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Small Animal Diseases

Relationship of obesity with clinical and laboratory changes in female dogs with mammary neoplasia[1]

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ABSTRACT.- Magalhães J.R., Guimarães J.B., Bonfim L.S., Carvalho C.F., Luz L.C., Cagnini D.Q., Regalin D. & Amaral A.V.C. 2020. **Relationship of obesity with clinical and laboratory changes in female dogs with mammary neoplasia.** *Pesquisa Veterinária Brasileira 40(7):536-545.* Rodovia BR-364 Km 195, Setor Parque Industrial 3800, Jataí, GO 75801-615, Brazil. E-mail: jessicarmedvet@gmail.com

Obesity may be associated with the onset of mammary tumors in women. In companion animals, these data are still scarce, mainly associating the clinic of the patient with laboratory and histological findings. This study aimed to evaluate the clinical and laboratory aspects of female dogs with mammary neoplasia, investigating whether they are related to obesity. Four groups of spayed or non-spayed female dogs were studied, divided into (G1) female dogs without mammary tumor and normal body condition score (BCS), (G2) female dogs without mammary tumor and obese, (G3) female dogs with mammary tumor and normal BCS and (G4) female dogs with mammary tumor and obese. BCS, canine body mass index (CBMI), blood count, glucose, total cholesterol, triglycerides and cytopathological and histopathological examinations were evaluated. Mixed-breed animals were the most prevalent in this study (67.5%). There was a limitation regarding the use of CBMI related to body proportionality. Considering the 28 tumors evaluated, carcinoma in mixed tumor was the most prevalent malignant histological type, while adenomyoepithelioma was the most prevalent benign histological type. It was possible to conclude that obesity was not related to clinical and laboratory changes in the female dogs affected with mammary neoplasias. In addition, no relationship was noted between BCS and CBMI with the histological type or malignancy of mammary neoplasia, since the malignant histological types were the most prevalent tumors in both the ideal score group and the overweight to obese group.

INDEX TERMS: Obesity, clinical change, laboratory change, female dogs, mammary neoplasia, dogs, bitches, cholesterol, glucose, triglyceride, mammary tumors.

RESUMO.- [**Relação da obesidade com alterações clínicas e laboratoriais em cadelas com neoplasia mamária.**] A obesidade pode estar associada ao aparecimento de tumores mamários em mulheres. Em animais de companhia, esse dado ainda é escasso, principalmente associando a clínica da paciente aos achados laboratoriais e histológicos. Este estudo objetivou avaliar os aspectos clínicos e laboratoriais de cadelas com neoplasia mamária, investigando se estão relacionadas à obesidade. Foram estudados quatro grupos de cadelas castradas ou não castradas, divididas em (G1) cadelas sem tumor de mama e Escore de Condição Corporal normal (BCS), (G2) cadelas sem tumor de mama e obesa, (G3) cadelas com tumor de mama e ECC normal e (G4) cadelas com tumor de mama e obesa. Foram avaliados o Escore de Condição Corporal (ECC), Índice de Massa Corporal Canina (IMC), hemograma, glicose, colesterol total, triglicerídeos e exames citopatológicos e histopatológicos. Os animais de raça mista foram os mais prevalentes neste estudo (67,5%). Houve uma limitação quanto ao uso do IMC relacionado à proporcionalidade corporal. Considerando os 28 tumores avaliados, o carcinoma em tumor misto foi o tipo histológico maligno mais prevalente, enquanto o adenomioepitelioma foi o tipo histológico benigno mais prevalente. Foi possível concluir que a obesidade não esteve relacionada a alterações clínicas e laboratoriais nas cadelas acometidas por neoplasias

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mamárias. Além disso, não foi observada relação entre BCS e CBMI com o tipo histológico ou malignidade das neoplasias mamárias, uma vez que os tipos histológicos malignos foram os tumores mais prevalentes no grupo escore ideal e no grupo com sobrepeso e obesidade.

TERMOS DE INDEXAÇÃO: Obesidade, alterações clínicas, alterações laboratoriais, cadelas, neoplasia mamária, cães, caninos, colesterol, glicose, triglicerídeos, tumores mamários.

INTRODUCTION

Mammary tumors are the most common types of neoplasia in female dogs, representing more than 50% of the cases, being 50% to 70% diagnosed as malignant, with metastatization capability for regional lymph nodes and lung. Consequently, the occurrence of mammary neoplasia in domestic animals is a subject addressed worldwide (Lana et al. 2007, Szczubial et al. 2012, Gundim et al. 2016, Dolka et al. 2018, Marconato et al. 2019). In addition, cancer is the leading cause of death in elderly dogs and the second largest cause in companion animals (Horta et al. 2012).

The awareness of health care for pets on the part of their guardians, along with the progress of Veterinary Medicine, has provided increased life expectancy, resulting in improved prevention of diseases, nutrition and therapeutics, in addition to increasing the need for the search for a better and more reliable prognosis (Horta et al. 2012, Matos et al. 2012, Silva et al. 2014). Nevertheless, concomitantly with the achievement of greater longevity, the cases of neoplasia diagnosed have increased (Portilho et al. 2015) because the more animals live, the greater their exposure to the carcinogenic factors that predispose to molecular changes with consequent disordered cell multiplication (Moreira et al. 2018).

Studies have shown a possible relationship between obesity and mammary neoplasia in female dogs (Lim et al. 2015, Chandler et al. 2017). According to German et al. (2006), the high ingestion of red meat had a positive correlation for the development of canine mammary tumors. For Lana et al. (2007), animals fed with home food had a higher risk of tumor development. For Cleary et al. (2010), obesity in female dogs decreases the latency period for detecting mammary tumors and increases their incidence. Kamiguchi et al. (2016) reported that obese animals fed with fatty diets have a higher risk of developing cancer.

Obese and oncological patients have a variety of systemic changes and, in oncological patients, such changes may result from the direct effect of tumor growth or paraneoplastic syndrome (Childress 2012). In obese patients, the increased pro-inflammatory cytokines may be associated with iron sequestration, with consequent reduction in the half-life of red blood cells and erythropoietin secretion, resulting in anemia (Mangieri 2009). Therefore, laboratory tests, such as blood count, are indispensable for detecting possible changes and assisting in the determination of prognosis and treatment, in order to provide better quality of life and survival (Garrido et al. 2015).

