Epidemiological study of canine mammary tumors: age, breed, size and malignancy

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ABSTRACT. A retrospective study was carried out on 858 female canine patients who were attended at the Surgery Service of the Clinical Veterinary Hospital of the University of Extremadura (Spain), during a period of 5 years (2008-2012). This study aims to determine the incidence of mammary tumors in the canine species of this region together with the analysis of several epidemiological variables (breed, age, and size) and the histopathological variable tumor malignancy. Three groups of patients were considered: (I) general group including all patients (n=858), (II) oncological patients (n=376), and (III) patients with mammary tumors (n=227). Oncology represented the most common pathology for female dogs (n=376, 43.82%), with mammary tumors being the most frequent neoplasms (n=227, 60.37%). Mixed breed dogs were more likely than purebred dogs to suffer from mammary tumors, with these tumors being more frequently malignant. It was also found that large-sized patients show a greater predisposition to malignant mammary tumors. In patients with tumors, age at diagnosis was significantly associated with a higher malignancy rate. The results of the study confirm the importance of oncology in the veterinary clinic in the region. Mammary tumors represent the most frequently diagnosed neoplasia in female dogs. Old age, mixed breed and large size predispose to its development and represent risk factors for malignancy.

Key words: epidemiology, malignancy, dog, mammary tumor.

INTRODUCTION

The incidence of cancer continues to increase worldwide both in humans and companion animals (Pinho et al 2012). Despite the seriousness of this process and the large number of studies performed, current medical research in this area continues to be hampered by the complex biology of cancer (Pinho et al 2012).

The studies of spontaneous neoplasia in companion animals, particularly in canine species, provides useful information in the research on comparative epidemiology, pathogenesis, and therapeutics, since dogs have a shorter lifespan and develop tumors similar to those diagnosed in humans regarding both morphological and biological behaviour (Marconato et al 2009). Although rodents have been used for the development of highly standardised cancer models, they are often limited to very specific aspects of spontaneous human cancer such as latency period, recurrence, and metastasis (Thamm and Dow 2009, Ranieri et al 2013). Canine species share a greater proportion of its genome with humans than rodents representing a powerful model to study human mammary tumors (Pinho et al 2012).

Mammary tumors are one of the most common neoplasms in female dogs (Rivera and von Euler 2011). These processes are very similar to those that occur in humans, although the incidence in the dog is three times higher (Moe 2001, Rivera and von Euler 2011). Daily, veterinarians assess patients with mammary tumors establishing their clinical stage, prognosis and treatment (Perez Alenza et al 2000). Recently, an increase in the diagnoses of malignant mammary tumors in female dogs has been observed (Salas et al 2015), probably linked to the increased exposure to oncogenic substances such as pyrethroids, that represent an important environmental pollution agent (Andrade et al 2010).

The aim of this study was to describe the incidence of mammary tumors in female dogs in the region of Extremadura, Spain, and to determine the epidemiological characteristics (age, breed and size) and their relationship with tumor incidence and malignancy.

MATERIAL AND METHODS

SAMPLE DESCRIPTION

In this study 858 female dogs attended in the Surgery Service of the Clinical Veterinary Hospital of the University of Extremadura (UEx) in Spain from 2008 to 2012 were evaluated. In order to homogenise the study population and considering that, in our practice, the age at diagnosis of mammary pathology is over 5 years old; we have only included in this retrospective study female canine patients above this age. This hospital is the only referral Veterinary Hospital in Extremadura, covering a region of 41,633 km\textsuperscript{2}, with a population of 1,072,884 inhabitants and a demographic density of 25.77 h/km\textsuperscript{2}; attending an annual average of 3,788 patients.
The patients included in this study were distributed into three groups according to their pathology:

Group I: includes all study patients (n=858); Group II: includes group I patients diagnosed with any type of tumor (n=376); Group III: includes group II patients diagnosed with mammary tumors (n=227).

The epidemiological and clinical data were collected from their medical records. Histopathological evaluation was performed after surgery in those animals with oncological processes. The epidemiological variables studied were breed (including the different groups of pure breeds according to the Fédération Cynologique Internationale (FCI 2013) and mixed breed), age and size (in accordance to their withers height: large size (> 50 cm) or medium-small (< 50 cm), as reported by the FCI Breeds Nomenclature (FCI 2013)). In groups II and III, the variable malignancy (benign or malignant tumors) was considered.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using the Statistical Package for Social Sciences, version 22.0 (SPSS, Chicago, USA), using descriptive analysis of variables, Shapiro-Wilk and Kolmogorov-Smirnov tests were used for assessing normality. Wilcoxon-Mann-Whitney (non-parametric contrasts for two independent samples), the Pearson Chi-Square test (to compare two discrete variables) and logistic regression were applied. A P-value of less than 0.05 was considered significant.

