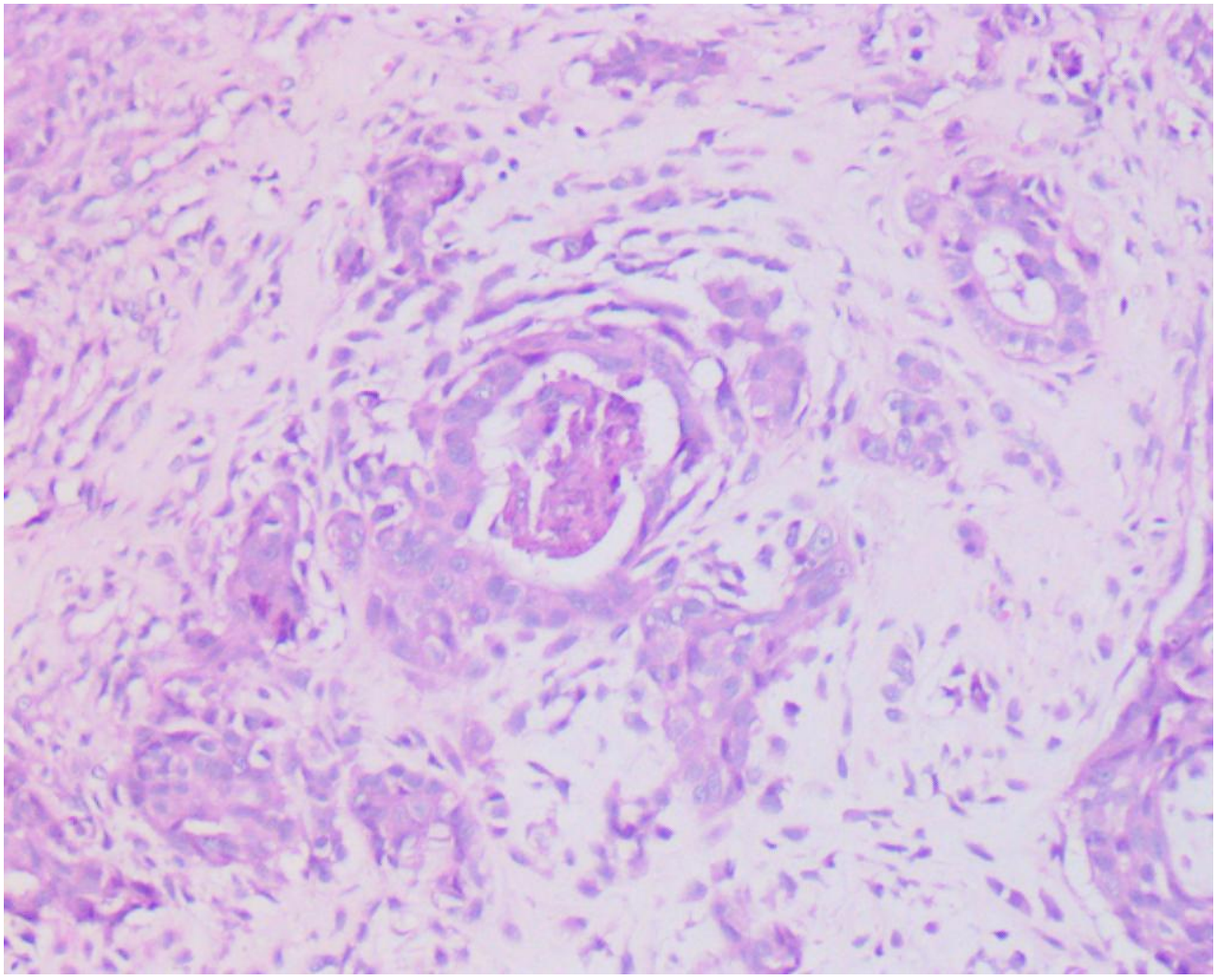

Figure 4. Optical micrograph of carcinoma in a mixed tumor. There is a proliferation of epithelial cells with invasive areas and a proliferation of mesenchymal tissue characterized by the formation of a pre-chondroid matrix (female dog, mammary gland. HE - 20X).

