Canine mammary tumors in Santos, Brazil: clinicopathological and survival profile

Neoplasias mamárias caninas em Santos, Brasil: perfil clinicopatológico e de sobrevida

Luiz Roberto BIONDI[1,2]; Luciana Boffoni GENTILE[1]; Alexandre Aparecido Mattos da Silva REGO[3]; Nathalia Pisciottano NORONHA[2]; Maria Lucia Zaidan DAGLI[1]

[1] Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Patologia, Laboratório de Oncologia Experimental e Comparada, São Paulo – SP, Brasil
[2] Universidade Metropolitana de Santos, Faculdade de Medicina Veterinária, Santos – SP, Brasil
[3] Pet Legal, Laboratório Privado de Patologia Animal, São Paulo – SP, Brasil

Abstract

Tumors of the mammary glands are the most common neoplasms in dogs in our country; however, there are few Brazilian reports dedicated to clinicopathological and survival studies about this disease. This report aims the clinical and pathological study of canine mammary tumors in the Santos Metropolitan Region, an area in Sao Paulo state with an estimated canine population of 120,000 animals. Data of 14,298 dogs were collected retrospectively from the medical records of the Veterinary Medical Teaching Hospital of the Metropolitan University of Santos – São Paulo – Brazil. During the study period, from records of 317 females with histopathological diagnosis of neoplasia, 170 were mammary epithelial lesions distributed in 13 benign tumors, 152 malignant (89.4% of diagnosis) and 5 non-neoplasic epithelial lesions (ductal hyperplasia). The highest prevalent malignant tumor was tubular carcinoma (38.2% of diagnosis) and Grade I tumors, corresponding to 73.0% of all diagnosis. The results have shown clinical staging of canine mammary neoplasms as an important prognostic survival factor and, in a multivariate analysis, tumor diameter, tumor grade, adjuvant chemotherapy and recurrence as covariates with predictive value for survival. Moreover, the high prevalence of tubular carcinoma qualifies the canine population of Santos as a promising model for the translational study of this disease.

Keywords: Clinical stage. Chemotherapy. Recurrence. Survival. Tumor diameter.

Resumo

Os tumores das glândulas mamárias são as neoplasias mais comuns em cadelas em nosso país; no entanto, são poucos os trabalhos brasileiros dedicados ao estudo clinicopatológico e de sobrevida nesta doença. O presente trabalho teve por objetivo o estudo clínico e patológico dos tumores mamários caninos na Região Metropolitana de Santos, uma área no estado de São Paulo com uma população canina estimada em 120 mil animais. Dados de 14.298 cães foram coletados retrospectivamente dos prontuários médicos do Hospital Veterinário da Universidade Metropolitana de Santos – São Paulo – Brasil. Durante o período do estudo, foram atendidas 317 fêmeas com diagnóstico histopatológico de neoplasia, dos quais, 170 se referiam a lesões mamárias epiteliais distribuídas em 13 tumores benignos, 152 malignos (89,4% dos diagnósticos) e 5 lesões epiteliais não-neoplásicas (hiperplasia ductal). O tumor mais frequente foi o carcinoma tubular (38,2% dos tumores malignos) e tumores de grau I, respondendo por 73,0% do total diagnosticado. Estudos de sobrevida apontaram para o estadiamento clínico das neoplasias mamárias caninas como importante fator prognóstico, e na análise multivariada, diâmetro do tumor, grau histológico, quimioterapia adjuvante e recorrência apresentaram-se como covariáveis com valor preditivo de sobrevida. Levando-se em conta a elevada prevalência de carcinoma tubular simples na população canina de Santos, pode-se considerá-la como promissor modelo translacional para o estudo da doença.

Palavras-chave: Estadiamento. Quimioterapia. Recidiva. Sobrevida. Diâmetro tumoral.

Introduction

Cancer is a leading cause of death in both humans and canines and their predictable behavior of malignancy and metastatic pattern qualify the naturally occurring cancer in dogs as a model for the study of breast cancer in humans, not possible with other animal model system (PINHO et al.,

Correspondence to:
Luiz Roberto Biondi
Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Patologia, Laboratório de Oncologia Experimental e Comparada
Av. Prof. Dr. Orlando Marques de Paiva, 87
CEP 05508-900, São Paulo, SP, Brasil
e-mail: lrbiondi@usp.br

Received: 21/11/2013
Approved: 05/09/2014

DOI: 10.11606/issn.1678-4456.v51i3p252-262

2012; PORRELLO; CARDELLI; SPUGNINI, 2006). Dogs are also exposed to the same factors that affect tumor initiation and progression in humans, such as age, hormones, nutritional factors, reproductive status and environmental factors (KHANNA et al., 2006; PAOLONI; KHANNA, 2007; UVA et al., 2009). Additionally, the genetic sequencing of canine genome highlighted its close relationship with the human genome (DAGLI, 2006; KHANNA et al., 2006; UVA et al., 2009), pointing out the involvement of the same oncogenes and signaling pathways as in human carcinogenesis (PINHO et al., 2012).

In some regions of the world, mammary gland tumors are the most common neoplasms in female dogs, a disease that represents a highly heterogeneous group in terms of morphology and biological behavior (GAMA; ALVES; SCHIMITT, 2008; NERURKAR et al., 1989). Although the prevalence of these tumors is decreasing in regions where preventive sterilization is performed, it still remains an important disease in veterinary medicine (SLEECKX et al., 2011). In developed countries, it is reported that about 50% of canine mammary tumors are malignant and the recognition of reliable prognostic factors is essential to identifying the individual risks of unfavorable clinical onset (CASSALI et al., 2011; DAGLI, 2006; NERURKAR et al., 1989) such as tumor size, histological type, histological grading and lymph node involvement (DAGLI, 2006; CASSALI et al., 2011; PÉREZ-ALENZA et al. 2000; YAMAGAMI et al., 1996).

The present study aims to assess the clinical and pathological behavior of canine mammary tumors and survival of female dogs related to this disease, in Santos Metropolitan Region, São Paulo State, an area with a human population of 1.6 million people according to 2010 Census of the Brazilian Institute of Geography and Statistics – IBGE (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2013) and a canine population estimated in 120,000 animals.

Material and Methods

Medical records of 14,298 dogs examined at the Veterinary Medical Teaching Hospital of the Metropolitan University of Santos between 2005 and 2010 were retrospectively reviewed for clinical diagnosis of mammary neoplasm.

Cases were eligible for inclusion in this study when main histological diagnosis of benign mammary lesion or mammary carcinoma was confirmed; if adequate follow-up information was available and the minimum follow-up of 18 months starting with mastectomy date was accomplished. Records of females with no mammary gland tumors, like sarcomas or round cell tumors, were not included.

