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Research Article

Exploring risk factors linked to canine lymphoma: a case-control study

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ABSTRACT

Environmental factors, largely influenced by human behavior, account for approximately 80 % of malignant tumors. Risk factors associated with non-Hodgkin's lymphoma (NHL) have been identified in various countries among both humans and domestic animals. This study aimed to investigate potential risk factors for NHL in dogs residing in the district of Porto, Portugal. A comprehensive survey comprising 70 questions was undertaken and given to 113 dog owners, including 55 cases and 58 controls. Our findings revealed that dogs weighing over 10 kg (OR=9.1, $p < 0.001$), purebred dogs (OR=2.4, $p = 0.037$), those with consuming homemade food (OR=2.7, $p = 0.03$), and fruits and vegetables (OR=2.8, $p = 0.022$) exhibited higher odds of developing lymphoma. Notably, dogs with lymphoma were exposed to a significantly higher mean smoking index compared to the control group (13.7, SD=12.5 vs. 8.4, SD=9.3, $p < 0.001$). These findings suggest that lymphoma risk in dogs seems to be influenced by a combination of innate (genetic) factors and modifiable environmental factors linked to owner habits. Nevertheless, further large-scale epidemiological studies are warranted to validate these results.

Introduction

Non-Hodgkin lymphoma (NHL) is a highly prevalent malignant neoplasm in both humans and companion animals. In canines, lymphoma accounts for approximately 90 % of hematopoietic neoplasms, with an estimated incidence of 21.7 cases per 100,000 dogs[1] and an age-adjusted incidence of 107 cases per 100,000 dogs.[2] This incidence surpasses the global standardized incidence of 9.8 cases per 100,000 individuals observed in humans.[3]

Canine lymphoma serves as spontaneous neoplasms that closely mimic the pathophysiology and clinical characteristics of human NHL, making them valuable models for studying the disease.[4] Research in dogs offers advantages such as reduced confounding factors present in human studies, including the influence of alcohol, occupational hazards, and migration-induced environmental variations.[5] Additionally, the shorter life expectancy of dogs results in shorter latency periods between exposure to risk factors and neoplastic development.[5] As dogs are increasingly living in close proximity to their owners, investigating the impact of environmental factors on their health could potentially reveal shared risk factors for human diseases.[5] Consequently, dogs can be regarded as sentinels for identifying environmental hazards that affect human health.[5-7]

Multiple factors have been associated with the development of NHL in both humans and dogs.[8] Environmental tobacco smoke (ETS), also known as secondhand smoke (SHS), consists of mainstream smoke exhaled by smokers and smoke emitted by burning tobacco, which becomes diluted with ambient air.[9] The International Agency for Research on Cancer (IARC) has identified 63 chemicals in SHS, 11 of which have

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proven carcinogenic effects on various types of cancer.[10] However, SHS is not the sole source of exposure for nonsmokers.[11] Smoke gases and particles from SHS can deposit, age, and persist in textiles, surfaces, and dust, forming what is referred to as thirdhand smoke (THS).[12] THS constituents may re-enter the gas phase or interact with oxidants and other atmospheric compounds, leading to the formation of secondary pollutants, some of which exhibit heightened toxicity.[11,12] Dogs, like children, are increasingly spending more time indoors[13] and are in closer proximity to the ground, making them more vulnerable to secondary and tertiary pollutants from cigarettes. Although attempts to establish a definitive causal association between SHS exposure and canine cancers have yielded inconclusive results thus far.[14-19] A study by Bertone et al. in 2002 reported a three-fold relative risk for lymphoma in cats exposed to ETS for five or more years compared to those in nonsmoking households.[20]

Our previous studies have demonstrated a geographical correlation between human and canine lymphoma in Porto, Portugal[21], as well as a link between tobacco exposure and high proliferative lymphoma[22]. Considering the risk factors and to broaden the previous studies' results, we conducted a case-control study aiming to identify associations between exposure factors and canine lymphoma in dogs diagnosed with and without lymphoma in the Porto district of northwestern Portugal.

