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Original

Canine mammary gland tumors: risk factors and their epidemiological influence in Manizales-Colombia

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ABSTRACT

Objective. To describe the prevalence of canine mammary gland tumors (CMT) in females in the municipality of Manizales-Colombia from 2014-2017. **Materials and methods.** A database of 15961 patients was consolidated. The variables analyzed were, breed, age, reproductive history, weight, diet type and clinical characterization of the tumor with the TNM (tumor-node-metastases) staging system. The statistical analysis include Pearson's chi-squared test with Yates correction for continuity and Fisher's exact test ($p<0.05$), relative risk and odds ratio (CI95%) and Kaplan-Meier estimator for survival analysis. **Results.** The incidence in purebred dogs was 79.14%, with a peak at the average age of 9.3 years old. Pearson's chi-squared test and the relative risk and odds ratios indicated a high risk for purebreds ($p=0.019$, 3.96/100, 1.64, respectively). Females of ages between 9 and 12 years old showed a 74% likelihood of developing a mammary tumor. No found significant relation to weight or reproductive stage but indeed a high association with homemade diet ($p<0.001$). The inguinal mammary pairs were the most affected (6.9%). The Kaplan-Meier estimate showed a higher survival of surgically-intervened patients, with 2013 days of survival after diagnosis with surgery compared to 1484 days without surgery. **Conclusions.** The study confirmed the relevance of risk factors, breed type, age, body condition and diet type in the mammary tumor presentation. Furthermore, it highlights the need for improving and integrating the veterinary diagnostic information systems, considering their importance in public health.

Keywords: Cancer; epidemiology; incidence; prevalence (*Source: CAB*).

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RESUMEN

Objetivo. Describir la prevalencia del tumor mamario canino (TMC) en hembras en el municipio de Manizales-Colombia durante 2014-2017. **Materiales y** mé**todos.** Se consolidó una base de datos de 15961 pacientes. Las variables analizadas fueron raza, edad, historia reproductiva, condición corporal, tipo de dieta, y caracterización clínica del tumor mediante el sistema de estadificación tumor-nódulo-metástasis (TNM). El análisis estadístico incluyó prueba de chi-cuadrado de Pearson con corrección de Yates, prueba exacta de Fisher ($p<0.05$), riesgo relativo, oportunidad relativa (IC95%) y análisis de supervivencia de Kaplan-Meier. **Resultados.** La incidencia en perros de razas puras fue 79.14%, con un pico promedio a la edad de 9.3 años. La prueba chi-cuadrado de Pearson, el riesgo y las oportunidades relativos mostraron un riesgo alto para razas puras ($p=0.019$, 3.96/100, 1.64, respectivamente). Hembras entre los 9 y 12 años mostraron una probabilidad del 74% de desarrollo de tumor mamario. No se encontró una relación significativa entre la masa corporal o estado reproductivo pero una elevada asociación con la dita casera ($p<0.001$). Los pares inguinales mamarios fueron los más afectados (6.9%). La estimación de Kaplan-Meier mostró una alta supervivencia para pacientes intervenidas quirúrgicamente, con 2013 días después del diagnóstico con cirugía en comparación con 1484 días sin cirugía. **Conclusiones.** El estudio confirma la relevancia de los factores de riesgo: tipo de raza, edad, condición corporal y tipo de dieta en la presentación de tumor mamario. Además, se destaca la necesidad de mejorar e integrar los sistemas de información de diagnóstico veterinario, considerando su importancia en la salud pública.

Palabras clave: Cáncer; epidemiologia; incidencia; prevalencia (*Fuente: CAB*).

INTRODUCTION

Canine mammary gland tumor (CMT) is one of the most common tumors in dogs (1) and one of the main causes of mortality, similarly, as occur in humans (2,3). In female dogs, 40% of tumors are of the mammary gland (4,5) and these are three times more frequent than in women (1,6). In particular, intact females are more susceptible (4,7,8,9,10,11).

Canine mammary tumors are the second most frequent type after skin tumors (12,13). There are several predisposing factors to the presentation of CMT. For instance, purebreds are more susceptible (9,10) and, among these, small breeds are more susceptible to CMT (7,10,14). Age-wise, the highest incidence of CMT is estimated from 8 to 10 years old (1,8,9,10,13,14,15,16).

