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Original

Survival in female dogs with mammary neoplasia

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ABSTRACT

Objective. To evaluate selected biological and histological factors associated with the survival of female dogs with mammary neoplasms. **Materials and methods.** An epidemiological study was performed based on the medical records of 72 female dogs diagnosed with mammary neoplasms. Associations between the characteristics of the female dogs, their reproductive status, the presence of parturition, the post-surgical survival time (m) and the final outcomes of the individual dogs were evaluated. Life tables and a Kaplan-Meier estimator were used for the statistical analysis of survival. **Results.** The female dogs evaluated were nulliparous (65.28%), with a mean age of 9.53±3.07 years and a weight varying between 1.5 and 49.1 kg (median 10.5 kg). The mortality incidence rate was 0.06 cases/patient/month (50 deaths/827.1 month of follow-up). Of the female dogs with mammary neoplasia, 37.50% survived, 15.28% were euthanized and 30.56% died. The risk of death was 60.5 cases/1,000 female dogs/month and the probability of post-surgical survival in months was 30.36%. Overall post-surgical survival was associated with the age, size and reproductive status of the female dogs; along with size, subtype, malignancy and TNM classification of the tumor ($p<0.05$). **Conclusions.** To ensure survival of canine females with mammary neoplasia early diagnosis by thorough physical examination and cytology, surgical removal when necessary, and timely consultation are necessary. Variables that are predictive of survival include the age and reproductive status of the animals, as well as the size of the tumor.

Keywords: Mammary cancer; biological factors; reproductive factors; survival (*Source: CAB*).

RESUMEN

Objetivo. Evaluar algunos factores biológicos e histológicos asociados con la supervivencia postquirúrgica de hembras caninas con neoplasias mamarias. **Materiales y métodos**. Se hizo un estudio epidemiológico basado en las historias clínicas de 72 hembras caninas diagnosticadas con neoplasias mamarias. Se evaluaron asociaciones entre las características de las hembras, el estatus reproductivo, presencia de partos, tiempo de supervivencia posquirúrgica (m) y la evolución final de las pacientes. Para el estudio de la supervivencia se usaron tablas de vida y el estimador Kaplan-Meier.

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Resultados. Las hembras evaluadas eran nulíparas (65.28%), con edad promedio de 9.53±3.07 años, peso variable entre 1.5 y 49.1 kg (mediana 10.5 kg). La tasa de incidencia en mortalidad fue de 0.06 casos/paciente/mes (50 muertes/827.1 mes de seguimiento). El 37.50% de las hembras caninas con neoplasia mamaria sobrevivió, el 15.28% sometidas a eutanasia y el 30.56% murió. El riesgo de morir fue de 60,5 casos/1.000 hembras/mes y la probabilidad de supervivencia postquirúrgica en meses fue 30.36%. La supervivencia posquirúrgica general se asoció a la edad, tamaño y estado reproductivo de la hembra; tamaño, subtipo, malignidad y clasificación TNM del tumor ($p<0.05$). **Conclusiones.** Para garantizar la supervivencia de las hembras caninas con neoplasia mamaria es necesario realizar un diagnóstico precoz por medio de examen físico minucioso y citología; la extracción quirúrgica cuando sea necesario y la consulta oportuna. Existen variables predictivas de la supervivencia como la edad y el estado reproductivo de los animales, así como el tamaño del tumor.

Palabras clave: Cáncer mamario; factores biológicos; factores reproductivos; supervivencia (*Fuente: CAB*).

INTRODUCTION

Some of the relationships that are addressed in a social context are those established between humans and companion animals, due to their close affective and emotional contact (1). The concern of owners for their pets has strengthened responsible pet ownership, the demand for veterinary services and the interest in the diagnosis and treatment of diseases that affect them (1,2).

