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Concordance between clinical presentation and histopathological staging canine mammary tumors

Concordancia entre la presentación clínica y la estadificación histopatológica en tumores mamarios caninos

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ABSTRACT

In this work it was examined the concordance between clinical staging and histopathological staging of mammary tumors in 32 female dogs. It was observed that the average age of presentation of the pathology was 9 years (ranged from 6 to 12 years). The most affected mammary glands were the caudal abdominal and the inguinal, 20 out of 32 female dogs had multiple tumors (62%), and 38% single tumors. Regarding the breeds, the most frequent ones were mixed breeds, Poodle, Cimarron (native breed of Uruguay) and Labrador Retriever. Of the 32 female dogs with breast tumors studied, 65% had histopathological diagnosis of malignant tumor, while 35% had benign tumors. Clinical staging data showed that 64% of the cases with benign tumors were in stage I (1 to 3 cm) and 36% were in stage II (3 to 5 cm). Among those diagnosed with malignant tumors, 10% were in stage V, 57% in stage III, 9% in stage II, and 24% in stage I. There were no animals in stage IV. The most frequently found malignant tumors were tubular carcinoma and complex carcinoma, followed by solid and tubulopapillary carcinomas. Within the benign tumors, complex adenoma was the most frequent, followed by benign mixed tumor and simple ductal papilloma. The concordance between clinical staging and histopathology was low, as we could observe both benign T2 (3 to 5 cm) and malignant T1 (1 to 3 cm) tumors.

Key words: Canine mammary tumors; mammary tumor histopathology; clinical staging in mammary tumors

RESUMEN

En el presente trabajo se evaluó la concordancia entre la estadificación clínica y la clasificación histopatológica de tumores de mamas en 32 perras. Se observó que la edad promedio de presentación de la patología fue de 9 años. Las mamas más afectadas fueron los abdominales caudales, e inguinales, 20 de las 32 perras presentaban tumores múltiples (62 %) y un 38 % tumores únicos. Con respecto a las razas, las más representativas fueron mestizos, Caniche, Cimarrón (raza autóctona de Uruguay) y Labrador. De las 32 perras estudiadas con tumores de mama, el 65 % (21) tuvieron diagnóstico histopatológico de tumor maligno, mientras que el 35 % (11) fueron catalogados benignos. Con los datos de la estadificación clínica se pudo observar que el 64 % de las perras que presentaron tumores benignos se encontraban en estadio I (1 a 3 cm) y 36 % se encontraban en estadio II (3 a 5 cm). Dentro de las perras que desarrollaron un diagnóstico de tumores malignos, el 57 % se encontraban en estadio III, el 9 % en estadio II y 24 % en estadio I. En el presente estudio no se obtuvieron animales que se encontraran en estadio IV y un 10 % estaban en estadio V de la enfermedad. Los tumores malignos encontrados con más frecuencia fueron el carcinoma tubular y el carcinoma complejo, seguido por el carcinoma sólido y el túbulo papilar. Dentro de los tumores benignos se destacó con mayor frecuencia el adenoma complejo seguido por el tumor benigno mixto y el papiloma ductal simple. Respecto de la concordancia entre la estadificación clínica y la histopatología, fue bajo dado que se observaron tumores benignos T2 (de 3 a 5 cm) y tumores malignos T1 (1 a 3 cm).

Palabras clave: Tumores mamarios caninos; histopatología de tumores de mama; estadio clínico en tumores mamarios

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INTRODUCTION

Breast tumors are the most frequent neoplasms in intact female dogs (*Canis lupus familiaris)* accounting for about half of oncologic cases [1, 2, 3]. They correlate with life expectancy and their incidence is significantly reduced by ovariohysterectomy (spaying) in young female dogs, as their development is clearly hormone-dependent. [4]. Compared to intact female dogs, the risk for malignant tumors in dogs spayed before the first estrus is 0.5%, it increases to 8% if spaying is performed after the first estrus, and to 26% if spaying is performed after the second estrus [4, 5, 6, 7]. When performed later, spaying does not reduce the risk of malignant tumors, although it does appear to be a reduced risk for benign tumors [8, 9, 10]. The average age of affected female dogs is 10 to 11 years (range: 2 to 16) [11]. Younger female dogs usually have benign tumors, while malignant tumors tend to occur at more advanced ages. Approximately half of the canine mammary tumors are malignant, and half of them have metastasized by the time of the initial diagnosis [10, 11].