Materials and methods

Study population

The study included dogs diagnosed with lymphoma confirmed through histopathological or cytological examinations between 2005 and 2016, whose owners resided in the Porto district, regardless of any minimum residency time. All types of lymphoma were included, irrespective of their subtype or anatomical location. The records were obtained from various sources, including the Laboratory of Animal Pathology at the School of Medicine and Biomedical Sciences (ICBAS), University of Porto; the Veterinary Hospital of the University of Porto – UPVet; the Veterinary Hospital Centre – CHV, and the Veterinary Reference Hospital Montenegro, the last two being private veterinary hospitals located in the city of Porto. To gather information the owners of dogs with lymphoma were contacted by telephone, email, or during in-person consultations.

The control group comprised healthy dogs aged four years or older, residing in the Porto district, regardless of any minimum residency time, and with no known neoplastic or infectious diseases. Dog owners in the control group were recruited through an online questionnaire distributed among the University of Porto's employee database, including faculty, technical staff, and administrators. This database consists of approximately 5,000 individuals living in the Porto district.

Risk factors

After an intensive revision of the literature, a certain number of risk factors common to humans and dogs were identified and selected: Age of onset[23, 24], sex[25, 26], breed[24, 27, 28], exposure to chemical agents[29-33], tobacco smoke[34-36] and the secondary and tertiary forms of smoking for dogs[5, 15, 18, 22]; magnetic fields[37], ionizing radiations[29, 38], air pollution[39-45], owner's occupation[46, 47], weight and body condition[48-52] and diet[46, 53].

Questionnaire

A questionnaire with 70 questions was prepared based on the previous review. The questionnaire consisted of five main sections (Table 1). Section 1 included: the identification of the dog and dog owners. Section 2 included: residence - address, zip code, neighborhood, place of residence, and type of residence. Section 3 addressed owner literacy, the proximity of dog to owners, date and origin of acquisition - shelter, breeder, pet shop, stray, or other - and years living at the same address. Section 4 contained information on the presence of a co-inhabitant (animal or human) with cancer. Section 5 concerned risk factors and was divided into two subsections. Subsection 5.1 was related to animal characteristics: age, sex, breed, breed size (small, medium, large; according to the Federation Cynologique Internationale – FCI, when available or based on the commonly accepted size of the breed), reproductive status, weight, weight category (< 10 kg, 10-25 kg, > 25 kg), body condition (subjectively assessed by the owner, supported by illustrative figures that were not based on any preexisting scoring system), hair and nose length, signals of anxiety and level of physical activity. Subsection 5.2 was related to exposure to environmental factors. Type of food, fruit/vegetable intake, water source, type of bowl, use and frequency of external parasite treatment, and history of tick infestation. Occupation of owner; home use and frequency of herbicides, fungicides, insecticides, rodenticides, and bleach. Proximity to high-voltage power lines (<1 kilometer); characteristics of the home - size, type of flooring, presence of curtains and rugs. Indoor pollution - use of fireplaces and incense sticks; exposure to tobacco smoke. Outdoor pollution: Type of neighborhood (rural/urban), proximity of forest fires, type and intensity of road traffic.

According to our previous results[22], the questionnaire placed special emphasis on data collection on exposure to tobacco smoke. Data were collected on the number of former or current roommates who smoked, the classification of daily cigarette consumption per person into four categories, the intensity of smoking in the past ten years, and the places where smoking was allowed at home. To estimate daily cigarette consumption per individual, participants were classified into four categories based on the number of cigarettes smoked per day: < 10, 11–20, 21–30, and > 30. For each category, the median value of the respective interval was used as the representative daily consumption for that individual.

Table 1
Variables included in the questionnaire.