Currently, it is estimated that one in four dogs over the age of two years dies of cancer (3). In order of frequency, the main neoplasms are those of cutaneous origin; Secondly, developing mammary gland tumors are described, which are the most frequent in older adult female dogs, with a prevalence that varies according to each region studied; for example in studies carried out in Denmark and Switzerland between 2005 - 2008, and 1955 – 2008 where a prevalence of 28 and 18.09% was reported respectively (4,5), while in Colombia, the University of Antioquia in 2007 and the National University of Colombia in 2012 presented reports of 14.64% (6) and 17.81% amongst all neoplasms (7). Breast cancer represents a problem in animal medicine due to the mortality of patients caused by tumor dissemination. This behavior helps to reaffirm the importance of histological diagnosis of this type of masses, which fundamentally requires qualified personnel with specialized training and early detection, without neglecting the existence of a direct relationship between diagnosis and short-term prognosis (8). In addition to this, in recent decades advances in the treatment of breast cancer have made it possible to prevent its local dissemination and metastasis by means of different therapeutic options that include surgery, radiotherapy, chemotherapy and some variations of these that offer good results in relation to quality of life and life expectancy (9,10).

Similarities have been described between female breast cancer in humans and mammary gland tumors in female dogs, where the hormonal predominance in development, histological characteristics, expression patterns of some molecular markers and an unpredictable clinical outcome stand out (7). For this reason, dogs are now considered a useful natural biological model for the study of neoplastic disease in humans, not only at the clinical and pathological level, but also in terms of prognosis and life expectancy after treatment (1). The prognosis and survival rate has been found to be similar to that observed in women with breast cancer (11).

Histology is the most important diagnostic test to classify and provide prognostic information on survival in dogs with mammary neoplasms. The study of prognostic factors and markers is highly relevant for veterinary physicians and oncologists, for clinical decision making, the selection of adjuvant therapies in reducing tumor recurrence and optimizing the healthy longevity of patients (3). In view of the aforementioned, the aim of the present research is to evaluate some biological and histological factors associated with the survival of female dogs with mammary neoplasms.

MATERIALS AND METHODS

Samples and monitoring. This study was approved by the Animal Use Ethics Committee at the University of Caldas, Colombia. In this study, 72 female dogs diagnosed with mammary neoplasms between January 2008 and April 2018 were evaluated. All patients underwent

surgical mastectomy as a treatment option. The histological criteria of the World Health Organization were used for the classification and diagnosis of canine mammary tumors. The criteria for inclusion was: a complete medical history with hemogram test and pre-surgical examinations, confirmation of mammary surgery and request for tumor biopsy, histopathological diagnosis report of the mammary tumor performed in the animal pathology laboratory of the University of Caldas by a veterinary pathologist (94.34%), reproductive history and monitoring of survival for at least twenty-four (24) months post-surgery.

Patient data was obtained through telephone interviews with owners and by reviewing the medical records available at the Diego Villegas Toro Veterinary Hospital at the University of Caldas. Overall post-surgical survival (PFS) was calculated and characterized as the time from tumor removal to the date of animal death/ euthanasia due to tumor-related causes and/ or at the end of the study (m); as well as final patient outcomes (survivor, death due to causes unrelated to mammary disease, euthanasia).

Variables evaluated. The variables evaluated were: a) body condition based on the body condition classification system proposed by Tvarijonaviciute et al (12), b), age at consultation (years), breed according to the 2013 "Federation Cynologique Internationale for pedigree dogs worldwide" (FCI) categorization, size and weight of the patient (dwarfs 5 kg or less; small between 5 and 14 kg; medium between 14 and 25 kg and large between 25 and 50 kg), reproductive status (spayed, non-spayed), births (nulliparous or multiparous), tumor type (benign and malignant), size of the tumor, the histological classification of carcinoma, the total number of mammary glands affected and the TNM classification.

Statistical analysis. Data analysis was performed with the statistical program STATA version 13.1 (College Station, Texas, USA). Descriptive analysis of categorical variables was performed. The normal distribution of the variables was evaluated by means of the Shapiro wilk test. PFS curves were constructed using the Kaplan-Meier method and differences in PFS time between patients and study variables were evaluated using the log-rank test. A statistical significance level of 95%, $p \leq 0.05$, was considered for all analyses.

RESULTS

Clinical and histological data. The mean age of the dogs was 9.53 ± 3.07 years (range: 2-16 years); other clinical and histological characteristics of the individuals are presented in Table 1. The clinical and blood chemistry parameters were found to be within the ranges considered normal for canine species.