This study aimed to evaluate whether obesity triggered clinical and laboratory changes in the female dogs affected with mammary cancer treated at the "Hospital Veterinário" of the "Universidade Federal de Goiás" - Jataí Regional Campus (HV-UFG-REJ).

MATERIALS AND METHODS

Animals. Forty female dogs with or without mammary neoplasia treated at the "Hospital Veterinário" of the "Universidade Federal de Goiás" - Jataí Regional Campus (HV-UFG-REJ) were selected.

Four groups were created, primarily selecting ten female dogs for each group based on physical examination, according to the following characteristics:

Group 1 (G1): ten female dogs with ideal body condition score (BCS), without mammary neoplasia;

Group 2 (G2): ten overweight to obese female dogs, without mammary neoplasia;

Group 3 (G3): ten female dogs with ideal BCS, with palpable mammary neoplasia;

Group 4 (G4): ten overweight to obese female dogs, with palpable mammary neoplasia.

The criteria to include animals of Groups 1 and 2, in this study, were: spayed or non-spayed female dogs, over 1 year old, of the canine species, without palpable mammary nodules and without predilection for breed. For Groups 3 and 4, inclusion criteria were: spayed or non-spayed female dogs, over 1 year old, of the canine species, with palpable mammary nodules. The exclusion criteria, regardless of the group, considered the presence of concomitant disease and, specifically for Groups 3 and 4, the non-approval, by the guardian, of the mastectomy surgical protocol as treatment. The guardians were informed about the project and signed a consent term for participation in the experiment. The project was approved by the Ethics Committee on the Use of Animals (CEUA-UFG) nr 017/17.

Anamnesis and physical evaluation. In the anamnesis, the guardian was asked about nutritional life (type of food provided and daily number of meals when the animal was a puppy and when adult), reproductive life (number and frequency of estrous cycle, number of pregnancies, history of contraceptive use and castration) and tumors, when applicable (onset and evolution time, aspect and location, occurrence and location of previous tumors).

Physical evaluation consisted of physical examination (heart rate, respiratory rate, rectal temperature and evaluation of visible mucosal coloration, and inspection and palpation of lymph nodes and mammary glands) and determination of body condition score BCS and canine body mass index (CBMI). The Body Condition Score (BCS) was performed as proposed by Laflamme (1997), using the scale from one to nine points. The CBMI was determined as proposed by Muller et al. (2008), which considers as anatomical reference points the length between the base of the atlanto-occipital joint and the area immediately behind hind limbs, leaning the measuring tape on the back of the animal and on the base of the tail. Measurement was obtained and calculated by the formula: CBMI = body weight (Kg)/(height in meter)^2.

Laboratory evaluation. In the laboratory evaluation, performed after the puncture of 10mL of blood from the jugular vein, the material intended for blood count was placed in a tube with ethylenediaminetetraacetic acid (EDTA) anticoagulant and, for glucose dosage, a tube with fluoride was used. For cholesterol and triglyceride evaluation, a tube without anticoagulant was used. Previous 12-hour fasting was requested for glucose, cholesterol and triglyceride evaluation.

Cytopathology. The cytopathological evaluation was performed before surgical procedure, by a trained professional, with the puncture of the nodules and reactive lymph nodes. The material was then stained with fast stain derived from Romanowsky (Fast Panoptic) and analyzed in an optical microscope. The criteria for sample classification were as described by Grandi (2017), including,

for example, cellularity, mitotic index, nuclear and cytoplasmic morphology, nucleus: cytoplasm ratio, presence of evident nucleoli, macronucleoli, chromatin pattern, anisocytosis, anisocariasis, presence of dysplasia, anaplasia and ratio of these findings in the population of cells evaluated. The cytopathological evaluation was not performed only in the female dogs who had already been treated by another Veterinary Physician.

Staging. The female dogs evaluated with mammary neoplasia underwent staging according to the TNM system (tumor size, infiltration in regional lymph nodes and distant metastasis), proposed by the World Health Organization and created by Owen (1980). Stages one, two and three are related to tumor size, while stage four is related to lymph node involvement and stage five to distant metastases. Thoracic radiography and abdominal ultrasonography were requested to determine the distance metastasis study. When the presence of multiple tumors was observed, the tumor with the highest classification according to Owen (1980) was considered for staging.

Treatment. Patients with mammary neoplasia were submitted to surgical treatment. The choice of the surgical technique varied according to each case, considering tumor size, location, lymphatic drainage and cytopathological result. Moreover, regardless of the surgical technique, the removal with a two-to-three-centimeter safety margin of normal tissue was recommended. All nodules removed were sent for histopathological evaluation, including safety margin tissues and inguinal lymph nodes. The axillary lymph nodes were only removed if reactive.

Histopathology. For histopathological evaluation, fragments were fixed in 10% buffered formaldehyde solution, with formaldehyde volume in the 10:1 ratio as of the size of the surgical specimen. After fixation and cleavage, surgical specimens were subjected to dehydration in alcoholic solution and diaphanization in xylol, and subsequently included in histological paraffin. A mechanical rotating microtome (LEICA RM 2245) performed 4µm cuts which were arranged in matte-end slides and subsequently stained with hematoxylin and eosin. The slides were evaluated in light microscopy (LEICA DM 750) and classified according to Goldschmidt et al. (2011), Cassali et al. (2014) and Cassali et al. (2017).

Statistical analysis. Statistical analyses were performed using the Sigma Plot version 12 software (Systat Software Inc., USA). After evaluating data distribution by the Shapiro-Wilk test, parametric data were analyzed by T Test, shown as mean (± standard error of the mean) and considered significant when $P<0.05$. The non-parametric data were analyzed by the Rank Sum Test, as median (first percentile and third percentile) and considered significant when $P<0.05$.

