

LIST OF ABBREVIATIONS

AT	Austria
CD	Cluster of differentiation
CH	Switzerland
CI	Confidence interval
cL	Canine lymphoma
DLBCL	Diffuse large B-cell lymphoma
DSBCL	Diffuse small B-cell lymphoma
ES	Spain
FC	Flow cytometry
FCI	Federation Cynologique Internationale®
FL	Follicular lymphoma
FNA	Fine needle aspirate
FR	France
H&E	Haematoxylin & Eosin
HIV	Human immunodeficiency virus
HPF	High power field
ICC	Immunocytochemistry
IHC	Immunohistochemistry
IT	Italy
KUSA	Kennel Union of Southern Africa
LBL	T-cell lymphoblastic lymphoma
MCL	Mantle cell lymphoma
MZL	Marginal zone lymphoma

NHL	Non-Hodgkin's lymphoma
NL	Netherlands
OR	Odds ratio
PARR	Polymerase chain reaction assay for antigen receptor rearrangement
PTCL-NOS	Peripheral T-cell lymphoma not otherwise specified
RBC	Red blood cell
REAL	Revised European-American Classification of Lymphoid Neoplasms
RR	Risk ratio
TZL	T-zone lymphoma
UK	United Kingdom
WHO	World Health Organisation

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SUMMARY

Breed, age, and sex predispositions for canine lymphoma (cL) have been reported for various locations. However, epidemiological information concerning cL in South Africa is scarce. The objective of the study was to describe the epidemiologic and histological features of cL and the frequency of World Health Organisation (WHO) classification subtypes in South Africa.

A retrospective case-controlled study was performed that included 342 cases, submitted between December 2018 - December 2020, with a histopathological diagnosis of cL, matched with non-cL control cases. Associations between cL and breed, sex and age were assessed using univariate and multivariable conditional logistic regression. Associations were reported as odds ratios (ORs) and significance set as $P \leq 0.05$.

Breed, in general, was significantly associated with cL, but age, sex, and neutering status were not. Median population age was 8 years, with a male to female ratio of 1.2:1. The Boerboel had an increased (OR = 3.25, $P = 0.004$) and the Yorkshire Terrier a decreased risk (OR = 0.17, $P = 0.004$) of developing cL. The Boerboel cL group ($n = 27$) had a younger median age of 6 years and a higher male to female ratio of 1.5:1. Immunophenotyping was performed on 119 (35%) cases, of which 82 (69%) were B-cell, 34 (29%) T-cell and 3 (2%) neither. World Health Organisation classification subtypes were available for 88 cases; of these 66 (75%) were diffuse large B-cell lymphoma (DLBCL) with remaining subtypes each $\leq 7\%$. All Boerboel cases ($n = 4$) that were WHO subtyped were DLBCL.

This study confirmed a breed predisposition for cL in the Boerboel, a South African mastiff-type dog, as previously reported. However, the lack of association between age, sex, and other breeds, and cL, was unexpected. The frequency of immunophenotypes and WHO classification subtypes were largely similar to findings in other locations. The study results suggest a possible predilection for B-cell lymphoma in the Boerboel.

Chapter 1

Introduction

1.1 Background

In veterinary oncology, lymphoma is one of the most frequently diagnosed and managed malignant neoplasms in dogs ¹. Lymphoma is a term used to broadly categorise several lymphoid cancer subtypes.

The most common form of canine lymphoma is termed multicentric lymphoma and involves a single to multiple lymph nodes. This type of lymphoma is seen in 73% to 82% of cases, of which the majority are of B-cell origin ^{2,3}. Extra nodal forms of canine lymphoma exist but are far less common than multicentric lymphoma. These include lymphoma of the skin (12-13%), gastrointestinal tract (2-7%), mediastinal (5%), spleen (2-5%), and tonsils (2%) ^{2,3}.

Clinical staging for multicentric lymphoma is done according the World Health Organisation (WHO) guidelines, considering the extent of lymph node and extra-nodal organ involvement and whether the patient is exhibiting any clinical signs ⁴.

