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ORIGINAL RESEARCH

An epidemiological study of canine lymphoma in South Africa

SB Harris,[1] EH Hooijberg,[1] SJ Clift,[2,3] GT Fosgate,[4] EC du Plessis,[5] Y Rautenbach[1]

[1] *Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, South Africa*
[2] *Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, South Africa*
[3] *Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, South Africa*
[4] *Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, South Africa*
[5] *IDEXX Laboratories (Pty) Ltd, South Africa*
Corresponding author, email: *sybrandharris@gmail.com*

Breed, age, and sex predispositions for canine lymphoma have been reported for various geographical locations. However, epidemiological information concerning canine lymphoma in South Africa is scarce.

The aim of the study was to describe the epidemiological features of canine lymphoma and the frequency of World Health Organization (WHO) classification subtypes in South Africa.

A retrospective, case-control study was performed that included 342 cases with a histopathological diagnosis of canine lymphoma matched with 342 canine non-lymphoma control cases. Associations between canine lymphoma and breed, age, sex, and neutering status were assessed using univariate and multivariable conditional logistic regression. Associations were reported as odds ratios and significance set as $p \leq 0.05$.

Breed was significantly associated with canine lymphoma, but not age, sex, or neutering status. Median population age was eight years, with a male-to-female ratio of 1.2:1. The Boerboel had an increased risk (OR = 1.63, CI = 1.02–2.62, $p = 0.002$) and the Yorkshire Terrier a decreased risk (OR = 0.59, CI = 0.38–0.93, $p = 0.050$) of having canine lymphoma. Immunophenotyping was performed on 119 (35%) cases, of which 82 (69%) were B-cell, 34 (29%) T-cell and three (2%) neither. WHO subtype was available for 88 cases; of these 66 (75%) were diffuse large B-cell lymphoma (DLBCL) with remaining subtypes each ≤ 7%.

This study identified a breed predisposition for canine lymphoma in the Boerboel, a South African mastiff-type dog, but significant associations were not detected for age, sex, and other breeds. The frequency of immunophenotypes and WHO subtypes was similar to previous studies in other locations.

Keywords: breed, dog, haemopoietic, neoplasia, signalment

Introduction

In veterinary oncology, lymphoma is one of the most frequently diagnosed and managed malignant neoplasms in dogs (Zandvliet 2016). Lymphoma is a term used to broadly categorise several subtypes of lymphoid neoplasms. Most dogs with lymphoma (73 to 82% of cases) present with generalised lymphadenomegaly, referred to as the multicentric form of lymphoma. The majority of these cases are of B-cell origin, and intermediate to high grade (Ponce et al. 2010; Vezzali et al. 2010; Zandvliet 2016).

The revised World Health Organization (WHO) canine lymphoma classification scheme, amongst others, is used for lymphoma subtyping (Harris et al. 1999; Valli et al. 2011). This classification system is based on histomorphological evaluation (growth pattern, relationship between neoplastic foci and non-neoplastic follicles, nuclear size, nuclear morphology, grade and immunophenotype) and almost 80% of all canine lymphomas are classified into one of five subtypes, namely: diffuse large B-cell lymphoma (DLBCL), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), T-zone lymphoma (TZL), T-cell lymphoblastic lymphoma (T-LBL) and marginal zone lymphoma (MZL) (Valli et al. 2011). A recent study identified and suggested the addition of another lymphoma subtype, currently referred to as diffuse small B-cell lymphoma (DSBCL). This lymphoma subtype is relatively common, has a diffuse pattern and consists of small to intermediate cells (Hughes et al. 2021).

Large epidemiological studies of canine lymphoma have been conducted throughout the world, identifying several breed, age group, sex, and neutering status risk factors for the development of this disease (Bennett et al. 2018; Comazzi et al. 2018; Jark et al. 2020; Van Rooyen et al. 2018; Villamil et al. 2010). In 2018, the first epidemiological canine lymphoma study in South Africa identified two South African breeds, the Boerboel and Rhodesian Ridgeback, at an increased risk of developing lymphoma (Van Rooyen et al. 2018). One breed, the Maltese Poodle, was identified as having a decreased risk. The Rhodesian Ridgeback has previously been associated with an increased risk of developing lymphoma (Bennett et al. 2018), but this was the first report for the Boerboel breed. In 2009, a South African case report was published suggesting a familial link of lymphoma in three related Rottweilers in the same household (Lobetti 2009).

Studies conducted in Australia, North America, Brazil, Europe and South Africa identified the following breeds as being predisposed to developing lymphoma or being overrepresented: Airedale Terrier, Basset Hound, Bernese Mountain Dog, Border Collie, Boxer, Bull Terrier, Bullmastiff, Cocker Spaniel, Corgi, English Bulldog, German Shepherd, Golden Retriever, Gordon Setter, Labrador Retriever, Mastiff, Rhodesian Ridgeback, Rottweiler,

Scottish Terrier, Saint Bernard and Vizsla (Bennett et al. 2018; Comazzi et al. 2018; Jark et al. 2020; Van Rooyen et al. 2018; Villamil et al. 2010).

Lymphoma is a neoplasm of middle-aged to older dogs with the age group of 6.1–9.0 years significantly predisposed in South Africa and in other countries (Coelho et al. 2019; Cunha et al. 2011; Ernst et al. 2016; Van Rooyen et al. 2018).

Contradictory results have been published concerning sex and neutering status associations with lymphoma. Some studies describe a protective hormonal phenomenon in females and intact animals, with neutered males being the most at risk of developing lymphoma (Bennett et al. 2018; Grüntzig et al. 2016; Villamil et al. 2010). In South Africa, males and neutered females had the highest risk of lymphoma (Van Rooyen et al. 2018). However, studies showing no sex or neutering status association also exist (Coelho et al. 2019; Cunha et al. 2011; Ernst et al. 2016; Ponce et al. 2010; Teske 1994).

There is limited knowledge about breed, age, sex, and neutering status associations with canine lymphoma in South Africa. Furthermore, histopathological, and phenotypical lymphoma characteristics have not yet been described in the dog population of this country. This study aimed to further investigate epidemiological features, in terms of breed, age, sex, and neutering status, associated with canine lymphoma in South Africa and to describe the lymphoma characteristics in this population.

Materials and methods

Study design

In this retrospective, case-control, observational study, histopathology reports of canine lymphoma biopsy samples submitted to the private veterinary laboratory, IDEXX (Kyalami, Gauteng, South Africa), were retrieved and analysed. The study timeframe was from December 2018 to December 2020.