RESULTS

Population studied

Most of the animals studied were mixed-breed, followed by the Dachshund breed, as shown in Table 1. When analyzing groups G1xG3 and G3xG4 as to breed, there was a statistical difference, since only two animals in G3 were mixed-breed. In addition, in the groups with mammary tumors, half of the patients were pure-breed.

When analyzing the age of the groups without mammary tumors, the mean observed was 4.9±2.9 for G1 (ideal BCS) and 6.8±1.3 for G2 (overweight to obese), while the mean of the groups with mammary tumors was 8.1±2.8 for G3 (ideal BCS) and 9.6±1.8 for G4 (overweight to obese).

Anamnesis and physical examination

When analyzing feeding frequency, as a puppy and then as an adult, there was a statistical difference between groups G2xG4, between the two stages of the patient´s life (Table 2). It was also observed that 87.5% (35/40) of the patients were fed with ration and 12.5% (5/40) were fed only with homemade food.

When analyzing the use of contraceptives in patients, it was observed that 25% (10/40) had already received application of the medication. Of those, three from G1, three from G3 and four from G4. When comparing them statistically, there was a difference between groups G2xG4 (Table 3), and G4

Table 1. Distribution according to breed of spayed or non-spayed female dogs, over 1 year old, with or without palpable mammary nodules

Breed	Total amount	%
Mixed breed	27	67.5%
Dachshund	5	12.5%
Shih Tzu	2	5
Blue Hiller	2	5
Pinscher	2	5
Siberian Husky	1	2.5%
Collie	1	2.5%
TOTAL	40	100%

Table 2. Feeding frequency when puppies and when adults*, of spayed or non-spayed female dogs, over 1 year old, with or without palpable mammary nodules, distributed in female dogs without mammary tumor and normal BCS (G1), female dogs without mammary tumor and overweight to obese (G2), female dogs with mammary tumor and normal BCS (G3) and female dogs with mammary tumor and overweight to obese (G4)

Groups	Puppy feeding frequency	Adult feeding frequency
G1	1.8 ± 0.6A	1.6 ± 0.5A
G3	1.3 ± 0.4A	1.3 ± 0.5A
G2	2.3 ± 0.6A,B	2.0 ± 0.7A.B
G4	1.3 ± 0.4A,B	1.6 ± 0.5A,B

Mean ± standard error of the mean; ^{A,B} different letters differ from each other ($P<0.05$) by T-Test; * according to information from guardians, obtained through the questionnaire.

Table 3. Use of contraceptives for spayed or non-spayed female dogs*, over 1 year old, with or without palpable mammary nodules, distributed in female dogs without mammary tumor and normal BCS (G1), female dogs without mammary tumor and overweight to obese (G2), female dogs with mammary tumor and normal BCS (G3) and female dogs with mammary tumor and overweight to obese (G4)

Groups	Use of contraceptives
G1	0 (0;1)
G3	0 (0;1)
G2	0 (0;0)A,B
G4	0 (0;1)A,B

Median (first percentile; third percentile); ^{A,B} different letters differ from each other ($P<0.05$) by the Rank Sum Test; * according to information from guardians, obtained through the questionnaire.

received a higher number of applications. As to the history of pseudocyesis, there was no statistical difference among groups, however 17.5% (7/40) of the female dogs showed the change at least once throughout life, being one from G2, two from G3 and four from G4.

All patients showed good general condition at physical examination, as heart rate, respiratory rate, rectal temperature and evaluation of visible mucosal coloration, and inspection and palpation of lymph nodes and mammary glands. In the BCS evaluation, comparing G1xG3 and G2xG4, no statistical differences were observed (Table 4), as expected. When CBMI was considered, there was a statistical difference between groups G2 and G4 (Table 4). When comparing BCS and CBMI evaluations, it was observed that 42.5% (17/40) of the female dogs had the same classification.

In the groups with mammary tumors, a total of 28 nodules were evaluated revealing that 65% (13/20) of the female dogs had only one palpable nodule, of which seven were from G3 and six from G4, while 35% (7/20) had two or more palpable mammary nodules, of which three were from G3 and four from G4.

When analyzing the location of mammary tumors, inguinal mammary glands constituted the most affected sites (42.86%), followed by caudal abdominal mammary glands (17.86%), cranial abdominal mammary glands (18.75%), caudal thoracic mammary glands (10.71%) and cranial thoracic mammary glands (12.5%). Among G3 patients, two had nodules in the cranial thoracic mammary gland, two in the caudal thoracic mammary gland, two in the cranial abdominal mammary gland, three patients in the caudal abdominal mammary gland and six in the inguinal mammary gland. Among G4 patients, one had nodules in the cranial thoracic mammary gland, two in the caudal thoracic mammary gland, three in the cranial abdominal mammary gland, two in the caudal abdominal mammary gland and six in the inguinal mammary gland.

Still in the macroscopic analysis of mammary tumors, it was observed that 40% (8/20) had tumor diameter smaller than three centimeters and, of those, three patients were from G3 and five from G4, 35% (7/20) between three and five centimeters, being four patients from G3 and three from G4 and 25% (5/20) larger than five centimeters, with three patients from G3 and two from G4. Two patients had ulcerated tumors (both from G3), three had inflamed tumors (two patients from G3 and one from G4), one had mammary secretion (G3) and there were two in which the development of the tumor already involved fascia and muscle (one patient from each group).

Laboratory evaluation

The evaluation of hematological parameters (blood count/leukogram) showed no statistical difference among groups (Table 5). No patient had anemia. However, 25% (10/40) had thrombocytosis, being two from G1, four from G2, one from G3 and three from G4. Other 7.5% (3/40) had lymphopenia, being one patient from G2, one from G3 and one from G4, sequentially. Another 10% (4/40) had leukopenia, corresponding to one patient from G1, two from G2 and one from G3. And 10% (4/40) had leukocytosis, being one patient from each group.