Canine lymphoma bears many similarities to human non-Hodgkin's lymphoma (NHL), in terms of the molecular mechanisms of tumorigenesis, occurrence of spontaneous disease and shared environmental risk factors. For these reasons, canine lymphoma has been proposed to be an exceptional model for human NHL ^{5,6}. Furthermore, classification schemes used by veterinary pathologists to classify canine lymphoma have followed schemes developed for the diagnosis of NHL in humans. Since 1966, ongoing advancements in the knowledge of human NHL classification have led to continuous change and development in veterinary classification schemes. These include, in chronological order of classification system development, the Rappaport, Luke-Collins (North America), Kiel (Europe), Working Formulation (North America) and updated Kiel (Europe) ^{1,7-10}.

Advances in immunohistochemistry (IHC) prompted the formation of the International Lymphoma Study Group to revise outdated schemes, whereafter one of the most fundamental shifts in NHL classification followed, through development of the Revised European-American Classification of Lymphoid Neoplasms (REAL) system ^{11,12}. Making diagnoses of lymphoma solely on histopathology was believed

to be inexact and a classification scheme with more holistic, expanded criteria for lymphoma as a disease was advocated for. The REAL classification gave origin to the WHO classification scheme for NHL in 1997 with great success in global standardisation of disease classification ¹³. This system includes all aspects of the disease process, namely cell type, topography, immunophenotype, molecular analysis and cytogenetics, rather than cell type alone.

The updated Kiel and WHO classification systems are both currently in use by pathologists worldwide. The aforementioned system can be applied to histological and cytological classification of lymphoma whereas the last-mentioned solely focusses on histological interpretation ^{2,3,14,15}. Because of high accuracy and great reproducibility between pathologists using the WHO classification system in humans and animals, it renders it superior to other classification systems ^{11,16}.

The WHO classification system is based on histomorphological evaluation and when this human classification system was applied to 300 canine lymphoma biopsies, almost 80% of all lymphomas could be classified into 1 of 5 subtypes, namely: diffuse large B-cell lymphoma (DLBCL) (145/300, 48%), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) (42/300, 14%), T-zone lymphoma (TZL) (38/300, 13%), T-cell lymphoblastic lymphoma (LBL) (12/300, 4%) and marginal zone lymphoma (MZL) (11/300, 4%) ¹⁵. A recent study identified and suggested a 6th WHO classification subtype to be added. This type of lymphoma is relatively common, has a diffuse pattern and consists of small to intermediate cells, temporarily termed diffuse small B-cell lymphoma (DSBCL) ¹⁷. Diffuse large B-cell lymphoma and the B-cell immunophenotype is most commonly seen in dogs ¹⁵. When diagnosing lymphoma in canine patients, subclassification of the lymphoma type is needed for more accurate prognostication and to develop better treatment regimens ^{15,16}.

Large epidemiological studies of canine lymphoma have been conducted throughout the world identifying several breeds, age group, sex, and neutering status risk factors for the development of this disease in canines. When some of these studies, conducted in Australia, North America, Brazil, Europe and South

Africa were reviewed, the following breeds were identified as being predisposed to developing lymphoma, or were overrepresented, in at least two or more of these studies (in alphabetical order): 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 Vizsla¹⁸⁻²².

When looking solely at South Africa, the first epidemiological canine lymphoma study in this country, according to the author's knowledge, was conducted in 2018²¹ identifying two South African breeds, the Boerboel and Rhodesian Ridgeback, at an increased risk of developing lymphoma. The Rhodesian Ridgeback has previously been associated with lymphoma¹⁸, but this was a first report for the Boerboel breed.

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^{21,23-25}.

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 canine lymphoma^{18,22,26}. In South Africa, males and neutered females had the highest risk of lymphoma²¹. Studies showing no sex or neutering status association also exist^{2,23-25,27-29}.

Despite the pioneer study in South Africa providing valuable epidemiological information about lymphoma in the dog population²¹, limited histological, phenotypic and WHO classification subtype information exists.