Conclusion

Carcinoma in a mixed tumor proved to be the most frequent neoplasm in the city of Rio Branco, Acre, Brazil, but other malignant tumors occurred in the sampled population, as well as benign tumors to a lesser extent.

This study demonstrates the high malignancy of mammary neoplasms, being one of the tumors with the largest casuistry in the veterinary clinic, and its occurrence is independent of sex, age, breed, and reproductive status. However, we can increase the chances of a better prognosis through clinical staging, helping guardians and clinicians within the veterinary routine.

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References

Bearss, J. J., Schulman, F. Y., & Carter, D. (2012). Histologic, immuno-histochemical and clinical features of 27 mammary tumours in 18 male dogs. *Veterinary Pathology, 49*(4), 602-607. doi: 10.1177/0300985811402843

Beauvais, J. M., Cardwell, J. M., & Brodbelt, D. C. (2012). The effect of neutering on the risk of mammary tumours in dogs: a systematic review. *Journal of Small Animal Practice, 53*(6), 314-322. doi: 10.1111/j.1748-5827.2011.01220.x

Carvalho, C. J. S., Quessada, A. M., Barbosa, S. R., Pires, L. V., Damasceno, K. A., Gamba, C. O., & Cassali, G.D. (2011). Carcinoma tubulo-papilar da glândula mamária em um cão macho Tubulo-papillary carcinoma of the mammary gland in a male dog. *Revista Portuguesa de Ciências Veterinárias, 4*(110), 577-580.

Cassali, G. D., Damasceno, K. A., Bertagnolli, A. C., Estrela-Lima, A., Lavalle, G. E., Di Santis, G. W., Nardi, A. B. de, Fernandes, C. G., Cogliate, B., Sobral, R., Costa, F. V. A., Ferreira, E., Salgado, B. S., Campos, C. B., D'Assis, M. J. M. H., Silva, L. P., Machado, M. C. A., Firmo, B. F., Nunes, F. C., & Nakagaki, K. Y. R. (2017). Consensus regarding the diagnosis, prognosis and treatment of canine mammary tumors: benign mixed tumors, carcinomas in mixed tumors and carcinosarcomas. *Brazilian Journal of Veterinary Pathology, 10*(3), 87-99. doi: 10.24070/bjvp.1983-0246.v10i3p87-99

Cassali, G. D., Jark, P., Gamba, C., Damasceno, K., Estrela-Lima, A., De Nardi, A., Ferreira, E., Horta, R. S., Firmo, B. S., Sueiro, F. A. S., Rodrigues, L. C. S., & Nakagaki, K. Y. R. (2020). Consensus regarding the diagnosis, prognosis and treatment of canine and feline mammary tumors - 2019. *Brazilian Journal of Veterinary Pathology, 13*(3), 555-574. doi: 10.24070/bjvp.1983-0246.v13i3p555-574

Cassali, G. D., Lavalle, G. E., Ferreira, E., Estrela-Lima, A., De Nardi, A. B., Ghever, C., Sobral, R. A., Amorim, R. L., Oliveira, L. O., Sueiro, F. A. R., Beserra, H. O., Bertagnolli, A. C., Gamba, C. O., Damasceno, K. A., Campos, C. B., Araujo, M. R., Monteiro, L. N., Nunes, F. C., Horta, R. S., ..., Torres, R. (2014). Consensus of the diagnosis, prognosis and treatment of canine mammary tumors - 2013. *Brazilian Journal of Veterinary Pathology, 7*(2), 38-69.

Chang, S. C., Chang, C. C., Chang T. J., & Wong, M. L. (2005). Prognostic factors associated with survival two years after surgery in dogs with malignant mammary tumors: 79 cases (1998-2002). *Journal of the American Veterinary Medical Association, 227*(10), 1625-1629. doi: 10.2460/javma.2005.227.1625

Cirillo, J. V. (2008). Chemotherapeutic treatment of mammary neoplasms in female dogs and cats. *Revista do Instituto de Ciências da Saúde, 26*(3), 325-327.