Regarding biochemical assessments, triglycerides and total cholesterol did not show statistical difference. However, glucose differed when comparing groups G1xG3 and G2xG4 (Table 6). In the individual evaluation of each group, 25% (10/40) showed increased triglycerides, being three patients from G1, two from G2, two from G3 and three from G4. Other 10% (4/40) showed increased total cholesterol, corresponding

Table 4. Determination of body condition score (bcs) and canine body mass index of spayed or non-spayed female dogs, over 1 year old, with or without palpable mammary nodules, distributed in female dogs without mammary tumor and normal BCS (G1), female dogs without mammary tumor and overweight to obese (G2), female dogs with mammary tumor and normal BCS (G3) and female dogs with mammary tumor and overweight to obese (G4)

Groups	BCS	CBMI
G1	5.0 ± 0.0^{A}	15.0 ± 3.3^{A}
G3	5.0 ± 0.0^{A}	14.3 ± 1.9^{A}
G2	7.6 ± 1.0^{A}	18.1 ± 3.0^{A,B}
G4	7.3 ± 0.7^{A}	14.2 ± 4.8^{A,B}

Mean ± standard error of the mean; ^{A,B} different letters differ from each other (P<0.05) by T-Test.

Table 5. Hematological Parameters (hematocrit, platelet, leukocyte, neutrophil and lymphocyte) of spayed or non-spayed female dogs, over 1 year old, with or without palpable mammary nodules, distributed in female dogs without mammary tumor and normal BCS (G1), female dogs without mammary tumor and overweight to obese (G2), female dogs with mammary tumor and normal BCS (G3) and female dogs with mammary tumor and overweight to obese (G4)

Groups	Hematocrit (%)	Platelet (/µL)	Leukocyte (/µL)	Neutrophil (%)	Lymphocyte (%)
G1	44.8±6.2^{A}	402.0±150.0^{A}	11.6±4.3^{A}	6.5±0.9^{A}	3.4±0.8^{A}
G3	47.5±5.0^{A}	433.6±99.0^{A}	12.5±5.2^{A}	10.6±2.1^{A}	2.6±0.5^{A}
G2	47.7±7.6^{A}	551.2±236.3^{A}	13.4± .6^{A}	10.1±1.2^{A}	3.3±0.7^{A}
G4	48.1±6.4^{A}	441.2±166.9^{A}	13.9±10.4^{A}	6.7±1.5^{A}	2.6±0.7^{A}

Mean ± standard error of the mean; ^{A,B} different letters differ from each other (P<0.05) by T-Test.

Table 6. Biochemical Parameters (triglycerides, total cholesterol, glucose) of spayed or non-spayed female dogs, over 1 year old, with or without palpable mammary nodules, distributed in female dogs without mammary tumor and normal BCS (G1), female dogs without mammary tumor and overweight to obese (G2), female dogs with mammary tumor and normal BCS (G3) and female dogs with mammary tumor and overweight to obese (G4)

Groups	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	Glucose (mg/dl)
G1	95.9 ± 88.5^{A}	172.4 ± 42.7^{A}	88.7 ± 7.0^{A,B}
G3	58.4 ± 34.5^{A}	194.7 ± 104.1^{A}	108.0 ± 26.4^{A,B}
G2	67.4 ± 56.9^{A}	210.4 ± 100.8^{A}	86.4 ± 31.5^{A,B}
G4	101.7 ± 74.7^{A}	183.4 ± 47.4^{A}	110.0 ± 12.8^{A,B}

Mean ± standard error of the mean; ^{A,B} different letters differ from each other (P<0.05) by T-Test.

to one patient from G2, two from G3 and one from G4. Another 7.5% (3/40) had increased glucose, being one patient from G2, one from G3 and one from G4, respectively.

Cytopathology

The cytopathological examination was performed in 13 patients, however two examinations had inadequate or insufficient cellularity. Of the patients who had the cytopathological examination performed, malignancy criteria attributed compatible with the histopathological examination were attributed to nine and the benign criteria to one, both in cytology and histology, determining the cytohistopathologic correlation of 90.9% (10/11). Only one patient had confronting cytological and histological results.

Staging

Of the 20 patients with mammary tumors, eight were classified as staging 1, six as staging 2, four as staging 3 and two as staging 4. No patient had distant metastasis, that is, there was no staging 5 classification. The two patients classified as staging 4 had metastasis in a regional inguinal lymph node, being one with squamous cell carcinoma, from G4 (Fig.1A and B) and the other with papillary carcinoma, from G3. No difference was observed between the groups when staging was considered.

Treatment

All patients with mammary neoplasia received surgical treatment. Of the 20 patients, 19 underwent the unilateral mastectomy technique, while the regional mastectomy technique was used in the patient with the benign cytological type. Twenty inguinal lymph nodes corresponding to the surgical excision side were evaluated. No axillary lymph node was evaluated, since they were not reactive at the time of surgery.

Histopathology

All mammary tumors found in the female dogs were histologically analyzed. Of the malignant mammary tumors, cases of carcinoma in mixed tumor, papillary carcinoma, tubular carcinoma, complex carcinoma, "in situ" carcinoma, intraductal papilloma and carcinoma, sclerosing carcinoma, carcinoma, malignant myoepithelioma and carcinosarcoma were observed (Fig.2A and B). As to malignancy, eight patients from G3 and nine patients from G4 had malignant histological types. The benign histological types were adenomyoepithelioma (Fig.3A and B), sclerosing adenoma, lactation adenoma and benign mixed tumor. Only one case showed another histological type not coming from the mammary gland, which was the squamous cell carcinoma, because a connection with cutaneous epidermis was found. One patient from G4 with a benign mixed tumor had bone and cartilaginous metaplasia and another patient from G3 with carcinoma in a mixed tumor had metaplasia in cartilage (Fig.4A and B). In total, seven patients had more than one histological type of mammary neoplasia, three from G3 and four from G4. Of the seven patients, three had more than one histological type in the same mammary gland evaluated, one from G3 and two from G4.

Histological types are shown in Table 7. No difference was observed between the groups when tumor malignancy was considered.

DISCUSSION

The fact that the patients were in good general condition at physical examination corroborates Petrov et al. (2014), in which most of the female dogs are clinically healthy when physically evaluated. The age mean in this study is within the range considered as of higher incidence to the onset of mammary neoplasia according to Munkhtuul et al. (2014) and Ariyarathna et al. (2018).

