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Exploring canine mast cell tumors: An investigation into demographic characteristics, and grading system analysis from a pathology lab data (2019–2021)

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ABSTRACT

Mast cell tumors (MCT) are among the most common neoplasia in dogs, representing up to 21 % of skin tumors. However, etiology and risk factors for its development remain unclear. This study aimed to reduce this knowledge gap by comprehensively analyzing 905 MCT cases diagnosed in Portugal between 2019 and 2021, using descriptive and inferential analyses. Most tumors affected the skin, with 69.9 % and 21.2 % classified as cutaneous and subcutaneous tumors, respectively. Only subcutaneous MCT exhibited female predisposition. Breed-specific analyses revealed male predominance in French Bulldogs and female predominance in Shar-Peis. Tumors in the extremities were the most prevalent (43.2 %, n = 183). Age-related characteristics varied by breed, with Pugs, Boxers, French Bulldogs, and Shar-Peis being diagnosed at younger ages. Logistic regression showed that age increased the likelihood of developing higher-grade cutaneous tumors ($p < 0.01$, OR=1.17, 95 % CI 1.02–1.21) and subcutaneous tumors with an infiltrative pattern ($p = 0.02$, OR=1.17, 95 % CI: 1.04–1.33). The estimated annual incidence risk for MCT in dogs from Lisbon and Setúbal districts is 3.1 cases per 10,000 dogs, and 3.0 for males and 3.2 for females. Compared to mixed-breed dogs, Boxers, Shar-Peis, and Golden Retrievers had significantly higher relative risks (7.1, 6.3, and 5.9, respectively, $p < 0.01$). Sex-specific relative risks showed Boxers with the highest values among males (9.9, $p < 0.01$) and Shar-Peis among females (8.0, $p < 0.01$). This study provides insights into canine MCT, emphasizing the importance of age, sex, and breed, as well as the need for tailored veterinary care that considers these demographic characteristics to enhance prevention, early detection, and management.

1. Introduction

Advancements in veterinary medicine and diagnostic techniques have significantly improved the identification and diagnosis of various cancers in dogs. Better pet care and expanded access to healthcare have contributed to increased longevity in dogs, which, in turn, increases the risk of age-related diseases such as cancer (Martins et al., 2021).

Canine mast cell tumors (MCT) are among the most common

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neoplasms in dogs, representing a significant portion of skin tumors (up to 21 %) (Villamil et al., 2011; Kiupel and Camus, 2019; Bae et al., 2020; Martins et al., 2022). Dysregulation of mast cell proliferation and function can lead to the development of MCT, which shows considerable variability in biological behavior and clinical outcomes. However, certain genetic mutations such as those affecting the c-KIT receptor gene (Webster et al., 2007; Chen et al., 2022) have been associated with MCT, emerging evidence suggests that demographic factors may also play a role in their development (Mochizuki et al., 2017; Śmiech et al., 2018; Pierini et al., 2019).

Despite its frequency, the etiology and risk factors associated with MCT development in dogs remain incompletely understood. Generally observed in adult/older animals, it is consensual that MCT poses an increased risk as age advances (Śmiech et al., 2018). Sex predilection remains a debated topic, with conflicting reports on whether there is any predisposition (Webster et al., 2007) or a potential female bias (Śmiech et al., 2018). Breed predisposition is well-established, with Boxers, Labrador Retrievers, Golden Retrievers, French Bulldogs, and Shar-Peis being some of the most frequently identified (Mochizuki et al., 2017; Śmiech et al., 2018, 2019; Reynolds et al., 2019; Pinello et al., 2022a). MCTs can develop in various organs; however, visceral MCT are considered rare and are usually associated with a poor prognosis (Takahashi et al., 2000). They are most often diagnosed on the skin, where they are further classified as cutaneous or subcutaneous (Kiupel et al., 2011; Thompson et al., 2011a, b). Cutaneous MCTs are usually graded using the two-tier (Kiupel's grading) and three-tier (Patnaik's) histological grading systems. In the three-tier system, Grade I tumors are confined to the dermis and interfollicular spaces, with well-differentiated cells, low cellularity, and easily identifiable cytoplasmic granules. Mitotic figures are either rare or absent. Grade II tumors affect the dermis, and possibly the epidermis or subcutaneous tissue, showing intermediate cellularity and differentiation, with moderate anisokaryosis, anisocytosis, and pleomorphism. Mitotic figures range from 0 to 2 per high-power field (HPF). Grade III tumors have poorly defined boundaries and high cellularity. The cells are poorly differentiated, with high anisokaryosis, anisocytosis, and pleomorphism, including giant cells. Cytoplasmic granules are either faint or absent. Mitotic figures range from 3 to 6 per HPF. The two-tier system divides tumors into low- and high-grade. High-grade tumors exhibit one or more of the following: karyomegaly, a mitotic count of over 7 in 10 HPFs, 3 or more multinucleated cells per 10 HPF, or more than 3 bizarre nuclei per 10 HPF. Low-grade tumors do not exhibit any of these characteristics.

Subcutaneous MCT are characterized following the classification of Thompson et al. (2011a), where the growth pattern is categorized based on the submacroscopic appearance of the tumor and can be divided into three classes: circumscribed, combined, and infiltrative. Grading is a crucial predictor of tumor behavior.

Several demographic factors have been linked to prognosis and tumor grading, with age being one of the most widely recognized factors because of the strong association between higher-grade tumors and older animals (Kiupel et al., 2005; Shoop et al., 2015; Reynolds et al., 2019; Treggiari et al., 2023). There is also some consensus regarding breed associations. For instance, Pugs are often linked to low-grade tumors (Reynolds et al., 2019), whereas Shar-Peis are associated with higher-grade tumors (Śmiech et al., 2018). However, the associations with other breeds remain inconclusive and require further investigation. There is a notable lack of consensus regarding sex and anatomical location. For sex, studies have yielded conflicting results, with some studies reporting that males are more predisposed to higher-grade tumors or worse prognoses (Kiupel et al., 2005; Mochizuki et al., 2017), whereas other studies have indicated no association (Shoop et al., 2015; Reynolds et al., 2019). Similarly, findings regarding the anatomical locations are inconsistent. While some studies have reported no association between tumor location and prognosis (Kiupel et al., 2005), other studies have suggested that certain locations, such as the inguinal region (Reynolds et al., 2019) or axilla (Śmiech et al., 2018), may be linked to higher-grade tumors. However, the lack of standardized reporting of this parameter often leads to conflicting results. Another complicating factor is the limited number of studies focusing exclusively on subcutaneous tumors, making it challenging to directly compare the findings with those of cutaneous MCTs (Thompson et al., 2011a). The complexities of sex, breed, age, and anatomical location in relation to tumor grading and development add to the intricacies of understanding MCT.

Therefore, an in-depth investigation of the epidemiology of MCT is essential to identify potential risk factors, comprehend disease mechanisms, and formulate preventive strategies. This knowledge could aid veterinarians and dog owners in recognizing high-risk individuals and implementing effective screening measures for early detection.

Thus, this study aimed to explore MCT epidemiology in dogs using primary data sourced from a comprehensive database from a pathology laboratory based in Lisbon, which includes a large number of diagnoses across Portugal, particularly from the Lisbon and Setúbal districts. This study will focus on comparing cutaneous and subcutaneous MCT, age-related characteristics, breed-specific patterns, grading, and anatomical characteristics, and will conclude with an estimated incidence risk analysis in the districts of Lisbon and Setúbal. This study offers an assessment of MCT characteristics, revealing associations that may not be evident in studies conducted in other countries, while providing insights into the epidemiology of MCTs within this specific regional context and filling a gap in regional research that can serve as a foundation for future large-scale nationwide studies.