Section	Variables
1	Identification of owner and animal
2	Address, postal code, city and district
	Type of residence (apartment/house)
	Neighborhood (rural/urban)
3	Owner literacy
	Level of proximity to the dog
	Dog's acquisition (shelter, petshop, other)
	Years lived at the current residence
4	Co-habitant (animal and/or person) with cancer
5	Risk factors
5.1	Related with animal characteristics
	Age, sex, breed, weight; weight category, size, reproductive status body condition (thin, normal, overweight, or obese), length of nose and hair; signals of anxiety (vocalizations, destructions, urination, and defecations at home) and level of physical activity.
5.2	Related with exposure to environmental factors
	More frequent type of food (homemade or commercial)
	Frequency of fruits/vegetables ingestion
	Water source (tap, well or both)
	Type of bowl (plastic, ceramic, stainless steel, glass, or clay)
	Use and frequency of external parasites treatment and history of tick infestation
	Owners' occupation
	Use and frequency of herbicides, fungicides, insecticides, rodenticides, and bleach at home.
	Live near high voltage power towers (<1 km).
	Home characteristics - size, floor type, presence of curtains and rugs
	Housing situation (indoor-only, indoor with garden access, or outdoor living)
	Indoor pollution: Use of fireplace and incense; Rooms allowed to smoke and dog's access; typology (size), type of floor, presence of curtains, rug, carpet and access to gardens. Time spent outsider the house.
	External pollution: Type of residence area and road traffic features.
	Smoking owners, number of smokers, number of cigarettes per smoker/day, number of years smoking, smoking in the house and proximity to dog when smoking.

Due to the lack of standardized questionnaires for assessing second- and third-hand smoke exposure in animals, the questions were adapted from two existing sources:[1] the Global Adult Tobacco Survey (GATS)[54], Section E, which addresses second-hand smoke exposure in humans, and[2] a questionnaire used to assess children's exposure to Environmental Tobacco Smoke (ETS) in Portugal[55].

Second-and third-hand smoke index

To assess the extent of secondhand and thirdhand smoke exposure in the dogs' living environment, a composite variable called "secondhand smoke" was created by summing the following individual variables: presence of smokers in the household (yes - 1 point), number of smokers in the household, average daily cigarette consumption per smoker (represented by the median value), presence of smoke throughout the entire house (yes - 1 point), unrestricted access of the dog to all areas of the house (yes - 1 point), presence of curtains (yes - 1 point), presence of carpets (yes - 1 point), frequent use of incense (yes - 1 point), frequent use of the fireplace (yes - 1 point), and type of house (apartment - 1 point).

Data analysis

The data analysis and statistical calculations were conducted using commercially available software, including Excel® and R. Shapiro-Wilk normality tests were performed for discrete variables, and either t-tests or Wilcoxon tests were employed based on the results. Descriptive statistics are reported as mean, standard deviation (SD), median, and interquartile range (IQR) as appropriate. For categorical variables, the results are presented as counts and percentages. The chi-square test was used when Cochran's criteria were met; otherwise, the Exact Fisher test was applied for each variable.

Univariate logistic regression analysis was utilized to determine the crude odds ratios (ORs) and corresponding 95 % confidence intervals (95 % CI) for the exposure factors. Variables with a p-value < 0.2 were selected for inclusion in the multivariable binomial logistic regression model. The model with the lowest Akaike Information Criterion (AIC) was considered the best-fit model.

A p-value lower than 0.05 was considered statistically significant. However, considering the sample size in this study, the following criteria were adopted: a p-value of <0.001 indicated a very strong association between the risk factor and the likelihood of developing lymphoma; a p-value below 0.01 suggested strong evidence; a p-value below 0.1 implied weak evidence or a trend, and a p-value of 0.1 or greater indicated insufficient evidence.

The study was approved by the bioethics authority for animal welfare (approval number 066/2014) of the Institute of Biomedical Sciences of Abel Salazar of the University of Porto on 12th October 2015.

Results

A total of 113 questionnaires were included in the analysis, consisting of 55 from dogs diagnosed with lymphoma (cases) and 58 from dogs without lymphoma (controls). Among these questionnaires, 41 (36.3 %) were completed through face-to-face interviews, 58 (51.3 %) were completed online, and 14 (12.4 %) were conducted via telephone interviews. Table 2 provides an overview of the categorical variables, while Table 3 presents the results and analysis for all discrete variables.