1.2 Aims and Objectives

The aim of the study was to describe the epidemiologic features in terms of breed, age, sex, and neutering status, of canine lymphoma in South Africa. Furthermore, we aimed to determine the lymphoma characteristics of this population.

The objectives were firstly to retrospectively investigate canine lymphoma histology reports to record and statistically analyse information on epidemiological data i.e., breed, age, sex, and neutering status.

Secondly, lymphoma characteristics like anatomical origin, grade, immunophenotype and WHO classification subtypes (at least DLBCL, PTCL-NOS, TZL, LBL, MZL and DSBCL) were recorded and descriptively analysed.

Lastly, the abovementioned findings were used to descriptively compare epidemiological and histological lymphoma data from South Africa to other canine studies in the rest of the world and to investigate whether the canine lymphoma population could be a feasible model when compared with non-Hodgkin's lymphoma in the human population of South Africa.

1.3 Hypotheses

It was hypothesised that significant associations would be seen with breed, age, sex and neutering status and the development of lymphoma in the study population and that epidemiological associations with lymphoma WHO classification subtypes would be seen and described. It was further hypothesised that the data obtained from the South African canine lymphoma population would follow trends in the rest of the world and that our findings would support using canine lymphoma as a model for studying NHL in humans, especially in South Africa.

1.4 Benefits

It was anticipated that the role of breed, age, sex, and neutering status in the development of canine lymphoma in South Africa would be identified. When compared to studies in the rest of the world, the similarity, or difference, in epidemiological risk factors in South Africa may identify or confute risk factors, whether they be environmental or genetic, in the pathophysiology of the disease process, specifically in this country. This information will be beneficial to small animal clinicians when presented with at-risk dogs with a clinical suspicion of lymphoma as it may encourage a more focussed diagnostic work-up, better prognostication and improved medical management of these patients.

The knowledge of this study will further benefit insurance companies, breeders, and pet owners by providing further information regarding at risk breeds, as there are big financial and emotional implications of insuring, breeding, and living with a companion animal that will require an increased level of veterinary medical intervention at some stage in their life.

The similarity or differences of the prevalence of lymphoma characteristics of canine vs. human lymphoma in South Africa may indicate the presence or absence of shared risk factors for the disease in both species. This may warrant or refute the idea of a One Health approach in canine and human lymphoma to better understand the disease in both species.

Lastly, the study serves as fulfilment of the principal investigator's requirements for a Master of Science degree.

Chapter 2

Literature Review

2.1 Anatomical Manifestations of Canine Lymphoma

Most ($\pm 77\%$) of canine lymphoma cases are classified anatomically as multicentric, with involvement of one or multiple peripheral lymph nodes ^{2,3,30}. This form of canine lymphoma may further be present in other haemopoietic organs such as the spleen, liver and/or bone marrow, which influences lymphoma grade ⁴. Extra-nodal forms exist which include cutaneous, gastrointestinal, mediastinal, splenic, hepatosplenic, hepatic, ocular, central nervous system and pulmonary lymphoma ¹.

Cutaneous lymphoma is typically of T-cell origin and is classified as either non-epitheliotropic or epitheliotropic, depending on whether there is invasion of the papillary dermis and epidermis or not. Epitheliotropic lymphoma affects the skin as well as mucocutaneous junctions and the oral cavity. This type of cutaneous lymphoma is further classified according to the WHO into three subtypes: mycosis fungoides, pagetoid reticulosis and Sézary syndrome (listed from most common to rare) ³¹.

Gastrointestinal lymphoma is mainly a primary neoplasia of the gastrointestinal tract (75%) and is of T-cell origin. It mostly affects the small intestine, followed by the stomach and colon. Primary gastrointestinal lymphoma does not seem to infiltrate the spleen and superficial lymph nodes as is seen with multicentric lymphoma but can affect the regional lymph nodes and liver ^{32,33}.

An enlarged thymus and/or cranial mediastinal lymph nodes are the main characteristics of mediastinal lymphoma. This type of lymphoma is associated with mainly a T-cell immunophenotype, and hypercalcaemia is commonly seen (10-40%) ^{27,34}.