The histopathological description and/or slides were reviewed when there was a presumptive histological diagnosis of lymphoma. Cases eligible for further WHO classification subtyping were similarly reviewed. Breed, age, sex, neutering status, and South African province of biopsy origin, where available, were recorded. Furthermore, histopathological diagnosis, and lymphoma characteristics i.e. sample site, tumour grade, immunophenotyping and WHO classification subtype, where available, were also recorded for all cases.

Study population

An unmatched case-control power analysis was performed to determine the minimum sample size using statistical freeware (OpenEpi). As the previous South African study also used data from the IDEXX database, and identified the Boerboel as a popular breed with increased odds of developing canine lymphoma in that population, the Boerboel was chosen for the power analysis calculation (Van Rooyen et al. 2018).

A 1:1 control to case ratio was used with a 95% two-sided confidence interval (CI), 80% power of detection and an odds ratio of 3. An appropriate total sample size of 488 was determined i.e. a minimum of 244 cases and 244 controls.

All dogs, regardless of breed, age, sex, and neutering status, with a histopathological diagnosis of any type of canine lymphoma were included in the study population.

Control inclusion criteria were any canine biopsy sample, not diagnosed as canine lymphoma, submitted to IDEXX Laboratories on the same day as the included lymphoma case.

Data collection

Lymphoma cases received by IDEXX Laboratories were retrieved by the following method: The IDEXX Microsoft SQL Server Database was searched with the search phrase "lymphoma" with the set search time range. This query filtered all samples accessioned by IDEXX Laboratories with a diagnosis or description containing the term "lymphoma" for the given time frame. The query results were copied into a commercial spreadsheet program, Microsoft Excel, containing the following information: laboratory number, patient signalment and pathologist.

All corresponding histopathology reports were extracted as a .pdf or .docx document from the company's electronic archive stored in Microsoft SQL Server. Both Microsoft Excel spreadsheet and the resultant histopathological reports were sent to the primary investigator (S.H.) for review. Non-canine cases were excluded from the filtered list, and subsequently all reports were reviewed by the primary investigator to include cases that met the inclusion criteria.

Control selection was done using the following method: Submission dates from the included lymphoma cases were used to extract all canine biopsy samples submitted to the laboratory on the given submission dates. The query results were copied into a Microsoft Excel spreadsheet and all canine lymphoma cases were removed from the control dataset.

All canine non-lymphoma case submissions during the study period were ordered in Microsoft Excel based on date of submission, and within date, using a random number. One control was randomly selected for each case matching on submission date thus creating a single data set for analysis. Controls were selected using lookup functions and reordering of the dataset based on new random numbers when multiple cases had the same submission dates.

Report review

Cases suggestive of lymphoma with an inconclusive morphological diagnosis underwent a series of report reviewing in a two-phased process: In the first phase lymphoma reports were reviewed by two specialist veterinary clinical pathologists (Y.R. & E.H.). In this review phase all cases that did not definitively meet the inclusion criteria during the primary investigator's review process were re-evaluated and either included, excluded, or if still ambiguous, set aside for a second review cycle.

The second phase of report reviewing was done by one veterinary anatomical pathologist (S.C.). Cases in this review process were either included or excluded. Cases were excluded if a definitive

diagnosis of canine lymphoma could not be made, by any of the three pathologists, based on the histopathological description.

WHO classification subtype review

Biopsies with a canine lymphoma diagnosis from a haemopoietic organ that underwent immunohistochemistry (IHC), but that were not further subtyped into a specific WHO class, were reviewed by a veterinary anatomical pathologist (S.C.) to assess eligibility for WHO classification subtyping. Tissue sections from canine lymphoma cases submitted to IDEXX Laboratories, where private practitioners requested immunophenotyping, all underwent IHC staining at the University of Pretoria's Veterinary Immunohistochemistry Laboratory.

The IHC reports and tissue sections were obtained using the following steps: The Veterinary Immunohistochemistry Laboratory's database (UVIS) was searched using the Query Tool, using the criteria "Species = Canine" and "Result Value Contains Lymphoma" for the set timeframe. This search query extracted all canine samples accessioned by the Veterinary Anatomic Pathology Laboratory, which the Veterinary Immunohistochemistry Laboratory forms part of, with a diagnosis containing the term lymphoma.

Reports were matched with the same case's IDEXX histopathological report and evaluated for evidence of immunophenotyping. The IDEXX histopathology report in conjunction with the IHC report were reviewed for WHO classification subtyping.

In cases where further subtyping was not possible by report reviewing, the tissue sections were extracted from the Veterinary Immunohistochemistry Laboratory's tissue section archive for slide review. Where the tissue sections could not be found in the archive, the original tissue blocks in formalin were requested and delivered from IDEXX to be sectioned and the immunophenotyping was repeated. The tissue sections were subjected to the application of pan-T- and B-lymphocyte markers, specifically CD3 (polyclonal rabbit antibody, Dako) and CD20 (polyclonal rabbit antibody, ThermoFisher), using optimised IHC protocols (Henning et al. 2020). In instances where confirmation of the B-cell phenotype was necessary, PAX5 (mouse monoclonal antibody, BD Biosciences) IHC was performed (Henning et al. 2020).

Furthermore, all DLBCL cases that were not further classified into immunoblastic or centroblastic subtypes, including cases that were diagnosed as DLBCL but were described as small cell lymphomas, were reviewed for further classification or re-classification by the same veterinary anatomical pathologist (Hughes et al. 2021).

The lymphoma grade assigned by the reporting IDEXX pathologist was reviewed by the primary investigator to ensure uniform grading using the mitotic count reported. The mitotic count per high power field (HPF, 400x magnification) was used to grade lymphoma as low (0–5), intermediate (6–10), or high (> 10) (Valli et al. 2011).

Methodologies

All samples submitted to IDEXX Laboratories were stained routinely with haematoxylin and eosin stain (H&E) and examined and reported by one of three veterinary pathologists as a diagnostic service to referring veterinarians. Cases that underwent additional IHC staining were examined and reported by one of the ten veterinary anatomical pathologists or residents at the university's Veterinary Anatomic Pathology Laboratory. All biopsy samples included as control cases were examined and diagnosed by the above-mentioned IDEXX pathologists.

Statistical data analysis

Dog breeds were classified into groups based on the Fédération Cynologique Internationale® (FCI) for Pedigree Dogs Worldwide. All breeds that contributed to 3% or more of the study population were also categorised individually for analysis. Dog age was categorised as < 5.0 years, 5.0–9.9 years, and ≥ 10.0 years for descriptive presentation and analysed as a continuous variable in the statistical analysis to improve the control of confounding and increase precision. Cases of lymphoma were categorised based on tumour grade, sample site, immunophenotype, and WHO classification subtype.