Animal-related risk associations with lymphoma

Sex and Age: No significant differences were observed in the sex distribution or age distribution of the animals included in this study (Tables 2 and 3). Furthermore, reproductive status was not found to be associated with the probability of developing lymphoma.

Weight and Size: A strong significant association was found between

Table 2
Descriptive analysis and univariable logistic regression results of categorical variables.

Variable	Cases (n=55)		Control (n=58)		Univariate analysis		
	n	%	n	%	OR	95 %CI	*P-value*
Animal characteristics							
Sex	*55*		*58*				
Female	25	40.0	28	48.3	Reference		-
Male	30	60.0	30	51.7	1.12	0.54-2.35	0.764
Reproduction status	*55*		*58*				
Intact	36	65.5	39	67.2	Reference		-
Spayed	19	34.5	19	32.8	1.08	0.50-2.37	0.845
Weight (Kg) (f)	*53*		*58*				0.004***
≤ 10	2	4.1	15	25.9	Reference		-
10 – 25	23	43.4	19	32.7	9.08	1.84-44.70	<0.001***
> 25	28	52.8	24	41.4	8.75	1.81-42.20	0.004***
Dog size	*44*		*34*				0.464
Small	3	6.8	4	11.7	Reference		-
Medium	8	18.2	9	26.5	1.20	0.20-6.99	0.999
Large	33	75.0	21	61.8	2.10	0.43-10.31	0.430
Breed	*55*		*56*				
Mixed-breed	11	20.0	22	38.0	Reference		-
Purebred dogs (vs mixed breed)	44	80.0	34	62.0	2.44	1.05-5.70	0.037**
Labrador Retriever)	11	20.0	10	17.2	2.20	0.86-5.64	0.165
Cocker Spaniel	4	7.3	4	6.9	1.99	0.30-12.82	0.433
Boxer	5	9.1	2	3.4	4.82	0.65-57.94	0.094*
Golden Retriever	4	7.3	1	1.7	7.51	0.64-410.30	0.068*
German Shepard	2	3.6	3	5.2	1.33	0.09-13.43	0.999
Body condition (f)	*55*		*57*				0.424
Underweight / thin	7	12.7	3	5.3	2.59	0.62-10.74	0.314
Normal	37	67.3	41	71.9	Reference		-
overweight/obese	11	20.0	13	22.8	0.94	0.38-2.35	0.999
Hair (f)	*55*		*57*				0.177
Long	11	20.0	10	17.5	Reference		-
Medium	16	29.0	26	45.6	0.56	0.19-1.61	0.300
Short	28	51.0	21	36.9	1.21	0.43-3.38	0.800
Length of nose	*55*		*58*				0.126
Long	7	12.7	6	10.3	1.47	0.46-4.72	0.562
Medium	39	70.9	49	84.5	Reference		
Short	9	16.3	3	5.7	3.77	0.96-14.87	0.064*
Lifestyle factors							
Type of water (multiple choice) (f)	*61*		*79*				0.294
Bottled	6	10.0	10	12.6	Reference		-
Tap	49	80.3	54	68.4	1.51	0.51-4.47	0.592
Rain	2	3.0	9	11.4	0.37	0.06-2.32	0.400
Well water	4	6.7	6	7.6	1.11	0.22-5.62	0.999
Type of food							
Homemade (f)	55		57				
No	36	65.4	48	84.2	Reference		-
Yes	19	34.5	9	15.8	2.74	1.05-7.87	0.029**
Commercial (f)							

(*continued on next page*)

Table 2 (*continued*)