Primary splenic lymphoma is far less common than lymphoma of the peripheral lymph nodes ¹⁶. Marginal zone lymphoma (MZL) and mantle cell lymphoma (MCL) are primary splenic B-cell lymphomas with MZL being the most common. The incidence of this neoplasm is unknown as only a few studies with small numbers exist ³⁵⁻³⁸. Other splenic lymphomas include follicular lymphoma (FL), DLBCL, natural killer cell lymphoma and PTCL-NOS ¹⁵. Splenectomy has shown favourable

outcomes in splenic lymphoma patients (without systemic involvement), without chemotherapy increasing survival outcome^{38,39}.

Hepatosplenic lymphoma is rare and has first been described in the literature in 2003⁴⁰. It is typically a lymphoma of T-cell origin, aggressive in nature and mainly involves the liver, spleen, and bone marrow with minimal peripheral lymphadenopathy^{40,41}. In one study that looked at 9 dogs with T-cell lymphoma and hepatic involvement, without peripheral lymphadenopathy, diagnosed hepatosplenic T-cell lymphoma in 7 dogs and hepatocytotropic T-cell lymphoma in the remainder of the two⁴².

Primary hepatic, ocular and nervous system lymphoma remain rare in dogs¹. Primary pulmonary lymphoma is rare but secondary involvement with multicentric lymphoma is commonly seen in this organ (reported 66% in one study)^{43,44}.

2.2 Clinical Presentation of the Lymphoma Patient

The clinical presentation of patients diagnosed with multicentric canine lymphoma are broad and non-specific. The most common clinical signs in decreasing commonality are: generalised lymphadenopathy, splenomegaly on abdominal palpation, inappetence, pyrexia, weight loss, dyspnoea and pallor⁴⁵.

During the clinical work up of a canine lymphoma patient, clinical haematology and biochemistry show a wide variety of non-specific abnormalities⁴⁵. The most common, but inconsistent, haematological abnormalities include a mild to moderate non-regenerative, normocytic, normochromic anaemia in 47% of cases, a leukogram of chronic inflammation in 53% of cases, and a mild, asymptomatic thrombocytopenia in 12-36% of cases^{45,46}. On serum biochemistry, 10-15% of patients diagnosed with canine lymphoma, mainly of T-cell immunophenotype, show hypercalcaemia^{1,47,48}. In patients with multicentric canine lymphoma, a common non-prognostic finding on urinalysis is a mild proteinuria postulated to be caused by an impairment of the glomerular permselectivity⁴⁹.

As 32-55% of canine lymphoma patients are reported to have bone marrow involvement, a bone marrow sample should ideally be evaluated in these patients

^{50,51}. A single bone marrow aspirate for cytological examination is sufficient to indicate involvement of the organ ⁵⁰.

Although diagnostic imaging modalities such as thoracic and abdominal radiographs, abdominal ultrasonography and computed tomography are not useful in providing a specific diagnosis of canine lymphoma, these modalities provide important information on staging of the disease and anatomical location guidance for biopsy taking and/or possible surgical treatment ⁵²⁻⁵⁴.

2.3 Diagnosis and Phenotyping of Lymphoma

Cytological diagnosis of canine lymphoma from a fine needle aspiration (FNA) biopsy can be definitive in high grade tumours but challenging in low grade tumours or atypical hyperplastic proliferations ¹⁰.

A recent study concluded that cytology can be used with high accuracy and pathologist confidence to make a diagnosis of lymphoma but that the accuracy and pathologist confidence decreased for further classification like grade and phenotype. Diagnostic accuracy for WHO classification subtype was variable and pathologist confidence was low for determining WHO classification subtype on cytology ⁵⁵.

The combinations of cytology and immunocytochemistry (ICC) ¹⁴, or cytology, flow cytometry (FC) and ICC combined ⁵⁶, have shown to be reliable methods for diagnosing and classifying lymphomas using the updated Kiel classification system ^{14,56}. Although immunophenotyping can be done with fair accuracy on FC of FNA biopsies, and together with cytology improves the accuracy of canine lymphoma diagnosis, FC generally falls short of diagnosing specific lymphoma subtypes ^{16,57}.