The clinical information obtained from medical records included sex, breed and weight, age at diagnosis, reproductive status and age at surgical sterilization, number and location of affected glands, pulmonary and lymph nodes involvement, laboratorial exams (blood count, renal and liver function tests), previous treatments, histological classification and tumor grade. Information about the survival time was performed by telephone contact with animal owners.

For statistical purposes, the animals were grouped into sizes: **large/giant:** > 25 kg; **medium size:** > 15 kg to < 25 kg; **small:** > 5 kg to < 15 kg and **toy** < 5 kg.

Clinical stage was assigned according to WHO TNM classification (OWEN, 1980). Tumor size was identified as the maximum diameter of the largest tumor in dogs with more than one tumor, as previous proposed (SORENMO et al., 2011). Distant metastasis, such as involvement of lungs was investigated by three-way thoracic radiography while lymph nodes involvement was investigated by cytological and/or histological examinations.

The slides of the tumors, previously classified according to Misdorp et al. (1999), were reevaluated according to the criteria proposed by Cassali et al. (2011), whereas histological grading was based on the Elston and Ellis method as described (CASSALI et al., 2011; GAMA; ALVES; SCHIMITT, 2008;

KARAYANNOPOULUS et al., 2005; SLEECKX et al., 2011). According to medical records, only fragments of the greatest tumor were considered for histopathological study, regardless to the number of affected glands.

Mammary gland tumor was considered cause of death only when unequivocal evidence such as euthanasia due to the metastatic disease or death by mammary gland neoplastic cachexia was reported by owners or clinicians.

The statistical analysis was performed using commercial software (Graphpad Prism® v.5.0 or Medcalc® v.12.3.0), considering statistically significant $P < 0.05$.

Overall survival time was calculated from the date of surgical removal of the tumor to the date of death, which was considered the endpoint of the study. Only animals with malignant mammary disease were computed in survival analysis. Kaplan-Meier method was used to compute overall survival time while the log-rank test was used to identify factors associated with survival after mastectomy. Deaths unrelated to the mammary gland tumors or animals that were lost to follow-up were considered censored for statistical purposes. The Cox proportional hazards model was used as univariate and multivariate analysis in search of factors potentially associated with survival. Variables of biological importance or that were found to be significant in univariate analyses were selectively included in the forward multivariate model.

Results

Of the 14,298 medical records of this study, there were 317 female with diagnosis of neoplastic disease including 170 mammary lesions from epithelial and myoepithelial origin, corresponding to 53.6% of all neoplastic diagnosis in this gender. Among the diagnoses of mammary cancer, 7.6% (13/170 females) had benign tumors diagnosis while malignant tumors accounted for 89.4% (152/170 females). The highest frequent histological tumor type was tubular carcinoma (38.2%) and grade I tumors, accounting for 73.0% of total diagnosis, as summarized in table 1.

Survival analysis related to malignant histological tumor type did not show any statistical difference between groups, as demonstrated in figure 1. However, the survival rate evaluating tumor grade as a predictor variable, showed a highly significant statistical difference between them, as shown in figure 2.

Table 1 – Distribution of mammary epithelial lesion by histological type and grade in Santos – Sao Paulo – Brasil – 2014

			Grade		
Lesion	**Type****	**Frequency**	**I**	**II**	**III**
NNEL*	Ductal hyperplasia	5 (2.9%)			
Benign	Adenoma	7 (4.1%)			
	Complex adenoma	4 (2.4%)			
	Mixed benign tumor	2 (1.2%)			
	Benign subtotal	13 (7.6%)			
Malignant	Tubular carcinoma	65 (38.2%)	54 (35.5%)	10 (6.6%)	1 (0.7%)
	Complex carcinoma	46 (27.1%)	35 (23.0%)	11 (7.2%)	
	Mixed carcinoma	16 (9.4%)	15 (9.9%)	1 (0.7%)	
	Solid carcinoma	14 (8.2%)		1 (0.7%)	13 (8.6%)
	Papillary carcinoma	9 (5.3%)	7 (4.6%)	2 (1.3%)	
	Ductal carcinoma *in situ*	2 (1.2%)			
	Malignant subtotal	152 (89.4%)	111 (73.0%)	25 (16.4%)	14 (9.2%)
	Total	**170 (100%)**			

* NNEL – Non-neoplastic epithelial lesion

** According to Cassali et al., (2011)

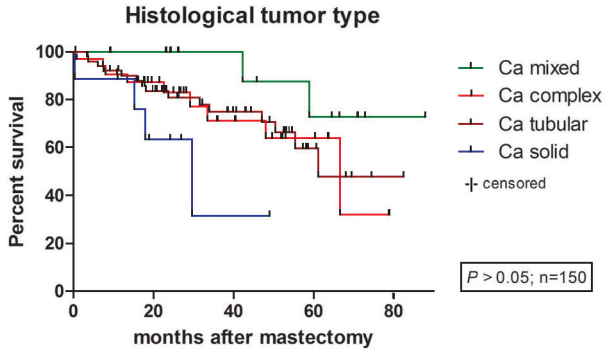

Figure 1 – Kaplan-Meier survival probability following mastectomy for 150 dogs grouped according to histological tumor type as a prognostic factor. Log-rank test (Mantel-Cox) $P = 0.1141$ Chi-square 5.950 DF = 3. Ductal carcinoma *in situ* not included
Source: (BIONDI et al., 2014)

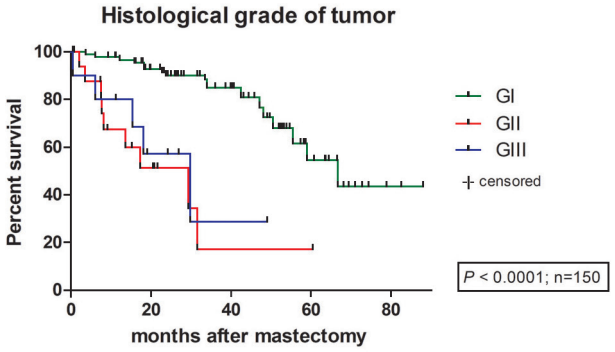

Figure 2 – Kaplan-Meier survival probability following mastectomy for 150 dogs grouped according to histological grading of malignant tumors as a prognostic factor. Log-rank test (Mantel-Cox) $P < 0.0001$ Chi-square 21.26 DF = 2. Ductal carcinoma *in situ* not included
Source: (BIONDI et al., 2014)

The mean age at diagnosis was 9.3 ± 2.32 years, with lower and upper limits of, respectively, 2.4 and 9.3 years.