Variable	Cases (*n*=55)		Control (*n*=58)		Univariate analysis		
	n	%	n	%	OR	95 %CI	*P-value*
No	6	10.9	2	3.5	Reference		-
Yes	49	89.1	55	96.5	0.30	0.02-1.77	0.158
Ingestion of fruits/ vegetables	*55*		*58*				0.022**
Never/rarely	21	38.2	37	63.8	Reference		-
Occasionally	19	34.5	13	22.4	2.58	1.06-6.24	0.030**
Often/every day	15	27.3	8	13.8	3.31	1.20-9.08	0.020**
Physical activity	*55*		*58*				0.481
Never/rarely	15	27.3	15	25.9	Reference		
Occasionally	10	18.2	16	27.6	0.62	0.22-1.81	0.390
Often/every day	30	54.5	27	46.5	1.11	0.46-2.69	0.820
Environmental factors							
Use of bleach	*55*		*58*				
Never/rarely	19	34.5	30	51.7	Reference		-
Occasionally/ often	36	65.5	28	48.3	2.03	0.95-4.33	0.066*
House near a road with heavy traffic (f)	*55*		*56*				
No	13	23.6	19	33.9	Reference		-
Yes	42	76.4	37	66.1	1.66	0.72-3.81	0.295
Living <1 km from medium / high voltage towers	*43*		*42*				
No	16	37.2	22	52.4	Reference		-
Yes	27	62.8	20	47.6	1.86	0.78-4.41	0.115
Others							
Cohabitant with cancer	55		58				
No	46	83.6	49	93.1	Reference		-
Yes	8	14.5	4	6.9	2.13	0.74-6.16	0.234
Tobacco smoke exposure							
Smoking owners	55		58				
No	24	43.6	33	56.9	Reference		-
Yes	31	56.4	25	43.1	1.71	0.81-3.59	0.159
Smoking in the entire house	*28*		*24*				
No	22	78.6	22	91.7	Reference		-
Yes	6	21.4	2	8.3	3.00	0.72-12.5	0.192
Dog's proximity when owner smokes (f)	*31*		*23*				0.130
Never/rarely	8	25.8	11	47.8	Reference		-
Occasionally	9	29.0	7	30.4	1.77	0.46-6.78	0.510
Often/ever	14	45.2	5	21.8	3.85	0.98-15.12	0.100

Chi-square test when Cochran criteria were verified. Otherwise, (f) Fisher Exact test was performed. **** $p < 0.001$ (very strong evidence)
*** $p < 0.01$(strong evidence)
** $p < 0.05$ and * $p < 0.1$ (weak evidence/trend).

weight and odds for lymphoma. Dogs with lymphoma were found to have a higher mean weight compared to the control group (Table 3). When stratified into three weight categories, the two categories of dogs weighing >10 kg exhibited a higher probability of developing lymphoma compared to the lower weight category ($P < 0.005$) (Table 2).

Regarding size, statistical significance was not reached due to a lack of information on the size of mixed breed dogs (Table 2).

Table 3
Descriptive analysis of discrete variables.

Variable	Cases ($n = 55$)		Controls ($n = 58$)		P-Value
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
Age (years)	9.6 (3.2)	10.0 (4-16)	8.6 (3.9)	8.0 (4-20)	0.095
Weight (kg)	28.2 (13.9)	30.0 (10-60)	22.1 (14.1)	23.0 (2-58)	0.026**
Years lived in the current residence	8.5 (4.1)	9.0 (5-11.5)	6.8 (3.8)	6.0 (4-9)	0.033**
Total number of smoker cohabitants	0.9 (0.8)	1.0 (0-4)	0.6 (1.0)	0.0 (0-3)	0.100
Total of cigarettes per day for all smokers	14.8 (11.3) (n = 30)	15.0 (5-50)	11.0 (6.4) (n = 25)	10.0 (5-25)	0.208
Second-hand exposure index	13.7 (12.5)	11.0 (1-58)	8.4 (9.3)	4.0 (0-41)	0.001*****

Wilcoxon signed rank test. **** $p < 0.001$ (very strong evidence).
*** $p < 0.01$(strong evidence).
** $p < 0.05$ and * $p < 0.1$ (weak evidence/trend).