2. Materials and methods

2.1. Study population

This study included MCTs diagnosed histopathologically at the DNAtech Veterinary Laboratory, located in Lisbon, Portugal, between 2019 and 2021. These MCT were further classified into cutaneous MCTs (MCTcut), subcutaneous MCTs (MCTsub), scrotal MCTs, and MCTs originating from non-cutaneous sites (such as, mammary glands, mucosal areas, mucocutaneous transition zones, spleen, and small intestine) (MCT_others). Diagnoses were conducted by three different pathologists and were sourced from 261 referral centers across 17 out of 18 districts, from Mainland Portugal, and from both autonomous regions of the Portuguese Atlantic archipelagos/islands (Azores and Madeira). This study was approved by the Animal Welfare Ethics Committee (ORBEA) of the University of Évora (approval number: GD/7087/2024).

2.2. Data collection

2.2.1. MCT cases

Clinicopathological data were extracted from histopathological reports encompassing variables such as age, breed, sex, anatomical location of the tumor, and the postal code of the referring clinic. Records were based on individual tumors. Complete surgical excision is the most common treatment approach for MCTs, making the duplication of records highly unlikely. Cases of duplication, which were almost nonexistent, were identified using the dog's laboratory identification number and removed.

Given the diversity of breeds in our study population and the absence of official publications categorizing breeds by group, we conducted a breed group analysis using the Fédération Cynologique Internationale (FCI) breed groups (FCI, 2024) (Table S1). MCTcuts were graded using the two-tier (Kiupel et al., 2011) and three-tier (Patnaik et al., 1984) grading systems, whereas MCTsub growth patterns were classified according to Thompson et al. (2011a). For skin tumors, anatomical locations were categorized into topographical regions, as suggested by Kiupel et al. (2005), including the head and neck, trunk, extremities, and perineal regions. Only breeds and locations with a sample size of > 10 were included in the inferential statistical analysis.

2.2.2. Canine population data

The canine populations registered in Lisbon and Setúbal, as documented in the Portuguese companion animal registry system (SIAC, 2024), served as the basis for our epidemiological analysis. The Sistema de Informação de Animais de Companhia (SIAC) is a national platform that registers pets based on microchips. In Portugal, microchipping and registration are mandatory, as per Decree No. 313/2003 and No. 82/2019. This created a comprehensive database for all dogs, regardless of their breed. SIAC is the most inclusive platform in Portugal, with each pet's microchip serving as a unique identifier. Its wide use and mandatory status ensure a broad and representative dataset of pets in Portugal. The estimated annual incidence risk (EAIR) was calculated using the following formula: total number of MCT cases in the specified regions divided by the total SIAC population in those regions divided by 3 (to reflect the 3-year time span of the records from 2019 to 2021). Lisbon and Setúbal were selected as the primary districts of interest because of their substantial representation in most of the collected samples.

Relative risks (RR) were computed by comparing the EAIR of the predominant breeds to that of mixed-breed dogs, using a 95 % confidence interval (CI) for accuracy and reliability.

2.3. Statistical analysis of MCT cases

Descriptive and inferential analyses were conducted using Excel and R (version R-4.3.2). Continuous variables were summarized using means with standard deviations (SD), and categorical variables were presented as frequencies and percentages.

To assess the normality of continuous variables, graphical methods such as histograms were employed, along with statistical tests, including the Shapiro–Wilk test and Bartlett test for homogeneity. Parametric tests were performed when the assumptions of normality and homogeneity of variance were met.

The independent samples *t*-test was used to compare continuous variables between the groups. Analysis of variance (ANOVA), followed by Tukey's test, was performed to compare the means across multiple groups. Non-parametric tests, such as the Kruskal–Wallis test, were used for non-normally distributed data, and Dunn's test was employed for comparisons involving multiple groups.

A one-proportion Z-test was used to evaluate potential deviations from the presumed proportion of 0.5. To examine whether age distributions differed across MCT subtypes (such as, grades and patterns), the Kolmogorov–Smirnov (K-S) test was applied. Odds ratios (OR) were calculated, and chi-square tests, including the corresponding 95 % CI, were conducted to analyze the association between the Kiupel grade and anatomical location.

Binomial logistic regression models were fitted to examine the relationships between the independent variables, MCTcut versus MCTsub, and Infiltrative versus Non-infiltrative (circumscribed + combined) MCTsub patterns. After adjusting for sex, these models incorporated all relevant variables (such as age, breed, FCI group, and anatomical location) in a forward model.

Statistical significance was set at $p < 0.05$ and was used for all comparisons.

3. Results

Among the 905 cases, 633 were classified as MCTcut (69.9 %) and 192 were identified as MCTsub (21.2 %). Of the remaining cases, 65 (7.2 %) were scrotal and 15 (1.7 %) involved mucosal or visceral sites. These included four in the mammary gland, three in the mucocutaneous transitional areas, two in the vulvar areas, two in the oral mucosa, two splenic, one in the third eyelid, and one in the small intestine.

3.1. Sex, breed, and anatomical location

Information on sex and breed was available for all the cases. No sex predilection was observed across any MCT or MCTcut. However, MCTsub showed a female predilection (57.3 %, n = 110; $p = 0.04$) (Table 1).

A total of 55 breeds were represented in all MCT cases. French Bulldogs had a significantly higher representation of males (62.5 %, n = 40; $p = 0.04$), whereas Shar-Peis were predominantly represented by females (81.2 %, n = 13; $p = 0.01$) (Table 1). Additionally, for all MCT, the "Terriers" FCI group was more represented by females ($p = 0.03$) (Table 1).

In terms of anatomical location (Table 1), the extremities were the most common site (n = 183, 43.2 %), followed by the trunk (n = 153, 36.1 %). The head and neck region (n = 65; 15.3 %) was ranked third, whereas the perineal region was the least common (n = 23, 5.4 %). No sexual predilection was identified, and the distribution of MCTcut per location was similar (Table 1). However, MCTcut exhibited a significantly higher proportion of tumors in the head and neck region in females, accounting for 34 cases (65.4 %). For MCTsub, the trunk was the most frequent location (n = 47, 47.5 %), with a notable female predilection for tumors in the extremities (n = 25, 71.4 %) (Table 1).

3.2. Age

The overall mean age at diagnosis was 8.3 years (SD=2.9) with no differences observed between sexes (females 8.3 years, SD=3.0; males 8.2 years, SD=2.9; $p = 0.80$) (Table 2). However, within breeds, male Golden Retrievers had a higher mean age at diagnosis (8.7 years, SD=2.8) than females (7.1 years, SD=1.5) ($p < 0.01$). This trend was consistent within the FCI breed groups, where male Retrievers displayed an older age at diagnosis (8.6 years, SD=2.5) than females (7.8 years, SD=2.4) ($p < 0.01$) (Table 2). Age information was missing for 47 cases.

Pugs, Boxers, French Bulldogs, and Shar-Peis demonstrated significantly lower ages at diagnosis ($p < 0.01$) than mixed-breed dogs (Table 2). In the FCI breed group analysis, "Mixed-breed," "Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattledogs," and "Companion and Toy Dogs" also had significantly lower ages at diagnosis ($p < 0.01$) than mixed-breed dogs (Table 2).

For MCTcut, the mean age at diagnosis was 8.1 years (SD=2.9) with no differences observed between sexes (Table 2). Pugs (6.2 years, SD=1.7), French Bulldogs (6.5 years, SD=2.5, $p = 0.52$), and Boxers (7.2 years, SD=2.4) had the lowest mean age, which was significantly lower than that of mixed-breed dogs (8.8 years, SD=3.3) ($p < 0.01$) (Table 2). Within the FCI breed groups, "Companion and Toy Dogs" and "Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattledogs" also presented the lowest mean ages compared to the mixed breeds ($p < 0.01$) (Table 2).

For MCTsub, the mean age at diagnosis was 8.6 years (SD=2.9) with no significant differences between sexes ($p = 0.62$) (Table 2). Owing to sample size limitations, inferential analyses of age and breed were conducted only for the FCI breed groups. Among these, "Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattledogs" exhibited the lowest mean age (6.8 years, SD=2.5), and the difference was significant compared to mixed-breed dogs ($p < 0.01$) (Table 2).