The study population consisted of different breeds with different reproductive status, predominantly non-castrated females (146/170), with mean age at sterilization surgery of 7 ± 3.42 – range of 0.5 to 14 years. Information about the regularity of estrus, pseudopregnancy and use of hormonal therapy for heat prevention did not show any constancy in medical records, and therefore it could not be assessed with certainty to be included in this study.

The greater relative frequency of mammary tumors was found in English cocker spaniel, boxer, German shepherd, and poodle female dogs (Table 2), with statistically significant association between breed and mammary tumor (G-Test 57.0507 DF 9 $P < 0.0001$), however, no significant statistical difference was found when comparing purebred and mongrel dog at Chi-square analysis (Chi-square 5.341 DF 1 P>0.05) or between animals grouped by size (p = 0.5857; Chi-square 1.937, DF 3, n = 152), age at sterilization (p = 0.9298; Chi-square 0.008; DF 1, n = 24) or previous castration (p = 0.1559; Chi-square 2.104, DF 1, n = 152) at survival analysis.

Regarding tumor presentation, 100/170 (58.82%) animals had more than one tumor at diagnosis, 22/170 (12.94%) had ulcerated tumors and 16/170 (9.41%) had tumors adhered to deep tissues. Mean tumor size was 4.76 ± 3.86 cm, with lower and upper limits respectively 1.0 and 25.0 cm. Survival analysis of maximum tumor size as predictive variable showed significant statistical difference with favorable prognosis among animals with tumors smaller than 5 cm in diameter, as showed in figure 3. However, survival analysis between females with single

Table 2 – Mammary neoplasm distribution among different breeds in Santos – São Paulo – Brasil – 2014

	Mammary tumor		
Breed	**non affected**	**affected (%)**	**TOTAL**
English cocker spaniel	269	19 (6.6)	288
boxer	144	9 (5.9)	153
German shepherd	266	14 (5.0)	280
poodle	940	41 (4.2)	981
pinscher	279	9 (3.1)	288
Labrador retriever	123	4 (3.1)	127
teckel	287	7 (2.4)	294
mongrel dog	2329	47 (2.0)	2376
rottweiler	287	4 (1.4)	291
other breeds	1322	11 (0.8)	1333
Total	**6246**	**165**	**6411**

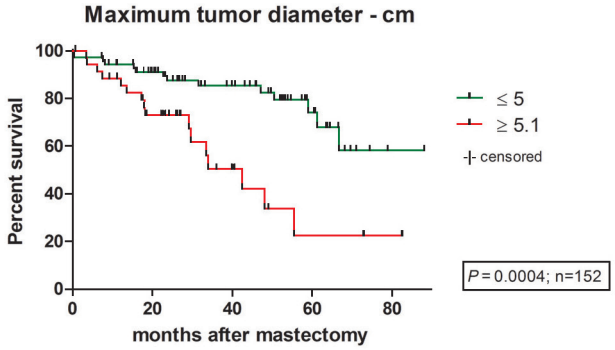

Figura 3 – Kaplan-Meier survival probability following mastectomy for 152 dogs grouped according to maximum tumor diameter as a prognostic factor. Log-rank test (Mantel-Cox) $P = 0.0004$ Chi-square 12.5 DF = 1
Source: (BIONDI et al., 2014)

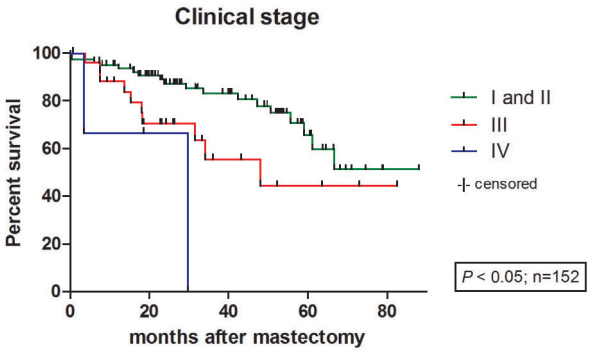

Figure 4 – Kaplan-Meier survival probability following mastectomy for 152 dogs grouped according to clinical stage based on WHO TNM method as a prognostic factor. Log-rank test (Mantel-Cox) $P = 0.0078$ Chi-square 9.700 DF = 2
Source: (BIONDI et al., 2014)

tumor presentation (63/152 females) or multiple presentations (89/152 females) did not show any statistical difference.

Although the presence of metastasis at diagnosis is considered an important prognostic factor and the mean survival time of animals with metastasis in this study was as small as 5 months, this variable was not considered for survival analysis purposes due to the limited number of animals within these conditions (three animals showed cutaneous metastasis of the mammary cancer, confirmed by cytology and histopathology – tubular carcinoma grade I and II and a complex carcinoma grade II respectively, and only two animals showed lung metastasis, diagnosed by thoracic radiographic examination – solid carcinoma grade III; mixed carcinoma grade II respectively). Once WHO TNM classification takes into account these parameters, distant and regional metastasis were both assessed by clinical staging. The survival analysis of the clinical stage as a predictive variable has shown significant statistical difference between stages, as established at figure 4.

Forty-eight animals (48/170; 28.23%) underwent total mastectomy, 122/170 (71.76%) underwent partial mastectomy and 93 females (93/170; 54.70%) underwent ovariohysterectomy simultaneously. Among intact animals, no significant difference was found at survival analysis between individuals that underwent simultaneously ovariohysterectomy from those ones, not submitted to this surgery. Also, no significant statistical difference at survival rate was found between animals submitted to radical or partial surgery.

The clinical follow-up showed recurrence or new mammary tumor presentation in nineteen (19/170; 12.5%) females after mastectomy. Survival analysis of this predictive variable revealed statistically difference, with a 19 times greater risk for the group of recurrence or new tumors, as showed in figure 5 (Hazard ratio 19.91; 95% CI of ratio = 7.904 to 50.17).