Breed: In our sample, mixed breed dogs accounted for the largest proportion, representing 29.2 % ($n = 33$) of the total. Among the identified breeds, the five most commonly represented were Labrador Retrievers at 18.5 % ($n = 21$), Cocker Spaniels at 7 % ($n = 8$), Boxers at 6.2 % ($n = 7$), German Shepherds at 4.4 % ($n = 5$), and Golden Retrievers at 4.4 % ($n = 5$). When compared to mixed-breed dogs, purebred dogs had a 2.44 increased risk of developing lymphoma. Golden retrievers and Boxers exhibited a trend ($p < 0.1$) to have lymphoma (Table 2).

No significant associations were observed between lymphoma and body condition and hair length. Nevertheless, dogs with short length of nose showed weak evidence of higher odds to develop lymphoma ($p < 0.1$) (Table 2).

Lifestyle Factors

Water, Food, and Physical Activity: The type of water and the level of physical activity were not found to be associated with lymphoma incidence (Table 2). Dogs fed homemade food ($p = 0.029$) and with regular consumption of fruits and vegetables ($p = 0.022$) had significantly increased probability of developing lymphoma (Table 2).

Environmental Factors: Although the statistical significance desired was not achieved, there appeared to be a weak association between frequent use of bleach in house cleaning and an increased likelihood of lymphoma, as indicated in Table 2. Factors such as residing in areas with heavy traffic or high voltage, as well as living in households where someone had a history of cancer, did not reach statistical significance in relation to the increased likelihood of developing lymphoma. The mean duration (in years) of residence was significantly longer in dogs diagnosed with lymphoma compared to those without the disease ($p = 0.033$) (Table 3). The remaining substances queried in the questionnaire did not garner a sufficient number of responses to warrant analysis.

Influence of Tobacco Smoking: None of the factors related to tobacco exposure analyzed separately reached the desirable statistical significance. Nevertheless, dogs frequently in proximity to smoking owners exhibited a weak association with lymphoma ($p = 0.1$) (Table 2), and the median total number of smokers in cohabitation was slightly higher in dogs with lymphoma (Table 3). However, when the various factors were aggregated into the Second-hand expossure index, a strong significant difference in values emerged between the groups with dogs with lymphoma showing a higher mean and median values when compared to the control group (Table 3 and Fig. 1).

Multivariate analysis

The subsequent multivariate logistic regression analysis confirmed

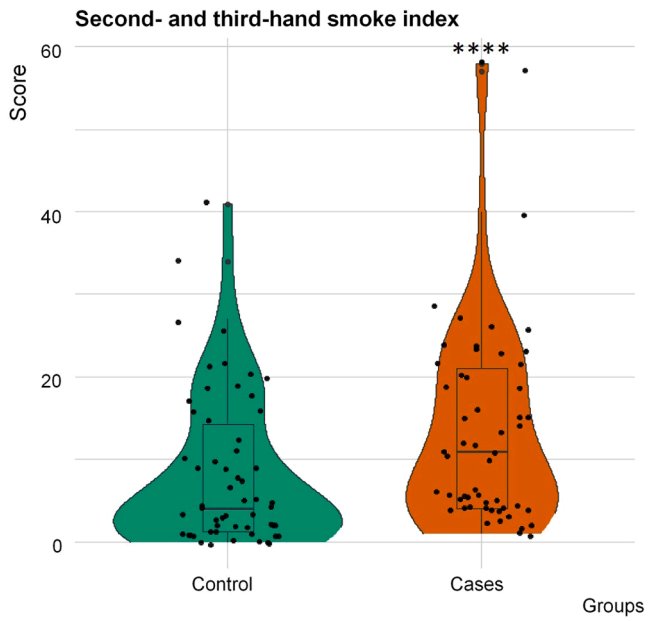

Fig. 1. Violin plot from the distribution of the Second-hand smoke Index among dogs without (control) and with lymphoma (case) groups. **** p-value <0.001.

that higher weight categories, purebreds, those fed homemade food, with frequent consumption of fruits and vegetables, and exposed to a high smoking index were identified as independent risk factors significantly associated with an elevated risk of lymphoma (Table 4).