When comparing the age at diagnosis by location (Table 2) across all MCT, the trunk exhibited the lowest age at diagnosis (7.9 years, SD=2.9), whereas the perineal region demonstrated the highest age (9.2 years, SD=2.3). However, no significant differences were observed between the groups (Table 2).

The MCTsub group had a significantly higher mean age than the MCTcut group (Table 2 and Fig. 1A). In addition, the two-sample K-S test indicated that the distributions were different (D=0.128; $p = 0.03$). This distinction was evident in the cumulative line charts (Fig. 1B). Logistic regression analysis adjusted for sex indicated that age was the only statistically significant factor affecting the likelihood of developing

Table 1
Distribution of mast cell tumors (MCT), cutaneous MCTs (MCT cut), and subcutaneous MCTs (MCT sub) based on sex, breed, Fédération Cynologique Internationale (FCI) breed groups, and anatomical location.

	All MCT			**MCT cut**			**MCT sub**		
	Total	F	M	Total	F	M	Total	F	M
n (%)	905	463 (51.2)	442 (48.8)	633	339 (53.5)	294 (46.5)	192	**110 (57.3)***	82(42.7)
Breed (n, %)	n (%$^{\text{in column}}$)	n (%$^{\text{in line}}$)	n (%$^{\text{in line}}$)	n (%$^{\text{in column}}$)	n (%$^{\text{in line}}$)	n (%$^{\text{in line}}$)	n (%$^{\text{in column}}$)	n (%$^{\text{in line}}$)	n (%$^{\text{in line}}$)
Mixed-breed	309 (34.1)	169 (54.7)	140 (45.3)	212 (33.5)	118 (55.6)	94 (44.4)	75 (39.1)	46 (61.3)	29 (38.7)
Labrador Retriever	197 (21.8)	100 (50.8)	97 (49.2)	134 (21.2)	72 (53.7)	62 (46.3)	49 (25.5)	25 (51.0)	24 (49.0)
Boxer	67 (7.4)	29 (43.3)	38 (56.7)	46 (7.3)	22 (47.8)	24 (52.2)	9 (4.7)	7 (77.8)	2 (22.2)
French Bulldog	64 (7.1)	24 (37.5)	**40 (62.5)***	45 (7.1)	22 (48.9)	23 (51.1)	7 (3.6)	2 (28.6)	5 (71.4)
Golden Retriever	51 (5.6)	19 (37.3)	32 (62.7)	40 (6.3)	14 (35.0)	26 (65.0)	10 (5.2)	5 (50.0)	5 (50.0)
Pit Bull	24 (2.6)	15 (62.5)	9 (37.5)	18 (2.8)	12 (66.7)	6 (33.3)	2 (1.0)	2 (100.0)	0
Shar-pei	16 (1.8)	**13 (81.2)***	3 (18.7)	10 (1.6)	8 (80.0)	2 (20.0)	5 (2.6)	4 (80.0)	1 (20.0)
Pug	13 (1.4)	3 (23.1)	10 (76.9)	12 (1.9)	3 (25.0)	9 (75.0)	1 (0.5)	0	1 (100)
Yorkshire Terrier	13 (1.4)	10 (76.9)	3 (23.1)	7 (0.1)	5 (71.4)	2 (28.6)	4 (2.1)	3 (75.0)	1 (25.0)
Beagle	10 (1.1)	7 (70.0)	3 (30.0)	5 (< 0.1)	4 (80.0)	1 (20.0)	4 (2.1)	3 (75.0)	1 (25.0)
Others	141 (15.1)	74 (52.5)	67 (47.5)	104 (16.4)	59 (56.7)	45 (43.3)	26 (13.6)	13 (50.0)	13 (50.0)
FCI Breed group									
Retrievers	254 (28.1)	123 (48.4)	131 (51.6)	180 (28.4)	90 (50.0)	90 (50.0)	59 (30.7)	30 (50.8)	29 (49.2)
Molossoid	187 (20.7)	83 (44.4)	104 (55.6)	127 (20.1)	65 (51.2)	62 (48.8)	30 (15.6)	17 (56.7)	13 (43.3)
Terriers	58 (6.4)	**37 (63.8)***	21 (36.2)	44 (6.9)	28 (63.6)	16 (36.4)	8 (4.2)	6 (75.0)	2 (25.0)
Companion	28 (3.1)	15 (53.6)	13 (46.4)	21 (3.3)	11 (52.4)	10 (47.6)	6 (3.1)	3 (50.0)	3 (50.0)
Pointing	23 (2.5)	10 (43.5)	13 (56.5)	17 (2.7)	7 (41.1)	10 (58.9)	4 (2.1)	2 (50.0)	2 (50.0)
Hounds	20 (2.2)	11 (55.0)	9 (45.0)	12 (1.9)	7 (58.3)	5 (41.7)	7 (3.6)	4 (57.1)	3 (42.9)
Spitz	11 (1.2)	5 (45.5)	6 (55.5)	9 (1.4)	4 (44.4)	5 (55.6)	1 (0.5)	1 (100.0)	0
Sheepdogs	11 (1.2)	8 (72.7)	3 (27.3)	9 (1.3)	7 (77.8)	2 (22.2)	2 (1.0)	1 (50.0)	1 (50.0)
Dachshunds	3 (0.3)	2 (66.7)	1 (33.3)	2 (0.3)	2 (100)	0	0	0	0
Anatomical location									
Head and Neck	65 (15.3)	40 (61.1)	25 (39.9)	52 (16.0)	**34 (65.4)***	18 (34.6)	13 (13.1)	6 (46.2)	7 (53.8)
Trunk	153 (36.1)	74 (47.0)	79 (53.0)	106 (32.6)	53 (50.0)	53 (50.0)	47 (47.5)	21 (44.7)	26 (55.3)
Extremities	183 (43.2)	103 (56.3)	80 (43.7)	148 (45.5)	78 (52.7)	70 (47.3)	35 (35.4)	**25 (71.4)***	10 (28.6)
Perineal Region	23 (5.4)	9 (39.1)	14 (60.9)	19 (5.9)	8 (42.1)	11 (57.9)	4 (4.1)	1 (25.0)	3 (75.0)

In bold and *$p < 0.05$, Z-test between sexes (vs. 0.5). The total number of cases in the FCI results was 904, owing to the exclusion of male Boerboel, a breed not recognized by the FCI.

Table 2
Age at diagnosis distribution (mean and standard deviation [SD]) of mast cell tumors (MCT), cutaneous MCTs (MCT cut), and subcutaneous MCTs (MCT sub) based on sex, breed, Fédération Cynologique Internationale (FCI) breed groups, and anatomical location.