The stablished staging system was proposed by Owen *et al.* [12] for the World Health Organization (WHO). It is based on the Classification of Malignant Tumors (TNM) system, by which animals are categorized into five stages according to tumor size and attachment to adjacent tissue (T), spread to nearby lymph nodes (N), and distant metastasis (M). Therefore, staging should include minimum database, consist al.ting of, chest X-ray (three images: dorsoventral, right laterolateral and left laterolateral), abdominal ultrasound, full blood count, serum biochemistry panel, urinalysis, and assessment of regional lymph nodes by palpation. If enlarged, axillary and inguinal lymph nodes should be palpated and aspirated for cytological analysis [11]

Clinical staging allows to define the extent of the disease and, consequently, establish a prognosis and treatment plan [13]. According to Vail *et al.* [14], the transition from T1 to T2 and from T2 to T3 worsens the prognosis of the disease and implies changes in the treatment. Regardless of tumor size, metastases in regional lymph nodes or distant metastases cause any clinical stage to raise to stage IV or V, respectively.

The WHO classification with respect to the histopathological study follows the differentiation process, starting with malignant tumors that most closely resemble the normal structure of the mammary gland and ending with poorly differentiated tumors with no glandular structure. Previous classifications did not include inflammatory carcinomas, because they were not considered to be a specific histological subtype, but just any breast carcinoma with marked inflammation [15]. In the new classification of Goldsmith *et al.* [16], inflammatory carcinoma became a specific entity. This histological classification of breast tumors in female dogs was validated in 2017 by Rasotto *et al.* [17], where a population of 229 female dogs was followed up for two years to determine survival times of each subtype, rate of local recurrence, and distant metastases [16, 17].

Among the reasons for performing the histopathological classification of breast tumors is the objective of evaluating the architecture of the neoplasm and the morphological variations of the nucleus, the histological grade, which represents a significant correlation with the aggressiveness of the tumor [18].

In Human Medicine, currently the most widely used classification system is the Nottingham classification system modified by Elston and Ellis [18], which has replaced previous subjective evaluations when the degree of tumor differentiation was estimated by the general appearance of the tumor. According to this system, determination of the histological grade is based on: the evaluation of the index of tubules formation (one point: more than 75% of the tumor is composed of tubules, two points: between 10 and 75% of tubular formations, and three points: the tubules occupy 10% or less of the tumor); nuclear pleomorphism (one point: small and regular nuclei; two points: moderate increase in size and variation of nuclei; three points: marked pleomorphism, with great variation in size and shape of nuclei); and mitotic count (one point: 0–8 mitoses, two points: 9–16 mitoses, and three points: more than 17 mitoses in 40X lens). The histological grade of the tumor is obtained through the sum of the scores, which results in a total number ranging between 3 and 9. The summary of the tumor grades is: 3–5 points: grade I; 6–7 points: grade II; 8–9 points: grade III. Anaplasia increases with increasing grade. Histological grade is considered as an independent prognostic indicator for primary breast tumor in women. The malignancy degree is based not only on the tumor type but also on the presence of significant cellular pleomorphism, mitotic index, the presence of necrotic areas, peritumoral and lymphatic invasion, and regional lymph node metastases [16, 18, 19].

Cases of undifferentiated carcinoma (grade III) had a 21-fold increased risk of death compared to cases of differentiated carcinoma (grade I and II). An increased risk (approximately 10-fold) was also associated with simple undifferentiated carcinomas compared with differentiated ones. The predictive value of histological grade was not influenced by the size of the tumor or the age of the dog at the time of mastectomy [20].

Regarding canines, recently, the number of veterinary investigators who have adopted the histological staging criteria proposed by Nottingham to evaluate mammary carcinomas in dogs has increased. According to the available literature, the use of clinical staging criteria TNM and evaluation of classical morphologic prognostic factors (tumor size, mitotic count, histologic grade and type, and lymphatic involvement), well established in humans, are useful in evaluating the prognosis of female dogs with mammary carcinomas. Therefore, diagnostic criteria need to be improved and standardized, and continued investment in the study of prognostic and predictive markers is needed for these factors to be routinely employed by veterinary pathologists and provided to clinicians and surgeons. The goal is not to subject patients to unnecessary aggressive treatment or to stop treating those who would benefit. The quality of life of the animal should always be prioritized [19].

The aim of this study was to determine the concordance between clinical findings and histopathological classification in breast tumors in dogs. There are still no studies that have evaluated the effectiveness of clinical staging as a predictor of histopathological classification.

