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A retrospective histopathological survey on canine lymphomas subtypes of Porto District

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Abstract

Background: Lymphomas are dogs' most common hematopoietic neoplasms and represent a heterogeneous group, as occurs in humans. Considering the role of dogs as models of human lymphomas and the geographical correlation of the cases of canine and human lymphoma, it is important to continuously assess the epidemiological distribution of lymphoma subtypes in dogs.

Aim: This study aimed to provide a survey of canine lymphoma subtypes diagnosed from 2005 to 2016 in the academic veterinary pathology laboratory of the University of Porto.

Methods: A total of 75 canine lymphomas diagnosed by histopathology in the Porto district were included. All cases were immunophenotyped by CD3 and PAX5, classified according to the current classification WHO and coded with Vet-ICD-O-canine-1.

Results: Mixed breed dogs were most common (28%), followed by Cocker Spaniels (12%), Boxers (9%), and Labrador Retrievers (6%). The mean age was 9.2 years (SD = 3.3) (10.7 years for small, 8.9 years for medium and large, and 5.7 years for giant breed dogs, $p < 0.05$). Regarding sex, there was no difference in frequencies or mean age. B-cell lymphomas were more common (57.4%) than T-cell lymphomas (37.3%), and 5.3% were classified as non-B/non-T-cell lymphomas. Of the cases, 49% had a multicentric distribution, followed by splenic (22%), cutaneous (12%), alimentary (12%), and extranodal (3%) forms. The most common B-cell subtypes were diffuse large B-cell lymphoma (DLBCL) (16.3%) and large immunoblastic lymphoma (14%), while T-zone lymphoma (21.4%) and intestinal lymphoma (18%) were the most common T-cell lymphoma subtypes.

Conclusion: Our study shows that the Porto district follows the international trend of higher prevalence of B-cell lymphomas in dogs, especially of the DLBCL subtype.

Keywords: Canine, Lymphoma, Histopathology, Portugal, Vet-ICD-O-canine-1.

Introduction

Lymphomas are one of the most prevalent neoplasias in dogs worldwide and represent a significant practical challenge because they are a heterogeneous group of tumors with distinct biological behavior, clinical course, and treatment response, thus paralleling with human lymphomas (Vail *et al.*, 2019). Such heterogeneity resulted in more than 30 entities listed in the World Health Organization (WHO) classification, each with several morphologic features and clinical outcomes (Valli *et al.*, 2011, 2015). Hence, the simple diagnosis of "lymphoma" is clearly insufficient when correct treatment is envisaged.

The accurate diagnosis and classification of lymphoma require (1) an exact location of the lesion, (2) appropriate selection and handling of sampled tissues, (3) evaluation of tissue microscopic architecture, (4) immunophenotyping, (5) clonality assessment, (6) assessment of cell size and nucleolus features, (7) mitotic index, (8) evaluation of invasion, and (9) clinical course (Valli *et al.*, 2011, 2013, 2015).

Several studies documented geographical differences in the prevalence of non-Hodgkin lymphoma (NHL) in humans (Muller *et al.*, 2005; Chiu and Hou, 2015). While follicular lymphomas are more prevalent in Western countries, T-cell lymphomas prevail in Asia, while in

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the Middle East, intestinal lymphomas are particularly common (Muller *et al.*, 2005). Epidemiologically, studies concerning geographical differences in canine lymphoma subtypes are increasingly relevant, both for the benefit of animals and for their characterization as models of human lymphoma (Marconato *et al.*, 2013; Ito *et al.*, 2014; Pinello *et al.*, 2019).

This study describes canine lymphomas diagnosed in the Porto district according to their main types and epidemiological characteristics to compare with the international distribution of this cancer.