	All MCT			MCT cut			MCT sub		
	F+M	F	M	F+M	F	M	F+M	F	M
Mean Age (SD)	8.3 (2.9)	8.3 (3.0)	8.2 (2.9)	8.1 (2.9)	8.2 (3.0)	8.1 (2.8)	8.6 (2.9)$^{\#}$	9.0 (2.7)	8.7 (2.9)
Breed									
Mixed-breed	8.9(3.3)^{d}	9.1 (3.2)^{a}	8.8 (3.5)^{a}	8.8 (3.3)^{c}	8.9 (3.3)^{b}	8.7 (3.3)^{a}	9.2 (3.2)^{a}	9.7 (2.9)^{a}	8.4 (3.5)^{a}
Labrador Retriever	8.3 (2.4)^{bd}	8.1 (2.5)^{ab}	8.5 (2.4)^{a}	8.0 (2.4)^{ac}	7.9 (2.5)^{ab}	8.2 (2.3)^{a}	8.9 (2.4)^{a}	8.9 (2.0)^{a}	8.8 (2.7)^{a}
Boxer	7.4 (2.5)^{abc}	6.9 (2.4)^{bc}	7.7 (2.5)^{a}	7.2 (2.4)^{ab}	7.1 (2.5)^{ab}	7.3 (2.4)^{a}	7.0 (2.5)^{a}	6.0 (2.0)^{a}	9.5 (1.6)^{a}
French Bulldog	6.8 (2.4)^{a}	6.4 (2.6)^{b}	7.1 (2.3)^{a}	6.5 (2.5)^{b}	6.5 (2.7)^{a}	6.5 (2.4)^{a}	7.3 (1.6)^{a}	6.0 (0.0)^{a}	8.0 (1.6)^{a}
Golden Retriever	8.1 (2.5)^{ad}	7.1 (1.5)^{ab}	8.7 (2.8)$^{a\#}$	8.0 (2.6)^{bc}	7.2 (1.5)^{ab}	8.5 (2.9)^{a}	8.5 (2.5)^{a}	7.0 (2.0)^{a}	10.0 (2.0)^{a}
Pit Bull	8.1 (2.8)^{ad}	7.6 (2.9)^{ab}	8.7 (2.5)^{a}	8.2 (3.1)^{bc}	7.9 (3.3)^{ab}	8.7 (2.9)^{a}	6.5 (0.7)^{a}	6.5 (0.7)^{a}	-
Pug	6.3 (1.8)^{ab}	5.3 (2.1)^{ab}	6.6 (1.7)^{a}	6.2 (1.8)^{ab}	5.3 (2.1)^{ab}	6.4 (1.7)^{a}	8.0 (0)^{a}	-	8.0 (0)^{a}
Shar-pei	6.4 (2.8)^{ab}	6.1 (2.5)^{bc}	7.3 (4.2)^{a}	6.7 (2.7)	6.4 (2.4)^{ab}	6.5 (2.4)^{a}	5.5 (2.6)^{a}	5.3 (3.2)^{a}	6.0 (0)^{a}
Yorkshire Terrier	9.2 (2.3)^{ad}	10.0 (1.8)^{ac}	6.7 (4.5)^{a}	8.9 (3.4)	10.6 (2.1)^{ab}	4.5 (3.5)^{a}	9.7 (1.5)^{a}	9.3 (1.5)^{a}	11.0 (0)^{a}
Beagle	10.3 (2.3)^{cd}	9.7 (2.6)^{ab}	11.6 (0.6)^{a}	9.2 (2.6)	8.5 (2.9)^{ab}	12.0 (0)^{a}	11.2 (0.5)^{a}	11.3 (0.6)^{a}	11.0 (0)^{a}
FCI Breed Group									
Retrievers	8.3 (2.4)^{ac}	7.8 (2.4)	8.6 (2.5)$^{\#}$	8.1 (2.5)^{ac}	7.8 (2.5)	8.3 (2.5)	8.8 (2.4)^{b}	8.6 (2.1)	9.0 (2.6)
Molossoid	7.1 (2.5)^{b}	7.1 (2.6)	7.4 (2.4)	7.2 (2.5)^{b}	7.3 (2.7)	7.1 (2.4)	6.8 (2.2)^{a}	6.6 (2.2)	7.2 (2.2)
Terriers	8.5 (2.8)^{bc}	8.7 (2.6)	8.1 (3.0)	8.6 (2.9)^{bc}	9.0 (2.7)	7.9 (3.2)	7.7 (2.5)	7.5 (2.4)	8.5 (3.5)
Companion	6.3 (1.8)^{b}	8.3 (3.2)	6.8 (2.2)	6.9 (2.8)^{ab}	7.6 (3.4)	6.2 (1.7)	9.5 (2.4)	10.3 (1.5)	8.7 (3.1)
Pointing	6.6 (1.9)	6.0 (2.4)	7.2 (1.3)	7.0 (1.7)	6.9 (2.2)	7.1 (1.5)	4.7 (2.5)	3.5 (2.1)	7.0 (0)
Hounds	10.3 (2.3)^{c}	9.5 (2.6)	8.7 (2.6)	8.5 (2.6)	9.2 (2.6)	7.8 (2.7)	9.6 (2.3)	10.0 (2.7)	9.0 (2.0)
Spitz	9.8 (4.0)	11.6 (5.1)	8.0 (2.4)	10.0 (4.7)	12.5 (5.4)	7.5 (2.5)	8.0 (0)	8.0 (0)	-
Sheepdogs	8.6 (3.1)	8.3 (3.3)	9.0 (4.0)	8.6 (3.8)	8.4 (3.6)	9 (5.7)	8.5 (0.7)	8.0 (0)	-
Dachshunds	9.0 (3.0)	7.0 (2.0)	12 (0)	7.0 (2.0)	7.0 (2.0)	-	-	-	-
Anatomical Location									
Head and Neck	8.2 (3.3)^{a}	8.1 (3.5)^{a}	8.4 (3.0)^{ab}	8.3 (3.4)^{a}	8.3 (3.6)^{a}	8.3 (3.0)^{a}	7.9 (2.7)^{a}	7.0 (2.2)^{a}	9.0 (3.5)^{a}
Trunk	7.9 (2.9)^{a}	8.2 (3.1)^{a}	7.5 (2.8)^{a}	7.9 (3.0)^{a}	8.1 (3.2)^{a}	7.6 (2.7)^{a}	7.9 (2.8)^{a}	8.5 (2.6)^{a}	7.3 (2.9)^{a}
Extremities	8.0 (2.9)^{a}	8.0 (3.0)^{a}	7.9 (2.7)^{ab}	7.7 (2.8)^{a}	7.8 (2.9)^{a}	7.7 (2.7)^{a}	9.0 (2.9)^{a}	8.7 (3.3)^{a}	9.9 (1.3)^{a}
Perineal Region	9.2 (2.3)^{a}	9.2 (2.4)^{a}	9.1 (2.3)^{ab}	9.2 (2.3)^{a}	9.1 (2.5)^{a}	9.2 (2.2)^{a}	9.0 (2.6)^{a}	10.0 (0)^{a}	8.5 (3.5)^{a}

^{a,b,c,d} ANOVA followed by Tukey's test; $p < 0.05$. $^{\#}p < 0.05$ for Student's *t*-test