MATERIALS AND METHODS

This work was carried out at the Hospital of the Faculty of Veterinary Medicine of the University of the Republic, Uruguay. It was approved by the Ethics and Animal Use Committee under No. 518.

The Hospital of the Veterinary School has the necessary facilities for clinical care and obtaining samples. Blood studies were made at the Clinical Analysis Laboratory (equipment CB30i Wiener-lab Group, Argentina). Blood samples were obtained (3 mL) from the cephalic vein with a 21 G butterfly by the veterinary nurse

This samples were divided into two tubes: 1 mL in Ethylenediaminetetraacetic acid (EDTA) tube for complete blood

count and the 2 mL remaining in a dry tube to determine creatinine urea and liver function.

Thoracic radiographs were obtained in a Vetter Rems 100 device (Argentina) and digitalized in a Kodak DirectView, Sistem Classic CR Caresream (Japan) positioning the patient in lateral decubitus and sternal decubitus, in order to get latero–lateral and dorso–ventral views. The images were evaluated by the veterinary radiologists of the Imaging Unit of Veterinary School.

Histopathology was performed at the Eastern Regional Laboratory (DILAVE MGAP), with an Olympus BX51 microscope (Japan) using hematoxylin and eosin (H–E) staining at 40X and 10X magnification.

The study included 32 female dogs admitted to the hospital over a one year period. The case selection criteria were intact (not spayed) female dogs with palpation–detectable mammary tumors. No distinction of breed was made. It was also took into account that the staging of the disease and pre–surgical studies would allow mastectomy (ASA I or II).

Case history was carried out through a survey that included data such as age, breed, and reproductive status, as well as tumor characteristics such as location, size, and whether it was single or multiple. In each patient, a thorough physical examination was carried out, including individual inspection and palpation of each breast of both chains, and of the regional lymph nodes (axillary and inguinal). Staging of the patients was performed using the WHO staging system. Pre–surgical evaluation included chest x–rays (left–lateral, right–lateral, and ventro–dorsal), blood count, blood biochemistry (renal and hepatic functional profile), and urinalysis. In all cases, surgical removal was the treatment of choice. Surgical techniques used were: regional mastectomy, unilateral or bilateral radical mastectomy. The choice of the appropriate technique depended on the size of the tumor, the number of affected mammary glands, the location, fixation to the surrounding tissues and health status of the patient. Surgeries were performed by the veterinary surgeon team from the Veterinary School. (FIG. 1)

Following this procedure, tumors were fixed in 10% formalin on paraffin–embedded 1.5 × 1.5 centimeters (cm) sections and stained with H–E.

For histological examination, tumor samples were in fixed in 4% neutral buffered formalin, embedded in paraffin, sectioned at 5–7 µm (microtome RM2125 RTS, Leica Microsystems) and stained with H–E (slide stainer Shandon Varistain 24–4). The rates of mitotic cells were calculated by counting them in several randomly high–magnification fields (40X) per tumor. The mammary tumors were histologically classified and grading according to the new system proposed by Goldschmidt *et al.* [16].

Statistical analysis

The evaluation of concordance between the clinical staging and the histopathological classification of the tumors was carried out using the weighted Kappa Index [21] since it is applicable to qualitative variables with more than 2 categories among which there is a hierarchical order (qualitative variables of ordinal scale). In this situation, there may exist varying degrees of agreement or disagreement between the clinical and histopathological evaluations. The weighted Kappa Index considers the agreement between the tests (clinical vs. histopathology) on the diagonal and penalizes (in a weighted way) the disagreement according to the distance to the diagonal of agreement.