Discussion

The key findings of this study reveal potential risk factors associated with the development of lymphoma in dogs, which may have implications for humans as well. The identified risk factors can be classified into two categories. Firstly, inherent characteristics of dogs such as higher weight and purebred dogs were identified as potential risk factors for lymphoma. Secondly, external factors including secondary and tertiary tobacco exposure, as well as homemade feeding and occasionally fruit consumption, were also found to be potentially associated with an increased risk of lymphoma. These findings shed light on the complex interplay between intrinsic and environmental factors in the development of lymphoma in both dogs and humans.

Our study revealed that the average age of dogs in the lymphoma group (9.6 years old) was consistent with findings from previous studies, which also reported similar mean ages without a sex predisposition.[21,56-58] Based on univariate analysis, our study identified higher odds of lymphoma in purebred dogs compared to mixed-breed. While the study did not allow for detailed analysis of specific breeds, there was a notable trend of increased risk in Boxers and Golden Retrievers, consistent with findings from previous research. .[27,40,57,59,60]

A recent large-scale study on breed predisposition in Europe,[61] found that large dogs have a higher risk of developing lymphoma compared to small and medium-sized dogs. This finding is consistent with our observation that dogs weighing >10 kg had a significantly greater likelihood of developing lymphoma. While the study demonstrated that heavier dogs have higher odds of lymphoma, no significant association was observed with body size. This may be due to the lack of detailed information on mixed-breed dogs. Since weight is often correlated with body size, this may lead to misinterpretation. For future research, it is recommended to include both weight and height measurements for all animals to more accurately assess the relationship between weight, body size, body condition and lymphoma risk.

Studies investigating the association between fruit and cruciferous vegetable consumption and non-Hodgkin lymphoma (NHL) have suggested that these dietary factors act as protective factors, particularly in women.[53] However, in our sample, we observed a contrasting finding, as the consumption of fruits and vegetables was associated with a higher risk of lymphoma in dogs whose diets included these food items. This discrepancy may be attributed to potential risk factors not captured by our questionnaire.

Dietary factors have been implicated in the etiology of NHL in humans, with studies highlighting the increased intake of trans fatty acids and a pro-inflammatory diet as potential contributors.[62] Surprisingly, our results indicated a higher likelihood of lymphoma in dogs fed a homemade diet. To the best of the authors' knowledge, no studies have yet established a correlation between the type of food (commercial or homemade) and an increased risk of cancer development in dogs. However, knowledge from human medicine regarding trans fatty acids and pro-inflammatory diets may suggest a similar role in the development of canine non-Hodgkin lymphoma. It is important to highlight that our study offers preliminary findings that could provide a foundation for larger-scale nutritional epidemiology studies to further investigate and either confirm or refute these initial observations.

Questions were asked about exposure to several groups of chemicals, including bleach, herbicides, insecticides, rodenticides, and asbestos, but only bleach use showed a weak association with lymphoma in dogs. Sodium hypochlorite, the active ingredient in bleach, is generally considered safe and non-carcinogenic. However, its use can result in the formation of volatile organic compounds (VOCs), which are carcinogenic when inhaled..[63] The closer proximity to the ground and sniffing habits of dogs may contribute to higher exposure to VOCs. Larger studies are needed to clarify this tendency.

The observation that dogs with lymphoma have lived at the same address for a longer duration compared to those without the disease could suggest potential environmental and geographical influences. However, it highlights a limitation of the study, as the direct impact of these environmental factors remains uncertain. In future research, it is essential that this variable is included in the matching criteria to accurately assess their role in the odds of lymphoma in dogs. In addition, collecting information on the owners' previous places of residence before moving to the Porto district would allow us to determine whether the dogs had lived in predominantly urban or rural environments, thereby enabling a more comprehensive assessment of their cumulative exposure time to each setting.

Although tobacco smoke contains carcinogens that have been extensively studied, most epidemiologic studies have failed to demonstrate a strong association between smoking and an increased risk of developing NHL in humans, with a positive correlation suspected only in follicular NHL.[34] In our sample, dogs whose owners smoked frequently near the dog and the number of smokers in the same house did not reach a significant association with lymphoma development. However, the index score used showed a strong significantly higher value for exposure in the dogs with lymphoma compared with the control group. The index

Table 4
Multivariable binomial logistic regression results, sex- and age adjusted, for variables significantly associated with diagnosis of lymphoma amongst dogs in Portugal.