Material and Methods

In a cross-sectional study, 75 formalin-fixed paraffin wax-embedded tissue samples of canine lymphomas were retrieved from the archives of the Laboratory of Veterinary Pathology of the School of Medicine and Biomedical Sciences, ICBAS—University of Porto. The cases were diagnosed between 2005 and 2016 and were chosen only from the samples from the District of Porto. Immunohistochemical evaluation of the lesions was performed in all samples with antibody anti-CD3 (rabbit polyclonal, Dako) for T-cells and antibody anti-PAX5 (monoclonal mouse, Leica Biosystems, Nussloch, Germany) for B-cells (Willmann *et al.*, 2009) using an indirect immunoperoxidase assay Novolink Polymer® (Leica Mycrosystems, Nussloch, Germany). All cells with the expected staining pattern (membranous/cytoplasmic for CD3 and nuclear for PAX-5) were considered positive, regardless of the staining intensity. Lymphomas were classified as B- or T-cell types if at least 70% of neoplastic cells were labeled for the respective immunomarkers or null (O) when there was no immunolabelling (Ponce *et al.*, 2010a). Histological subtypes were assigned based on tissue architecture, cell morphological characteristics (size, nucleus, and nucleoli characteristics), and mitotic index, according to the criteria defined by the WHO classification (Valli *et al.*, 2015). The cases in which it was impossible to determine a classification were denominated No Other Specified (NOS). Each record was classified according to the anatomical localization (topography) and histological type (morphology) using the Vet-ICD-O-canine-1 classification system (Pinello *et al.*, 2022).

Data were collected on the breed, sex, and age of the animals. Pedigreed dogs were categorized by size (small, medium, and large). Mixed breed dogs were categorized by weight when available: small <11 kg, medium: 11.1–23 kg, large: 23.1–40 kg, and giant: >40 kg. Descriptive analysis was performed for the breed, sex, breed size, immunophenotype, morphology, and topography. Continuous data were tested for normality by the Shapiro–Wilk test. Numerical summaries included frequencies, percentages, mean with respective standard deviation (SD), and median with minimum and maximum values. *T*-test (for two categories) and analysis of variance (ANOVA) (more than two categories) followed by multiple comparison Tukey tests to compare differences between age groups were performed to describe differences in the mean age. B- and T-cell proportions were calculated, and the crude odds ratios (OR) were used to find the over-representation of immunophenotype (T-cell/B-cell). A Chi-squared test was used to assess *p*-values.

Statistical significance was considered for a *p*-value less than 0.05. The analyses were performed using IBM SPSS Statistics software (Version 27).

Ethical approval

The study was approved by the Organism responsible for the Animal Welfare of the Institute of Biomedical Sciences of Abel Salazar of the University of Porto (ORBEA ICBAS-UP) on October 12, 2015 (approval number 066/2014).

Results

The overall mean age of lymphomas was 9.2 years (SD = 3.3), with no difference between the sexes. In addition, the distribution of lymphomas in dogs (Table 1) showed that there was no sex predisposition, and there were no differences in the age of diagnosis or the proportion of B- and T-cell proportions.

Lymphomas were evenly distributed in three age categories: 0–5 years, 5–10 years, and older than 10 years. More than 88% occur after 5 years old. The highest T-cell proportion occurs between 5 and 10 years old, however not statically significant (Table 1).

Regarding breed size, the highest proportion of the cases belongs to large dogs (Table 1). Compared to the group without size information (mixed breed dogs), there were no differences in the T-/B-cell proportions. However, when grouped, small and medium-sized dogs had a higher proportion of B-cell lymphomas, whereas the opposite was true in large and giant dogs ($p = 0.004$). In addition, gigantic dogs had a statistically lower mean age at the time of the diagnosis than all others ($p < 0.05$) (Table 1).

Regarding breeds, mixed-breed dogs were the most common, followed by Cocker spaniels, Boxers, and Labrador retrievers (Table 1). The mean and median ages were similar in all breeds but differed in proportions of B- and T-cell lymphomas, with Boxers presenting 7.5-fold higher odds of having a T-cell lymphoma.