Categorical data were presented as percentages and mid-P exact 95% confidence intervals (CIs) using statistical freeware (OpenEpi). The association between lymphoma and signalment was assessed using univariate conditional logistic regression. Multivariable conditional logistic regression was used to evaluate the effect of breed while accounting for dog age, sex, and neutering status, which were forced into all statistical models to adjust for potential confounding. Multivariable models were fit using backwards elimination until all remaining breeds or breed groups were significantly associated with lymphoma case status.

Mixed breeds were used as the referent group during the univariate breed group analysis. For the univariate individual breed analysis, all other dog breeds, except the breed under investigation, were used as the referent group. This included mixed breeds. For the multivariable analysis, the referent group were all excluded breed groups or individual breeds, respectively, including mixed breeds.

Statistical modelling was performed using commercial software (IBM SPSS Statistics Version 27, International Business Machines Corp., Armonk, NY, USA) with associations reported as odds ratios (ORs) and significance set as $p \leq 0.05$.

Results

Case review outcome

Of the 548 cases extracted from the IDEXX software, 307 cases met the inclusion criteria and were included during the initial report review process performed by the primary investigator. Fifty-seven cases, where lymphoma was listed as a major differential diagnosis but not as the definitive diagnosis, were identified for further review. After the report review process, the canine lymphoma data set was finalised, totalling 342 included cases.

All cases that underwent immunophenotyping and lacked a final morphological diagnosis or further classification were selected for classification review. Thirty-two cases qualified for the review by a veterinary anatomical pathologist. Nineteen cases were further classified by simply reviewing the histopathological and IHC report. The remaining 13 cases were identified for slide review.

Eleven archived slides could be retrieved. Two cases were not found, and the paraffin blocks were requested from IDEXX Laboratories to recut and repeat the IHC stains. One case had intermingled CD3- and CD20-positivity and additional PAX5 IHC was requested. A final diagnosis was made and/or further classification was done on all 13 slide review cases.

Control population

Three hundred and forty-two controls were selected matching the date of submission of the lymphoma biopsy to IDEXX Laboratories, totalling the study population at 684 dogs. Of these 342, eight did not have a diagnosis assigned but signalment data were available. Diagnoses were grouped into the following four categories: neoplastic, inflammatory, both neoplastic and inflammatory, hyperplastic, and miscellaneous. Diagnoses categorised as neoplasia included all abnormal cell proliferation, whether benign or malignant and comprised more than half (n = 217/342, 63%) of diagnoses in the control group. The second most common disease process was inflammatory (n = 74/342) accounting for > 20% of the biopsies (Table I).

Table I: Absolute numbers and prevalence of the underlying disease process of biopsies in the control population (n = 342)

Disease process	Absolute numbers (n)	Prevalence (%) (95% CI)
Neoplastic	217	63 (58–68)
Inflammatory	74	22 (18–26)
Both neoplastic and inflammatory	14	4 (2–7)
Hyperplasia	12	4 (2–6)
Miscellaneous	17	5 (3–8)
No diagnosis	8	2 (1–4)
Total	342	100

n = absolute numbers in the control group, CI = confidence interval

Epidemiological factors

Breed

Fifty-one different breeds, including mixed breeds, were identified in the lymphoma group of the study (n = 342). Breeds comprising ≥ 3% in the lymphoma population included: Mixed (n = 64, 19%), Boerboel (n = 27, 8%), Jack Russell Terrier (n = 23, 7%), German Shepherd Dog (n = 15, 4%), Labrador Retriever (n = 15, 4%), Rottweiler (n = 15, 4%), Dachshund (n = 14, 4%), Beagle (n = 11, 3%), and Rhodesian Ridgeback (n = 11, 3%).

Sixty-three different breeds were identified in the control population (n = 342), including mixed breeds. Breeds that comprised ≥ 3% of the control population included: Mixed breed (n = 46, 13%), Jack Russell Terrier (n = 29, 8%) Labrador Retriever (n = 23, 7%), Dachshund (n = 19, 6%) and Yorkshire Terrier (n = 19, 6%) (Table II). The absolute breed numbers per FCI group for all breeds in the study are set out in Table II.

Based on the univariate analysis, breed in general was significantly associated with lymphoma ($p < 0.001$). All individual breeds that comprised 3% or more of the entire population were compared to all other breeds (Table III).

The Boerboel showed statistically significant increased odds (OR = 3.25, CI = 1.47–7.18, p = 0.004) of developing lymphoma when compared to all other breeds. When comparing the Yorkshire Terrier to all other breeds, there were statistically lower odds of lymphoma (OR = 0.17, CI = 0.05–0.57, p = 0.004) (Table III).

Multivariable analysis of the individual breeds, while accounting for age, sex, and neutering status, were consistent with the results

Table II: Breed distribution of the study population within each FCI group

Breed Group	N(L)	N(C)	Breed	n(I)	n(c)
1 – Sheepdogs and Cattle dogs (Except Swiss Cattle Dogs)	26	25	Australian Cattle Dog	0	1
			Australian Shepherd	1	1
			Border Collie	6	9
			Bouvier Des Flandres	1	1
			Collie (rough/smooth)	1	1
			Dutch Shepherd Dog	0	1
			German Shepherd Dog	15	9
			Miniature American Shepherd	0	1
			White Swiss Shepherd Dog	2	1
2 – Pinscher and Schnauzer – Molossoid and Swiss Mountain and Cattle Dogs	81	50	Kangal Shepherd Dog (Anatolian)	0	1
			Bernese Mountain Dog	0	1
			Boerboel[a]	27	7
			Boxer	9	6
			Bulldog	8	5
			Bullmastiff	6	4
			Italian Cane Corso	0	1
			Dobermann	2	2
			Great Dane	5	2
			Miniature Pinscher	1	1
			Rottweiler	15	9
			Schnauzer (all types)	6	9
			Shar Pei	2	2

Table II: Breed distribution of the study population within each FCI group (*Continued*)