Early sterilization greatly reduces the susceptibility of developing CMT (17) and increases the survival rate (7,14). Ovarian steroid hormones or exogenous products, such as medroxyprogesterone acetate, stimulate the proliferation of the mammary tissue and, consequently, increase the risk of CMT (8,17,18). Another relevant risk factor is excess weight and obesity, which markedly increase the risk of CMT (19). Although other authors have not found this association (20). Homemade diets rich in fat is another risk factor (8,19). Many of these risk factors are shared with humans, in particular, lifestyle, type of diet/obesity, and hormonal birth control therapies (21,22).

Although CMT is a relatively common disease, in several places the databases are not consolidated, represents a challenge for data retrieval (23). Many clinical records are incomplete, ambiguous, or do not indicate the definitive diagnosis or the length of survival of the patients (24). The main objective of this study was to describe the prevalence of CMT in females and males in the municipality of Manizales-Colombia from 2014-2017.

MATERIALS AND METHODS

Data retrieval. We consolidated a database from clinical records between 2014 and 2017 provided by eight veterinary care centers in the city of Manizales (Caldas, Colombia). Access to the clinical records was done with signed consent from the animal owners and/or veterinarians. The constructed database was filtered to establish four groups of patients: Group I: all patients reported from consultations. Group II: patients from group I with a clinical diagnosis. Group III: patients from group II with any type of tumor affection. Group IV: it includes patients

from group III with a mammary gland tumor. The variables analyzed in the females diagnosed with CMT were breed (25), and breed type (purebred or mixed-breed), age, weight, type of diet, reproductive stage, hormonal treatments, and clinical characterization of the tumor (type, size, number of affected mammary glands, affected location, and metastasis).

We estimated the rate of presentation of tumor pathologies each year and determined the most affected organ system, as well as the annual number of females diagnosed with CMT. The topographic classification was done based on the Classification of Disease for Oncology System (ICD-O) (26). We determined the disease prevalence according to breed, age, and weight (normal, overweight, or obese based on the breed). In dogs, overweight is considered being 15% more than the "optimal weight" (27,28) and obese when it exceeds 30% (29).

The clinical characterization of the tumors was determined based on the TNM staging system (30). The histopathological findings were adapted according to Cassali et al (31). The data were consolidated in a MS Excel® spreadsheet. We also searched the bank of histopathological slides and histopathological reports databases of the consulted veterinary attention centers. We made a photographic register with a Leica ICC50 HD camera system and analyzed the images with ImageJ software (Wayne Rasband, National Institutes of Health, USA). Finally, we confirmed by telephone, the survival time from the initial diagnosis and surgical procedure of the females.

Statistical analysis. The relationship between the variables breed, age, weight, type of diet, and reproductive stage was determined using Pearson's chi-squared test with Yates correction for continuity and Fisher's exact test for continuous data with $p<0.05$. We also estimated epidemiological indices including incidence rate (relative risk – RR) for the female population over six years old with a definitive diagnosis and odds ratio – OR with a confidence interval of 95% (CI95%). The RR was calculated as the total number of canine females per 100 females-years divided by the total number of diagnosed females. The OR was estimated using 2x2 tables for each the study variables. The data was consolidated in a MS Excel® spreadsheet and analyzed using the R statistical package v.3.5.3 (R Foundation for Statistical Computing, Vienna, Austria). The survival analysis was performed using the Kaplan-Meier estimator ($p<0.05$) with GraphPad Prism v.8.2.0 software (GraphPad Software Inc., San Diego, CA).

RESULTS

General prevalence. The consolidated database (2014-2017) allowed distributing the patients into four groups. Group I (n=20815); Group II (n=15961), 15258 canines; Group III, tumor alterations (n=403), female and male canines; Group IV, females with CMT diagnosis (n=139) (Table 1). We classified the data into 30 categories for each tumor alteration (ICD-O), in addition to a non-determined category. The general prevalence of the tumor alterations was 2.52% (403/15961). The most affected system during 2014-2017 was the mammary gland (code C50), 34.5%, followed by the skin (C44), 88 cases (21.8%). We found 139 alterations of the mammary gland that corresponded to CMT. We excluded those of different origin. The general prevalence of CMT was 0.87% (139/15961).