B-cell lymphomas were diagnosed most frequently (57.4%), followed by T-cell lymphomas (37.3%). The mean ages of dogs affected by B-cell lymphomas were slightly higher than for T-cell and null lymphomas; however, the difference did not reach statistical significance (Table 1).

The predominant anatomic types (Table 2) were multicentric (49.3%), followed by splenic (22.7%), cutaneous and gastrointestinal lymphomas (12.0% each), and extranodal with fewer cases (3.0%). The mean age of dogs with splenic lymphomas (11 years old) was significantly higher than that of the other

Table 1. Descriptive analysis of canine lymphomas subtypes (B and T cells) cases by sex, age, breed size, and breeds.

	n	%	Mean Age (SD)	Median age (min-max)	B-cell (*n*, %)	T-cell (*n*, %)	OR (T/B-cell) (95% CI)	*p*-value
Sex								
Female	41	54.7	9.4 (3.2) [a]	10 (2–16)	24 (63.2)	14 (36.8)	ref	–
Male	34	45.3	8.9 (3.0) [a]	9 (3–14)	19 (57.6)	14 (42.4)	1.2 (0.49–3.28)	0.630
Age (years)								
0–5	9	12.0	2.3 (0.5)	2.0 (2–3)	6 (75.0)	2 (25)	ref	-
>5–10	32	42.7	7.0 (1.3)	7.0 (5–9)	15 (48.4)	16 (51.6)	3.2 (0.56–18.39)	0.181
>10	34	45.3	11.8 (1.7)	11.5 (10–16)	22 (68.7)	10 (31.3)	1.3 \(0.23–7.98)	0.732
Breed size								
Small	8	10.7	10.8 (2.4) [a]	10 (8–14)	5 (62.5)	3 (37.5)	1.1 (0.18–6.54)	0.936
Medium	20	26.7	8.9 (3.0) [a]	8 (5–15)	17 (85.0)	3 (15.0)	0.3 (0.06–164)	0.325
Large	28	37.3	8.9 (3.0) [a]	10 (2–16)	11 (42.3)	15 (57.7)	2.4 (0.64–9.39)	0.184
Gigant	4	5.3	5.7 (3.7) [b]	6 (2–9)	1 (33.3)	2 (66.7)	3.6 (0.26-50.3)	0.736
No information	15	20.0	10.4 (2.8) [a]	11 (6–15)	9 (64.3)	5 (35.7)	ref	-
Breed (top 5)								
Mixed breed	21	28.0	9.5 (3.0) [a]	10 (5–15)	15 (75.0)	5 (25.0)	ref	-
Cocker spaniel	9	12.0	9.5 (3.4) [a]	9 (5–15)	6 (66.7)	3 (33.3)	1.5 (0.27–8.34)	0.641
Boxer	7	9.3	9.0 (2.7) [a]	10 (4–12)	2 (28.6)	5 (71.4)	7.5 (1.09–51.5)	0.031*
Labrador Retriever	5	6.7	9.6 (4.15) [a]	9 (5–16)	1 (25.0)	3 (75.0)	9.0 (0.75–107.3)	0.180
Golden Retriever	4	5.3	9.2 (2.2) [a]	10 (6–11)	1 (33.3)	2 (66.7)	6.0 (0.44–81.2)	0.431
Immunophenotype								
B-cell	43	57.4	9.7 (3.0) [a]	10 (3–15)				
T-cell	28	37.3	8.8 (3.0) [a]	9 (2–16)				
Null	4	5.3	7.2 (4.1) [a]	8 (2–11)				

*$p < 0.05$, chi-squared test.
NOS: no other specified.
Letters: Differences between groups; *T*-test and ANOVA followed by Tukey test, $p < 0.05$.

Table 2. Descriptive analysis of canine lymphoma by anatomic forms: frequency, percentage, mean age with respect to SD, median age with the minimum and maximum values.