Kim et al. (2016), when analyzing the characteristics of mammary tumors according to age groups, found that the histological types of worse prognosis tended to develop in dogs with advanced age. In this study, only three elderly animals had tumors with benign histological features, revealing that most of female dogs in this age group had tumors with malignant features, corroborating the authors.

It was observed that most of the patients evaluated were mixed-breed, followed by the Dachshund, Shih Tzu, Blue Hiller,

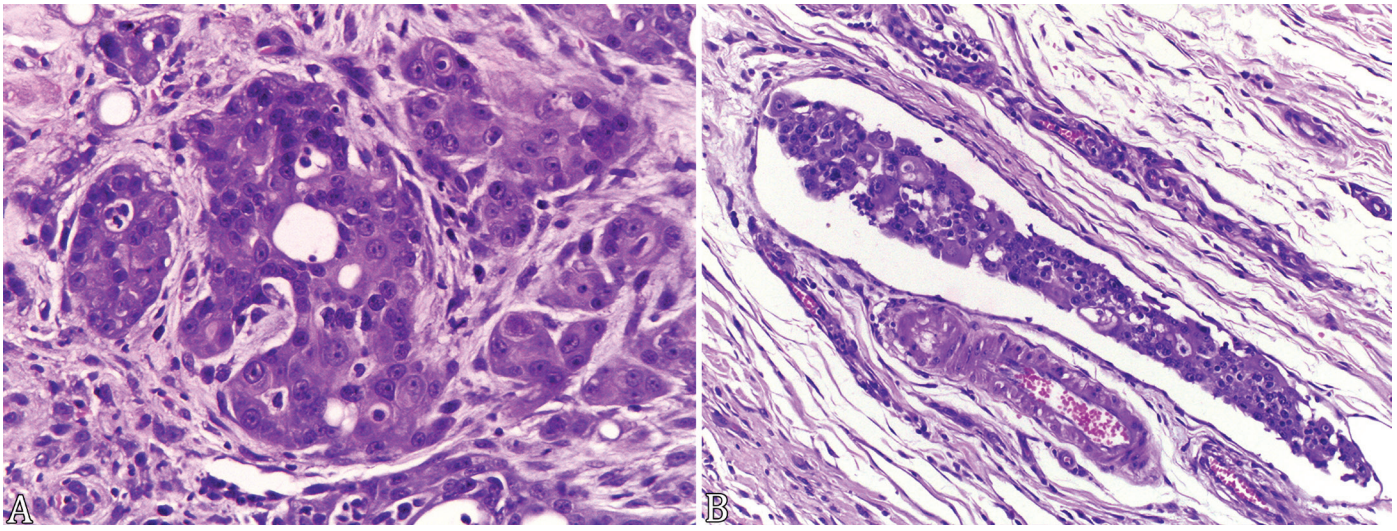

Fig.1. Canine, mixed-breed, 10 years old, Squamous cell carcinoma with inguinal lymph node metastasis. (**A**) Tissue invasion area of the neoplastic squamous epithelium (black arrow). HE, obj.40x. (**B**) Invasion of lymphatic vessels by neoplastic cells (black arrow). HE, obj.20x.

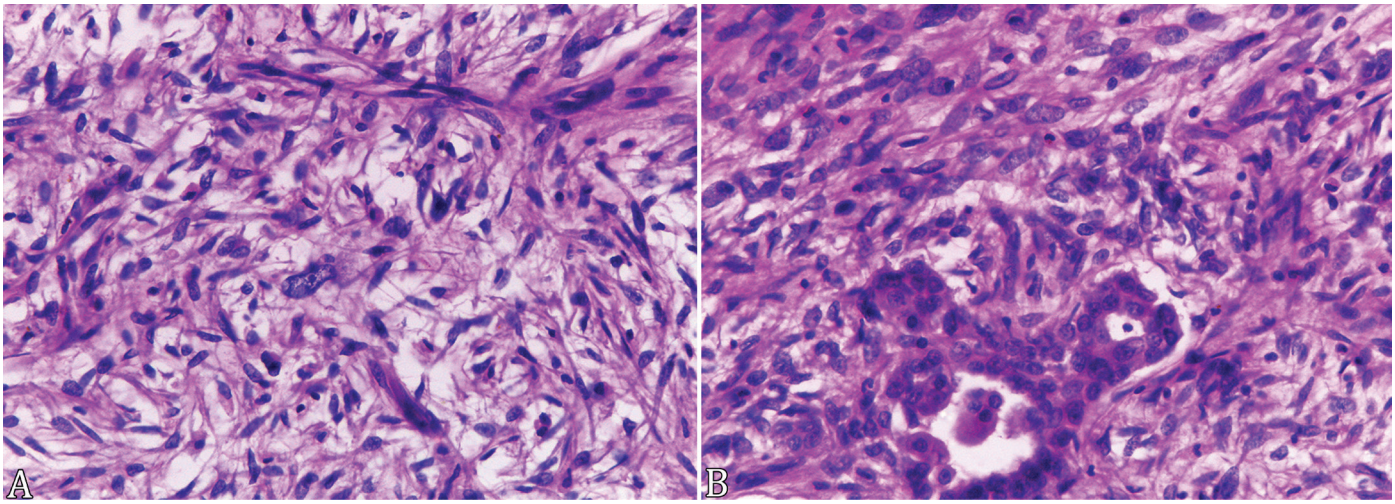

Fig.2. Canine, Dachshund, 6 years old, Carcinosarcoma. (**A**) Malignant mesenchymal proliferation, with high cellularity, showing a macronucleus with irregular borders and evident nucleolus in the black circle. (**B**) Epithelial component (black arrow) amid the highly cellular sarcomatous area. HE, obj.40x.