Prevalence by breed, age, and body condition. Purebreds were more affected (79.14%) compared to mixed-breeds (20.86%) (Table 2). The most affected breeds were French Poodle (24%), Pinscher (10%), Miniature Schnauzer, Cocker Spaniel, Beagle, and Labrador Retriever (Table 2). The mean age of the patients was 9.3 years old (SD= ±3.68) and the median was 10 years. The age range was Xmin 1year - Xmax 16 years. The most reported age ranges were 10 to 12 years old (n=24; 17.3%), followed by 7 to 9 years (n=21; 15.1%). Females older than 12 years (n=13; 9.4%), 4 to 6 years old (n=7; 5%) and less than 4 years old (n=6; 4.3%). We found that 48.9% (n=68) of the clinical records did not describe the age of the patient. Moreover, 75.5% (n=80) were patients with normal weight, 10.4% (n=11) were overweight, and 15 patients were obese (n=15; 14.1%). We did not include mixed-breeds (n=29).

Pearson's chi-squared test with Yates' correction for continuity showed a chi-squared value of 5.4287 ($p=0.019$) for breeds, indicating a positive relationship between breed and CMT. This result agrees with the breed-wise RR of 3.96/100-females, indicating a high risk (OR=1.64). Fisher's test showed a strong relationship between CMT and age ($p<0.001$). The ages from 9 to 12 years showed the highest significant risk for CMT (OR 2.81). We did not find a significant relationship between body mass (weight) and the presence of CMT ($p=0.051$).

Table 1. Tumor alterations by organ system in canines of Manizales, Colombia from 2014-2017.

Sitie	Topographic Code (ICD-O)[a]	Total (%)		Female (%)		Male (%)	
Tongue, UP	C02	1	0.2	1	0.2		0
Mouth floor	C04	1	0.2		0	1	0.2
Mouth UP	C06	18	4.0	8	1.8	10	2.2
Nasopharynx	C11	2	0.4	1	0.2	1	0.2
Small intestine	C17	1	0.2	1	0.2		0
Anus and anal canal	C21	2	0.4		0	2	0.4
Liver and intrahepatic ducts	C22	9	2.0	2	0.4	7	1.6
Other digestive organs (intestine, GI)	C26	1	0.2	1	0.2		0
Bronchi and Lung	C34	1	0.2	1	0.2		0
Heart, mediastinum, pleura	C38	1	0.2	1	0.2		0
Bones, joints, cartilage	C40	1	0.2	1	0.2		0
Bones, joints, cartilage, UP	C41	2	0.4	1	0.2	1	0.2
Hematopoietic and reticuloendothelial system	C42	3	0.7	1	0.2	2	0.4
Spleen	C42.2[a]	8	1.8	1	0.2	7	1.6
Skin	C44	88	19.7	37	8.3	51	11.4
Connective, subcutaneous and other tissue	C49	8	1.80	6	1.3	2	0.4
Mammary gland	C50	139	40.8	139	31.4		0
Vulva	C51	1	0.2	1	0.2		0
Vagina	C52	23	5.2	23	5.2		0
Uterus	C55	1	0.2	1	0.2		
Ovaries	C56	1	0.2	1	0.2		0
Penis	C60	25	5.6		0	25	5.6
Prostate gland	C61	4	0.9		0	4	0.9
Testicles	C62	13	2.9		0	13	2.9
Kidney	C64	2	0.4	1	0.2	1	0.2
Eyes and annexes	C69	5	1.1	1	0.2	4	0.9
Brain	C71	2	0.4	1	0.2	1	0.2
Thyroid gland	C73	1	0.2		0	1	0.2
Lymph nodes	C77	1	0.2		0	1	0.2
Unknown Primary Site	C80	1	0.2		0	1	0.2
Undetermined	ND	37	8.3	26	5.8	11	2.5
Total		403	100	257	63.8	146	36.2

[a]ICD-O: Structure of the topographic code. Site=(C00), sub-site=(.0).

Table 2. Breed-wise morbidity from CMT in canines of Manizales, Colombia, according to the classification by Salt et al (25).

Breed type (Kg)	Breed	Total cases	Proportion(%)
I (< 6.5)	Chihuahua	1	1
	Maltese	1	1
	Pinscher	14	10
	Yorkie	1	1
	Yorkshire Terrier	4	3
Total		21	15.1
II (6.5 to < 9)	Jack Russell Terrier	1	1
	Pekingese	1	1
	Miniature schnauzer	5	4
	Shih Tzu	2	1
Total		9	6.5
III (9 to < 15)	French bulldog	2	1
	Cocker Spaniel	5	4
	Pug	1	1
	Beagle	5	4
	Boston terrier	1	1
	Fox Terrier	1	1
	French Poodle	34	24
Total		49	35.3
IV (15 to < 30)	Basset Hound	3	2
	English bulldog	3	2
	Pit Bull	4	3
Total		10	7.2
V (30 to < 40)	Golden Retriever	4	3
	Labrador Retriever	6	4
	German shepherd	4	3
	Siberian Husky	4	3
Total		18	12.9
VI (40+)	Rottweiller	3	2
Total		3	2.2
Other	Mongrel	29	21
Total		29	20.9
Total		139	100

The chi-squared test indicated a relationship between CMT and homemade food diet ($p<0.001$) and a RR=1.8 showed a high risk for this variable. We did not find a relationship for CMT and the reproductive stage ($p=0.913$), OR=1.47, indicating it is a non-significant risk factor. Although, this could be attributed to the few reports on the reproductive stage of the patients (Table 3).