	N	%	Mean age (SD)	Median age (min-max)
Multicentric	37	49.3	8.6 (3.1) [a]	9.0 (2–16)
Splenic	17	22.7	11.0 (2.5) [b]	11 (6–15)
Alimentary	9	12.0	9.3 (3.7) [a, b]	9.5 (2–14)
Cutaneous	9	12.0	8.2 (2.4) [a]	8.0 (5–10)
Extra nodal	3	3.0	7.0 (2.8) [a]	-

Letters: Differences between groups, ANOVA followed by Tukey test, $p < 0.05$.
For entities with less/equal three cases, the median age was not calculated.

anatomic forms, except for the alimentary form ($p < 0.05$).

Regarding the subtypes of lymphomas (Table 3), the most common subtype of B-cell lymphoma was large diffuse cell (DLBCL) (16.3%), followed by large cell immunoblastic lymphoma and splenic marginal zone lymphoma (8.0%) and NOS (6.7%). In the T-cell lymphoma group, the most common were T-zone lymphoma (TZL) (21.4%), intestinal lymphoma (17.9%), NOS (14.3%), and large anaplastic lymphoma (14.3%).

Considering the subtypes per anatomic types (Table 4), DLBCL, large cell immunoblastic lymphoma, and TZL were the most predominant in MC lymphomas ($n = 6$, 16.2% each). Splenic marginal zone B-cell lymphoma was the most frequent in the splenic

Table 3. Descriptive analysis of canine lymphoma subtypes: frequencies, proportions, mean age with respect to SD, and median age with minimum and maximum values.

Lymphomas subtypes, Vet-ICD-canine-1 code	*N*	%	Mean age (SD)	Median age (min-max)
B-cell lymphomas	43	57.3	9.7 (3.1)	10.0 (3–15)
DLBCL, 9,680/3	7	16.3	7.6 (2.9) [a]	7.0 (3–11)
Large cell immunoblastic lymphoma, (9684/3)	6	13.9	9.0 (3.6) [a]	8.0 (6–13)
Splenic marginal zone B-cell lymphoma, (9689/3)	6	13.9	10.7 (3.3) [a]	11.0 (6–15)
B-cell lymphoblastic leukemia/lymphoma, (9811/3)	4	9.3	8.0 (2.6) [a]	7.0 (6–11)
Follicular lymphoma, NOS, (9673/3)	3	6.9	9.7 (3.2) [a]	–
Mantle cell lymphoma, (9673/3)	3	6.9	11.3 (4.0) [a]	–
T-cell-rich large B-cell lymphoma, (9688/3)	2	4.6	9.0 (0) [a]	–
B-cell lymphoma, NOS, (9591.1/3)	2	4.6	11 (2.7) [a]	–
Marginal zone lymphoma, NOS, (9699/3)	2	4.6	11.0 (1.4) [a]	–
Lymphoplasmacytic lymphoma, (9671/3)	2	4.6	10.0 (5.7) [a]	–
Extranodal marginal zone lymphoma of MALT, (9699.2/3)	1	2.3	9.0 [a]	
T-cell lymphomas	28	37.3	8.8 (3.0)	9.0 (2–16)
T-zone lymphoma (TZL), nodal, (9702.1/3)	6	21.4	7.7 (1.8) [a]	7.5 6–10)
Intestinal T-cell lymphoma (enteropathy associated), (9717/3)	5	17.9	8.2 (2.5) [a]	8.5 (5–11)
T-cell lymphoma, NOS, (9591.2/3)	4	14.3	8.2 (4.6) [a]	9.0 (2–13)
Anaplastic large cell lymphoma (T-cell and null cell type), systemic, (9714/3)	4	14.3	10.0 (2.8) [a]	9.0 (8–14)
Precursor cell lymphoblastic lymphoma, NOS, (9727/3)	3	10.7	9.7 (2.5) [a]	-
Cutaneous epitheliotropic lymphoma, (9700/3)	3	10.7	9.0 (3.6) [a]	-
Peripheral T-cell lymphoma, NOS, (9702/3)	2	7.1	7.0	-
Angioimmunoblastic T-cell lymphoma, (9705/3)	1	3.6	16.0	-
Null cell	4	5.3	7.2 (4.1)	8.0 (2–11)
Total	75	100	9.2 (3.1)	9.0 (2–16)

For entities with less and equal three cases, the median age was not calculated.
Letters: Differences between groups, ANOVA followed by Tukey test, $p < 0.05$.