Table 1. Some descriptive characteristics of female dogs and general characteristics of mammary neoplasms.

Variable		Frequency (n)	Percentage (%)
Size	Dwarf < 5 kg	10	13.89
	Small 5-14 kg	29	40.28
	Medium 14-25 kg	9	12.50
	Large 25-50 kg	19	26.39
Reproductive Status	whole	34	47.22
	spayed	30	41.67
Parity	Nulliparous	47	65.28
	Multiple births	24	33.34
Type of Tumor	Benign	14	19.44
	Malignant	39	54.17
Mammary gland affection	Single Incidence (1 gland)	21	29.17
	2 glands	19	29.39
	3 glands	6	8.33
	4 glands	9	12.50
	5 glands	5	6.94
	6 glands	2	2.78
	7 y 8 glands	1	1.39
Tumor Stages and TNM Classification	Stage III (T3N0M0)	18	25.00
	Stage I (T1N0M0)	15	20.83
	Stage II (T2N0M0)	13	18.06
	Stage IV (T3N1M0)	10	13.89
	Stage IV (T2N1M0)	6	8.33
	Stage IV (T1N1M0)	3	4.17

Fifty-three slices of mammary tumors from 72 female dogs studied were analyzed. The size of the major axis of the tumor masses ranged from 0.5 to 15 cm, with a median of 4.15 cm and an IQR of 3 cm; malignant tumors predominated (size: 0.5 to 11 cm, mean 4.64 cm), compared to benign tumors (size: 2 and 8 cm, mean 5.54 cm). The most frequent benign tumors were diferent types of adenoma (50.01%; n=7), adenosis (7.14%, n=1) and others (7.14%, n=1), while the most common malignant tumors were carcinoma (79.49%, n=31) and mixed

tumor (7.69%, n=3). Regarding carcinomas, the tubular type predominated (41.94%, n=13), followed by papillary (16.13%, n=5).

Evaluation of post-surgical survival. At the end of the study 37.50% (n=27) of the female dogs with mammary neoplasia were still alive, 15.28% (n=11) were euthanized (72.73% due to causes associated with neoplastic disease) and 30.56% (n=22) had died; there was no information recorded for 16.67% (n=12). Median time to progression of neoplastic breast disease was 3.5 m; median PFS was 18.5 m. The mortality rate was 0.06 cases/patient/month (50 deaths/827.1 month follow-up). The risk of death was 60.5 cases/1,000 females/month and the probability of PGS was 30.36 m.

Figure 1A shows the relationship between PGS by months in 56 female dogs and their survival rate by the end of the study. It is observed that the mortality of the female dogs increased over the follow-up time and, therefore, the probability of PGS decreased. During the initial 30 d of follow-up, a mortality rate of 14.28% (n=8) was recorded, representing a decrease in the probability of PGS by 85.71%. By the middle of the follow-up period (month 12), 44.64% (n=25) of the females dogs had died; by the end of the study the PGS probability was 30.36%, (n=17). Figure 1B shows PGS according to the type of mammary neoplasia (benign/malignant). In the first month post-surgery, the probability of PFS for females with malignant neoplasms was 78.79% (n=7 deaths), while during the same period of time in the group of female dogs affected with benign neoplasms only one death occurred (PGS= 90.91%). The probability of PGS at the end of the follow-up period, was higher in female dogs affected by malignant mammary neoplasms (36.36%, n=12 Vs 9.09%, n=1).