**RESULTS AND DISCUSSION**

In this study, we analysed the relevance of mammary tumors in female dogs and the influence of 4 variables (breed, age, size, and tumor malignancy). Neutering status and spay age were not analysed in this study because only 11.69% of the patients were spayed at the time of diagnosis and none of them before the fourth estrous. There are not many records about the incidence of mammary tumors in dogs (Evengall et al 2005). Our results support the importance of oncological pathology in female dogs with a prevalence of 43.8% (95 percent of confidence interval, CI_{95%}: 40.48-47.12%). In addition, the prevalence of mammary tumors within tumor-bearing patients (figure 1) was 60.37% (CI_{95%}: 55.42-65.31%). These results agree with other studies in which the most common tumor type in the female dog corresponded to neoplasms of the mammary gland (Lana et al 2007, Merlo et al 2008, Choi et al 2016). As several authors point out, the fact that mammary tumors together with skin tumors represent the most frequent veterinary consultations might be because both locations are easily observed and accessed by both the owner and the veterinarian (Vascellari et al 2009, Dobson 2013, Gruntzig et al 2015). Thus, in this study, skin tumors are the second most frequent type of tumors diagnosed in female dogs (17.02%) as shown in figure 1.

The incidence of mammary tumors according to their histological type and stage of the patient is shown in tables 1 and 2, respectively. We observed a high impact of malignant mammary tumors (88.13%) (CI_{95%}: 83.92%, 92.34%). Our results do not agree with those observed by other authors, who detected much lower percentages of histological malignancy, with studies showing an incidence between 30% (Salas et al 2015) and 50% (Gilbertson et al 1983). The reason for this might be that our referral hospital is the only one in the entire region of Extremadura and patients with clinical signs of malignancy are habitually attended at the hospital, whereas those patients with small tumors, with slow growth ratio and with no visible signs of malignant disease are frequently treated by local veterinarians. As reported by Olivera et al (2003), we cannot exclude that neutering status is related to the high percentage of malignancy observed in our study with only 11.69% of spayed females; in addition, Vascellari et al (2016) observed a significantly proportion of complex carcinoma in intact females. However, we cannot exclude the role of other factors such as environmental pollution (e.g. pesticides and fertilizer) as the responsible for the high incidence of malignant tumors in the region. Thus, 64.74% of our patients were classified in stages II to V (table 2).

Regarding the risk factors that favour the appearance of the different types of tumors, it was possible to observe that the onset age of the disease does not have an effect on the type of oncological process developed (mammary tumor versus other tumors; P=0.073). In group II (oncological patients) age is directly correlated with tumor malignancy (P=0.012). In mammary tumors our results confirm previous reports suggesting a remarkable increase in mammary neoplasms in females between 6 and 10 years, with an average age of 10 years. The highest peak of presentation is reached at 10 years old which is followed by a marked decrease in the incidence of tumors of the
Table 1. Distribution of patients with mammary tumors according to histological type.

| Histological type* | n  | %   |
|--------------------|----|-----|
| Benign             | 21 | 11.86 |
| Malignant          | 156 | 88.13  |
| Complex carcinoma  | 37 | 23.72 |
| Simple carcinoma   | 49 | 31.41 |
| Mixed malignant tumors | 19 | 12.18 |
| Special types of carcinoma | 17 | 10.9 |
| Other carcinomas   | 34 | 21.79 |

* Patients (n=50) without typified histological diagnosis have not been included.

Table 2. Distribution of patients with malignant mammary tumors according to their staging.

| Staging                     | n  | %   |
|-----------------------------|----|-----|
| Stage I ($T_1 N_0 M_0$)     | 55 | 35.26 |
| Stage II ($T_2 N_0 M_0$)    | 48 | 30.77 |
| Stage III ($T_3 N_0 M_0$)   | 32 | 20.51 |
| Stage IV ($T_4 N_0 M_0$)    | 13 | 8.33 |
| Stage V ($T_4 N_1 M_1$)     | 8  | 5.13 |

Tumor staging was established using the TNM system. T: size of the primary tumor and its spread into nearby tissue; N: spread of cancer to nearby lymph nodes; M: presence of metastasis to distant organs.

The relationship between the age of onset of canine mammary tumors and the animal size was analysed in this study, observing a trend between the size of the patient and the age at diagnosis of mammary tumors ($P=0.058$). This is in line with the results of Sonnenschein et al (1991) who suggested that animals of larger breeds have, by their very nature, a shorter lifespan, aging faster than animals of smaller breeds and, therefore, present neoplasms at an earlier age.