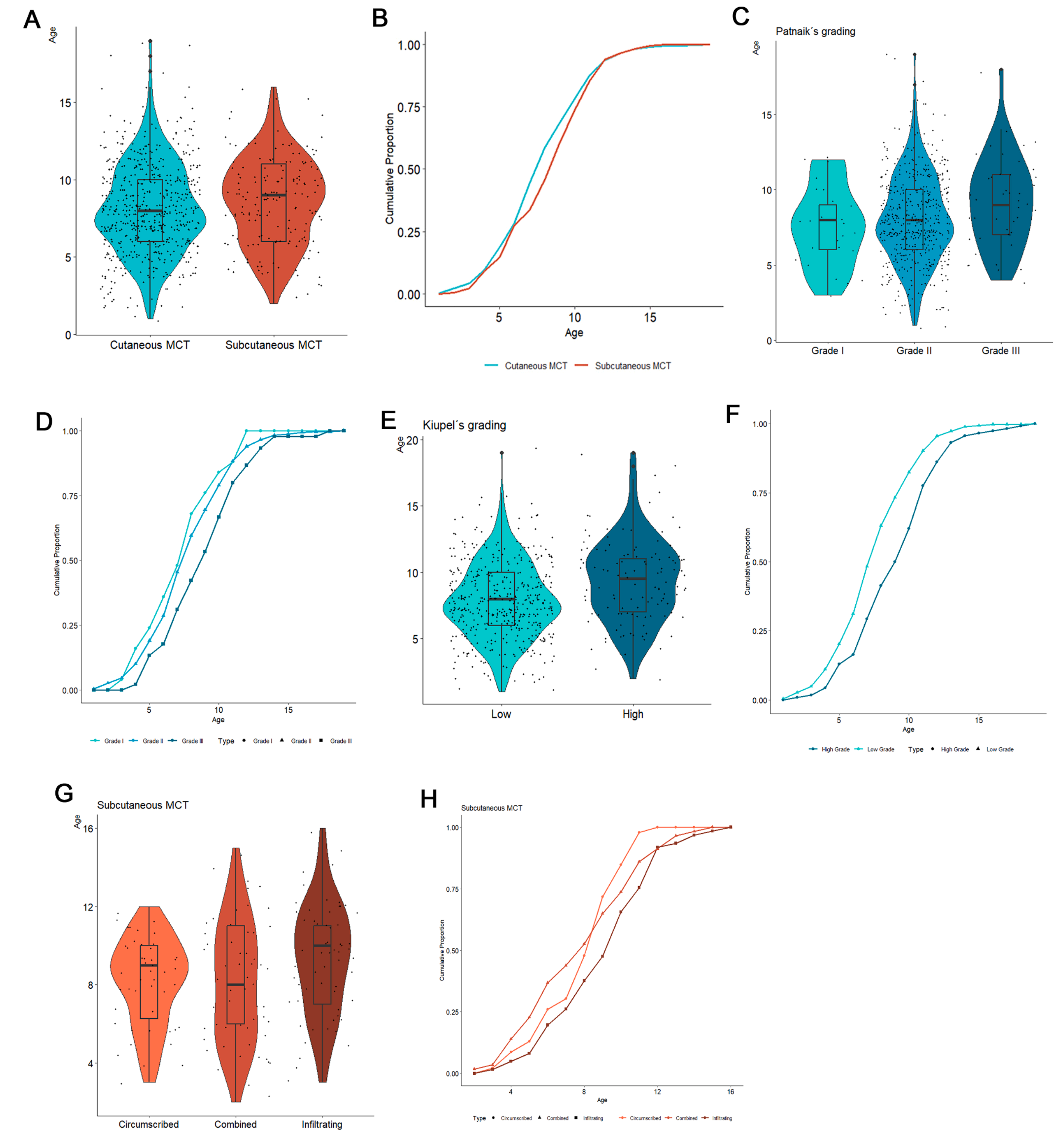

Fig. 1. Violin plots and cumulative line charts for mast cell tumors. A-B: Cutaneous versus subcutaneous mast cell tumors. C-D: Comparison of Patnaik's grading. E-F: Low versus high Kiupel's grades for cutaneous mast cell tumors. G-H: Comparative analysis of subcutaneous mast cell tumor patterns. *p < 0.05 for *t*-test. #p < 0.05 for the K-S test.

MCTsub versus MCTcut ($p < 0.01$, OR=1.08, 95 % CI: 1.01–1.12).

3.3. Cutaneous mast cell tumors

3.3.1. Patnaik's grading (Patnaik et al. 1984)

Data on Patnaik's grading were available for the 633 cases of MCTcut. Grade II tumors were the most predominant (n = 558, 88.1 %), followed by Grade III tumors (n = 46, 7.2 %) (Table 3). No statistically significant associations were identified between grading and sex (data not shown) ($p > 0.05$) or breed ($p > 0.05$). However, significant age-related differences emerged across the tumor grades. Grade III tumors were associated with a higher mean age at diagnosis (9.2 years, SD=3.0) than grade II (8.1 years, SD=2.9; $p = 0.03$) and Grade I (7.6 years, SD=2.6; $p = 0.02$) tumors (Table 3). Additionally, as shown in Figs. 1C and 1D, the K-S test confirmed that the age distributions across grades were statistically distinct (D=0.72; $p < 0.01$).

Table 3
Patnaik's grading distribution for cutaneous mast cell tumors per breed (n, %) and age (mean age and standard deviation [SD]). Only breeds with more than 10 cases were considered.

Breed	Grade I			Grade II			Grade III		
	n	% per breed	Mean Age (SD)	n	% per breed	Mean Age (SD)	n	% per breed	Mean Age (SD)
Mixed-Breed	4	1.9	7.7 (2.1)	185	87.3	8.7 (3.3)	23	10.8	9.7 (3.3)
Labrador Retriever	8	6.0	7.6 (2.1)	120	89.6	8.0 (2.4)	6	4.4	9.5 (2.3)
Boxer	2	4.3	8.0 (0.0)	40	87.0	7.1 (2.5)	4	8.7	7.8 (2.6)
French Bulldog	0	0.0	-	41	91.1	6.5 (2.6)	4	8.9	6.5 (1.7)
Golden Retriever	2	5.0	6.5 (0.7)	37	92.5	8.1 (2.6)	1	2.5	10.0 (0.0)
Pit Bull	2	11.1	11.0 (1.4)	15	83.3	7.6 (3.1)	1	5.5	11.0 (0.0)
Pug	2	16.7	3.5 (0.7)	10	83.3	6.7 (1.3)	0	0.0	-
Shar-pei	2	20.0	8.0 (5.7)	7	70.0	6.6 (2.5)	1	10.0	5.0 (0.0)
Total	29	4.6	7.6 (2.6)^{x}	558	88.1	8.1 (2.9)^{x}	46	7.3	9.2 (3.0)^{y}

% - Proportions of gradings per breeds.
$^{x, y}$ Student's *t*-test, $p < 0.05$, comparing between grades.

3.3.2. Kiupel's grading (Kiupel et al. 2011)

Data on Kiupel's grading were available for the 633 MCTcut cases, with 80.4 % (n = 509) classified as low-grade tumors and 19.6 % (n = 124) classified as high-grade tumors (Table 4). No statistically significant associations were found between tumor grade and sex ($p > 0.05$) (data not shown) or breed ($p > 0.05$). Notably, unlike most other breeds, French Bulldogs exhibited a very similar age at diagnosis for both high- and low-grade tumors (p = 0.79).

Age distribution analyses revealed significant differences: high-grade tumors had a higher mean age at diagnosis (9.4 years, SD=3.1) compared to low-grade tumors (7.8 years, SD=2.8; $p < 0.01$), based on *t*-test results (Table 4 and Fig. 1E). A distinct age distribution was confirmed using the Kolmogorov–Smirnov (K-S) test (D=0.53; p = 0.01) (Fig. 1E and F). In the logistic regression adjusted for sex, age was the only statistically significant factor associated with the likelihood of developing a high-grade tumor over a low-grade tumor ($p < 0.01$, OR=1.17, 95 % CI: 1.11–1.31).

In terms of anatomical location (Table 5), the head exhibited an increased probability of developing high-grade tumors when compared to the trunk (OR=2.28; p = 0.04; 95 % CI: 1.02–5.08). No other statistically significant differences were observed when various locations were compared, and no associations were identified between location and other factors.

3.4. Subcutaneous MCT patterns (Thompson et al. 2011a)

Data on these patterns were available for the 192 MCTsub cases. Infiltrative tumors were the most prevalent (n = 73, 38.0 %), followed by combined (n = 67, 34.7 %) and circumscribed (n = 52, 27.1 %) tumors (Table 6). There was no significant association between these patterns and sex (p = 0.96).

Regarding pattern and age, the K-S test for comparing age distributions found no differences between patterns (Fig. 1G and H). However, the *t*-test comparing infiltrative and non-infiltrative patterns (circumscribed + combined) showed significant differences, with the infiltrative pattern being associated with an older mean age (p = 0.02) (Table 6). The results of the logistic regression adjusted for sex indicated that age was the only factor that increased the probability of developing a tumor with an infiltrative pattern (p = 0.02, OR=1.17, 95 % CI: 1.04–1.33).

Mixed-breed dogs and Labrador Retrievers showed a higher percentage of tumors with an infiltrative pattern, whereas circumscribed MCTsubs tended to appear more frequently in the head and neck at a higher mean age (Table 6).

3.5. Population analysis

The districts with the highest representation were Lisbon (n = 369, 40.8 %), Setúbal (n = 139, 15.4 %), and Leiria (n = 83, 9.2 %) (Figure S1).

The EAIR was calculated for the Lisbon and Setúbal districts. Our database included 508 MCTs from these regions, over three years, with 249 cases in females and 259 in males. The registered SIAC population for Lisbon and Setúbal included 547,995 dogs, consisting of 263,568 females and 284,427 males. This data represents an EAIR for MCTs in the Lisbon and Setúbal regions of 3.1 cases per 10,000 dogs—3.0 cases per 10,000 for males and 3.2 cases per 10,000 for females.