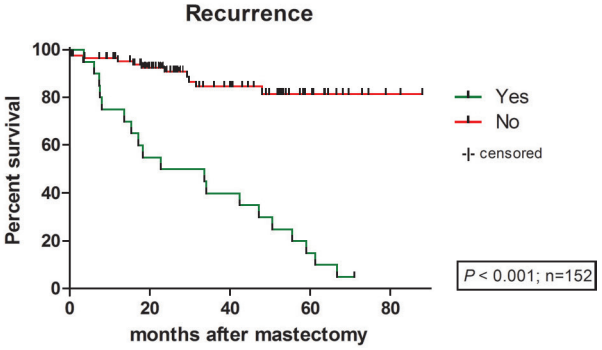

Figure 5 – Kaplan-Meier survival probability following mastectomy for 152 dogs grouped according to recurrence or new tumor presentation as a prognostic factor. Log-rank test (Mantel-Cox) $p < 0.0001$ Chi-square 40.26 DF = 1 HR 19.91 95% CI of ratio 7.904 to 50.17
Source: (BIONDI et al., 2014)

Chemotherapy was performed in seventeen females that were submitted to adjuvant antineoplastic treatment with doxorubicin plus cyclophosphamide (TODOROVA et. al., 2005). There was statistically significant difference between treated and untreated groups according to log-rank test, as shown in figure 6.

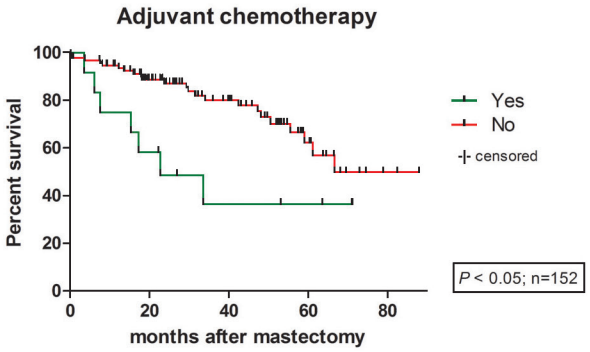

Figure 6 – Kaplan-Meier survival probability following mastectomy for 152 dogs grouped according to adjuvant or neoadjuvant chemotherapy as a prognostic factor. Log-rank test (Mantel-Cox) *P* = 0.0104 Chi-square 6.650 DF = 1 HR 4.907 95% CI of ratio 1.453 to 16.58
Source: (BIONDI et al., 2014)

At the end of the study, 42/170 (24.70%) animals were still alive, 80/170 (47.0%) had died and 48/170 (28.23%) were missing in the follow-up. Amongst the deaths, 33/80 (41.25%) were attributed to mammary cancer, 9/80 (11.25%) to others tumors developed after mastectomy (liposarcoma, hemangioma, epidermoid carcinoma, fibrosarcoma, hemangiosarcoma, and osteosarcoma) and the remaining (38/80; 47.5%) to other causes as natural aging, heart failure or renal failure.

There was no hematological or biochemical alteration that could be statistically related to mammary tumor and/or to the clinical course of the disease.

Cox multivariate proportional hazard model performed for selected covariate showed a highly significant statistical difference with P < 0.0001 on overall model fit (Table 3). Animals with tumors larger than 5 cm in diameter showed 72.1% greater likelihood to death than animals with smaller tumors. The same was observed regarding tumor grade, with 75.5% and 90.1% probability of death, respectively for grades II and III, as well as to neoadjuvant chemotherapy and recurrence, with a likelihood respectively of 73.2 and 91.8% of death compared to control animals (untreated/no recurrence).

Discussion

Cancer clinicopathological studies in veterinary medicine are very important tools to better understand the behavior of cancer disease in a particular population and set further strategies for cancer control.

With this work, in a retrospective study of 6 years and based on a minimum 18 months follow-up, we were able to identify the specific frequency of different histological types of mammary tumors in female dogs in our region, the significant prevalence of malignant mammary tumors in this population and the importance of clinical staging and histologic tumor grade as prognostic factors on this disease.

Table 3 – Cox proportional hazard model for selected covariates. Canine mammary neoplasm in Santos – São Paulo – Brasil – 2014

Covariate	b	SE	P	Exp(b)	95% CI of Exp(b)
Maximum tumor size = > 5 cm	0.9497	0.4685	0.0426	2.5848	1.0368 to 6.4442
Grade = II	1.1272	0.4884	0.0210	3.0869	1.1911 to 8.0000
Grade = III	2.2119	0.6740	0.0010	9.1326	2.4537 to 33.9909
Adjuvant chemotherapy = Yes	1.0026	0.4768	0.0355	2.7253	1.0756 to 6.9049
Recurrence = Yes	2.4147	0.4539	<0.0001	11.1864	4.6167 to 27.1049

P < 0.0001 for overall model fit. Forward method

The prevalence of mammary tumors diagnosis in the population of this study (53.6%) is in agreement with previous reports, which describe canine mammary neoplasm as a highly prevalent disease in dogs, ranging from 26 to 73% (ANDRADE et al., 2010; MUNSON; MORESCO, 2007; OLIVEIRA FILHO et al., 2010; PÉREZ-ALENZA et al., 2000). However, the high frequency of malignant tumors among the diagnosis of mammary neoplasia in this population is opposed to the literature that describes the disease in developed countries as around 50% (SLEECKX et al., 2011; SORENMO, 2003) but is in agreement with Brazilian reports. In fact, Toríbio et al. (2012), who studied a population of 132 female dogs carriers of mammary tumors from Salvador – Bahia – Brazil, observed a 90.9% malignant tumor frequency, similar to the results previously described by Filgueira, Araújo and Silva (2005), who studied 35 female dogs with this disease in Ceará – Brazil, and showed a 70.4% frequency of adenocarcinomas, and by Oliveira Filho et al. (2010) who have obtained similar results in a study in Rio Grande do Sul – Brazil. Although this observation could be attributed to the high average age at which the animals were sterilized, one must consider that there is a growing concerning with the association between cancer and environmental pollutants in humans and studies have shown some pesticides acting as xenoestrogens and its relation with the increased incidence of cancer (SNEDEKER, 2001). Once the Port of Santos has an historic relation with environmental contamination, it could in principle be another explanation for the higher incidence of malignant mammary neoplasm in this population. Indeed, Zago et al. (2005) studying the mortality from breast cancer in women in the metropolitan area of Santos, found a significant difference between the mortality in this population, with 25/100,000 cases per year versus 9.7/100,000 in other Brazilian regions, attributing this difference to soil and water contamination by organochlorines and dioxins in that region. In the veterinary filed, also Andrade et al. (2010), who have studied by chromatography the concentration of pyrethroids in the adipose tissue adjacent to mammary tumor in female dogs, warned to the high levels of contamination (33.3%) by different types of pyrethrins in this kind of tissue.