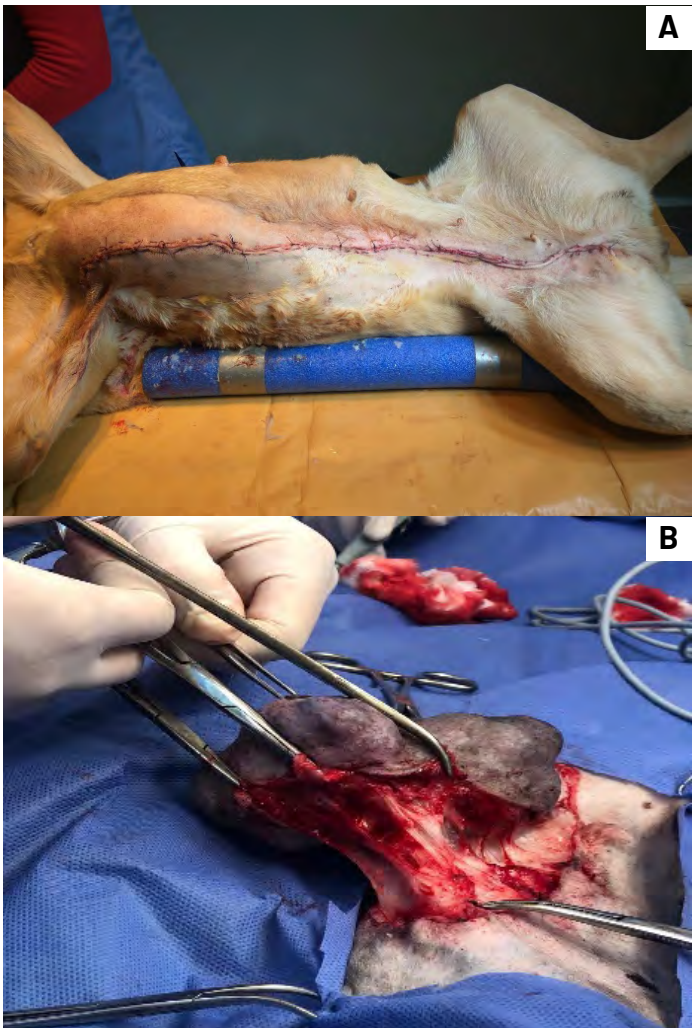

FIGURE 1 (A). Unilateral radical mastectomy. B) regional mastectomy

RESULTS AND DISCUSSION

Results in mammary tumors

The age of presentation of mammary tumors in this study ranged from 6 to 12 years with an average of 9 years. There was no differences in tumor location between both mammary chains, 51% of tumors were located in the right–side chain, while 49% were distributed in the left–side chain. The breasts with most tumors were the inguinal ones (11/32), followed by caudal abdominal (8/32), cranial abdominal (8/32), and caudal thoracic (4/32) breasts. In the case of cranial thoracic breasts, a single tumor was found in just one patient. In 62% of the cases (n=20), the presentation of the tumors was multiple. No female presents benign and malignant tumors within the same mammary chain but this can be attributed to the low number of the sample, and in 38% of the cases (n=12) they were single tumors. In terms of tumor malignancy, 35% (n=21) were malignant, while 11% (n=65) were benign. Of the female dogs with multiple tumors, 15 were malignant and 4 benign, of those with single tumors, 7 were malignant and 5 benign. Clinical staging data showed that 64% of female dogs with benign tumors had stage I (1 to 3 cm), and 36% had stage II (3 to 5 cm). Among the female dogs diagnosed with malignant tumors, 57% were

in stage III, 9% in stage II, and 24% in stage I. In this study, no animals were found in stage IV (lymph node metastases) which was discarded by cytology, and 10% were in stage V disease (lung metastases).

Histopathology of mammary tumors

Among malignant tumors, the most frequents in this study were simple tubular carcinoma and complex carcinoma, both with equal numbers (n=4 each), followed by solid and tubulopapillary papillary tubule carcinomas (n=2 each) and, with one case of each type, by simple carcinoma, cystic papillary carcinoma, comedocarcinoma, anaplastic carcinoma, mixed carcinoma, intraductal papillary carcinoma, malignant myoepithelioma, lipid-rich carcinoma, and inflammatory carcinoma (FIGS. 2, 3,4 and 5).

Following the classification of Goldsmith *et al.* [16], of the 21 malignant tumors, malignant epithelial tumors accounted for 85.7% (18/21), whereas those of special types accounted for 14.3% (3/21).