Cytology can therefore be used to determine a diagnosis of lymphoma and as a screening for grade and phenotype but needs confirmation on histopathology, IHC/ICC and/or FC ⁵⁵. Furthermore, histopathology on biopsy samples is required to diagnose indolent and low-grade lymphomas and histopathology further facilitates lymphoma subclassification based on established histomorphological criteria ^{16,36}.

Polymerase chain reaction assay for antigen receptor rearrangement (PARR) has been advocated as an alternative to immunophenotypic characterisation of canine lymphoma via IHC or FC but has been deemed inferior to the above-mentioned two methods⁵⁸. Using PARR for lymphoma staging has been described, but due to its high sensitivity it causes significant stage migration, for example: when PARR was performed on dogs in stages I-III, (i.e. no blood or bone marrow involvement), neoplastic cells at these locations were detected with PARR, which shifted these dogs to stage V⁵⁹. Some infections, for example *Ehrlichia canis*, are associated with clonal T-cell rearrangement, and neoplastic conditions, like acute myeloid leukaemia, can cause false positive results with PARR due to the non-neoplastic expansion of one of two lymphocyte clones⁶⁰. Thus, clinical staging and antibody-based immunophenotyping have been proven to be superior for disease prognostication and lymphocyte phenotyping, respectively^{58,61}.

Although some studies¹⁶ advocate that veterinary oncology should follow human medicine in only making a diagnosis of lymphoma on histopathology, other studies show great agreement between PARR and IHC (69%) and FC and IHC (94%)⁵⁸.

2.4 Clinical Staging

A clinical staging system for multicentric canine lymphoma has been developed by the WHO to describe the disease extent (Table 2.1)^{4,29}.

Table 2.1: Clinical staging for multicentric canine lymphoma according to the World Health Organisation (WHO)⁴.

Stage	
I	Single node or lymphoid tissue in single organ (excluding bone marrow)
II	Regional involvement of multiple lymph nodes (\pm tonsils)
III	Generalised lymph node involvement
IV	Stage I-III with involvement of liver and/or spleen
V	Stage I-IV with involvement of blood or bone marrow
Substage	
a	Absence of systemic signs
b	Presence of systemic signs (fever, >10% weight loss, hypercalcaemia)

The WHO-staging system takes into consideration the number of lymph nodes involved, extra nodal involvement, for example liver, spleen, blood and/or bone marrow, and whether the patient exhibits any clinical signs of disease ⁴. This system justifies the importance of developing a proper minimum database when working up a canine lymphoma patient.

Although routine serum biochemistry and immunophenotyping are not listed in the staging criteria, it is advised to also run these tests because of the negative impact that the T-cell immunophenotype and hypercalcaemia may have on prognosis ^{28,48,62}. However, the association between hypercalcaemia and a poorer prognosis is conflicting ⁶³. Imaging modalities, which can aid in disease staging, should be decided on an individual patient basis as it is not included in the WHO staging system.

2.5 Canine Lymphoma Classification and Grading

Before 1993 classification of canine lymphoma was a topic of confusion and debate because of different criteria used by the Rappaport, Luke-Collins (North America), Kiel (later updated Kiel; Europe) and the Working Formulation (North America) systems ¹². Due to the need for greater uniformity in the application of consistent criteria, the International Lymphoma Study Group developed the REAL classification system which formed the basis of the revised WHO lymphoma classification system that is used today ^{13,64}.

The WHO lymphoma classification system is based on the identification of specific histomorphological features which include the following ¹⁵:

- nodular (focal/multifocal) versus diffuse (sheet-like) growth pattern
- relationship between neoplastic foci and residual non-neoplastic lymphoid follicles
- nuclear size - small (<1.5x the diameter of a red blood cell (RBC)), intermediate (1.5-2x the diameter of an RBC), or large (>2x the diameter of an RBC)

- detailed nuclear morphology, which includes nuclear shape, chromatin pattern and presence/absence and/or prominence of nucleoli
- the number of mitoses in a single high-power field (HPF) (400x magnification)
- the immunophenotype (T- or B-cell)

The mitotic count per HPF is used to grade the lymphoma as low, intermediate, or high. Lymphomas with 0 to 5 mitoses per HPF field are graded as low grade, 6 to 10 are graded as medium grade, and greater than 10 are graded as high grade ¹⁵. Later literature ⁶⁵ reported that two studies by Valli et al. ^{15,16}, 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. Histological consensus on canine lymphoma grading remains unclear, to the authors knowledge.