Although our findings about survival related to histological tumor type is in disagreement with current reports (GOLDSCHMIDT et al., 2011; PÉREZ-ALENZA et al., 2000; SLEECKX et al., 2011) the distribution for each histological type (Table 1 and Figure 1) and the grading of histological malignancy (Figure 2), associating Grade III as the worst prognosis, is in according to previous reports and as expected (GOLDSCHMIDT et al., 2011; KARAYANNOPOULUS; KALDRYMIDOU; CONSTANTINIDIS, 2005; PÉREZ-ALENZA et al., 2000; SORENMO et al., 2011). On the other hand, we found a higher frequency of simple tubular tumors, followed by complex carcinomas, unlike Peña et al. (2013), who found greater number of complex tumors and adenosquamous carcinoma in a study that evaluated 65 female dogs with mammary tumors and Santos et al. (2013), who found a greater number of solid and complex carcinomas in a cohort of 80 female dogs with spontaneous mammary gland tumors.

The average age at diagnosis (9.3 ± 2.32 years) corroborate published reports that associated mammary gland tumors to middle-aged and old bitches (DALECK et al., 1998; PEÑA et al., 2013; SLEECKX et al., 2011; SORENMO et al., 2011). Although we also found statistical significance at the analysis of survival based on age (data not included), we decided, unlike Peña et al. (2013) disregard this variable as a prognostic factor, since age, by itself, is an unfavorable survival factor regardless of the neoplastic mammary disease.

The findings regarding mammary gland cancer and breed association are in agreement with current reports as discussed by Sorenmo et al. (2011), who considered that mammary tumors may occur in any female dog of any breed and the reported breed

vary somewhat depending on where these studies originate, as pointed by this work, where some pure breeds showed an increased risk. However, animal distribution by size to assess survival rate did not show any statistical significance or trend, unlike Itoh et al. (2005) and Peña et al. (2013) who found an increased survival in small breed animals, probably due to differences between the studied populations.

As described, tumor size was significantly associated with prognosis, with the worst course of the disease associated with higher than 5 cm diameter tumors. These data are in better agreement with the reports of Chang et al. (2005) and Yamagami (1996) who also conducted a long term study and found favorable prognosis for females carrying tumors smaller than 5 cm. Also Ferreira et al. (2009) who studying tumor size and prognostic markers in 120 female dogs with mammary neoplasia, found lower survival rates and lower expression of progesterone receptors in those animals with tumors classified as T3 in the TNM system, ie, those larger than 5 cm in diameter and a higher expression of the proliferation marker MIB1 (68%) in this kind of tumor, with significant difference between T1 and T3 tumors. In opposition, Peña et al. (2013) and Santos et al. (2013) considerate for statistical purposes, the limit of 3 cm for tumor diameter.

Multiple versus simple tumor presentation has shown no significant difference in survival analysis, in disagreement with Pérez-Alenza et al. (1997), who associated disease free survival with multiple malignant tumor presentation. According to medical records, when at multiple presentation, the criterion for choice was the largest tumor size, making us unable to evaluate the different histological types that animals with more than one tumor may have presented. Taking into account that animals with multiple presentations are more exposed to tumor progression, it is possible that this finding is not consistent with the reality and was biased by the above mentioned criterion (SORENMO et al., 2009, 2011).

The survival analysis of the clinical stage has shown significant statistical difference between stages, consistent with current literature (CASSALI et al., 2011; CHANG et al., 2005; KARAYANNOPOULUS; KALDRYMIDOU; CONSTANTINIDIS, 2005; PEÑA et al., 2013; SLEECKX et al., 2011; YAMAGAMI et al., 1996) pointing this variable as an important and inexpensive tool available to clinicians. However it is remarkable the small number (5/170; 2.94%) of animals that developed metastatic disease, ie, stage grouping IV, as a result of neoplastic mammary disease. Whereas the follow-up was mainly based on phone contact, it is possible that among the deaths attributed by owners to breast tumors, have also been due metastatic disease, underestimating this observation.

An important issue that concerns clinicians is the value of simultaneous ovariohysterectomy at mastectomy. Statistical analysis indicated no significant difference between animals underwent to simultaneously ovariohysterectomy from that ones not submitted to surgery on survival rates, findings in agreement with Cassali et al. (2011) and as previously reported by Morris et al. (1998), who studied 90 bitches followed for two years after mastectomy and grouped in spayed before the diagnosis, spayed simultaneously with surgical tumor removal and left entire. However this observation is disagreement with Sorenmo, Shofer and Goldschmidt (2000), who studied the effect of spaying and the timing of spaying on survival in 137 dogs with mammary gland tumors, grouped according to spay status and spay time, and considered ovariohysterectomy an effective adjunct to tumor removal in dogs with mammary gland carcinoma and the timing of ovariohysterectomy an important factor influencing survival. However, the authors did not evaluate the effect of simultaneously ovariohysterectomy and mastectomy. Another conflicting finding was reported by Peña et al. (2013), who described a better prognosis on non-spayed females. Nonetheless, the authors did not

explain its apparently conflicting findings. Actually, in a systematic review regarding mammary tumors and neutering, Beauvais, Cardwell and Brodbelt (2012), evaluating papers published about this issue, concluded that there is no element that can support the recommendation of castration in reducing the risk of mammary tumors or that age at castration may influence this effect.

We did not also find any advantage between females submitted to partial or total mastectomy. In fact, the best choice for surgical treatment of mammary cancer in dogs (radical or partial excision/ simultaneous castration) is still controversial. In a study that evaluated 99 female dogs undergoing partial or radical mastectomy, although there was no difference in survival between the groups, the authors warn that 58% of the animals submitted to partial removal have shown new mammary neoplasms in the ipsilateral chain, requiring caution in the decision of keeping the mammary glands intact in these animals (STRATMANN et al., 2008), corroborating ours findings where animals with recurrence or presentation of new tumors showed a worse prognosis and a four time greater risk.

Regarding adjuvant chemotherapy we had an interesting finding once untreated animals have presented a greater survival when compared with those animals submitted to adjuvant chemotherapy. This contradictory finding could be attributed to the unfavorable clinical preoperative conditions of these animals once the choice for chemotherapy inclusion is directed for those animals that have greater scores at clinical staging and/or unfavorable histopathology diagnosis. In addition, Cassali et al. (2011) highlights the limited information regarding the benefits of adjuvant chemotherapy in the treatment of mammary cancer in bitches nowadays. Indeed, in a recent work conducted from 2003 to 2012, Tran, Moore and Frimberger (2014), evaluating retrospectively 94 animals with mammary carcinoma treated with surgery (58 animals) and adjuvant chemotherapy (36 animals), did not find statistically significant difference between groups treated and not treated with chemotherapy.