Clinical aspects. Twenty-four clinical records provide reports of TNM staging the left mammary ridge being the most compromised. The inguinal pairs are the most affected (pair 5: 18.2% left and 15.6% right). For the variable diameter of the primary tumor (T) of the TNM staging system, we found that T1 (<3cm diameter) is the most reported with 38.46%, followed by T3 (3-5cm, 29.23%), and T2 (> 5cm, 20%). T4 (inflammatory carcinoma) comprises tumors adhered to the skin (6.15%), as well as non-fixed (4.62%) and 1.54% fixed to the muscle. Absence of compromise to the regional lymph node-LN (N) and distant metastasis (M) was 70.8% (N0, M0), N1 and M1 (29.2%) for both. The predominant Clinical Stage (CS) was CS-V (25%), followed by CS-I, CS-III, and CS-IV (20.8%), respectively, and CS-II, 12.5%.

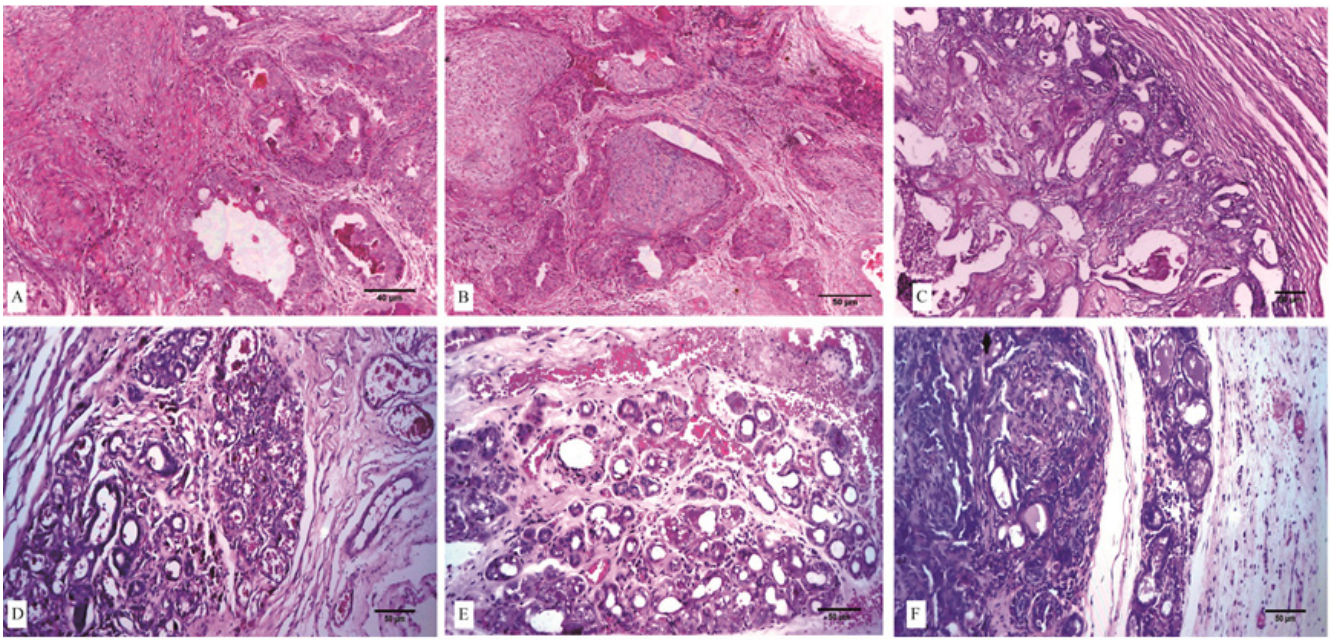
Of the histopathological findings, 18% were benign neoplasia, non-determined (ND) hyperplasia, mammary adenoma, and fibroadenoma. On the other hand, 5% were malignant neoplasia, mammary carcinoma-ND, papillary carcinoma, tubular carcinoma, and mixed tumor carcinoma. Figure 1 shows histopathological diagnostic slides of some of the females included in this study. Unfortunately, 77% were CMT reports with no definitive histopathological diagnosis. Metastases to the lung and the LN were reported in 31% and 38% of cases, respectively.