($n = 6$, 35.3%). Intestinal T-cell lymphoma (enteropathy associated) and malignant lymphoma (non-Hodgkin), NOS subtypes were the most frequent diagnosis in the alimentary lymphoma type ($n = 3$, 33.3% each). And cutaneous epitheliotropic lymphoma was the most frequent subtype in the skin.

Figure 1–C shows the unusual diagnosis of angioimmunoblastic T-cell lymphoma with prominent capillaries through the node and the corresponding immunostaining. Figure 1D–F shows a case diagnosed as follicular B-cell lymphoma, grade 2. Figure 1G–I represents an intestinal marginal zone lymphoma of the mucosa-associated lymphoid tissue type (MALT).

Discussion

This study shows that in dogs from the district of Porto, Portugal, diagnosed with lymphoma between 2005 and 2016, (1) there was no sex predisposition and no difference in mean age between the sexes, (2) the size/breed of the dogs was inversely related to age at diagnosis, (3) the highest mean age at diagnosis was found in splenic lymphomas, (4) DLBCL lymphomas predominated, followed by TZLs.

The WHO classification for canine lymphomas (Valli *et al.*, 2015) used in this study requires information on the cell phenotype, location, and histologic architectural pattern of the lesion (Valli *et al.*, 2015). These parameters correspond to those of the WHO classification for human lymphomas and thus allow comparative oncological studies.

Unlike previous studies that verified a higher male proportion (Gavazza *et al.*, 2001; Ponce *et al.*, 2010b; Kimura *et al.*, 2011; Vail *et al.*, 2012), our series consisted mainly of females, although there was no statistical difference. In addition, the mean age at diagnosis was slightly higher than that reported by other authors (9.2 years as opposed to a range of 5.9–9 years) (Ponce *et al.*, 2010b; Vezzali *et al.*, 2010; Vail *et al.*, 2012; Aupperle-Lellbach *et al.*, 2022).

Table 4. Distribution of canine lymphoma subtypes diagnosed per anatomical location.

Anatomical type Subtypes	*n*	%
Multicentric lymphoma	37	49.3
DLBCL	6	16.2
TZL, nodal	6	16.2
Large cell immunoblastic lymphoma	6	16.2
Precursor cell lymphoblastic lymphoma, NOS	3	8.1
Lymphoplasmacytic lymphoma	2	5.4
Null lymphoma	2	5.4
Intestinal T-cell lymphoma (enteropathy associated)	2	5.4
B-cell lymphoblastic leukemia/lymphoma	2	5.4
Anaplastic large cell lymphoma (T-cell and null cell type), systemic	2	5.4
Marginal zone lymphoma, NOS	2	5.4
Follicular lymphoma, NOS	1	2.7
T-cell-rich large B-cell lymphoma	1	2.7
Peripheral T-cell lymphoma, NOS	1	2.7
Angioimmunoblastic T-cell lymphoma	1	2.7
Splenic	17	22.7
Splenic marginal zone B-cell lymphoma	6	35.3
Malignant lymphoma (non-Hodgkin), NOS	5	29.4
Mantle cell lymphoma	3	17.6
Follicular lymphoma, NOS	2	11.8
Null lymphoma	1	5.9
Alimentar	9	12.0
Malignant lymphoma (non-Hodgkin), NOS	3	33.3
Intestinal T-cell lymphoma (enteropathy associated)	3	33.3
Anaplastic large cell lymphoma (T-cell and null cell type), systemic	1	11.1
Null lymphoma	1	11.1
Extranodal marginal zone lymphoma of MALT	1	11.1
Cutaneous	9	12.0
Cutaneous epitheliotropic lymphoma	3	33.3
B-cell lymphoma, NOS	2	22.2
Peripheral T-cell lymphoma, NOS	1	11.1
Malignant lymphoma (non-Hodgkin), NOS	1	11.1
Anaplastic large cell lymphoma (T-cell and null cell type), systemic	1	11.1
B-cell lymphoblastic leukemia/lymphoma	1	11.1
ExtraNodal	3	4.0
B-cell lymphoblastic leukemia/lymphoma	1	33.3
T-cell-rich large B-cell lymphoma	1	33.3
DLBCL	1	33.3
Total	75	100.0