Breed Group	*N*(L)	*N*(C)	Breed	*n*(l)	*n*(c)
3 – Terriers	62	84	Airedale Terrier	2	1
			Bedlington Terrier	0	1
			Bull Terrier	7	7
			Cairn Terrier	0	1
			Fox Terrier (smooth/wire)	3	2
			Irish Soft-Coated Wheaten Terrier	1	0
			Jack Russell Terrier	23	29
			Kerry Blue Terrier	1	1
			Pitbull Terrier[b]	10	10
			Scottish Terrier	5	3
			Staffordshire Bull Terrier	6	10
			Yorkshire Terrier	4	19
4 – Dachshunds	14	19	Dachshund	14	19
5 – Spitz and Primitive Types	1	4	Chow Chow	0	2
			German Spitz (Pomeranian)	0	2
			Siberian Husky	1	0
6 – Scent Hounds and Related Breeds	31	19	Basset Hound	8	2
			Beagle	11	9
			Bloodhound	1	1
			Dalmatian	0	1
			Rhodesian Ridgeback	11	6
7 – Pointing Dogs	5	14	German Pointing Dog (short-/wire haired)	0	6
			Irish Setter (red/red and white)	0	1
			English Pointer	0	1
			Hungarian Pointer (Vizsla) (short-/wire haired)	1	0
			Weimaraner	4	6
8 – Retrievers – Flushing Dogs – Water Dogs	34	41	Cocker Spaniel (American/English)	7	5
			Golden Retriever	6	10
			Labrador Retriever	15	23
			Retriever (Flat-/curly coated)	1	2
			Spaniel (unknown)	3	1
			Springer Spaniel (English/Welsh)	2	0
9 – Companion and Toy Dogs	12	29	Boston Terrier	2	5
			Cavalier King Charles Spaniel	1	0
			Chihuahua	1	4
			French Bulldog	1	2
			Griffon (Belge/Bruxellois)	0	1
			Maltese (Poodle)[c]	2	8
			Papillon	1	0
			Pekingese	0	3
			Poodle	0	4
			Pug	2	0
			Shih Tzu	2	2
10 – Sighthounds	1	8	Irish Wolfhound	0	1
			Greyhound	1	3
			Whippet	0	4
11 – Mixed Breed	66	49	Africanis	2	3
			Mixed	64	46
No breed assigned	9	0		9	0
Total	342	342		342	342

N(L) = absolute number of animals per FCI group of the lymphoma group, *N*(C) = absolute number of animals per FCI group of the control group, *n*(l) = absolute breed numbers in the lymphoma group, *n*(c) = absolute breed numbers in the control group, FCI = 'Fédération Cynologique Internationale®.
[a] The Boerboel is not FCI recognised but is grouped together with its ancestral breeds in Group 2.[b] The name "Pit Bull Terrier" is given to dogs in South Africa with the phenotypic characteristics of both the American Pit Bull Terrier (not FCI recognised) and the American Staffordshire Terrier (FCI recognised), and they are grouped as Pit Bull Terriers in Group 3. [c] The "Maltese Poodle" name is given to dogs in South Africa with phenotypic characteristics of a long-haired, white, toy breed, which might include the Maltese (FCI recognised) and any crossbreed with the same characteristics.

of the univariate analysis. Boerboels (OR = 1.63, CI = 1.02–2.62, $p = 0.002$) were more likely to have lymphoma compared to all other breeds while Yorkshire Terriers (OR = 0.59, CI = 0.38–0.93, $p = 0.05$) were less likely to have lymphoma (Table IV).

In the univariate analysis, no FCI breed group had a significant positive association with canine lymphoma, but four breed groups, namely Terriers (Group 3), Pointing Dogs (Group 7), Companion and Toy breeds (Group 9) and Sighthounds (Group 10), had statistically significant ($p \leq 0.05$) lower odds of developing lymphoma; the ORs for all four groups were 0.5 or less (with 95% CIs not encompassing an OR of 1.0) (Table III).

The Pinscher, Schnauzer, Molossoids, Swiss Mountain and Cattle Dogs group (Group 2) had a moderate OR of developing lymphoma compared to the other breed classification groups

Table III: Univariate associations between dog signalment and a diagnosis of lymphoma from a single veterinary histopathology laboratory

Variable	Level	Parameter estimate ($\hat{\beta}$)	Odds ratio (95% CI)	*p*-value
Breed group				< 0.001
1	Sheepdogs & Cattle Dogs	-0.248	0.78 (0.40–1.52)	0.464
2	Pinscher and Molossoid	0.247	1.28 (0.75–2.20)	0.370
3	Terriers	-0.666	0.51 (0.30–0.88)	0.015
4	Dachshunds	-0.552	0.58 (0.25–1.35)	0.204
5	Spitz and Primitive Types	-0.812	0.44 (0.04–5.49)	0.527
6	Scent Hounds and Related Breeds	0.191	1.21 (0.57–2.56)	0.616
7	Pointing Dogs	-1.412	0.24 (0.08–0.75)	0.014
8	Retrievers and Water Dogs	-0.471	0.63 (0.34–1.14)	0.126
9	Companion and Toy Dogs	-1.247	0.29 (0.13–0.63)	0.002
10	Sighthounds	-2.453	0.09 (0.01–0.72)	0.024
	Mixed Breeds	Referent		
Breed				
	Boerboel	1.179	3.25 (1.47–7.18)	0.004
	All other breeds	Referent		
	Labrador Retriever	-0.470	0.63 (0.33–1.19)	0.153
	All other breeds	Referent		
	Jack Russell Terrier	-0.357	0.70 (0.40–1.22)	0.210
	All other breeds	Referent		
	Dachshund	-0.348	0.71 (0.34–1.48)	0.356
	All other breeds	Referent		
	German Shepherd	0.310	1.36 (0.63–2.97)	0.435
	All other breeds	Referent		
	Rottweiler	0.405	1.50 (0.67–3.34)	0.321
	All other breeds	Referent		
	Yorkshire Terrier	-1.792	0.17 (0.05–0.57)	0.004
	All other breeds	Referent		
Age				0.703
	< 5 years	Referent		
	5–9.9 years	-0.008	0.99 (0.64–1.52)	0.969
	≥ 10 years	0.151	1.16 (0.72–1.89)	0.544
	Continuous (year)	0.025	1.03 (0.98–1.08)	0.330
Sex				
	Female	-0.241	0.79 (0.58–1.06)	0.113
	Male	Referent		
Neuter status				
	Intact	-0.220	0.80 (0.58–1.11)	0.186
	Neutered	Referent		

CI = confidence interval

($p \leq 0.05$) in the multivariable analysis (Table V). Terriers (Group 3), Pointing Dogs (Group 7) and Companion and Toy Dogs (Group 9) had significantly lower odds of developing lymphoma with ORs < 0.6 and $p \leq 0.05$ (Table V).

Age

When age was grouped into three categories, 52/342 (15%) of the lymphoma animals were < 5.0 years, 149/342 (44%) animals ≥ 5.0 and < 10.0 years and 99/342 (29%) animals ≥ 10.0 years of age. Forty-two out of 342 animals (12%) had no age recorded on the report (Table VI).