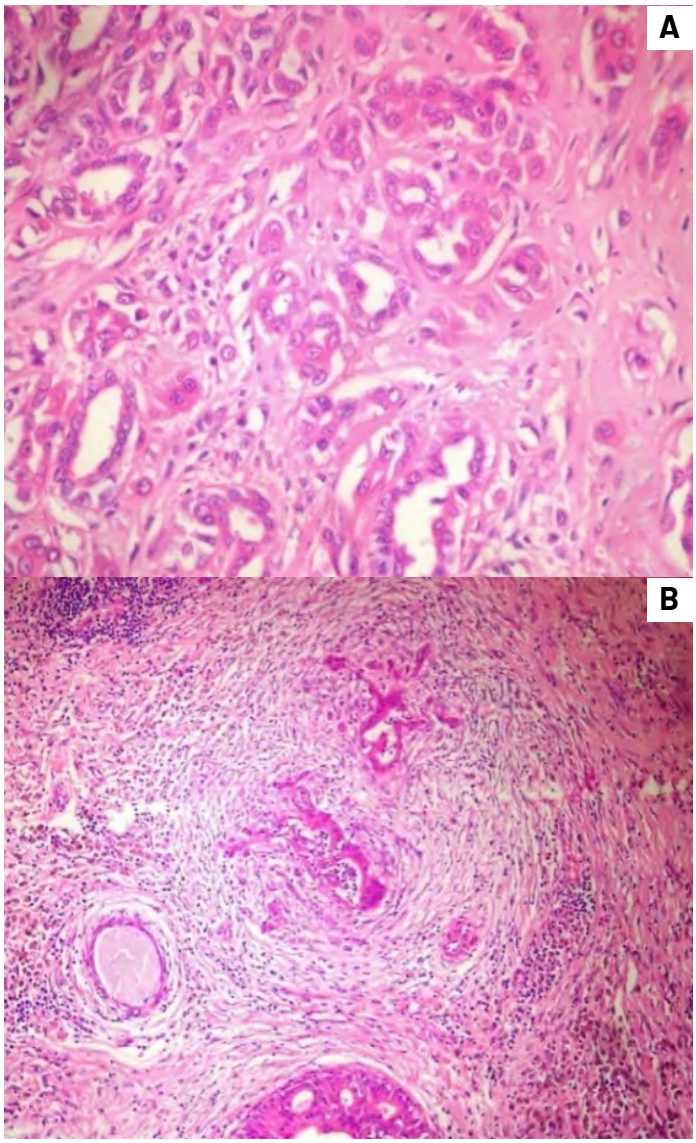

FIGURE 2. A: Histopathological sample of a simple tubular carcinoma, H-E staining 10X. B: Histopathological sample of a complex carcinoma, H-E staining 10X

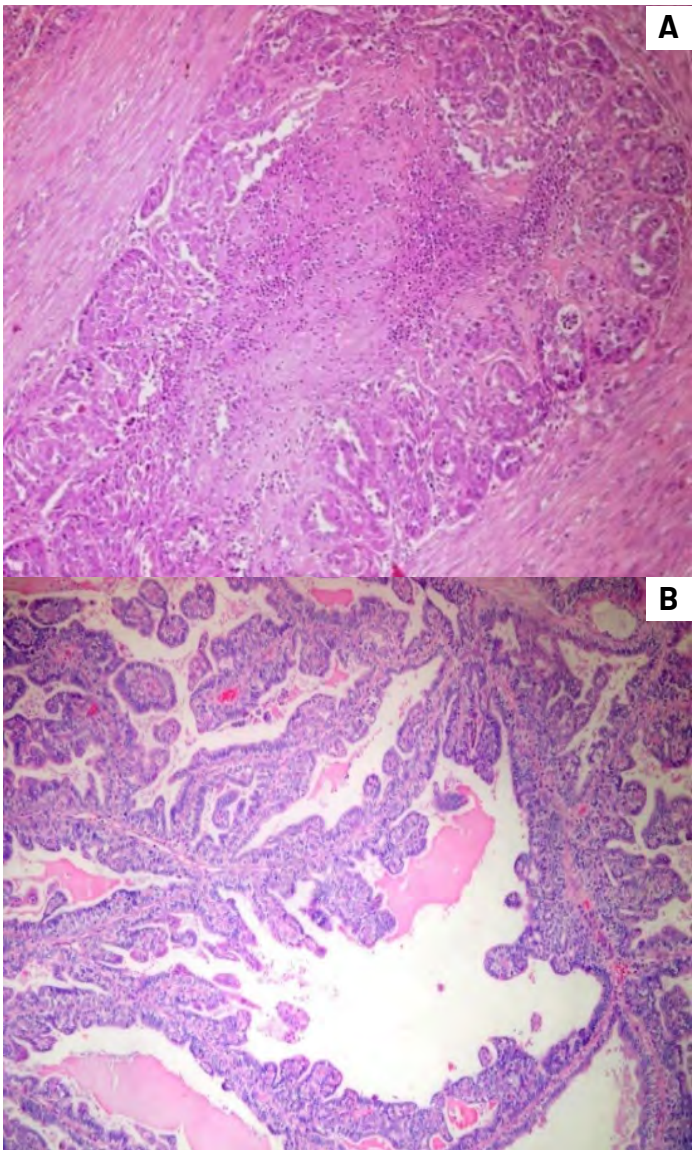

FIGURE 3. A: Comedocarcinoma 40X. B: Tubulopapillary carcinoma. Hematoxilyn and eosin staining 10X