The Kaplan-Meier curve indicated a higher survival of patients that were surgically intervened, showing a survival period of 2013 days after diagnosis with surgery compared to 1484 days without surgery. The survival period after surgery was 2005 days. We did not observe significant differences for both analyses ($p=0.31$ and 0.183, respectively) (Figure 2). The analysis of the clinical records was challenging, particularly due to incomplete records and unclear reports of the clinical findings.

Table 3. Odds Ratio (OR) with a 95% confidence level (95%CI), probability and risk for patients diagnosed with CMT. Variables breed, age, weight, type of diet, reproductive stage.

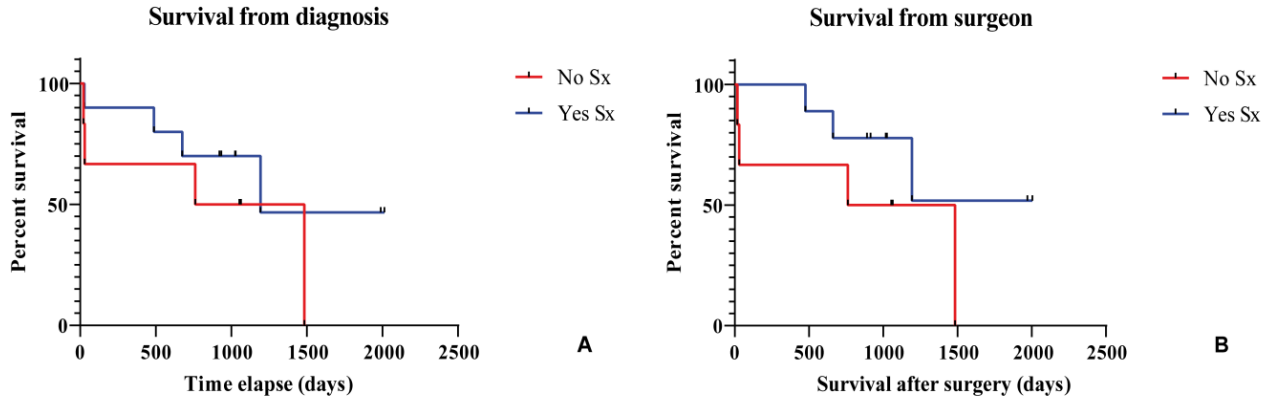
Category	CMT		Total	OR	95% CI	Probability
	Diseased	Healthy				
Breed						
Pure	103	233	336	1.64[a]	1.08-2.49	62%
Mongrel	26	73	99	0.61[b]	0.40-0.92	38%
Age (Years)						
0-4	19	167	186	0.3[b]	0.16-0.56	23%
5-8	19	167	186	0.36[b]	0.18-0.71	26%
9-12	22	80	102	2.81[b]	1.42-5.58	74%
≥ 13	19	55	74	1.33[c]	0.59-3.03	57%
Weight						
Normal	80	65	145	0.64[c]	0.39-1.03	39%
Overweight / Obesity	26	22	48	1.57[c]	0.97-2.56	61%
Feed Type						
Balanced feed	27	176	203	0.14[b]	0.08-0.26	12%
Mixed / homemade food	14	14	28	7.05[a]	3.81-13.04	88%
Reproductive state						
Intact	32	130	162	1.47[c]	0.68-3.16	60%
Spayed	7	66	73	0.68[c]	0.32-1.47	40%

Meaning: [a.] Risk factor, statistically significant; [b.] Protection factor, statistically significant; [c.] Not significant



(A) Tubular carcinoma with *in situ* areas. H&E. 10x. Pleiomorphic epithelial cells and mitotic figures present. Loss of the basal layer continuity. (B) Mixed tumor carcinoma. H&E. 40x. (C) Tubular carcinoma. H&E. 10x. Epithelial proliferation in tubule configuration. (D). Adenosis. H&E. 10x. Periductal tissues altered and lobular dilation. (E) Adenosis with hemorrhage. H&E. 10x. (F) Benign mixed mammary tumor. H&E. 10x. Mesenchymal proliferation and high epithelial cellularity.