When measuring PGS, differences were found between the female dogs according to histopathological classification (type) of carcinoma, TNM classification (staging) and size of the neoplasm (length in centimeters). The histopathological classification (type) of carcinoma allowed us to observe that post-surgical survival was higher in the female dogs with a histopathological diagnosis of complex type carcinoma (24 months), when compared with those affected by the tubular type (19 months) and the mixed type (1 month) (p=0.036), an aspect associated with higher mortality rates (2A). A similar situation occurred with the TNM staging method and the average time of PGS, since in female dogs affected with neoplasms of stage T3N1M0 (stage IV) the average post-surgical survival time was lower (8 months) than that found in female dogs with stage T2N0M0 (stage II) (23 months) (p= 0.000); It could also be observed that it was also lower in female dogs with stage T2N1M0 (stage IV) (2.5 months) when compared to female dogs with stage T3N0M0 (stage III) whose average survival was 18.16 months (higher) (Figure 2B). With respect to the size of the neoplasms (length of the major axis) taken into account by length ranges in 2-centimeter intervals, it was shown that female dogs with neoplasms with a length between 2.1 - 4 cm had a longer survival at the end of the follow-up period (21.83 months) compared to female dogs affected by larger mammary neoplasms (0.0011) (Figure 2C).

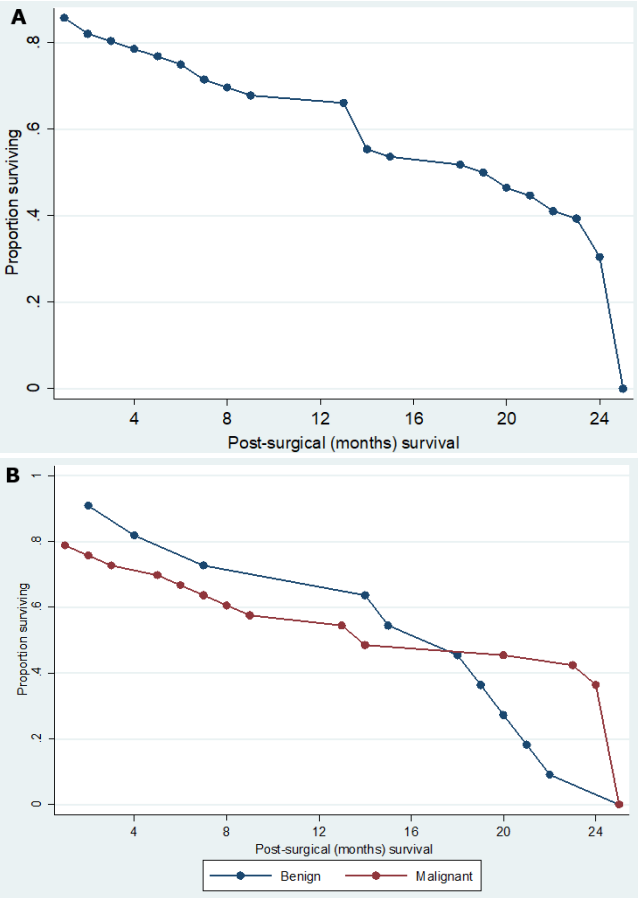

Figure 1. A: Lifetime Table of overall post-surgical survival in months for female dogs with mammary neoplasia during follow-up. B: Life Table of Post-surgical Survival in months according to classification of mammary neoplasia (benign/malignant) in canine females.