Boxers, Shar-Peis, and Golden Retrievers exhibited the highest RR per breed compared to mixed-breed dogs, followed by French Bulldogs, Labrador Retrievers, and Pit Bulls (Fig. 2A). Sex-specific RR patterns also showed notable differences. Among males (Fig. 2B), Boxers had the

Table 4
Kiupel's grading distribution for cutaneous mast cell tumors per breed (n, %) and age (mean age and standard deviation [SD]). Only breeds with more than 10 cases were considered.

Breed	Low-Grade			High-Grade			OR_{HG}	95 % CI	p-value
	n	% per breed	Mean Age (SD)	n	% per breed	Mean age (SD)			
Mixed-Breed	160	75.5	8.2 (3.1)	52	24.5	10.5 (3.2)*	ref	-	-
Labrador Retriever	107	79.8	7.8 (2.3)	27	20.2	8.8 (2.6)	0.78	0.46–1.31	0.36
Boxer	37	80.4	6.8 (2.3)	9	19.6	8.6 (2.7)*	0.75	0.34–1.65	0.47
French Bulldog	36	80.0	6.4 (2.7)	9	20.0	6.7 (1.6)	0.77	0.35–1.70	0.52
Golden Retriever	36	90.0	8.1 (2.6)	4	10.0	7.3 (2.5)	0.34	0.12–1.01	0.07
Pit Bull	17	94.4	8.0 (3.1)	1	5.6	11.0 (0)	0.18	0.02–1.39	0.07
Pug	11	91.7	6.0 (1.7)	1	8.3	8.0 (0)	0.28	0.04–2.22	0.20
Shar-pei	7	70.0	7.8 (2.7)	3	30.0	4.0 (1)	1.32	0.33–5.28	0.98
Total	509	80.4	7.8 (2.8)	124	19.6	9.4 (3.1)*	-	-	-

% - Proportions of gradings per breeds.
* *t*-test comparing the mean age of the grades, with a significance level of $p < 0.05$
OR_{HG}: Odds ratio for high-grade versus low-grade

Table 5
Kiupel's grading distribution for cutaneous mast cell tumors by anatomical location.

	Total		High-Grade		Low-Grade				
Anatomical Location	n	%	n	%	n	%	OR_{HG}	95 % CI	p-value
Extremities	148	45.5	26	17.6	122	82.4	1.20	0.61–2.37	0.60
Trunk	106	32.6	16	15.1	90	84.9	ref	-	-
Head and Neck	52	16.0	15	28.8	37	71.2	2.28	1.02–5.08	**0.04**
Perineal Region	19	5.9	5	26.3	14	73.7	2.01	0.34–6.35	0.38
Total	325	100	62	19.1	263	80.9	-	-	-

OR_{HG}: Odds ratio for high versus low-grade. The lower total number of cases compared to that in Table 4 is because of missing information in the reports regarding the anatomical location.

Table 6
Distribution of subcutaneous mast cell tumor patterns by breed and anatomical location (n, %), along with age statistics (mean and standard deviation [SD]).

	Circumscribed			Combined			Infiltrative		
	n	%	Mean Age (SD)	n	%	Mean Age (SD)	n	%	Mean Age (SD)
Total $_{n}$= 192	52	27.1	8.2 (2.2)^{x}	67	34.9	8.1(3.1)^{x}	73	38.0	9.3 (2.8)^{y}
Breed									
Mixed-Breed	23	30.7	8.2 (2.5)	18	24.0	9.1 (3.9)	34	45.3	10.0 (2.9)
Labrador Retriever	11	22.4	8.7 (1.4)	13	26.5	8.6 (2.8)	25	51.0	9.1 (2.6)
Golden Retriever	5	50.0	8.2 (2.6)	3	30.0	10.0 (0.0)	2	20.0	8.5 (3.5)
Boxer	1	11.1	5.0 (0.0)	7	77.8	7.3 (2.6)	1	11.1	-
French Bulldog	0			4	57.1	7.0 (2.0)	3	42.9	8.0 (0.0)
Anatomical location									
Head and Neck	7	53.8	10.0 (1.7)^{x}	4	30.8	6.0 (2.6)^{y}	2	14.5	7.0 (0.0)
Trunk	11	23.4	7.2 (2.1)	16	34.0	7.9 (3.1)	20	42.6	8.2 (3.0)
Extremities	7	20.0	9.4 (2.2)	10	28.6	7.5 (2.9)^{x}	18	51.4	10.1 (2.7)^{y}
Perineal Region	0	0.0	-	2	50.0	11.0 (0.0)	2	50.0	8.0 (2.8)

$^{x, y}$ Student's *t*-test, $p < 0.05$, comparing the patterns in line.

highest RR (9.9, 95 % CI: 6.7–15.3, $p < 0.01$). Conversely, among female dogs (Fig. 2C), Shar-Peis demonstrated the highest RR (8.0, 95 % CI: 3.7–17.3, $p < 0.01$).

4. Discussion

This study included 905 cases of MCTs in dogs and aimed to provide an in-depth analysis of their frequency, classification, and associated factors. Most cases were identified as MCTcut, whereas approximately 20 % of the cases were identified as MCTsub. Breed-specific analyses revealed intriguing patterns, including male predominance in French Bulldogs and female predominance in Shar-Peis. Age-related trends also emerged, with some breeds, such as Pugs, Boxers, French Bulldogs, and Shar-Peis, showing significantly lower ages at diagnosis. Examination of tumor grades also highlighted age as a significant factor, with higher-grade tumors in MCTcut and infiltrative patterns in MCTsub, both of which were associated with an older mean age at diagnosis.

RR analysis showed that Boxers, Shar-Peis, and Golden Retrievers had a higher RR for the development of MCT. Additionally, sex-specific RR analysis identified distinct results, with Boxers having the highest RR among males and Shar-Peis among females.

In summary, this study offers insights into various aspects of MCT in dogs, including the EAIR, age-related characteristics, geographical distribution, and breed-specific patterns, and highlights different risk factors, particularly age and breed, in the development of these tumors. Numerous studies have highlighted MCTs as one of the most prevalent neoplasms in dogs (Villamil et al., 2011; Shoop et al., 2015; Baioni et al., 2017; Pinello et al., 2022b). In this study, more than 90 % of the cases were cutaneous or subcutaneous MCT, supporting the consensus in the literature that the skin is the primary organ affected by this tumor, with extracutaneous occurrences generally considered rare (Martins et al., 2022; Takahashi et al., 2000). This high percentage of skin tumors can also be partly attributed to the fact that tumors affecting this organ are more readily observed, potentially leading to more frequent diagnoses.

In the present study, we considered the canine population on the national pet register platform (SIAC) as the denominator, providing a more accurate assessment of MCT occurrence. Most cases were observed in Lisbon and Setúbal districts. Our estimated EAIR (3.1 per 10,000 dogs) emphasizes the importance of understanding and addressing this canine tumor for both veterinary professionals and pet owners.

Only MCTsub showed a female predilection. This finding aligns with some literature but contradicts other reports. Śmiech et al. (2018) suggested a predisposition in females, and White et al. (2011) described that spayed females are more susceptible to MCT development than intact females. Notably, when encompassing all MCT in our study, no sex predilection was observed, which is consistent with the results of previous studies (Misdorp, 2004; Webster et al., 2007; Shoop et al., 2015; Aupperle-Lellbach et al., 2022). However, the relative risk analysis suggested potential breed-dependent sex differences. In contrast to the previous studies, our investigation revealed no correlation between the grading and sex. Notably, the descriptions in the literature can be conflicting, as exemplified in a study by Reynolds et al. (2019), in which no sexual predisposition for higher grades was found; this contradicted the observations of Mochizuki et al. (2017), who indicated a subtle tendency for males to develop high-grade tumors. In 2005, Kiupel et al. (2005) reported that male dogs exhibited a reduced survival duration compared to females. Similarly, a previous study observed a more favorable prognosis in female dogs treated with a multi-agent chemotherapeutic protocol than in male dogs (Gerritsen et al., 1998). Canine MCT have been identified with estrogen and progesterone receptors, which potentially account for the survival disparity between sexes; however, the precise role of sex steroid hormone receptors in canine MCT remains unclear (Gerritsen et al., 1998). Future investigations are imperative to validate sex-specific differences in survival among dogs with MCT and to elucidate the contribution of hormones and hormone receptors to their development. A limitation of our study was the absence of information on the neuter status, further complicating the comparison of the results.