Figure 1. Photomicrographs of tissue after hematoxylin and eosin staining (H&E), showing histopathology of mammary lesions in canines (Manizales, Colombia). Scale bars equal 40 or 50 µm.



Sx: Surgery

Figure 2. Kaplan-Meier survival curve. Female canines with CMT. Difference in survival between groups without (A) and with (B) surgery after diagnosis.

DISCUSSION

General prevalence. Females and males diagnosed with tumor lesions accounted for 63.8% and 36.8%, respectively, which agrees with a previous study (32) reporting 58% lesions for females and 39% for males. We found that alterations of the mammary gland are the most frequent cause of consultation (1,5,12,13,33,34,35).

Prevalence by age, breed, and body condition. The prevalence of CMT was higher in purebreds, consistent with the results of other studies (9,10,23,24,35). Medium-sized and small breeds are more frequently diagnosed with CMT than large breeds (10,35). French Poodle is the most common among the purebreds, a finding that agrees with previous studies conducted in the same city (36) and other cities of Colombia (12,13). Cocker Spaniel and Beagle also show high prevalence (10). For large breeds, Labrador Retriever, Golden Retriever, and German

Shepherd were the most reported (9,11,12,32). The presence of CMT and its frequency in certain breeds greatly depends on the popularity of the breeds, degree of ownership, and trends for certain breeds in a given area. For this reason, no general tendency was observed. However, in small breeds, an earlier diagnosis of tumor alterations is more frequent since these breeds are more easily managed by their owners.

The mean age was 9.3 years with a higher prevalence between 10 and 12 years old. Our findings agree with previous studies (11,36,37) and with age ranges reported by different authors, 9-11 years old (4,20), 6-10 years (9,35), 7-11.9 years (33), 10-11.9 years (34), 8-11 years (32), and 8-10 years (13). In particular, six years old is known as the "age of cancer" (1,15,19). Furthermore, the incidence of the disease increases with age, with maximum peaks between 9 and 10 years old (1,35) and decreases around 12 years old. Another peak is observed at approximately 13 years old and there are also exceptional cases before 5 years old. The age of 10 in canine females is considered equivalent to 58 in women, and these are the mean ages of cancer presentation in both groups (38).

Clinical aspects and prevalence. The inguinal pairs (pair 5) were the most diagnosed with CMT (16.9%). Several findings indicate that pairs 4-5 were the most susceptible to CMT presentation (7,10,37). CMT is less frequent in the cranial thoracic glands. The affected area can involve a single gland (10) or the inguinal and cranial glands simultaneously (24). The patients showed greater compromise of the left side (7), but these findings differ from other studies (e.g., 9). Conventionally, the size of the mammary glands tends to decrease from the cranial to the caudal; in particular, the thoracic glands are the smallest, the abdominal glands are intermediate, and the inguinal are the largest. However, tumor presentation depends on other factors as well, including a change in the lymphatic drainage pattern through lymphangiogenesis during CMT development (39).

The diameters T1 and T3 were the most frequent, although we did not find patterns for diameter or clinical stage that indicated a dominance of any (13,35) since both displayed relatively similar percentages of presentation for the patterns of metastasis, LN or distant (7).

Among benign tumors, we mainly found tumors of epithelial origin such as benign mixed tumors, complex adenomas, fibroadenomas, and papillary adenomas (11). The most frequently diagnosed malignant tumors of epithelial origin were carcinoma in mixed tumor, tubular carcinoma and, papillary carcinoma. Other authors have also reported these tumors as the most prevalent (12). Inflammatory lesions can be confused with tumor alterations (40). Histopathologically, mammary inflammations are characterized by mononuclear and mixed infiltrates. Small and hard masses are usually benign, while large masses that tend to ulcerate are generally malignant (9). The survival rate is favored by surgical intervention (7,17), yet it can also be affected by other factors.

This study provides an update of the epidemiological variables of CMT and discusses the risk factors that affect the development of the disease. The findings are highly relevant considering the importance of canines as sentinels for CMT in humans. We identify the need and opportunity of continuing to develop epidemiological studies that correlate environmental factors with patterns of CMT occurrence. Furthermore, the registration, management, and definitive histopathological diagnosis of mammary neoplasia must be improved. This can be achieved by optimizing and integrating medical record storage systems to enable data management and analysis at the population level. Unless this is accomplished, it will be difficult to determine the real impact of CMT on public health.

Conflict of Interests

The authors declare that there are no conflicts.

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