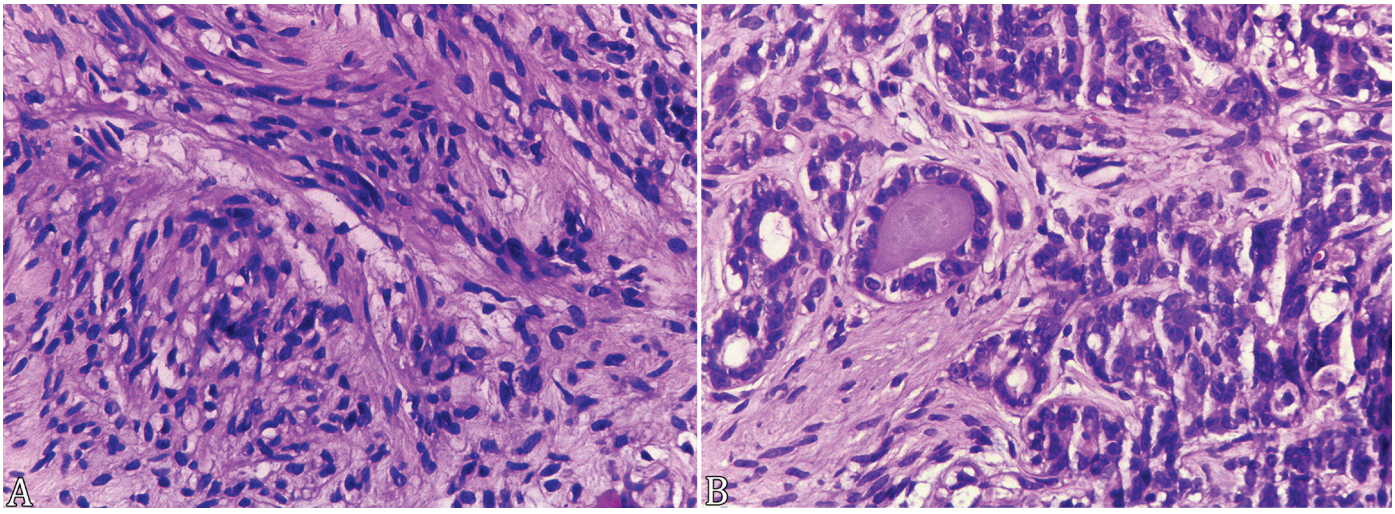

Fig.3. Canine, mixed-breed, 11 years old, Adenomyoepithelioma. (**A**) Mesenchymal cell proliferation with few morphological changes. (**B**) Area of proliferation of well-differentiated epithelial cells. HE, obj.40x.

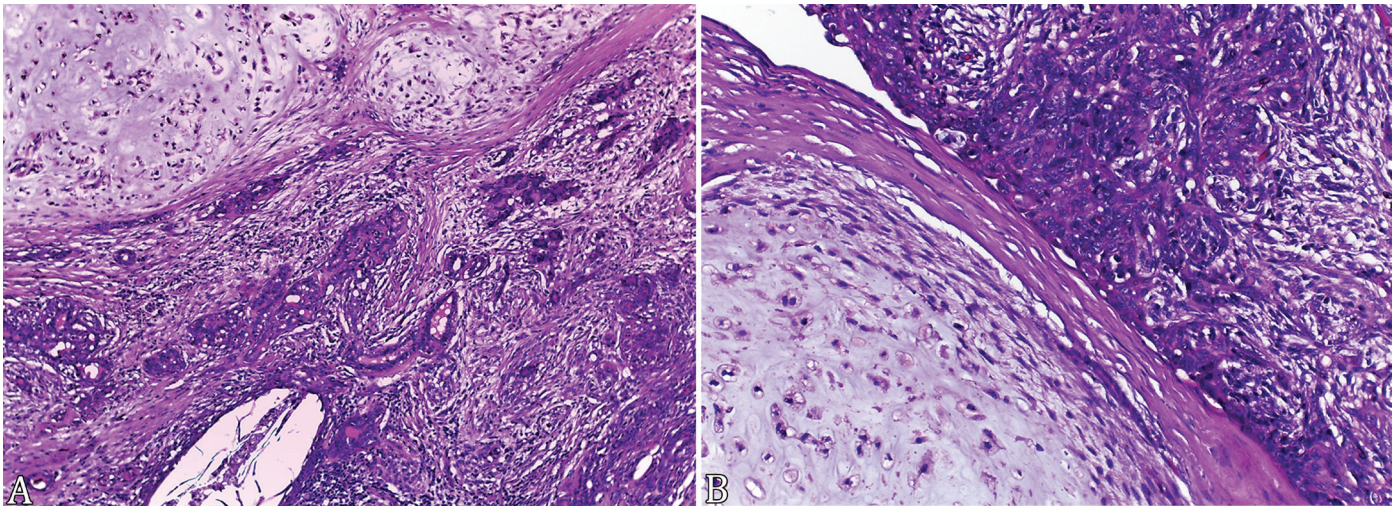

Fig.4. Canine, Dachshund, 5 years old, Carcinoma in mixed tumor with cartilaginous metaplasia. (**A**) Presence of neoplastic epithelial (black arrow) and mesenchymal (red arrow) components. HE, obj.10x obj. (**B**) Detail showing the transition between the area of metaplasia (black arrow) and the carcinoma (red arrow). HE, obj.20x.

Pinscher, Siberian Husky and Collie breeds. Ariyarathna et al. (2018) evaluated 74 female dogs and, of those, 39 were mixed-breed, followed by German Shepherd and Dachshund as the most prevalent breeds. Marconato et al. (2019) studied 32 female dogs and, of those, 21 were pure-breed (Bichon Frisé, Poodle and Golden Retriever) and 11 were mixed-breed. This breed pattern variation is possibly due to regional and country variations. For Sorenmo et at. (2011), mammary tumors in female dogs can occur in any breed.

Among overweight or obese female dogs, the group with mammary neoplasia (G4) was fed less frequently throughout the day when compared to the group without mammary neoplasia (G2). However, no relationship between feeding frequency and tumor onset was found in the literature. It is known that weight management and nutrition should receive attention from Veterinary Physicians and be included in the routine of preventive examinations and care (Bartges et al. 2017). In addition, the etiology of obesity involves genetic, biological, environmental and behavioral interactions, which also predisposes animals to organic dysfunctions (Chandler et al. 2017).