Among the histopathological grade I malignant tumors, It was found only one female dog with an intraductal papillary carcinoma. Among the histopathological grade II, It was found seven female dogs with the following tumor types: tubulopapillary carcinoma, complex carcinoma, mixed carcinoma, simple tubular carcinoma and lipid-rich carcinoma. As for histopathological grade III, there were 13 female dogs with the following carcinomas, in order of frequency: simple tubular carcinoma, complex carcinoma, solid carcinoma, inflammatory carcinoma, anaplastic carcinoma, malignant myoepithelioma and papillary cystic carcinoma. (TABLE I). Four of these female dogs with malignant tumors were ulcerated. None of the tumors was attached to deep planes. Among the benign tumors, complex adenoma (8 patients) was the most frequent, followed by benign mixed tumor (two patients) and simple ductal papilloma (one patient).

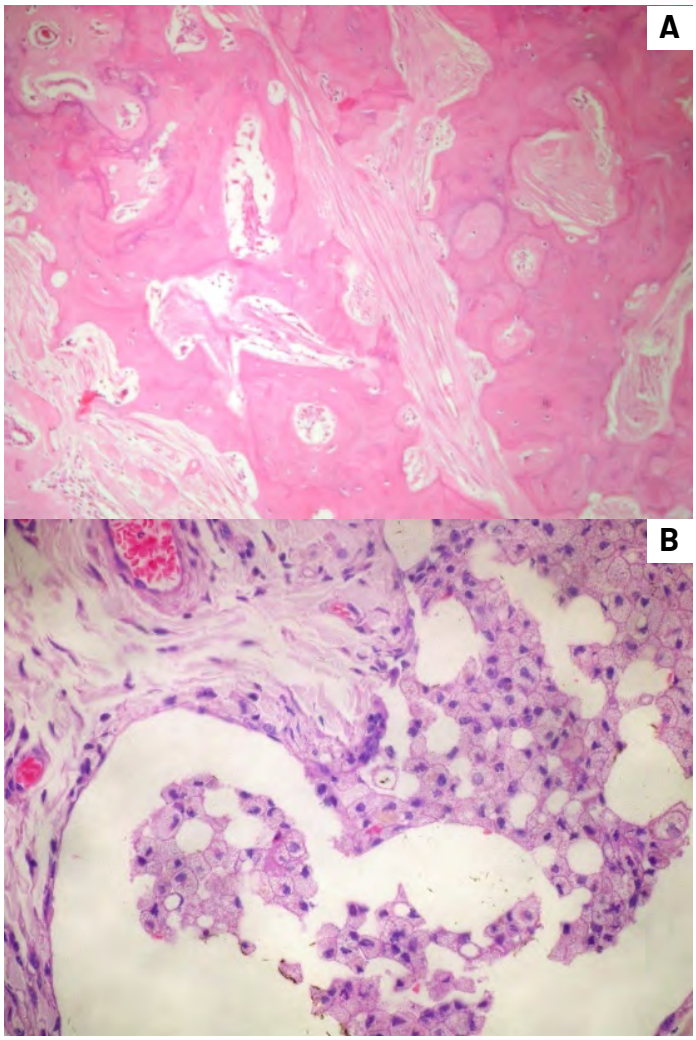

FIGURE 4. A: Histopathology of a mixed carcinoma, 40X. B: Lipid–rich carcinoma. H–E staining 10X

TABLE I
Relationship between Histopathological Type and Histopathological Grade

Histopathological type	Histopathological grade		
	Grade I (n)	Grade II (n)	Grade III (n)
Inflammatory carcinoma	–	–	1
Comedocarcinoma	–	–	1
Carcinoma mixed type	–	2	–
Carcinoma tubular simple	–	1	3
Carcinoma complex	–	1	1
Carcinoma anaplastic	–	2	2
Carcinoma solid	–	–	2
Malignant myoepithelioma	–	–	1
Intaductual papillary carcinoma	1	–	–
Lipid rich carcinoma	–	1	–
Carcinoma cystic papilar	–	–	1
Total	1	7	13