Cogliati, B. (2015). Patologia geral das neoplasias. In M. M. Jericó, J. P. D. Andrade, Neto, & M. M. Kogika (Eds.), *Tratado de medicina interna de cães e gatos.* (Cap. 8, pp. 1485-1509). Rio de Janeiro.

Daleck, C. R., & Nardi, A. B. de. (2016). *Oncologia em cães e gatos* (2a ed.). Roca.

Dias, M. L. D. M., Andrade, J. M. L., Castro, M. B. D., & Galera, P. D. (2016). Survival analysis of female dogs with mammary tumors after mastectomy: epidemiological, clinical and morphological aspects. *Pesquisa Veterinária Brasileira, 36*(3), 181-186. doi: 10.1590/S0100-736X2016000300006

Dobson, M. J., & Lascelles, B. D. X. (2011). *BSAVA manual of canine and feline oncology* (3nd ed.). British Small Animal Veterinary Association. https://www.cabdirect.org/cabdirect/abstract/20113066528

Elston, C. W., & Ellis, I. O. (1991). Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology, 19*(5), 403-410. doi: 10.1111/j.1365-2559.1991.tb0029.x

Feliciano, M. A. R., Silva, A. S., Peixoto, R. V. R., Galera, P. D., & Vicente, W. R. R. (2012). Clinical, histopathological and immunohistochemical study of mammary neoplasm in bitches. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 64*(5), 1094-1100. doi: 10.1590/S0102-09352012000500002

Ferreira, E., Bertagnolli, A. C., Cavalcanti, M. F., Schmitt, F. C., & Cassali, G. D. (2009). A relação entre o tamanho do tumor e a expressão de marcadores prognósticos em tumores mamários caninos benignos e malignos. *Veterinary Comparative Oncology, 7*(4), 230-235.

Fonseca, C. S., & Daleck, C. R. (2000). Neoplasias mamarias em cadelas: influência hormonal e efeitos da ovario-histerectomia como terapia adjuvante. *Ciência Rural, 30*(4), 731-735. doi: 10.1590/S0103-84782 000000400030

Han, J.-H., Kyeong-Soo K., & Jae-Hoon K. (2016). Mammary gland tumors in three male dogs. *Korean Journal of Veterinary Research, 56*(.4), 229-232. doi: 10.14405/kjvr.2016.56.4.229

Kamiguchi, I. E., Moreira, I. M., Silva Trindade, F. da, Zahn, F.S ., Hataka, A., & Sousa Rocha, N. (2016). Mammary neoplasms in female dogs: identification of cytopathological criteria for malignancy. *Journal of Cytology and Histology, 7*(1), 1-5. doi: 10.4172/2157-7099.1000392

Lana, S. E., Rutteman, G. R., & Withrow, S. J. (2007). Tumors of the mammary gland. In S. J. Withrow, & D. M. Vail (Eds.), *Withrow & MacEwen´s, small animal clinical oncology* (4a ed., pp. 619-636). St. Louis. Elsevier. doi: 10.1016/b978-072160558-6.50029-0

Martins, A. M. C. R. P. F., Tamaso, E., & Guerra, J. L. (2002). Retrospective review and systematic study of mammary tumors in dogs and characteristics of the extracellular matrix. *Brazilian Journal Veterinary Research and Animal Science, 39*(1), 38-42. doi: 10.1590/S1413-95962002000100007

Martins, D. C., & Ferreira, A. M. R. (2003). Marcadores prognósticos como um auxílio à conduta clínico- cirúrgica em uma cadela apresentando múltiplos nódulos mamários. *Acta Scientiae Veterinariae, 31*(3), 189-191. doi: 10.22456/1679-9216.17176

Misdorp W, Else RW, Hellmén, E. (1999). Histological classification of mammary tumors of the dog and the cat. 2nd Series. Vol VII. Washington DC: Armed Forces Institute of Pathology, American Registry of Pathology and the World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology. p. 1-59.