Regarding physical evaluation, Gama et al. (2016) noted that out of 30 dogs evaluated by BCS, 23.3% of the animals were classified as obese, 43.3% as overweight, 23.3% as ideal and 10% as lean. In this study, no lean female dog was observed according to BCS.

When comparing BCS and CBMI evaluations, 42.5% (17/40) of female dogs had the same classification, however, it was observed that three female dogs from G4 received different classifications, and one female dog was classified as lean in CBMI and as obese in BCS and two other female dogs pointed out as ideal in CBMI were also classified as obese in BCS. This is possibly because such female dogs were small, being one mixed-breed and other two of the Pinscher breed. Moreover, CBMI does not consider body proportionality and body fat distribution (Muller et al. 2008, Gama et al. 2016).

Table 7. Results of histological types of mammary nodules of spayed or non-spayed female dogs, over 1 year old, distributed according to histological classification

Histological type (benign)	Amount (total 28 nodules)	%
Adenomyoepithelioma	4	14.29%
* Sclerosing adenoma and Lactation adenoma	1	3.57%
Benign mixed tumor	1	3.57%
Histological type (malignant)	**Amount (Total 28 nodules)**	**%**
Carcinoma in mixed tumor	6	21.43%
Papilliferous carcinoma	3	10.72%
Tubular carcinoma	3	10.72%
Complex carcinoma	2	7.14%
In situ carcinoma	2	7.14%
*Papilloma and intraductal carcinoma	2	7.14%
Sclerosing carcinoma	1	3.57%
*Carcinoma and malignant myoepithelioma	1	3.57%
Carcinosarcoma	1	3.57%
Histological type (other)	**Amount (total 28 nodules)**	**%**
Squamous cell carcinoma	1	3.57%

* Patients with different histological types in the same mammary gland.

According to Muller et al. (2008), of the 246 dogs evaluated, most of them had ideal body condition (53.4%) and 39.5% were overweight to obese. Gama et al. (2016), when evaluating the body condition of dogs using BCS and CBMI, observed that 53.3% of the animals evaluated had the same classification. In this study, BCS and CBMI were not related to the histological type of mammary neoplasia, because both in the group with ideal score and in the overweight to obese group, the malignant histological types were more prevalent.

The hematological parameters did not differ statistically between the groups, however, some of the patients had laboratory changes. In the groups with mammary tumors, two patients had lymphopenia and leukocytosis, one with ideal score and another obese. Four patients had thrombocytosis. Garrido et al. (2015) also did not find significant changes in blood cell counts beyond the reference interval in female dogs, especially those with mammary tumors.

Silva et al. (2014) evaluated 161 female dogs with mammary tumors and found that 24.2% (39/161) of the patients had changes in blood count. They also observed a positive correlation between anemia and the progress of the disease as well as a positive correlation between leukocytosis and the progression of the oncological disease.

For Childress (2012), hematological changes are frequent in oncological patients, which may be a direct result of the tumor or secondary to the paraneoplastic syndrome. However, in this experiment, there were no female dogs with distant metastasis, patients in which most of the hematological changes would probably be observed.

There was no statistical difference in the laboratory evaluation of cholesterol and triglycerides. However, it would be opportune to develop other studies with larger sample sizes, in order to verify if there is any trend of parameter change involving obesity and the presence of mammary neoplasia. Hyperglycemia triggers the activation of TNF-α, a proinflammatory cytokine also present in obese patients, consequently increasing the survival of neoplastic cells in hypoxic conditions (Bail et al. 2016). In the biochemical parameters, there was a statistical difference among groups regarding glucose, and higher values were analyzed in the groups with mammary tumors, especially in G4, in which the patients were also obese.

Pro-inflammatory cytokines increase with inflammation, a process triggered by the tumor itself, favoring the proliferation and survival of tumor cells (Szczubial et al. 2018). In patients with increased glycemic level, no peritumoral inflammatory process was observed macroscopically, however, cytokines stimulate endothelial migration and proliferation since the onset of the tumor in order that it develops and, as this tumor grows, the vascular supply also develops (Carvalho et al. 2016).

The use of contraceptives is associated with cases of benign hyperplasia and neoplasia. Such changes may predispose the mammary tissue to malignant mutations and trigger the onset of malignant neoplasia. The regular use of these drugs increases in three times the risk of carcinoma onset (Ferreira & Amorim 2003, Oliveira Filho et al. 2010, Petrov et al. 2014).

In this study, it was observed that the groups with mammary neoplasia received more contraceptive applications (17.5%), corroborating the literature.

Feliciano et al. (2012) evaluated 60 female dogs, of which 13.37% had received contraceptives. Kamiguchi et al. (2016), when conducting the study with 20 female dogs, noted that 10% of the patients had a history of hormone administration for contraceptive purposes, which could indicate a low correlation with the development of mammary tumors. Ariyarathna et al. (2018) studied 74 female dogs and none of them had used contraceptives to prevent pregnancy. In the evaluation of 134 female dogs performed by Canadas et al. (2018), only 3% (4/134) received contraceptive medication.

In the oncological evaluation of the patients, the mean time of tumor progression until the appointment was 210 days (Ribas et al. 2012). In this study, 55% of the patients had already developed mammary tumors for more than 180 days when brought to the appointment. The guardians of nine animals from the study by Ariyarathna et al. (2018) admitted that, although they had observed mammary nodules in the female dogs before the appointment, they did not seek specific care because they thought the tumors were harmless. The delay in seeking for specialized veterinary assistance is related to the development of malignancy and worsening in the prognosis (Kamiguchi et al. 2016).

Coleto et al. (2018) evaluated 59 female dogs and 56% of them had a single nodule, 27.1% had two nodules, 11.9% had three nodules, 3.4% had four nodules and 11.9% had seven nodules. As for location, the inguinal mammary gland was also the most affected, corresponding to 37.1% of the cases. Medeiros (2017) states that the last pairs of mammary glands are more affected than the first pairs, however, the location of the tumors does not seem to affect the prognosis, results similar to those observed in this study.