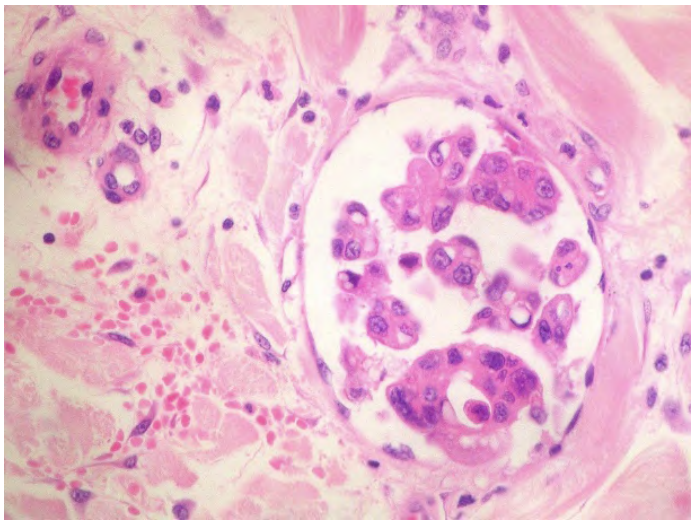

FIGURE 5. Histopathology of lymphatic emboli located in the dermis of an inflammatory carcinoma. H–E 40X

Concordance analysis between diagnostic methods.

The estimate of Cohen's weighted Kappa index, with a 95% confidence interval, was: k = 0.48 [0.16 – 0.81]. Since this index is given values between 0 and 1, in the present case the degree of agreement observed was weak to moderate. When analyzing the possible cause of the low concordance obtained between the tests, It was found that the proportion of agreement observed was Po = 12/32= 0.375 (37.5%), which indicates a low linear association between the clinical and histopathological diagnoses.

In the following Table (TABLE II) the tumors in each stage were shown.

TABLE II
Concordance Analysis between Diagnostic Methods: Histopathological Vs Clinical

	HG 0	HG 1	HG 2	HG 3
CS 0	0	0	0	0
CS 1	7	0	2	3
CS 2	4	0	2	0
CS 3	0	1	3	10

Number of tumors classified in each stage according to the two diagnostic methods simultaneously (clinical and histopathological). GH 0 was used for benign tumors. Clinical stages 4 and 5 were linked to stage 3 due to their low n. The proportion of agreement (accuracy) is 12/32= 37.5%, which indicates a low linear association, for which the concordance between the clinical and histopathological diagnosis is low. CS: clinical stage; HG histopathological grade.

In first place, regarding the age of presentation of mammary tumors, it was between 6 and 12 years, with an average of 9 years. TABLE III.

TABLE III
Distribution Of Benign And Malignant Tumors Classified By Age

Age (years)	Benign	Malignant	Total
6	1	2	3
7	0	0	0
8	3	4	7
9	1	1	2
10	2	7	9
11	2	0	2
12	2	7	9
Total	11	21	32

These values agreed with those of various authors [10, 11, 14, 22, 23]. It has been previously reported that two-thirds of mammary tumors occur in the caudal abdominal and inguinal breasts, probably due to their greater volume of breast tissue, while thoracic breast tumors are less frequent [14, 19, 24]. In this paper, the data agree with what has been previously described**.** Only one of the 32 female dogs studied had a single tumor in the right caudal thoracic mammary gland. Four had tumors in the caudal thoracic mammary glands, but these were cases of multiple tumors involving several breasts in the chain. In the remaining dogs, the breasts more frequently affected were the caudal abdominal and the inguinal breasts. This leads to consider the fact that at least 50% of female dogs with mammary neoplasms have multiple tumor masses [11]. In the present study, 20 of the 32 female dogs had multiple tumors, accounting for 62% of the cases. There is no consensus regarding the breeds with the highest incidence of breast tumors, the risk varies according to the study and geographic location [10, 11, 25, 26, 27]. Burrai *et al.* [23] observed that small breeds had predominantly benign tumors, particularly in Yorkshire Terrier, while malignant tumors were more frequently detected in German Shepherd dogs. Here, it was observed a higher percentage of benign tumors in the Poodle and German Shepherd breeds; this may be due to the previously mentioned factors, and it should also be taken into account that, as this paper had a low number of animals of each breed, these data may not be comparable.

Histopathological diagnosis before surgery is not a common practice in Veterinary Medicine, except in Veterinary Reference Centers but it is performed after surgical removal. This is because there are usually multiple tumors that could correspond to different mammary neoplasms, therefore, it would be necessary to take one sample from each one of them. With regard to fine needle aspiration (FNA), it should be added that there is a great cellular heterogeneity, which could give rise to a discrepancy in the case of samples as small as those obtained by this method. Another point to bear in mind is that regardless of the outcome of the histopathological analysis, the therapeutic modality will be the same (surgical). FNA would be indicated only for cases of differential diagnosis such as lipomas, mastocytomas, and inflammatory carcinomas [13]. Due to the above stated reasons, in the Specialized Oncology Clinic of the Veterinary Hospital of the University of the Republic, mammary tumors are diagnosed by histopathology after the surgical procedure, since neither routine FNA nor breast biopsies are performed.