Morris, J. S., Nixo, C., Bruck A., Nasir, L., Morgan, I. M., Philbey, A. W. (2008). Immunohistochemical expression of TopBP1 in feline mammary neoplasia in relation to histological grade, Ki67, ERa and p53. *Vet J.*, (175), 218-226.

Nardi, A. B. de, Rodaski, S., Rocha, N. S., & Fernandes, S. C. (2009). Mammary neoplasms. In C. R. Daleck, A. B. Nardi, de, & S. Rodaski (Eds.), *Oncologia em cães e gatos* (Cap. 25, pp. 371-383). São Paulo.

Nunes, F. C., Campos, C. B., Teixeira, S. V., Bertagnolli, A. C., Lavalle, G. E., Cassali, G. D. (2018). Epidemiological, clinical and pathological evaluation of overall survival in canines with mammary neoplasms. *Arquivo Brasileiro de Medicina Veteterinária e Zootecnia, 70*(6), 1714-1722 doi: 10.1590/ 1678-4162-10217

Oliveira, J. C., Fº., Kommers, G. D., Masuda, E. K., Marques, B. M. F. P. P., Fighera, R. A., Irigoyen, L. F., & Barros, C. S. L. (2010). Retrospective study of 1647 mammary gland tumors in dogs. *Pesquisa Veterinária Brasileira, 30*(2), 177-185. doi: 10.1590/S0100-736X2010000200014

Owen, L. N. (1980). *TNM classification of tumours in domestic animals.* World Health Organization.

Papazoglou, L. G., Basdani, E., Rabidi, S., Patsikas, M. N., & Karayiannopoulou, M. (2014). Current surgical options for mammary tumor removal in dogs. *Journal Veterinary Science Medical, 2*(1), 1-6 doi: 10.13188/2325-4645.1000007

Peña, L., Andrés, P. J. de, Clemente, M., Cuesta, P., & Pérez-Alenza, M. D. (2013). Canine mammary tumors: a review and consensus of standard guidelines on epitelial and myoephitelial phenotype markers, HER-2, and hormone receptor assessment using immunohistochemistry. *Veterinary Patology, 50*(1), 94-105.

Queiroga, F., & Lopes, C. (2002). Tumores mamários caninos: pesquisa de novos fatores de prognóstico. *Revista Portuguesa de Ciências Veterinárias, 97*(543), 119-127.

Ribas, C. R., Dornbusch, P. T., Faria, M. R., Wouk, A. F. P. F., & Cirio, S. M. (2012). Relevant clinical alterations in dogs whit staged mammary neoplasms. *Archives of Veterinary Science, 17*(1), 60-68.

Ribeiro, L. G. R., Damasceno, K. A., Costa, J. M., Neto, D'Assis, M. J. M. H., Costa, A. T., Silva, N. S., Aguiar, P. H. P., Cassali, G. D., & Estrela-Lima, A. (2009). Expressão da Cox-2 nos carcinomas mamários de cadelas. *Veterinária em Foco, 6*(2), 134-139.

Sleeckx, N., Rooster, H., Kroeze, E. J. B. V., Ginneken, C. V., & Brantegen, L. V. (2011). Canine mammary tumours, an overview. *Reproduction of Domestic Animals, 46*(6), 1112-1131. doi: 10.1111/j.1439-0531.2011.01816.x

Sorenmo, K. U., Kristiansen, V. M., Cofone, M. A., Shofer, F. S., Breen, A. M., Langeland, M., Mongil, C. M., Grondahl, A. M., Teige, J., & Goldschmidt, M. H. (2009). Canine mammary gland tumors - a histological continuum from benign to malignant; clinical and histopathological evidence. *Veterinary and Comparative Oncology, 7*(3), 162-172. doi: 10.1111/j.1476-5829.2009.00184.x