Another difference from previous results was the fact that Cocker Spaniels were the second most common dog breed after mixed breeds, similar to a recent German study (Aupperle-Lellbach *et al.*, 2022). However, this position is usually occupied by Boxers (Ponce *et al.*, 2010b; Kimura *et al.*, 2011). It is important to note that such differences in breed disposition between studies could be due to differences in the popularity and

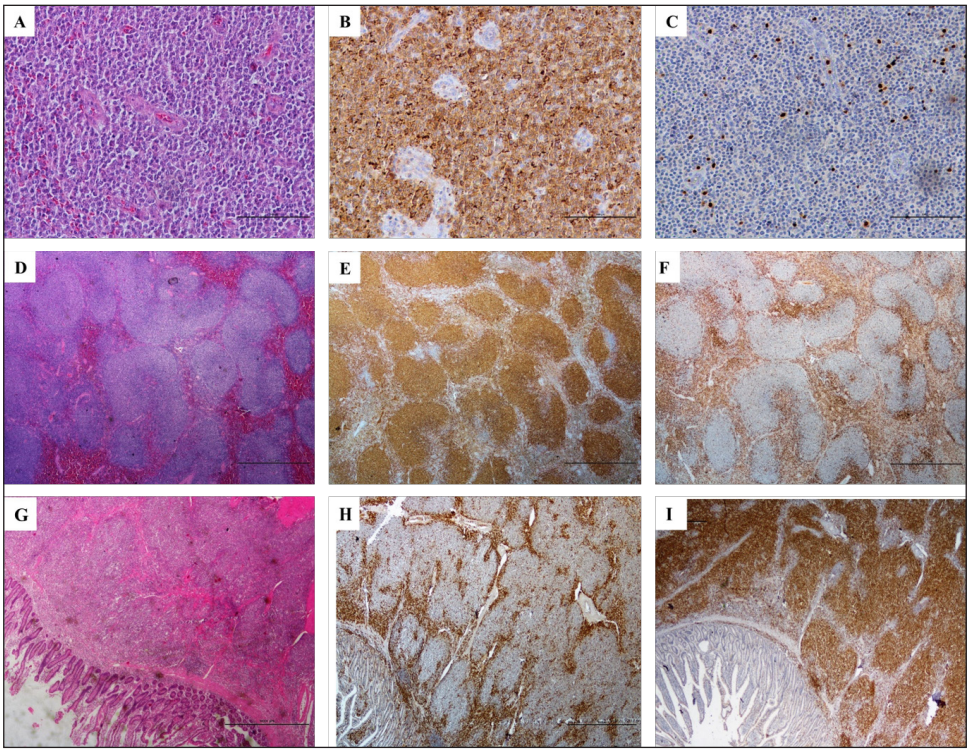

Fig. 1. Photomicrographs of canine lymphoma. A, B, and C: T-cell large angioimmunoblastic lymphoma. Lymph node. A: Prominent capillaries spread through the node. B: CD3 positive immunolabelling. C: PAX5 immunolabeling. D, E and F: B-cell center follicular lymphoma (grade 2). Lymph node. D: Large follicles, lack of mantle cell cuff. E: Neoplastic B-cells uniformly immunolabelled for anti-PAX5. F: CD3 immunolabeling. G, I, and H: B-cell MALT lymphoma. Intestine. G: Mucosa-associated lymphoid tissue composed predominantly of sheets and coalescent nodules of small lymphoid cells. I: PAX 5 positive immunolabeling of more than 90% of the neoplastic. cells. H: CD3 immunolabelling of a few T cells presented in the margins of the neoplastic lymphoid nodules.

number of certain breeds in different countries, an issue that only will be dismissed with the animal census.

Consistent with previous studies (Priester, 1967), most boxers had T-cell lymphomas, with the multicentric form being the most common (Ponce *et al.*, 2010b; Vezzali *et al.*, 2010; Kimura *et al.*, 2011). However, the second most common anatomic location was the splenic form; in contrast to most publications, the cutaneous and alimentary forms were more common than the splenic form (Vezzali *et al.*, 2010). However, it is important to note that some authors did not consider the classification of splenic lymphomas (Vail *et al.*, 2012; Valli *et al.*, 2013, 2015).

The immunophenotypic differentiation of B or T lymphomas has become a fundamental classification and prognostic tool (Ponce *et al.*, 2004, 2010b; Valli *et al.*, 2013). Traditionally, T-cell lymphomas are inevitably linked to a poor prognosis, whereas B-cell lymphomas were associated with a better prognosis (Ponce *et al.*, 2010b; Valli *et al.*, 2013). This generalization was, however, losing strength as other characteristics, namely subtypes, locations, and histological grades, were shown to have a higher prognostic value (Valli *et al.*, 2006; Aresu *et al.*, 2015). However, immunophenotypes remain of great importance in epidemiological studies, and human lymphomas have shown different risk factors among subtypes (Fisher and Fisher, 2004; Ekstrom-Smedby, 2006).