The percentage of female dogs with malignant tumors (65%) in this study is slightly above those reported by other authors, which range between 43 and 51% [1, 2, 3, 23]. According to Ferreira *et al.* [28], most lesions larger than 5 cm (T3) are malignant, with higher proliferation rate than those of smaller tumors (T1, T2); these data are consistent with our findings in this study, where 66.7% of T3 tumors were malignant. According to Camargo *et al.* [29], regarding tumor size, T3 lesions were predominantly associated with carcinosarcomas, while T1 and T2 lesions were more frequent in benign mixed tumors and mixed carcinomas within malignant tumors. Burrai *et al.* [23] observed a 2.3- and 3.6-fold increase in the odds of a malignant tumor when moving from T1 to T2 and from T2 to T3, respectively. These authors evaluated 1,866 female dogs with single breast tumors and observed 73% T1, 16.6% T2, and 10.3% T3. 89.65% of the canine mammary tumors were between 0 and 5 cm, and 63.76% of the malignant tumors were smaller than 3 cm, of which 62.5% were classified as simple carcinomas. T2 and T3 types included 21. 32% and 14.91% of mammary tumors. These data are consistent with the findings of this work, where we also observed both T1 and T2 malignant tumors, and the most observed histopathological type was also simple carcinoma. Within T1, it was found both benign and malignant tumors. Among the benign tumors, by decreasing order of frequency it was found in first place complex adenoma, followed by benign mixed tumor and ductal papilloma.

Among the malignant tumors, it was observed mixed carcinoma, comedocarcinoma, and complex carcinoma. In clinical stage T2, it was also observed malignant and benign tumors. Malignant tumors were mixed carcinoma and complex carcinoma, while complex adenoma was the most representative benign tumor. There were no tumors classified as carcinosarcoma; the most representative T3 tumors were simple tubular carcinoma and papillary tubular carcinoma, followed by inflammatory carcinoma, anaplastic carcinoma, malignant myoepithelioma, complex carcinoma, papillary intraductal carcinoma, and lipid-rich carcinoma. We also observed a high number of malignant breast tumors smaller than 1 cm, thus suggesting the need to reconsider the size (T) parameter in the TNM system, paving the way for the development of tools for clinical research and control by assessing risk factors for small-sized tumors. These T1 tumors were found to be high grade, and were classified as comedocarcinomas and solid carcinomas. When comparing female dogs with stage I and II tumors with those staged in stage V, the latter had a worse prognosis, coinciding with Karayannopoulou *et al.* [20]. In this study, two of the female dogs were in stage V at the time of diagnosis, with complex carcinoma and papillary cystic carcinoma. Among the female dogs with malignant tumors, four had ulcerated tumors, which are often considered as an indicator of malignancy. This is controversial, since according to Hellmén *et al.* [30] ulceration and necrosis are two features that have been suggested as indicators of increased tumor aggressiveness. But it is also possible to think that they may be often due to self-induced trauma, ischemia, or skin infection, characteristics that are not necessarily associated with an aggressive biological behavior of the tumor.

According to Vail *et al.* [14], the evaluation of regional lymph nodes has a major impact on the survival of dogs with canine mammary tumors (CMT), as they show a significant decrease in survival compared with those who tested negative for lymph node metastases. In this study, no patients with lymph node metastases were found.

Two of the female dogs had lung metastases, coinciding with the literature where it is said that lungs are the most frequent site of distant metastases [14, 31].

As mentioned above, with the results of the statistical analysis it was found a moderate agreement regarding the concordance between clinical and histopathological staging. Cohen's weighted Kappa index, with a 95% confidence interval, was k = 0.48 [0.16 – 0.81]. These data are consistent with histopathological results, since it was found both malignant T1 (1 to 3 cm) tumors and benign T2 (3 to 5 cm) tumors.

CONCLUSIONS

In the present work, the concordance between clinical aspect of the tumor and histopathology was low, since malignant tumors of small size (classified as T1: 1 to 3 cm) and benign tumors of low size (classified as T2: 3 to 5 cm) were found. It seems that clinically there is a tendency to classify tumors towards greater severity. Studies with a larger population size are required to obtain more consistent conclusions.

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