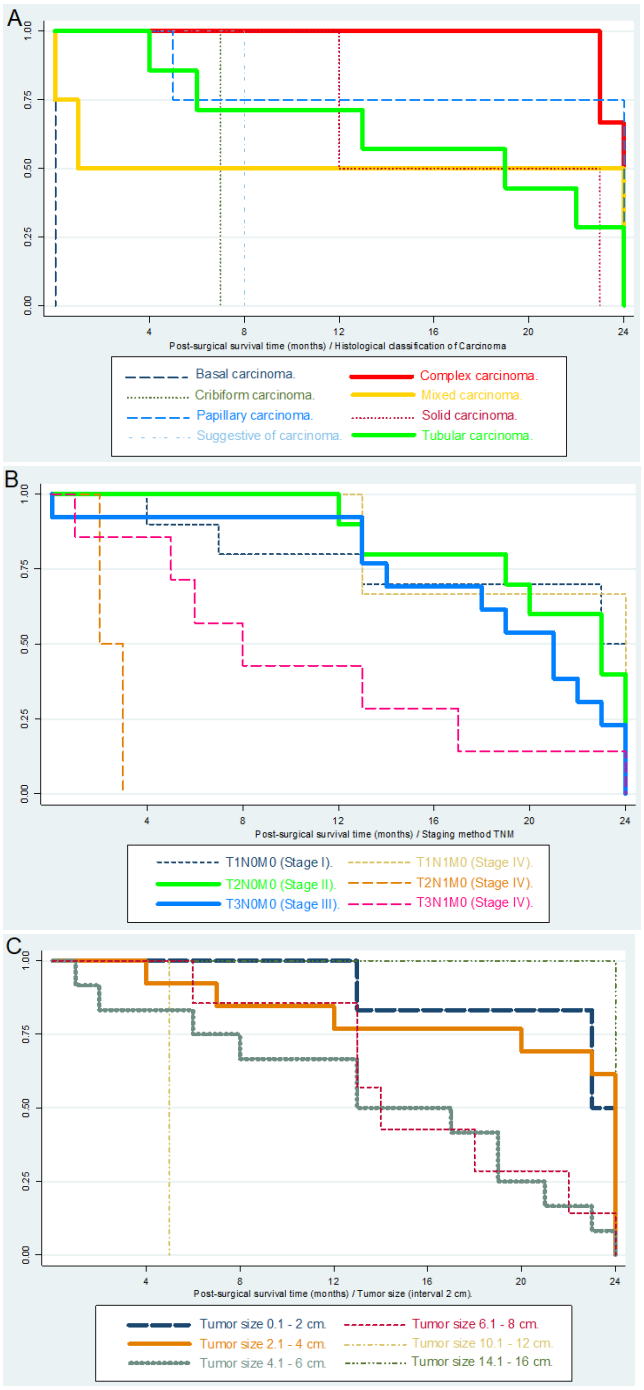

Figure 2. Kaplan-Meier survival curves. A: Survival analysis and its relationship with the type of carcinoma. B: Survival analysis and its relationship to TNM classification in canine females with mammary neoplasia. C: Survival analysis and its relationship with the size (2cm range) of the neoplasm.

Female dogs with benign tumors were diagnosed at 96 m of age, while those with malignant neoplasms were diagnosed at 120 m of age. Female dogs with one affected mammary gland had a higher GFR (21 m; p=0.000) compared to patients with two, three (20.33 m) and four (14.67 m) affected glands. Likewise, nulliparous female canines presented a higher GSP (23 months; p= 0.019) than patients who reported between 1 and 3 births (15.70 m) and > 4 births (6.67 m). At month 23. 50% of the nulliparous females were still alive, whereas at month 6 of post-surgery, 50% of the canines with > 4 births had died.

DISCUSSION

The age of female dogs is considered an important risk factor for cancer susceptibility (13). In this study, the average age of the patients at the time of consultation was 9.53 years, which is in consistent with other authors (14,15); it also supports the idea that canine breast cancer is a late onset disease with moderate lethality (3), explained perhaps because the cells of old canines have undergone more cell cycles and have been exposed to carcinogenic factors for a longer time, than the cells of young animals (16). In addition, it has been observed that with age cases of mammary neoplasms in female dogs are parallel as it increases, especially in the case of malignant neoplasms above 7 years of age (14).

Studies demonstrate the influence of a genetic component in the susceptibility to neoplasia, however, the contribution on receptor expression, proteins and molecular markers of this type of tumors has been valuable primarily in prognosis and survival (17). Nevertheless, a Swedish study of more than 80,000 insured female dogs found that predisposition of certain breeds may play a role, as large female dogs that develop mammary tumors may have a more uncertain prognosis than small breeds (18). In this study, small-sized patients were found to have mammary neoplasms more frequently, which is in agreement with other authors (17,19).

Several studies suggest that the relative risk for the development of mammary tumors increases in accordance with the number of the patient's estrous cycles (20). Several associations have been identified between ages, spaying and reproductive mammary hormonal effects in canines with mammary tumors; where it is inferred that if spaying is done at an advanced age, there is a higher risk of presenting malignant tumors (21). However, researches warn that spaying at an adult age (when the tumor appears clinically), does not seem to have preventive value against breast cancer,

but it does prevent the appearance of benign tumors and uterine and ovarian pathologies (cystic endometrial hyperplasia, pyometras, hematometras, mucometras, hydrometras, ovarian tumors) (22,23).