In this study a high incidence of tumors in purebred dogs was observed, which is likely to reflect the large number of purebreds attended at this particular Hospital. The evidence for the increased frequency of purebred dogs that suffer from mammary tumors (64.32%) is consistent with the findings of several studies (Perez Alenza 2012, Perez Alenza et al 2000, Evengall et al 2005, Vascellari et al 2016). However, Karayannopoulou et al (1989) observed the contrary and these discrepancies were attributed to the differences in the populations analysed (Perez Alenza et al 2000). Moreover, it was found that breed affects the type of tumor developed ($P=0.000$), with approximately 1.32 times (OR) being more likely to have a mammary tumor than any other oncological process if the patients are mixed-breeds. It was also observed that breed affects tumor malignancy in group II ($P=0.000$) and III ($P=0.000$), concluding that the likelihood to develop a malignant tumor in mixed-breeds compared to purebred patients is approximately 1.47 and 1.76 times, respectively. There is no explanation for this observation; further studies are required to identify those factors influencing tumor malignancy in mixed-breeds compared to purebred dogs. Otherwise, age at diagnosis of any type of tumor (group II) and mammary tumors (group III) in this study did not differ between mixed-breed or purebred dogs ($P=0.092$ and $P=0.436$, respectively).

In this study, the group of breeds with the highest incidence of mammary tumors is Retrievers-Flushing Dogs-Water Dogs (breed distribution of female dogs with mammary tumors is shown in figure 2). These results are similar to those observed by Perez Alenza et al (2000). However, other studies describe an increased risk for Teckels

Table 3. Distribution of patients with benign and malignant mammary tumors according to their age, breed and size.

|          | Benign tumors n (%) | Malignant tumors n (%) | p value | OR       |
|----------|---------------------|------------------------|---------|----------|
| Age ≤8   | 14 (66.67%)         | 67 (42.95%)            | 0.065   |          |
| Age >8   | 7 (33.33%)          | 89 (57.05%)            |         |          |
| Pure breeds | 17 (80.95%)   | 100 (64.10%)           | 0.000   | 1.76*    |
| Mixed breeds | 4 (19.05%)    | 56 (35.90%)            |         |          |
| Large size | 3 (14.71%)        | 41 (26.28%)            | 0.000   | 2.34**   |
| Medium-small | 18 (85.71%)  | 115 (73.72%)           |         |          |

* times more likely to develop a malignant tumor in mixed-breeds.
** times more likely to develop a malignant tumor in large size.
and Molossoides (Moe 2001), Grazing breeds (Sorenmo et al. 2005) and Poodle Dog group (Lana et al. 2007). Our results could be explained to a great extent by the outstanding presence of Retrievers-Flush dogs-Water Dogs in our region due to the fact that hunting plays an important role in the regional economy. Thus, 14.6% of all patients treated in the Surgery Service at the Veterinary Clinical Hospital of the UEx belong to this group, supporting our assertion.

On the other hand, the majority (66.1%) (CI95%; 59.94%, 72.26%) of female dogs with mammary tumors were medium-small sized; furthermore, the size of animals influenced the development of a particular type of neoplasia (P=0.000), being 0.49 times (OR) more likely to have a mammary tumor if the female dog is large than if it is medium-small sized dog.

Finally, animal size affected the tumor malignancy in groups II and III (P=0.000), with approximately 0.85 times (OR) more likely to have a malignant tumor (group II) and 2.34 times more likely to be a malignant mammary tumor (group III) when the patients are large sized dogs. These results agree with the findings of Itoh et al. (2005) in mammary tumors and differ with Salas et al. (2015) who found no association when the occurrence of malignant tumors was compared to the size category, suggesting that there might be an effect caused by the different classification system used to group patients according to their size.

It is concluded that oncological pathology is the most frequent clinical condition observed in female dogs attended at the Surgery Service of the Veterinary Clinical Hospital of the UEx and mammary tumors represent the most common neoplasia in female dogs. Mixed-breed and large size represent risk factors for malignancy. Likewise, older age should be considered as a risk factor for the development of malignant neoplasia.

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REFERENCES

Andrade F, Figueroa F, Bersano P. 2010. Malignant mammary tumor in female dogs: environmental contaminants. Diagn Pathol 5, 45.

Choi JW, Yoon HY, Jeong SW. 2016. Clinical outcomes of surgically managed spontaneous tumors in 114 client-owned dogs. *Immune Network* 16, 116-125.

Dobson JM. 2013. Breed-predispositions to cancer in pedigree dogs. *Vet Sci* 2013, 941275.

Egenwall A, Bonnett BN, Ohagen P, Olson P, Hedhammar A, et al. 2005. Incidence of and survival after mammary tumors in a population of over 80,000 insured female dogs in Sweden from 1995 to 2002. *Prev Vet Med* 69, 109-127.