The deaths related to non-neoplastic diseases as well as the number of deaths from other diseases are in accordance to reports in the literature (BENJAMIN; LEE; SAUNDERS, 1999; STRATMANN et al., 2008). In fact, cancer has been considered a chronic disease in humans, and studies in the last decade have pointed to important differences between incidence and mortality, especially in patients with breast cancer (ALBERT; BENJAMIN; SHUKLA, 1994).

One of the limitations of our work was the difficulty to compare studies of survival and prognosis, once endpoints and different evaluation times are used between them. On average, several reports described studies with 18 to 24 month follow-up, whereas this study was based on 72 months follow-up, possible explanation for different findings with the current literature.

The results obtained in this study allow us to conclude that clinical staging of canine mammary neoplasms is an important and inexpensive prognostic factor for canine breast cancer. Furthermore, Cox multivariate proportional hazard model, associating tumor diameter, tumor grade, adjuvant chemotherapy and recurrence demonstrate that these covariates have great predictive value regarding the likelihood of death.

Moreover, the high prevalence of mammary malignant tumors, particularly tubular carcinoma, qualifies the canine population of Santos Metropolitan Region as a promising model for translational cancer studies.

Acknowledgements

This study is part of the thesis developed by Luiz Roberto Biondi in the Experimental and Comparative Pathology Program of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo, Brazil. The project was supported by grants from the Fundacao de Amparo a Pesquisa do Estado de Sao Paulo, FAPESP, Process number 2011/14177-4.

References

ALBERT, R. E.; BENJAMIN, S. A.; SHUKLA, R. Life span and cancer mortality in the beagle dog and humans. **Mechanisms of Ageing and Development**, v. 74, n. 3, p. 149-159, 1994. Available from: <http://www.sciencedirect.com/science/article/pii/0047637494900868>. Viewed: 18 Sept. 2013. doi: http://dx.doi.org/10.1016/0047-6374(94)90086-8.

ANDRADE, F. H. E.; FIGUEIROA, F. C.; BERSANO, P. R. O.; BISSACOT, D. Z.; ROCHA, N. S. Malignant mammary tumor in female dogs: environmental contaminants. **Diagnostic Pathology**, v. 5, n. 45, 2010. Available from: <http://www.diagnosticpathology.org./content/5/1/45>. Viewed: 18 Mar. 2014. doi: http://dx.doi.org/10.1186/1746-1596-5-45.

BEAUVAIS, W.; CARDWELL, J. M.; BRODBELT, D. C. The effect of neutering on the risk of mammary tumours in dogs – a systematic review. **Journal of Small Animal Practice**, v. 53, n. 6, p. 314-322, 2012. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1748-5827.2011.01220.x/abstract;jsessionid=9768748BC9E7255F7169D9313C5E6A8E.f03t02>. Viewed: 5 May 2014. doi: http://dx.doi.org/10.1111/j.1748-5827.2011.01220.x.

BENJAMIN, S. A.; LEE, A. C.; SAUNDERS, W. J. Classification and behavior of canine mammary epithelial neoplasms based on life-span observations in beagles. **Veterinary Pathology**, v. 36, n. 5, p. 423-436, 1999. Available from: <http://vet.sagepub.com/content/36/5/423>. Viewed: 25 Oct. 2013. doi: http://dx.doi.org/10.1354/vp.36-5-423.

CASSALI, G. D.; LAVALLE, G. E.; DE NARDI, A. B.; FERREIRA, E.; BERTAGNOLLI, A. C.; ESTRELA-LIMA, A.; ALESSI, A. C.; DALECK, C. R.; SALGADO, B. S.; FERNANDES, C. G.; SOBRAL, R. A.; AMORIM, R. L.; GAMBA, C. O.; DAMASCENO, K. A.; AULER, P. A.; MAGALHÃES, G. M.; SILVA, J. O.; RAPOSO, J. B.; FERREIRA, A. M. R.; OLIVEIRA, L. O.; MALM, C.; ZUCCARI, D. A. P. C.; TANAKA, N. M.; RIBEIRO, L. R.; CAMPOS, L. C.; SOUZA, C. M.; LEITE, J. S.; SOARES, L. M. C.; CAVALCANTI, M. F.; FONTELES, Z. G. C.; SCHUCH, I. D.; PANIAGO, J.; OLIVEIRA, T. S.; TERRA, E. M.; CASTANHEIRA, T. L. L.; FELIX, A. O. C.; CARVALHO, G. D.; GUIM, T. N.; GUIM, T. N.; GARRIDO, E.; FERNANDES, S. C.; MAIA, F. C. L.; DAGLI, M. L. Z.; ROCHA, N. S.; FUKUMASU, H.; GRANDI, F.; MACHADO, J. P.; SILVA, S. M. M. S.; BEZERRI, J. E.; FREHSE, M. S.; PAES DE ALMEIDA, E. C.; CAMPUS, C. B. Consensus for the diagnosis, prognosis and treatment of canine mammary tumors. Brazilian **Journal of Veterinary Pathology**, v. 4, n. 2, p. 153-180, 2011.

CHANG, S. C.; CHANG, C. C.; CHANG, T. J.; WONG, M. L. Prognostic factors associated with survival two years after surgery in dogs with malignant mammary tumours: 79 cases (1998-2002). **Journal of the American Veterinary Medical Association**, v. 227, n. 10, p. 1625-1629, 2005. Available from: <http://avmajournals.avma.org/doi/abs/10.2460/javma.2005.227.1625>. Viewed: 23 Sept. 2013. doi: http://dx.doi.org/10.2460/javma.2005.227.1625.

DAGLI, M. L. Z. The search for suitable prognostic markers for canine mammary gland tumors: a promising outlook. **Veterinary Journal**, v. 177, p. 3-5, 2006. Available from: <http://www.sciencedirect.com/science/article/pii/S1090023307003681>. Viewed: 27 Sept. 2013. doi: http://dx.doi.org/10.1016/j.tvjl.2007.10.015.

DALECK, C. R.; FRANCESCHINI, P. H.; ALESSI, A. C.; SANTANA, A. E.; MARTINS, M. I. M. Aspectos clínicos e cirúrgicos do tumor mamário canino (Canine mammary neoplasia: clinical and surgical evolution). **Ciência Rural**, v. 28, n. 1, p. 95-100, 1998. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84781998000100016>. Viewed: 25 Sept. 2013. doi: http://dx.doi.org/10.1590/S0103-84781998000100016.