In our study, 55 breeds were represented, with Labrador Retrievers, Golden Retrievers, Boxers, Shar-Peis, and French Bulldogs being the most frequently affected, consistent with previous findings (Śmiech

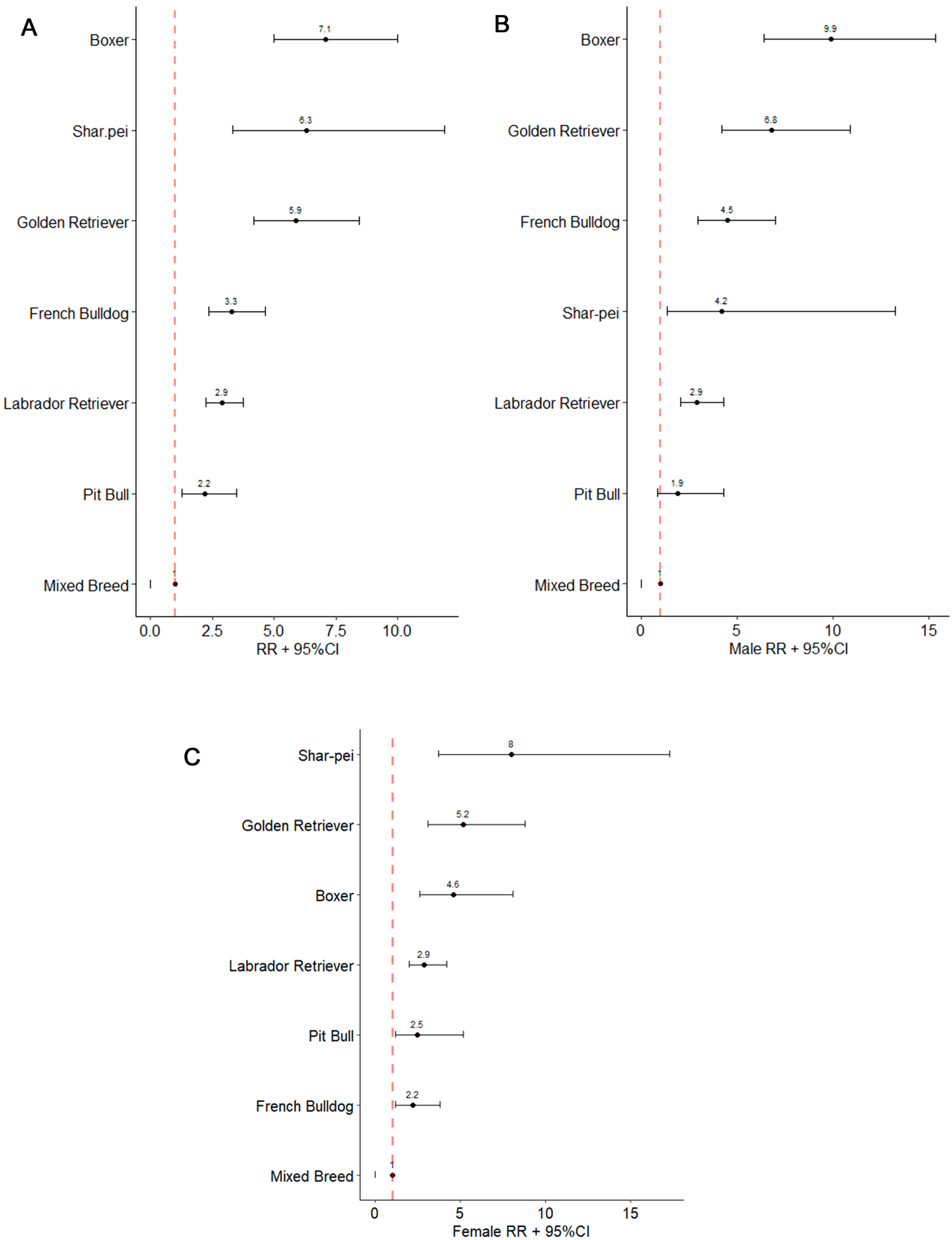

Fig. 2. Forest plot for the breeds with higher relative risk (RR) when compared with mixed-breed dogs. Each line depicts the 95 % confidence interval, with point estimates. A. RR for all mast cell tumors. B: RR for mast cell tumors in male dogs. C: RR for mast cell tumors in female dogs. *p < 0.05 for *t*-test. #p < 0.05 for the K-S test.

et al., 2019; Martins et al., 2021). Analysis of the FCI breed groups suggests a potential familial or genetic predisposition among these breeds. Risk analysis revealed that Boxers, Shar-Peis, and Golden Retrievers exhibited the highest RR, consistent with the findings reported by Martins et al. (2021), a study based on data from a laboratory in Northern Portugal, and Reynolds et al. (2019), emphasizing an elevated susceptibility to MCT in these specific breeds. In contrast, when aligning these outcomes with those of Mochizuki et al. (2017), in which the Parson Russell Terrier, Staffordshire Bull Terrier, American Staffordshire Terrier, Boxer, and Pug had the highest RR, the slight disparity in rankings might be attributed to the relative lack of popularity of these breeds in Portugal, as evidenced by the limited representation of these breeds in the present study. The prominence of Retrievers in this study can be attributed to the demographic composition of the research sample, as Retrievers are among the most prevalent dog breeds in Portugal. In terms of age, Molossoid breeds such as Boxers, French Bulldogs, Pugs, and Shar-Peis were shown to be more likely to develop tumors at younger ages. Mochizuki et al. (2017) noted a similar trend in Bulldog-associated breeds, including Boxers, French Bulldogs, English Bulldogs, American Staffordshire Terriers, Staffordshire Bull Terriers, and Boston Terriers. Breed-specific data of Rafalko et al. (2023) for Bulldogs, Boxers, Vizslas, French Bulldogs, and Boston Terriers also indicated median ages at cancer diagnosis of at least two years younger than the weight-predicted ages, suggesting that genetic factors may contribute to earlier cancer onset in these breeds. A recent study by Geraz et al. (2024) on dog life expectancy in Portugal found that the average lifespan of dogs of all breeds, including mixed breeds, born in the country is approximately 8.91 years. Crossbred dogs have a life expectancy of 9.48 years, and Yorkshire Terriers have the longest lifespan of 11.7 years. In contrast, French Bulldogs have the shortest lifespan at 6.29 years. Smaller breeds have the highest average lifespan at birth (9.52 years), followed by medium-sized breeds (9.26 years) and larger breeds (8.53 years). Breeds with a life expectancy under 7 years include six large breeds (Spanish Mastiff, Transmontano Mastiff, Neapolitan Mastiff, Great Dane, and Cane Corso) and the French Bulldog. McMillan et al. (2024) also highlighted significant variation in longevity across breeds. The breeds with the highest risk of early death included the Caucasian Shepherd Dog, Presa Canario, Cane Corso, Saint Bernard, Bloodhound, Affenpinscher, Neapolitan Mastiff, and French Bulldogs. Longevity also varied by breed size: small and medium-sized breeds had median survival times of 12.7 and 12.5 years, respectively, whereas large breeds had a shorter median survival of 11.9 years. Notably, only French Bulldogs were highlighted in our study for developing tumors at younger ages. Pugs (small breed), Shar-Peis (medium breed), and Boxers (large breed) were not indicated to have a higher risk of early death because of decreased life expectancies, which suggests that the fact that these breeds develop tumors at younger ages may not necessarily be related to shorter lifespans.