In the control group, 65/342 (19%) animals were < 5.0 years, 175/342 (51%) were ≥ 5.0 and < 10.0 years and 102/342 (30%) were ≥10.0 years of age (Table VI).

The median lymphoma group age (range: 1.0–15.0) and control group age (range: 0.4–20.5) were both 8.0 years. Median age of the Boerboel canine lymphoma population was 6.0 years (range: 3.0–15.0 years). The majority of Boerboel cases (16/27, 59%) were in the ≥ 5.0 and < 10.0 years group and 8/27 (30%) individuals were < 5.0 years old.

Table IV: Multivariable associations between breed, while accounting for age, sex and neutering status, and a diagnosis of lymphoma from a single veterinary histopathology laboratory

Variable	Level	Parameter estimate ($\hat{\beta}$)	Odds ratio (95% CI)	*p*-value
Breed				
	Boerboel	1.357	1.63 (1.02–2.62)	0.002
	Yorkshire Terrier	-1.287	0.59 (0.38–0.93)	0.050
	Other breeds	Referent		
Age				
	Continuous (yr)	0.035	1.04 (0.98–1.10)	0.219
Sex				
	Female	-0.176	0.84 (0.60–1.17)	0.297
	Male	Referent		
Neuter status				
	Intact	-0.143	0.87 (0.60–1.25)	0.441
	Neutered	Referent		

CI = confidence interval

Table V: Multivariable associations between breed group, while accounting for age, sex and neutering status, and a diagnosis of lymphoma from a single veterinary histopathology laboratory

Variable	Level	Parameter estimate ($\hat{\beta}$)	Odds ratio (95% CI)	*p*-value
Breed group				
2	Pinscher and Molossoid	0.490	1.63 (1.02–2.62)	0.042
3	Terriers	-0.501	0.61 (0.38–0.96)	0.033
7	Pointing Dogs	-1.368	0.26 (0.08–0.78)	0.016
9	Companion and Toy Dogs	-1.001	0.37 (0.17–0.81)	0.013
10	Sighthounds	-2.085	0.12 (0.02–1.02)	0.052
	Other breeds	Referent		
Age				
	Continuous (yr)	0.038	1.04 (0.98–1.10)	0.192
Sex				
	Female	-0.190	0.83 (0.59–1.17)	0.278
	Male	Referent		
Neuter status				
	Intact	-0.203	0.82 (0.56–1.19)	0.290
	Neutered	Referent		

CI = confidence interval

There was no significant association between age and a diagnosis of lymphoma ($p = 0.703$) (Table III).

Sex and neuter status

In the lymphoma population, 177/342 (52%) dogs were recorded as males, 148/342 (43%) as females and 17/342 (5%) had no information regarding sex (Table VI). The male to female ratio was 1.2:1 in the lymphoma population and 1:1.1 in the control population. The Boerboel population had a higher male to female ratio of 1.5:1. Of the 148 females, 57 were recorded as neutered and of the 177 males, 54 were recorded as neutered.

Sex and neutering status as predisposing factors for developing canine lymphoma were analysed and no significant association could be found ($p = 0.113$ and $p = 0.186$, respectively) (Table III).

Lymphoma characteristics

Grade

Two hundred and forty-six out of 255 (97%) haemopoietic tissue biopsies were assigned a grade. Fourteen cases from other tissue origins (skin/subcutaneous/mucosal) were inconsistently graded by the reporting veterinary anatomical pathologists. Of all 260 cases that underwent grading, 111 (43%) cases were intermediate grade, 71 (30%) cases were assigned a low grade and 78 (27%) a high grade (Table VIII).

Sample site

Two hundred and fifty four out of 342 (75%) canine lymphoma cases included in this study were biopsies of haemopoietic tissues, with lymph nodes being the most sampled organ (n = 228, 89%) in this group. Splenic biopsies were far less

Table VI: Distribution of variables in lymphoma (*n* = 342) and control (*n* = 342) groups within the study population (*n* = 684)

Variable	Lymphoma group – *n*(l)	Control group – *n*(c)	Study Population (*N*)
Age			
< 5.0 years	52	65	117
≥ 5.0 and < 10.0 years	149	175	324
≥ 10.0 years	99	102	201
Unknown	42	0	42
Total	**342**	**342**	**684**
Sex			
Male	177	166	343
Female	148	176	324
Unknown	17	0	17
Total	**342**	**342**	**684**
Neutering status			
Neutered	111	132	243
Male	*54*	*58*	*112*
Female	*57*	*74*	*131*
Intact	214	210	424
Male	*123*	*108*	*231*
Female	*91*	*102*	*193*
Unknown	17	0	17
Total	**342**	**342**	**684**

n(l) = absolute numbers in the lymphoma group, *n*(c) = absolute numbers in the control group, *N* = absolute numbers in the study population

Table VII: Absolute numbers and prevalence of lymphoma sampling sites. Total *n* = 342

Sample site	*n*	Prevalence (%) (95% CI)
Haemopoietic	254	75 (70–79)
Lymph node	228	67 (62–72)
Spleen	15	4 (3–7)
Other	11	3 (2–6)
Skin/subcutis/mucosal	**72**	**21 (17–26)**
Skin	48	14 (11–18)
Subcutaneous	5	2 (1–3)
Mucocutaneous/Mucosal	17	5 (3–8)
Other	2	1 (0–2)
Other	**14**	**4 (2–7)**
Total	340	100

n = absolute number in the lymphoma group, CI = confidence interval

common (*n* = 15, 6%). Other tissue sampling sites (*n* = 11, 3%) with a diagnosis suggestive of a haemopoietic tissue origin included biopsies from masses in the perianal, flank, axilla, penile, and gastric anatomical locations.

Seventy-two biopsies were diagnosed as cutaneous, subcutaneous, and mucosal lymphomas, comprising 21% of biopsy submissions (Table VII).

Other lymphomas were located in the cerebellum (*n* = 1), intestine (*n* = 5), liver (*n* = 2), mediastinum (*n* = 1), nasal cavity (*n* = 1), rectum (*n* = 3) and kidney (*n* = 1).

Thirty-seven cases had multiple sampling sites recorded of which 24/37 (65%) were multiple lymph nodes, 6/37 (16%) were a mass or lesion and regional lymph nodes, 4/37 (11%) were different combinations of biopsies of the cutis, subcutis, mucocutaneous and/or muscle, 2/37 (5%) were a combination of gastric and tonsillar biopsies and 1/37 (3%) were biopsies of several internal organs. The sample site for each case was classified in the data set based on the organs where the diagnosis of lymphoma was made.