FERREIRA, E.; BERTAGNOLLI, A. C.; CAVALCANTI, M. F.; SCHMITT, F. C.; CASSALI, G. D. The relationship betwen tumor size and expression of prognostic markers in benign and malignant canine mammary tumors. **Veterinary and Comparative Oncology**, v. 7, n. 4, p. 230-235, 2009. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1476-5829.2009.00193.x/abstract>. Viewed: 15 Oct. 2013. doi: http://dx.doi.org/10.1111/j.1476-5829.2009.00193.x.

FILGUEIRA, K. D.; ARAÚJO, R. W. B.; SILVA, L. D. M. Características histopatológicas de neoplasias mamárias em cadelas. **Ciência Animal**, v. 15, n. 2, p. 119-121, 2005.

GAMA, A.; ALVES, A.; SCHMITT, F. Identification of molecular phenotypes in canine mammary carcinomas with clinical implications: application of the human classification. **Virchows Archive**, v. 453, n. 2, p. 123-132, 2008. Available from: <http://link.springer.com/article/10.1007%2Fs00428-008-0644-3>. Viewed: 18 Oct. 2013. doi: http://dx.doi.org/10.1007/s00428-008-0644-3.

GOLDSCHMIDT, M.; PEÑA, R.; RASOTTO, R.; ZAPULLI, V. Classification and grading of canine mammary gland tumors. **Veterinary Pathology**, v. 48, n. 1, p. 117-131, 2011. Available from: <http://vet.sagepub.com/content/48/1/117.full> Viewed: 18 Nov. 2013. doi: http://dx.doi.org/10.1177/0300985810393258.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). **Censo Demográfico 2010**. Rio de Janeiro, 2010. Available from: <http://censo2010.ibge.gov.br/>. Viewed: 18 Nov. 2013.

ITOH, T.; UCHIDA, K.; ISHIKAWA, K.; KUSHIMA, K.; KUSHIMA, E.; TAMADA, H.; MORITAKE, T.; NAKAO, H.; SHII, H. Clinicopathological survey of 101 canine mammary gland tumors: differences between small-breed dogs and others. **Journal of Veterinary Medical Science**, v. 67, p. 345-347, 2005. Available from: <https://www.jstage.jst.go.jp/article/jvms/67/3/67_3_345/_article>. Viewed: 7 Oct. 2013. doi: http://dx.doi.org/10.1292/jvms.67.345.

KARAYANNOPOULUS, M.; KALDRYMIDOU, E.; CONSTANTINIDIS, T. C.; DESSIRIS, A. Histological grading and prognosis in dogs with mammary gland carcinomas: application of a human grading method. **Journal of Comparative Pathology**, v. 133, n. 4, p. 246-252, 2005. Available from: <http://www.sciencedirect.com/science/article/pii/S0021997505000654>. Viewed: 13 Sept. 2013. doi: http://dx.doi.org/10.1016/j.jcpa.2005.05.003.

KHANNA, C.; LINDBLAD-TOH, K.; VAIL, D.; LONDON, C.; BERGMAN, P.; BARBER, L.; BREEN, M.; KITCHELL, B.; McNEIL, E.; MODIANO, J. F.; NIEMI, S.; COMSTOCK, K. E.; OSTRANDER, E.; WESTMORELAND, S.; WITHROW, S. The dog as a cancer model. **Nature Biotechnology**, v. 24, p. 1065-1066, 2006. Available from: <http://www.nature.com/nbt/journal/v24/n9/full/nbt0906-1065b.html>. Viewed: 18 Oct. 2013. doi: http://dx.doi.org/10.1038/nbt0906-1065b.

MISDORP, W.; ELSE, R. W.; HELLMÉN, E.; LIPSCOMB, T. P. **Histological classification of mammary tumors of the dog and the cat**. Geneva: World Health Organization, 1999. v. 2, p. 16-25.

MORRIS, J. S.; DOBSON, J. M.; BOSTOCK, D. E.; O'FARRELL, E. Effect of ovariohysterectomy in bitches with mammary neoplasms. **The Veterinary Records**, v. 142, n. 24, p. 656-658, 1998. Available from: <http://veterinaryrecord.bmj.com/content/142/24/656>. Viewed: 13 Sept. 2013. doi: http://dx.doi.org/10.1136/vr.142.24.656.

MUNSON, L.; MORESCO, A. Comparative pathology of mammary gland cancers in domestic and wild animals. **Breast Disease**, v. 28, p. 7-21, 2007.

NERURKAR, V. R.; CHITALE, A. R.; JALNAPURKAR, B. V.; NAIK, S. N.; LALITHA, V. S. Comparative pathology of canine mammary tumours. **Journal of Comparative Pathology**, v. 101, n. 4, p. 389-397, 1989. Available from: <http://www.sciencedirect.com/science/article/pii/0021997589900224>. Viewed: 21 Sept. 2013. doi: http://dx.doi.org/10.1016/0021-9975(89)90022-4.

OLIVEIRA FILHO, J. C.; KOMMERS, G. D.; MASUDA, E. K.; MARQUES, B. M. F. P. P.; FIGHERA, R. A.; IRIGOYEN, L. F.; BARROS, C. S. L. Estudo retrospectivo de 1.647 tumores mamários em cães. **Pesquisa Veterinária Brasileira**, v. 30, n. 2, p. 177-185, 2010. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-736X2010000200014>. Viewed: 18 Nov. 2013. doi: http://dx.doi.org/10.1590/S0100-736X2010000200014.

OWEN, L. N. **The TNM classification of tumors in domestic animals**. Geneva: World Health Organization, 1980. 53 p.

PAOLONI, M. C.; KHANNA, C. Comparative oncology today. **Veterinary Clinics of North America: Small Animal Practice**, v. 37, n. 6, p. 1023-1032, 2007.

PEÑA, L.; ANDRÉS, P. J.; CLEMENTE, M.; CUESTA, P.; PÉREZ-ALENZA, D. Prognostic value of histological grading in noninflammatory canine mammary carcinomas in a prospective study with two-year follow-up: relationship with clinical and histological characteristics. **Veterinary Pathology**, v. 50, p. 94-105, 2013. Available from: <http://vet.sagepub.com/content/50/1/94>. Viewed: 2 May 2014. doi: http://dx.doi.org/10.1177/0300985812447830.

PÉREZ-ALENZA, M. D.; PEÑA, L.; DEL CASTILLO, N.; NIETO, A. I. Factors influencing the incidence and prognosis of canine mammary tumours. **The Journal of Small Animal Practice**, v. 41, n. 7, p. 287-291, 2000.

PÉREZ-ALENZA, M. D.; PEÑA, L.; NIETO, A. L.; CASTAÑO, M. Clinical and pathological prognostic factors in canine mammary tumors. **Annalli dell'Istituto Superiore di Sanità**, v. 33, n. 4, p. 581-585, 1997.