In the current study it was found that 65.28% of the canine females with mammary neoplasia were nulliparous. In canines, a higher incidence of mammary neoplasia has been observed in nulliparous females or females with few litters, compared to females that have had numerous litters (24), or that have been intensively used for breeding from an early age (25). Likewise, other studies show that the number of litters decreases when female dogs are older than 7 years, thus age could also influence the development of mammary tumors (19,26) between seasons of the year, in the distribution of matings and whelpings, litter size, pup deaths, and sex ratio in domestic dogs. Furthermore, we wanted to examine the effects of age and parity of the bitch at the time of whelping on litter size, as well as the effect of litter size on gestational length. A final aim was to investigate the fertility and frequency of whelping problems in a private kennel of Drever dogs. Data from the Swedish Kennel Club (SKK. In this study, nulliparous canine females present a higher PGS, which contradicts what was reported by Sirven (17), who found that the development or not of gestations and their number does not favor the establishment of a prognosis.

Several studies report higher mortality in female dogs diagnosed with malignant mammary neoplasia (15). However, in this study the PGS at the end of monitoring was higher in female dogs with malignant neoplasms (36.36%, n=12) versus female dogs affected by benign neoplasms (9.09%, n=1) probably due to the profound interest caused by female dogs affected by malignant neoplasms for follow-up treatment and the small number of female dogs in follow-up assessment affected by benign neoplasms, results that are discordant with those reported by Pedraza-Ordoñez (27), Cruz Amaya et al (15) and Salas-Araujo et al (19), but are in agreement with that described by Waters et al (3), who in a prospective cohort study found no evidence that breast cancer presented an adverse impact to the longevity of female dogs. Likewise, other studies have identified that the histological grade of the mammary tumor, is an independent predictor of the survival times of canines with malignant mammary neoplasms, because the histological criteria could be too stringent and could lead to an overestimation of malignancy (28). Additionally, there are differences in pathological features and biological behavior among carcinomas, as well as intra-observer differences in studies, which may affect the results (29).

Some parameters have also been identified that, when taken together, can offer significant indicators of survival, as is the case of the impact on the prognosis and disease-free survival of female dogs affected by mammary neoplasms when characterized by histopathological classification, tumor staging, histological grade and clinical characteristics of the neoplasm and of the female dogs (6). In this research, patients with complex, tubular and papillary type mammary carcinoma survived longer, compared to females with solid and mixed type carcinomas, results in discordance with those presented by Salas-Araujo et al (19), Sirven (17) and Gamazo (30). This discrepancy may be due to the heterogeneity of the number of cases evaluated, the geographical location of the studies and the biological differences of types to predict patient prognosis (31). However, other authors suggest that to ensure a more accurate prognosis veterinarians require, in addition to tumor classification, other specifics such as tumor immune phenotype and hormone receptor expression. However, as drawbacks they cite limitations in applying these in daily clinical practice (6).

When carrying out the analysis of the association between PGS and tumor size in the current study, it was found that patients with neoplasms with a length between 2.1 -4 cm had a higher PGS at the end of monitoring (21.83 months). Salas-Araujo et al (19) point out in their study that patients with tumors smaller than 2 cm had a longer survival than those with tumors larger than 6 cm, which allows considering tumor size as an important prognostic factor (p=0.009). These same results have been obtained in studies of breast cancer in women, where mortality increased with increasing tumor size and thus survival of the patient's decreased (32).

It is concluded that in order to guarantee the PGS of canine females suspected of presenting mammary neoplasia, it is vital that the veterinarian performs a thorough physical examination and early diagnosis; surgical

removal in the event that it is necessary and support for owners so that they can undertake a timely consultation. There is no agreement among authors on the degree of malignancy when implementing histotypes, which represents a limitation for prognostic studies. Nevertheless, histological assessment remains the test of choice in the diagnosis of canine mammary tumors and variables that are predictive of survival include age, reproductive status of female dogs and tumor size.

Conflicts Interest

The authors declare no conflict of interest.

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