	Predictor	β	SEβ	OR	P-value
	Intercept	−4.04	0.961		<0.001
Weight category	10 – 25kg	2.542	0.894	12.71	0.004
	>25kg	1.858	0.916	6.41	0.042
Breed	Purebred	1.082	0.612	2.95	0.077
Type of food	Homemade feeding	1.052	0.570	2.86	0.065
Fruits and vegetables	Occasionally	1.057	0.537	2.87	0.049
Smoking	Second-hand smoke index	0.059	0.024	1.06	0.015

SE: Standard error.

score combines information on dog owners' smoking habits, smoking intensity, and proximity to dogs when smoking, as well as the house characteristics that may increase exposure to tertiary tobacco smoke. In a previous study by our group, exposure to tobacco smoke was also associated with proliferative activity of canine lymphoma, not subtypes.[22] In view of these results, the hypothesis of smoke exposure and canine lymphoma should be further investigated. To date, the authors are not aware of a standardized scale to assess the intensity of smoke exposure in dogs living with smokers. The index proposed in this study is derived from questionnaires developed by the World Health Organization[54] and research conducted on children.[55] While it is recommended for use in larger studies, further validation is necessary.

Limitations and validity

In this study, the classification of dogs with lymphoma as positive cases was based on a comprehensive clinical examination and confirmed through laboratory diagnostics, ensuring the reliability of the diagnosis. However, a limitation of the study is that the absence of lymphoma in control dogs relied on the owner's assessment of having a healthy dog, which introduces the possibility of false negative controls.

The relatively small number of lymphoma cases ($n = 55$) can be attributed to the inclusion criteria, which specified that participants must reside in the District of Porto. The control group size was adjusted to match the case group size. It is important to note that the selection of controls could potentially impact the representation of certain variables, such as food exposure or proximity to high-voltage towers. The sample of owners included in the control study may not fully reflect the entire population of the Porto region. Another limitation is that dogs with lymphoma have spent a significantly longer average duration living in the same household compared to those without lymphoma, raising potential biases in environmental factors. Future studies should consider pairing participants based on this variable.

Another limitation of this study was the inclusion of all lymphoma subtypes as a single group, despite the potential differences in etiology, pathogenesis, and environmental risk factors that may exist among them.

A further limitation of this study is that the 'years lived in current residence', although potentially relevant, was not incorporated into the logistic regression model. Future research should consider integrating this variable to provide a more comprehensive understanding of the relationships under examination.

The limited sample size, potential lack of representativeness in the control group and the lack of matched pairs are significant limitations that affect the external validity and power of the study. However, the authors believe that the reported information is still valuable, especially considering this is currently the only available study in Portugal addressing this topic. The study provides important insights and identifies relevant variables that should be considered in future studies to further advance the understanding of canine lymphoma in the region.

Conclusion

In conclusion, our study suggests that higher weight dogs, purebred dogs, regular consumption of fruits and vegetables, and a homemade food diet may be associated with an increased likelihood of developing lymphoma. Additionally, the study highlights the negative impact of secondary and tertiary tobacco smoke exposure using an index with different factors. These findings provide valuable insights into potential interplay of risk factors for lymphoma in dogs. However, it is important to acknowledge that larger studies are necessary to validate and confirm these results. Further research will contribute to a more comprehensive understanding of lymphoma development in dogs and help inform preventive strategies and interventions.

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CRediT authorship contribution statement

Katia Pinello: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Liliana Leite-Martins:** Investigation. **Hugo Gregório:** Investigation. **Filipe Oliveira:** Investigation. **Katia C. Kimura:** Methodology, Investigation. **Maria Lúcia Z. Dagli:** Methodology. **Augusto de Matos:** Supervision, Conceptualization. **João Niza-Ribeiro:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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