In the Boerboel canine lymphoma group, 21/27 (78%) were from haemopoietic tissue. The remaining biopsies constituted 5/27 (19%) from the skin and 1/27 from the subcutaneous tissue.

Immunophenotype

One hundred and fourteen out of 342 (33%) biopsies underwent IHC, and five biopsies were assumed to be either B- or T-cell, based on characteristic histopathological features (4 MZL, 1 TZL). Eighty-two out of 119 (69%) cases were of B-cell origin and 34/119 (29%) were of T-cell origin (Table VIII). Four of the 27 (15%) Boerboel cases were immunophenotyped and all of them stained positive for B-cell lymphoma.

Three cases (one cutaneous and two lymph node biopsies) did not stain with either the CD3 or CD20 antibodies, and further staining was declined by the owners.

WHO Classification Subtype

The most common WHO classified lymphomas, by decreasing prevalence, were DLBCL (66/88, 75%), MZL (6/88, 7%), PTCL-NOS (6/88, 7%), TZL (5/88, 6%), and T-LBL (2/88, 2%) (Table VIII). DSBCL, not currently a recognised WHO classification subtype, was present in three cases (3/88, 3%). Of all the DLBCL biopsies, 52/66 (79%) were subclassified as centroblastic and the

Table VIII: Distribution of canine lymphoma grade, sampling site, immunophenotype, and WHO classification subtype across provinces in South Africa. Total *n* = 342

Variable	Overall		Gauteng		Western Cape		KwaZulu-Natal		Other provinces	
	n	Percentage (95% CI)	*n*	Percentage (95% CI)	*n*	Percentage (95% CI)	*n*	Percentage (95% CI)	*n*	Percentage (95% CI)
Tumour grade										
Low	78	30 (25–36)	43	28 (22–36)	13	32 (19–47)	10	31 (17–49)	12	33 (19–50)
Intermediate	111	43 (37–49)	67	44 (37–52)	17	41 (27–57)	11	34 (20–52)	16	44 (29–61)
High	71	27 (22–33)	41	27 (21–35)	11	27 (15–42)	11	34 (20–52)	8	22 (11–38)
Total	260	100	151	100	41	100	32	100	36	100
Sampling site										
Haemopoietic	254	75 (70–79)	148	74 (67–79)	41	71 (58–81)	31	84 (69–93)	34	77 (63–88)
Skin or mucosal	72	21 (17–26)	43	21 (16–27)	15	26 (16–38)	5	14 (5–27)	9	20 (10–34)
Other origin	14	4 (2–6)	10	5 (3–9)	2	3 (1–11)	1	3 (0.1–13)	1	2 (0.1–11)
Total	340	100	201	100	58	100	37	100	44	100
Immunophenotype										
B-cell	82	69 (60–77)	46	69 (57–79)	18	67 (48–82)	7	58 (30–83)	11	85 (58–97)
T-cell	34	29 (21–37)	20	30 (20–42)	7	26 (12–45)	5	42 (17–70)	2	15 (3–42)
Neither	3	3 (1–7)	1	1 (0.1–7)	2	7 (1–22)	0	0 (0–22)	0	0 (0–21)
Total	119	100	67	100	27	100	12	100	13	100
WHO classification										
DLBCL	66	75 (65–83)	40	83 (71–92)	12	71 (46–88)	4	40 (14–71)	10	77 (49–94)
DSBCL	3	3 (1–9)	1	2 (0.1–10)	0	0 (0–16)	2	20 (4–52)	0	0 (0–21)
MZL	6	7 (3–14)	1	2 (0.1–10)	3	18 (5–41)	1	10 (0.5–40)	1	8 (0.4–32)
PTCL-NOS	6	7 (3–14)	1	2 (0.1–10)	1	6 (0.3–26)	3	30 (8–62)	1	8 (0.4–32)
T-LBL	2	2 (0.4–7)	1	2 (0.1–10)	1	6 (0.3–26)	0	0 (0–26)	0	0 (0–21)
TZL	5	6 (2–12)	4	8 (3–19)	0	0 (0–16)	0	0 (0–26)	1	8 (0.4–32)
Total	88	100	48	100	17	100	10	100	13	100

n = absolute number in the lymphoma group, CI = confidence interval, WHO = World Health Organization, DLBCL = diffuse large B-cell lymphoma, DSBCL = diffuse small B-cell lymphoma, MZL = marginal zone lymphoma, PTCL-NOS = peripheral T-cell lymphoma not otherwise specified, T-LBL = T-cell lymphoblastic lymphoma, TZL = T-zone lymphoma

remaining 14/66 (21%) were immunoblastic. The four Boerboel cases that underwent immunophenotyping were also further classified. All were DLBCL, of which three cases were subclassified as centroblastic and the remaining one as immunoblastic.

Evaluation for statistical associations between canine signalment and immunophenotype and/or WHO classification subtype was not possible due to the low number of cases that underwent further IHC and subtyping.

Geographical location

The three provinces in South Africa that yielded the most lymphoma biopsy submissions were Gauteng (*n* = 202, 59%), Western Cape (*n* = 58, 17%) and KwaZulu-Natal (*n* = 37, 11%). Lymphoma characteristics, namely tumour grade, sampling site, immunophenotype and WHO classification, by geographical location for these three provinces were evaluated and are shown in Table VIII.

Discussion

In this study, although age, sex and neutering status did not show significant associations, breed was identified as an overall risk factor for developing canine lymphoma. Apart from the Boerboel and Yorkshire Terrier, there was no statistically significant association identified for the most commonly presented breeds in this study. This finding was unexpected as lymphoma associations for multiple different breeds in South Africa have previously been identified. These include: Belgian Shepherd, Bull Terrier, Boerboel, Border Collie, Boxer, English Bulldog, Rhodesian Ridgeback, Basset Hound, Bullmastiff, Labrador Retriever, Kerry Blue Terrier, Mastiff, Newfoundland and Schipperke (Van Rooyen et al. 2018).