In this study, French Bulldogs were the only breed displaying a nearly identical average age for both higher- and lower-grade tumors. This observation aligns with previous findings on mammary tumors in this breed (Carvalho et al., 2023), suggesting promising opportunities for breed-related cancer studies.

In terms of the association between breeds and tumor grading, no correlations were found between breeds and tumors with higher grades or infiltrative patterns. This lack of association with breed contrasts with previous studies, such as those byReynolds et al. (2019) and Śmiech et al. (2018), in which the Shar-Pei breed was found to have an elevated risk of high-grade tumors. Conversely, Pugs have been reported to have a higher likelihood of developing low-grade tumors. In our study, although Pugs were not associated with low-grade tumors, they stood out as one of the breeds with the earliest age at diagnosis. This highlights the potential influence of age on the development of both low- and high-grade tumors, reinforcing the role of age as a significant factor (Martins et al., 2021; McNiel et al., 2006; Reynolds et al., 2019). Notably, for cutaneous and subcutaneous tumors, the most affected breeds were similar, suggesting that breed may not be a determining factor for the development of cutaneous and subcutaneous lesions. With regard to the anatomical location, no associations were identified between any of the MCTs and any of the factors. In the case of cutaneous MCT, a slight tendency was observed in females to have more lesions on the head and neck, whereas subcutaneous MCT lesions tended to occur on the extremities and more frequently in females. Regarding grading and prognosis, an elevated OR for developing a higher-grade tumor was identified only in Kiupel's grading of cutaneous tumors located on the head and neck, an observation that does not align with most published data. A comparison of anatomical location associations across studies presents a notable challenge. Kiupel's 2005 findings aligned with those of previous investigations by Bostock in 1973 (Bostock, 1973), emphasizing the absence of a significant association between selected tumor locations and survival time. Conversely, Reynolds et al. (2019) suggested a higher likelihood of high-grade tumors in the inguinal and head regions, a trend partially corroborated by the present study. In our study, the axillary area, as highlighted by Śmiech et al. (2018), was grouped under the thoracic location, potentially contributing to discrepancies in the observations. A primary barrier to making these comparisons is the lack of standardized reporting of the anatomical locations. This study followed the classification suggested by Kiupel et al. (2005); however, variations in reporting practices across studies may have led to conflicting results. Future studies should strive to document the association between anatomical location and other demographic factors, emphasizing the need for standardized reporting practices.

The mean age at diagnosis of MCTs in our study aligns with the findings of previous research, reinforcing the understanding that MCT is a tumor primarily affecting older dogs (Shoop et al., 2015; Mochizuki et al., 2017; Pierini et al., 2019). The likelihood of developing MCT has been associated with age, with dogs aged over 10 years having a 41 times higher risk than those under 10 years old (Reynolds et al., 2019). Age emerged as the main variable among those tested, influencing both the probability of developing tumors with a higher-grade and an infiltrative pattern. This correlation with prognosis is well documented, with studies by Treggiari et al. (2023) and Kiupel et al. (2005) linking older age to poorer outcomes. MacFarlane et al. (2016) also associated older age with an elevated risk of high-grade tumors. This age-related trend may be explained by several possibilities, one of which is that recurrent tumors in dogs with incompletely excised MCT tend to be of the same or higher-grade than the primary tumors, potentially because of additional genetic mutations over time (Mochizuki et al., 2017). Another possibility is that the accumulation of mutations in the genomic DNA of older animals increases their susceptibility to cancer. It is plausible to assume that older dogs have an increased likelihood of harboring multiple transformed mast cells, leading to an increased probability of the diagnosis of one or more MCT with a possible worse prognosis at the time of assessment (Kiupel et al., 2005). The consistent association between older age and higher-grade suggests that the genetic factors contributing to disease development may differ from those determining malignancy (Reynolds et al., 2019). Age has also emerged as a factor influencing the probability of developing cutaneous versus subcutaneous tumors. This could be attributed to the depth and concealment of subcutaneous tumors, potentially causing delays in diagnosis and contributing to a slight increase in the age at diagnosis. Further research is required to confirm this distinction. Another notable finding on age came from a study of MCT cases in dogs under 12 months of age, indicating predominantly favorable outcomes, in contrast to the behavior of similar MCT in older dogs. This suggests the potential necessity of refining the prognostic criteria based on a dog's life stages (Rigas et al., 2020).

When focusing on grading using the Patnaik and Kiupel systems, as seen in many prior studies, most tumors in this study are categorized as Patnaik grade II and exhibit a low Kiupel grade (Kiupel et al., 2011; Sabattini et al., 2015; Martins et al., 2021). In subcutaneous MCTs, the prevailing pattern was infiltrative, similar to that reported in previous studies (Thompson et al., 2011a; Gill et al., 2020). Comparing these results with the existing literature presents challenges, particularly for subcutaneous MCTs, owing to the limited number of studies focusing specifically on this type. The scarcity of studies specifically dedicated to this type of MCT hinders the comparison of results, particularly regarding the growth patterns. In previous studies, these tumors have often been grouped with Patnaik grade II cutaneous MCTs, primarily because of their location (Thompson et al., 2011a). Nevertheless, in alignment with the latest consensus that highlights the distinct categorization of MCTs into cutaneous and subcutaneous types (Willmann et al., 2021), this distinction was emphasized in our study.

4.1. Limitations

Lisbon and Setúbal were chosen for the population analysis because of the high number of samples originating from these regions. This selection bias can be explained by the location of DNAtech in Lisbon and the larger network of veterinary hospitals and clinics in the surrounding areas that sent samples for histopathological analysis. It is important to acknowledge that the samples were obtained exclusively from a single diagnostic laboratory. However, based on the data analyzed by Vet-OncoNet (data not shown), DNATech cases represented a substantial proportion of the diagnoses within these districts. Thus, the considerable sample size likely reflects the broad representation of these regions.

The potential limitations of underreporting both tumor cases and population data must also be acknowledged. However, using the number of animals registered in the SIAC as the denominator is considered the most reliable approach and the best available method because it functions as a national census in Portugal. We anticipate that mandatory microchipping and increased public awareness of the importance of tumor diagnosis will contribute to more comprehensive registration over time. This study is the first to calculate an EAIR for this specific geographical area, offering insights into the impact of MCT in these regions and making a valid contribution to our understanding of this common neoplasia. Nevertheless, broader, multicenter, and ideally nationwide studies are essential to validate and deepen the findings presented in this study.

5. Conclusions

In our study, age and breed emerged as key animal-related factors influencing the development of MCTs in dogs. The impact of age is evident in its association with both the probability of developing an MCT with a higher-grade or infiltrative pattern and the likelihood of developing cutaneous versus subcutaneous tumors. These findings are consistent with those of previous studies that have identified age as a prognostic factor. Furthermore, breed-specific predispositions contribute significantly to the occurrence of MCT, emphasizing the importance of breed characteristics in the understanding and management of this tumor. However, breed does not seem to affect the probability of developing either a cutaneous or subcutaneous lesion on the skin. Variations in genetic makeup among different breeds, as well as trends in inbreeding levels in some breeds, may play a role in the susceptibility to and clinical presentation of MCTs. Integrating age and breed into diagnostic and prognostic assessments may be essential for a more comprehensive understanding of the development in dogs. These findings emphasize the need for tailored approaches in veterinary care, considering demographic factors such as age and breed, to enhance the prevention, early detection, and management of MCTs in canine populations.

CRediT authorship contribution statement

J. Catarino: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **K. Pinelloe:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **J. Niza-Ribeiro:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **J. Santos:** Writing – original draft, Methodology, Data curation. **R. Payan-Carreira:** Writing – original draft, Methodology, Conceptualization. **J. Reis:** Writing – original draft, Conceptualization. **P. Faísca:** Writing – original draft, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors state no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2025.106416.

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