PINHO, S. S.; CARVALHO, S.; CABRAL, J.; GÄRTNER, F. Canine tumors: a spontaneous animal model of human carcinogenesis. **Translational Research**, v. 159, n. 3, p. 165-172, 2012. Available from: <http://www.sciencedirect.com/science/article/pii/S1931524411004130>. Viewed: 14 Aug. 2013. doi: http://dx.doi.org/10.1016/j.trsl.2011.11.005.

PORRELLO, A.; CARDELLI, P.; SPUGNINI, E. P. Oncology of companion animals as a model for humans: an overview of tumor histotypes. **Journal of Experimental & Clinical Cancer Research**, v. 25, n. 1, p. 97-106, 2006.

SANTOS, A. A.; LOPES, C. C.; RIBEIRO, J. R.; MARTINS, L. R.; SANTOS, J. C.; AMORIM, I. F.; GÄRTNER, F.; MATOS, A. J. Identification of prognostic factors in canine mammary malignant tumours: a multivariable survival study. **BMC Veterinary Research,** v. 9, 2013. Available from: <http://www.biomedcentral.com/1746-6148/9/1>. Viewed: 6 May 2014. doi: http://dx.doi.org/10.1186/1746-6148-9-1.

SLEECKX, H. R.; KROEZE, E. J. B. V.; GINNEKEN, C. V.; BRANTEGEN, L. V. Canine mammary tumours, an overview. **Reproduction in Domestic Animals**, v. 46, n. 6, p. 1112-1131, 2011. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0531.2011.01816.x/pdf>. Viewed: 24 Sept. 2013. doi: http://dx.doi.org/10.1111/j.1439-0531.2011.01816.x.

SNEDEKER, S. M. Pesticides and breast cancer risk: a review of DDT, DDE, and dieldrin. **Environmental Health Perspectives**, v. 109, p. 35-47, 2001, Supplement 1.

SORENMO, K. Canine mammary gland tumors. **Veterinary Clinics of North America: Small Animal Practice**, v. 33, n. 3, p. 573-596, 2003.

SORENMO, K. U.; KRISTIANSEN, V. M.; COFONE, M. A.; SHOFER, F. S.; BREEN, A. M.; LANGELAND, M.; MONGIL, C. M.; GRONDAHL, A. M.; TEIGE, J.; GOLDSCHMIDT, M. H. Canine mammary gland tumours; a histological continuum from benign to malignant; clinical and histopathological evidence. **Veterinary Comparative Oncology,** v. 7, n. 3, p. 162-172, 2009. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1476-5829.2009.00184.x/abstract>. Viewed: 3 Oct. 2013. doi: http://dx.doi.org/10.1111/j.1476-5829.2009.00184.x.

SORENMO, K. U.; RASOTTO, R.; ZAPPULLI, V.; GOLDSCHMIDT, M. H. Development, anatomy, histology, lymphatic drainage clinical features, and cell differentiation markers of canine mammary gland neoplasms. **Veterinary Pathology**, v. 48, p. 85-97, 2011. Available from: <http://vet.sagepub.com/content/48/1/85>. Viewed: 8 Sept. 2013. doi: http://dx.doi.org/10.1177/0300985810389480.

SORENMO, K. U.; SHOFER, F. S.; GOLDSCHMIDT, M. H. Effect of spaying and timing of spaying on survival of dogs with mammary carcinoma. **Journal of Veterinary Internal Medicine**, v. 14, n. 3, p. 266-270, 2000.

STRATMANN, N.; FAILING, K.; RICHTER, A.; WEHREND, A. Mammary tumor recurrence in bitches after regional mastectomy. **Veterinary Surgery**, v. 37, n. 1, 82-86, 2008. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1532-950X.2007.00351.x/abstract>. Viewed: 19 Oct. 2013. doi: http://dx.doi.org/10.1111/j.1532-950X.2007.00351.x.

TODOROVA, I.; SIMEONOVA, G.; SIMEONOV, R.; DINEV, D. Efficacy and toxicity of doxorubicin and cyclophosphamide chemotherapy in dogs with spontaneous mammary tumours. **Trakia Journal of Sciences**, v. 3, n. 5, p. 51-58, 2005.

TORÍBIO, J. M. M. L.; LIMA, A. E.; MARTINS FILHO, E. F.; RIBEIRO, L. G. R.; D'ASSIS, M. J. M. H.; TEIXEIRA, R. G.; DAMASCENO, K. A.; CASSALI, G. D.; COSTA NETO, J. M. Caracterização clínica, diagnóstico histopatológico e distribuição geográfica das neoplasias mamárias em cadelas de Salvador, Bahia. **Revista Ceres**, v. 59, n. 4, p. 472-433, 2012. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0034-737X2012000400001&lng=pt&nrm=iso&tlng=en>. Viewed: 5 May 2014. doi: http://dx.doi.org/10.1590/S0034-737X2012000400001.

TRAN, C. M.; MOORE, A. S.; FRIMBERGER, A. E. Surgical treatment of mammary carcinomas in dogs with or whithout postoperatice chemotherapy. **Veterinary and Comparative Oncology,** 2014. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/vco.12092/abstract>. Viewed: 6 May 2014. doi: http://dx.doi.org/10.1111/vco.12092. NO PRELO

UVA, P.; AURISICCHIO, L.; WATTERS, J.; LOBODA, A.; KULKARNI, A.; CASTLE, J.; PALOMBO, F.; VITI, V.; MESITI, G.; ZAPPULLI, V.; MARCONATO, L.; ABRAMO, F.; CILIBERTO, G.; LAHM, A.; MONICA, N.; RINALDIS, E. Comparative expression pathway analysis of human and canine mammary tumors. **BMC Genomics**, v. 10, n. 135, p. 1-20, 2009. Available from: <http://www.biomedcentral.com/1471-2164/10/135>. Viewed: 25 Sept. 2013. doi: http://dx.doi.org/10.1186/1471-2164-10-135.

YAMAGAMI, T.; KOBAYASHI, T.; TAKAHASHI, K.; SUGIYAMA, M. Prognosis for canine malignant mammary tumours based on TNM and histologic classification. **The Journal of Veterinary Medical Science**, v. 58, n. 11, p. 1079-1083, 1996.

ZAGO, A.; PEREIRA, L. A.; BRAGA, A. L.; BOUSQUAT, A. Breast cancer mortality in women of Southern Brazil, 1980-1999. **Revista de Saúde Pública**, v. 39, p. 641-645, 2005.