The Boerboel, a unique South African mastiff-type breed, has only recently been identified as having an increased risk of developing canine lymphoma (Emikpe et al. 2016; Van Rooyen et al. 2018). Although the Boerboel's exact lineage is not clear, the Bullenbeisser, English Bulldog, English Mastiff and Bullmastiff, all part of the Molosser-type dogs, have all been described as part of the Boerboel breed's direct progenitors (Grabe 1995). Several studies have shown a clear breed association with these ancestry breeds of the Boerboel and lymphoma (Edwards et al. 2003; Ernst et al. 2016; Keller et al. 1993; Onions 1984; Priester & McKay 1980; Van Rooyen et al. 2018; Villamil et al. 2010). The English Bulldog was shown to have a significantly high prevalence of lymphoma (RR = 2.2; $p < 0.01$) and the Boxer had the highest risk ratio (RR) of all dogs (RR = 4.5; $p < 0.001$) in one study (Priester & McKay 1980). Furthermore, a high incidence of lymphoma has been described in the Bullmastiff breed (5 000 per 100 000 dogs) with a familial distribution (Onions 1984:909–912). These associations were

also seen in a later study where the Boxer ($p = 0.002$), Bullmastiff ($p = 0.002$) and English Bulldog ($p = 0.012$) were identified as having a significantly higher incidence of lymphoma (Edwards et al. 2003). In South Africa, the Boxer, English Bulldog, Bullmastiff and Mastiff were previously shown to be at an increased risk ($p < 0.05$) (Van Rooyen et al. 2018).

This study confirms the findings of Van Rooyen et al. who first described the Boerboel's predisposition to lymphoma (Van Rooyen et al. 2018). Additionally, the Boerboel has been shown to have a predisposition for mast cell and spindle cell tumours in another South African study (Tompkins et al. 2020).

Furthermore, this study found that Yorkshire Terriers have a decreased risk of developing lymphoma. This phenomenon has previously been reported in literature from Australia, the United Kingdom, Switzerland and North America (Bennett et al. 2018; Edwards et al. 2003; Grüntzig et al. 2016; Villamil et al. 2010). Previously, Yorkshire Terriers in South Africa were reported to have an OR of < 1.00 but this was not statistically significant (Van Rooyen et al. 2018). A Swiss study examining the role of breed, age, sex, neutering status, and body size in tumour development, found that the Yorkshire Terrier had not only a decreased OR for lymphoma development but also for mast cell tumours, haemangiomas/haemangiosarcomas and osteomas/osteosarcomas. Additionally, this breed had a decreased overall risk of tumour development when compared to crossbreeds. In the same study, the Yorkshire Terrier was grouped as a small breed to assess body size in tumour development, and although the small breed group was less likely to develop most types of neoplasms, an increased risk for the development of mammary and endocrine gland tumours was noted (Grüntzig et al. 2016). These findings might indicate that genetics and body size play a protective role in the development of lymphoma, and other neoplasms, in this breed.

While the following breeds constituted ≥ 3% of the lymphoma population in the study, they did not demonstrate significant associations with the development of canine lymphoma. Notably, these breeds are frequently linked to canine lymphoma in existing literature. Increased or decreased risk of developing lymphoma has been described in each of the overrepresented breeds: Jack Russell Terrier (Bennett et al. 2018; Day & Whitbread 1995), Labrador Retriever (Bennett et al. 2018; Priester & McKay 1980; Van Rooyen et al. 2018) and Dachshund (Bennett et al. 2018; Teske 1994; Villamil et al. 2010). Furthermore, an increased risk of developing lymphoma has been found in the German Shepherd Dog (Comazzi et al. 2017; Cunha et al. 2011) and Rottweiler (Bennett et al. 2018; Coelho et al. 2019; Comazzi et al. 2017; Cunha et al. 2011; Pastor et al. 2009; Villamil et al. 2010).

The Maltese (Poodle) Toy breed was previously found to have a decreased risk of developing lymphoma which was not confirmed in this study (Van Rooyen et al. 2018; Bennett et al. 2018; Villamil et al. 2010). The Maltese Poodle had an absolute number of 2 in the lymphoma group of this study and was included in the FCI Group 9 (Companion and Toy Breeds) with a total number of 10 dogs included in this group. This FCI group had significantly decreased odds of developing lymphoma. This might partially support the finding of Van Rooyen et al. The Maltese Poodle was also found to have a decreased risk for follicular and round cell tumours in another South African study. The reason was hypothesised to be due to genetic heterogeneity as this breed is most probably assigned to any long haired, white, Toy breed dog in South Africa. The Maltese Poodle has an unknown pedigree and is not registered with the Kennel Union of Southern Africa (KUSA) (Tompkins et al. 2020).

The median age of the Boerboel lymphoma group was 6.0 years, which was younger than the entire canine lymphoma population's median age (8.0 years). Ninety percent of the Boerboel lymphoma group were in the young to middle-aged groups (< 10.0 years). Most of the Bullmastiff lymphoma cases in the study by Van Rooyen et al. fell into the four to six-year age group but no mention of the Boerboel ages was made. When compared to the existing literature on canine lymphoma age in the ancestry breeds of the Boerboel, a higher prevalence was seen in younger age groups for the English Bulldog (≤ 3 years) and the Bullmastiff (≥ 4 and ≤ 6 years) (Edwards et al. 2003). Although the lymphoma population exhibited age prevalence patterns consistent with other studies and various neoplasms, it is noteworthy to highlight the slight increase in the prevalence of lymphoma in younger age groups within the Boerboel breed.

In a large study in North America investigating various variables associated with survival time in canine lymphoma, 992 biopsy samples were included and grade was determined using the WHO classification system (Valli et al. 2013). Of the cases included 51% were high grade, 32% low grade, 12% intermediate grade, and 5% were benign hyperplasia or a benign neoplasm such as thymoma (Valli et al. 2013). These findings differ substantially from the findings in this study where 27% of cases were high grade, 30% of cases were assigned a low grade and 43% an intermediate grade. Later literature, applying the WHO classification scheme to a large cohort of canine lymphoma nodal biopsies, reported grade as an average of the mitotic count over 10 HPFs rather than in a single field as per the WHO recommendations (Avallone et al. 2021; Valli et al. 2013; Valli et al. 2011). Histological consensus on canine lymphoma grading remains unclear, to the authors' knowledge, and accounts for great inter-pathologist grading variability worldwide.

This study showed that canine lymphoma demonstrates a similar clinical presentation (haemopoietic vs. skin/subcutis/mucosal vs. other organs) in South Africa when compared to reports in other parts of the world (Table VII) (Pittaway et al. 2019; Ponce et al. 2010; Vezzali et al. 2010).