It is known that tumor size is variable when it comes to mammary neoplasia. Dias et al. (2016) noticed that almost half of the animals evaluated had tumors smaller than three centimeters (48.95%), 22.38% had tumors between three and five centimeters and 28.67% had tumors larger than five centimeters. This study corroborates that, since 40% (8/20) of the patients had tumor size smaller than three centimeters. Coleto et al. (2018) showed that the size of the tumor influenced the occurrence of metastasis, strengthening the importance of diagnosis and early treatment of mammary tumors.

It is also common for mammary neoplasia to be associated with skin ulcers and local inflammatory reaction, with secretion in mammary chains and involvement of fascia and musculature (Cassali et al. 2014). Peritumoral skin ulcers were observed in 27% of the dogs studied by Ariyarathna et al. (2018) and 20.3% had adherences in the underlying tissues. In this study, these occurrences were lower.

The cytopathological examination is a safe and inexpensive method with satisfactory sensitivity and specificity for malignancy differentiation in mammary tumors, in addition to being considered an ideal method for identification of metastasis in lymph nodes (Cassali et al. 2014, Munkhtuul et al. 2014). In this study, 84.61% of the samples analyzed had adequate cellularity, which corroborated the detection of malignant and/or benign features in cytology, and 90.9% had the diagnosis of tumor behavior compatible with histopathology. Dolka et al. (2018) found correspondence between the two methods in 86.3%, which differed little from the data found in this study.

The final diagnosis of mammary tumors is also achieved by histopathological examination, considered gold standard also to classify histological types (Sorenmo et al. 2011). Malignant neoplasias were more prevalent in this study, as well as in the study by Horta et al. (2014) when evaluating 391 lesions, of which 56% were malignant neoplasias and 31% were benign neoplasias. Dolka et al. (2018) analyzed 18 tumors and, of those, 75.3% were malignant and 24.7% benign. O diagnóstico de tumores malignos e benignos, neste estudo, foi levado em consideração devido ao baixo número amostral, porém considera-se relevante o desenvolvimento de outras pesquisas para avaliar as características de cada tipo histológico.

Horta et al. (2014) pointed out in the study that benign types corresponded to 23.1% as papilloma and 17.4% as adenoma. Dias et al. (2016) considered the benign mixed tumor (16.78%), followed by adenoma (5.59%) and fibroadenoma (0.7%). This study differs from the previous two, in which 14.29% were adenomyoepithelioma, 3.57% benign mixed tumor and 3.57% sclerosing adenoma along with lactating adenoma. Benign mixed tumors are usually characterized by the presence of benign epithelial, myoepithelial and mesenchymal elements, with cartilage and/or bone formation (Cassali et al. 2017). This finding was observed in the female dog with this histological type, which had the two types of bone and cartilaginous metaplasia.

As of malignant tumors, Kamiguchi et al. (2016) found the papilliferous carcinoma histological type in 90% of the samples. Gundim et al. (2016) found tubular carcinoma, followed by carcinoma in a mixed tumor. Canadas et al. (2018) found that, of 144 tumors, the most frequent type was complex carcinoma (49/144). For Nunes et al. (2019), 75% (682/904) of the cases were carcinoma in a mixed tumor, which corroborates this study. In addition, other histological types were found, however in lesser number when compared to carcinoma in a mixed tumor.

Carcinoma in mixed tumor is the most common neoplasia of the mammary gland in female dogs with complex histological pattern, as it has components of epithelial, myoepithelial and mesenchymal origin (Cassali et al. 2014, Nunes et al. 2019). Microscopically, carcinomas in mixed tumor develop through a malignant epithelial component and a benign mesenchymal component, which may be of cartilaginous, bony or adipose tissue (Goldschmidt et al. 2011, Cassali et al. 2017). Greater cellularity and considerable pleomorphism of the epithelial part, with high mitotic index, necrotic points and infiltrative pattern should be observed to differentiate this type from mixed benign tumors (Goldschmidt et al. 2011). In this study, one of the patients with carcinoma in mixed tumor developed metaplasia in cartilage.

The two patients had inguinal lymph node metastasis confirmed by histopathological examination, one from G4 with squamous cell carcinoma and the other from G3 with papillary carcinoma. This finding influences clinical staging, conferring worse prognosis of patients, since patients with metastases in regional lymph nodes have decreased survival (Santos et al. 2015, Medeiros, 2017).

Moreover, in the patient in which squamous cell carcinoma was found, a connection with the cutaneous epidermis was

confirmed. There is a special type of squamous cell carcinoma that affects the mammary glands, which is histologically similar to the one that occurs in the skin, however there is no connection with the cutaneous epidermis. The neoplasia that affects the mammary gland is uncommon in the canine species, being usually infiltrative and disseminated by lymphatic route (Goldschmidt et al. 2011, Cassali et al. 2014). In this case, we noticed the importance of histopathological diagnosis, because the neoplasia, by macroscopic evaluation, consisted of a mass with involvement of the left inguinal mammary gland, with firm consistency, irregular surface and mobility.

In this study, one patient from G3 had the carcinosarcoma histological type. It is a mixed neoplasia with epithelial and mesenchymal malignant components, uncommon for mammary gland, with poor prognosis, since the metastasis of the epithelial component occurs by lymphatic route to regional lymph nodes and the lung, and the metastasis of the mesenchymal component occurs by hematogenous route to the lung (Goldschmidt et al. 2011, Cassali et al. 2017). The patient had already had the tumor for three years and the guardian reported slow growth. No presence of neoplastic cells was observed in the histological evaluation of the inguinal lymph node.

CONCLUSIONS

Obesity did not trigger clinical and laboratory changes in female dogs with mammary cancer.

However, it was observed that the female dogs in the overweight to obese groups had a higher number of changes in hematological and biochemical parameters, especially the female dogs of the group that also had mammary tumors, observing thrombocytosis, lymphopenia, leukocytosis, leukopenia and hyperglycemia, even at 12-hour fasting.

BCS and CBMI were not related to the malignancy of the mammary tumor, since, both in the group of overweight and obese female dogs and in the group of lean to ideal female dogs, the most observed histological types were those with malignant features.

Conflict of interest statement.- The authors have no competing interests.

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