The most frequent B-cell lymphoma was diffuse large cell (DLBCL), in agreement with the literature (Valli *et al.*, 2015). The DLBCL category consists of a mixture of immunoblast and centroblast populations and includes the centroblastic, immunoblastic, NOS, and "T-rich" variants, differentiable by the number of immunoblasts (Valli *et al.*, 2011, 2015). In Vet-ICD-O-canine-1, the centroblastic form is coded as "related" under the DLBCL, while the immunoblastic form has its own code. The "T-rich" variant, most common in horses and cats although poorly reported in dogs (Valli *et al.*, 2015), can be mistaken for T-cell lymphoma due to its high number of T-cells and the fact that centroblasts and immunoblasts (B-cells) may represent less than 10% of the cell population (Valli *et al.*, 2015).

Among T lymphomas, the predominant subtype was the T-zone, followed by the intestinal lymphoma, similar to previous studies (Vezzali *et al.*, 2010; Valli *et al.*, 2011, 2013). The cutaneous T-cell lymphomas of this study were classified as epitheliotropic, characterized by being indolent, sometimes multifocal, and locally widespread, sometimes referred to as mycosis fungoides (Valli *et al.*, 2015).

Finally, the results found for null lymphomas (noB/noT) are in agreement with the literature, representing less than 5% of the cases (Caniatti *et al.*, 1996; Fournel-Fleury *et al.*, 1997; Zandvliet, 2016).

In conclusion, the results of this study follow the international trend of a higher proportion of B-cell lymphomas in dogs, especially of the DLBCL subtype**.**

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Conflict of interest

The authors declare that there is no conflict of interest.

Author Contributions

KP, JNR, and AJM participated in the study's inception and design. MS, PDP, and KP performed the lymphomas diagnostic and classification. In addition, KP procured digital photographs, data, and statistical analyses. KP, JNR, AJM, MS and PDP were involved in manuscript preparation.

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