A higher B-cell prevalence in the South African canine lymphoma population (69%) with a concurrent lower T-cell prevalence (29%) (Table VIII) was found in this study. In a study in North America that investigated B- and T-cell prevalence in 1 263 dogs, B-cell immunophenotype had a prevalence of 61% and T-cell 39% (Modiano et al. 2005). Similar prevalences were seen in studies in Brazil (B-cell 62.5%, T-cell 37.5%) (Coelho et al. 2019), Germany (B-cell 79.4%, T-cell 20.6%) (Ernst et al. 2016), Italy (B-cell 68.2%, T-cell 31.8%) (Gavazza et al. 2008), France (B-cell 63.8%, T-cell 35.4%) (Pastor et al. 2009) and in a multicentre European study which included the United Kingdom (B-cell 65.7%, T-cell 34.3%) (Comazzi et al. 2018). Of interest is that an equal 50% prevalence

for B- and T-cell lymphoma was seen in the canine lymphoma population in Bangkok (Rungsipipat et al. 2014), which might indicate a different phenotypic profile based on geographical location.

A North American study by Valli et al. (2011) evaluated the consistency and accuracy of the WHO subtype classification scheme. In this study 248/300 of the biopsies could be classified into six groups, namely: DLBCL (145/300, 48%), MZL (11/300, 4%), PTCL-NOS (42/300, 14%), TZL (38/300, 13%), T-LBL (12/300, 4%); and 20/300 (7%) of cases were not lymphoma. In comparing the prevalence of WHO classification subtypes to the findings of this study, a higher proportion of biopsies were categorised as DLBCL, while the PTCL-NOS and TZL classification groups showed a lower prevalence in South Africa. However, further South African studies are warranted to ascertain whether this disparity represents a genuine geographical distinction.

During the review process, three cases in this study were diagnosed as DSBCL, a separate entity not currently included in the WHO subtype classification (Hughes et al. 2021). In the study by Hughes et al. (2021), DSBCL were typically graded as intermediate or high with a median age of 11 years. In our study, the three cases identified as DSBCL had a lower grade and younger median age. It is difficult to interpret the differences found in DSBCL characteristics and patient signalment due to the low number of cases in our study. The information for the histological classification of DSBCL was not available at the time of initial biopsy diagnosis (2018–2020) and therefore the conclusion can be made that some cases qualifying for a DSBCL diagnosis might have been misclassified under other existing classification groups, for example DLBCL.

Investigation into regional differences in lymphoma characteristics in South Africa revealed that KwaZulu-Natal had lower numbers of intermediate grade and slightly higher numbers of high-grade lymphoma compared to other provinces and the canine lymphoma population as a whole. Furthermore, a lower number of B-cell lymphomas were seen, especially DLBCL, and higher numbers of T-cell lymphomas, especially PTCL-NOS in this province. This might indicate a higher probability of dogs in KwaZulu-Natal developing more malignant T-cell lymphomas. However, negative risk factors previously investigated and associated with lymphoma development, such as exposure to sites of radioactive waste, pollution, and waste incineration, cannot clearly be identified for the province of KwaZulu-Natal exclusively and the significance of this finding is questionable (Pastor et al. 2009).

Although a retrospective case-control study has many advantages, the limitations of such a study design influence the interpretation of the study results. Such limitations encompass the fact that data and samples were not originally collected for research purposes, consequently giving rise to issues related to missing data (Cohen 2011). In this study, the recording of neutering status was inconsistent on submission reports from private veterinarians and, although the association was analysed, the results between neutering status and lymphoma are less precise for this reason. However, missing data are most likely randomly distributed throughout the lymphoma and control population, making bias less of a concern.

Information bias is a common limitation of case-control studies. A large proportion of dogs in South Africa are bred by informal breeders and are not registered with KUSA. This results in breeds being assigned to dogs by veterinarians, breeders and owners based on phenotypic characterisation, rather than by breed registration, which may cause non-differential misclassification (Tompkins et al. 2020). The breed variable might therefore be subject to information bias and bias results towards the null hypothesis (underestimation).

It is essential to note that the findings of this study cannot be extrapolated to represent the entire dog population in South Africa. The study's reliance on a commercial laboratory's dog population means it primarily includes dogs owned by households in higher income brackets that have access to advanced veterinary care. It is estimated that more than half of the dogs in South Africa are either homeless or residing in low-income rural communities and will have minimal representation in a commercial laboratory population (Mars Petcare 2020; Stats S.A. 2017; Tompkins et al. 2020). The characteristics of this rural dog population are different compared to dogs from affluent areas (Hergert et al. 2018; Rautenbach et al. 1991). This should not influence the risk evaluation of breeds in this study but does suggest that a large percentage of local dog breeds in the country were not represented and therefore could not be evaluated.

A further potential limitation of the study is that not all lymphoma cases underwent a slide review to standardise characterisation. All lymphoma reports were meticulously reviewed by the primary investigator, and the subsequent review of ambiguous reports was thorough and performed by pathologists. While reviewing all the slides by a single person could have provided standardisation, it might also have introduced bias into the study.

Additionally, using only one commercial laboratory's population situated in Gauteng, from where 59% of canine lymphoma biopsy submissions originated, biased the data to be more representative of Gauteng rather than the entire South Africa. This, again, has minimal effect on the presented results of the study as the control group was selected from the same laboratory population and the risk factor analysis remains unbiased.

Lastly, cases readily diagnosed by cytology were not included in the study's epidemiological information and this might have biased phenotypic prevalence data.

Conclusion

In this study, a breed predisposition for canine lymphoma was established in the Boerboel, a South African mastiff-type dog. Conversely, this study revealed that Yorkshire Terriers do not exhibit a predisposition for developing lymphoma.

Although limited, data from this study results might suggest a predilection for B-cell lymphoma in the Boerboel, especially DLBCL, with disease onset potentially being younger in this breed.

The frequency of immunophenotypes was similar to findings in other geographical locations, but WHO classification subtypes, and tumour grade differed from two large cohort studies using the same criteria in North America. A higher prevalence for DLBCL and fewer high-grade tumours were found in South Africa. However, consensus on histological grading under the WHO classification scheme needs to be reached.

Future, ideally prospective, studies need to be conducted in South Africa with a wider representation of dogs presented at welfare and state funded clinics to be more representative of the entire country's population. Therefore, a national multicentre study is proposed.

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Conflict of interest

The authors declare they have no conflicts of interest that are directly or indirectly related to the research.

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Ethical approval

The author/s declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.

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ORCID

SB Harris https://orcid.org/0009-0001-0942-813X
EH Hooijberg https://orcid.org/0000-0002-4367-799X
SJ Clift https://orcid.org/0000-0003-1368-1215
GT Fosgate https://orcid.org/0000-0002-9432-0042
EC du Plessis https://orcid.org/0000-0001-5651-5765
Y Rautenbach https://orcid.org/0000-0002-9798-1874

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