FCI, Fédération Cynologique Internationale. 2013. *FCI dog exhibition regulations and supplementary regulations for World and Section Exhibitions*. F.C.I., Thuin, Belgium.

Gilbertson SR, Kurzman ID, Zachrau RE, Hurvitz AI, Black MM. 1983. Canine mammary epithelial neoplasms: biologic implications of morphologic characteristics assessed in 232 dogs. *Vet Pathol* 20, 127-142.

Gruntzig K, Graf R, Hassig M, Welle M, Meier D, et al. 2015. The Swiss Canine Cancer Registry: a retrospective study on the occurrence of tumours in dogs in Switzerland from 1955 to 2008. *J Comp Pathol* 152, 161-171.

Itoh T, Uchida K, Ishikawa K, Kushima K, Kushima E, et al. 2005. Clinicopathological survey of 101 canine mammary gland tumors: differences between small-breed dogs and others. *J Vet Med Sci* 57, 345-347.

Karayannopoulou M, Kaldymidou E, Dessiris A. 1989. Some epidemiological aspects of canine mammary tumors. Treatment and prognosis. *Bulletin of the Hellenic Veterinary Medical Society* 40, 111-121.

Lana SE, Rutteman GR, Withrow SJ. 2007. Tumors of the Mammary Gland. In: Withrow SJ, Vail DM (eds). *Small Animal Clinical Oncology*. 4th ed. Saunders, Philadelphia, USA, Pp 619-636.

Marconato L, Leo C, Girelli R, Salvi S, Abramò F, et al. 2009. Association between waste management and cancer in companion animals. *J Vet Intern Med* 23, 564-569.

Merlo DF, Rossi L, Pellegrino C, Ceppi M, Cardellino U, et al. 2008. Cancer incidence in pet dogs: findings of the Animal Tumor Registry of Genova, Italy. *J Vet Intern Med* 22, 976-984.

Misdorp W. 2002. Tumors of the Mammary Gland. In: Meuten DJ (ed). *Tumors in Domestic Animals*. 4th ed. Iowa State Press, Iowa, Pp 575-606.

Moe L. 2001. Population-based incidence of mammary tumours in some dog breeds. *J Reprod Fertil Suppl* 57, 439-443.

Olivera LO, Oliveira RT, Loretti A, Rodrigues R, Driemeier D. 2003. Aspectos epidemiológicos da neoplasia mamária canina. *Acta Scientiae Veterinariae* 31, 105-110.

Perez Alenza MD. 1994. Influencia de la nutrición, alteraciones genéticas y aspectos clínicos en los tumores mamarios caninos. *Disertación PhD*, Universidad Complutense de Madrid, España.

Perez Alenza MD, Pena L, del Castillo N, Nieto AI. 2000. Factors influencing the incidence and prognosis of canine mammary tumours. *J Small Anim Pract* 41, 287-291.

Pinho SS, Carvalho S, Cabral J, Reis CA, Gartner F. 2012. Canine tumors: a spontaneous animal model of human carcinogenesis. *Transl Res* 159, 165-72.
Ranieri G, Gadaleta CD, Patruno R, Zizzo N, Daidone MG, et al. 2013. A model of study for human cancer: Spontaneous occurring tumors in dogs. Biological features and translation for new anticancer therapies. *Crit Rev Oncol Hematol* 88, 187-197.

Rivera P, von Euler H. 2011. Molecular biological aspects on canine and human mammary tumors. *Vet Pathol* 48, 132-146.

Salas Y, Márquez A, Diaz D, Romero L. 2015. Epidemiological Study of Mammary Tumors in Female Dogs Diagnosed during the Period 2002-2012: A Growing Animal Health Problem. PLOS ONE. 10(5): e0127381.

Sonnenschein EG, Glickman LT, Goldschmidt MH, McKee LJ. 1991. Body conformation, diet, and risk of breast cancer in pet dogs: a case-control study. *Am J Epidemiol* 133, 694-703.

Sorenmo KU, Kristiansen VM, Cofone MA, Shofer FS, Breen AM, et al. 2009. Canine mammary gland tumours; a histological continuum from benign to malignant; clinical and histopathological evidence. *Vet Comp Oncol* 7, 162-172.

Thamm D, Dow S. 2009. How companion animals contribute to the fight against cancer in humans. *Vet Ital* 45, 111-120.

Vascellari M, Baioni E, Ru G, Carminato A, Mutinelli F. 2009. Animal tumour registry of two provinces in northern Italy: incidence of spontaneous tumours in dogs and cats. *BMC Vet Res* 5, 39.

Vascellari M, Capello K, Carminato A, Zanardello C, Baioni E, et al. 2016. Incidence of mammary tumors in the canine population living in the Veneto region (Northeastern Italy): Risk factors and similarities to human breast. *Prev Vet Med* 126, 183-189.