Most canine lymphoma cases can be classified into five major subtypes according to the WHO classification scheme, with a sixth subtype being proposed, and each of these subtypes are briefly described below ^{15,17}:

2.5.1 Diffuse Large B-Cell Lymphoma (DLBCL)

This type of lymphoma comprised almost half of all canine lymphoma cases in the study by Valli et al. ¹⁵ (n = 145/300, 48%) and is also the most common subtype of NHL found in humans ⁶⁶. On IHC, DLBCL is cluster of differentiation (CD) 3 negative and strong positive for CD20 and CD79. Architecturally, the neoplastic B-cells are arranged in diffuse sheets, and peripheral sinus compression, lymph node capsule thinning, peri-nodal invasion of neoplastic cells, and overall destruction of the normal nodal structures can be seen ¹⁵.

A distinct histological feature of DLBCL is the scant cytoplasm and large, mostly round, nuclei uniformly throughout. Diffuse large B-cell lymphoma can be further classified based on the number and location of their nucleoli. Large B-cells with a single central nucleolus are termed immunoblastic and those with multiple, peripherally located nucleoli are termed centroblastic. Both types are often present in one patient and are only classified as immunoblastic if >90% of nucleoli are of

this type ^{2,15,16}. Human studies have described the immunoblastic subtype to have a worse prognosis and this finding may be worth investigating in the canine population ^{67,68}.

Another low-grade subtype exists, termed T-cell rich large B-cell lymphoma, where neoplastic B-cells with a low mitotic count are found between a predominant non-neoplastic T-cell population with ample fine stroma present. This subtype is however not common in dogs ¹⁵.

2.5.2 Peripheral T-Cell Lymphoma Not Otherwise Specified (PTCL-NOS)

Fourteen percent (n = 42/300) of canine lymphoma cases are classified as PTCL-NOS in the study by Valli et al ¹⁵. The definition of peripheral in this context refers to its extrathymic location. This subtype includes all T-cell lymphomas that are not well classified due to lack of further differentiation based on cellular size and topographic distribution ¹⁵.

Peripheral T-cell lymphomas have a diffuse proliferation of neoplastic T-cells, staining positive for CD3 and negative for CD20 and CD79, with a variable mitotic rate and cell morphology. Peripheral T-cell lymphomas are further classified into nodal (more common) and extra-nodal (less common). The architectural changes of the nodal form include focally obliterated and compressed sinuses, thinning of the capsule, diffuse paracortical expansion and perinodal infiltration of the neoplastic cells ¹⁵.

Oval or cleaved nuclei are often seen in the nodal form of PTCL. High-endothelial venules are associated with this subtype, and it is suggestive of PTCL when there is a pleomorphic background population of non-neoplastic cells including macrophages and eosinophils ¹⁵.

Extra-nodal forms are found in the skin, subcutis, liver and intestinal tract and neoplastic cells commonly invade the vasculature ¹⁵.

Differentiating PTCL from lymphoblastic lymphomas of the same cell size can be done based on the following cell characteristics: Lymphoblastic lymphomas are

often intermediate size, have an indistinct chromatin pattern, high mitotic count, and inconspicuous nucleoli ¹⁵.

High grade T-cell lymphoma with plasmacytoid appearance and T-cell immunoblastic lymphoma are two unusual types of T-cell lymphomas that have been described in the literature. These subtypes are difficult to diagnosed based on morphology alone, however, both have a high mitotic index and aggressive clinical behaviour ^{2,69}.

2.5.3 T-Zone Lymphoma (TZL)

T-zone lymphoma is a nodal lymphoma, mostly found in the submandibular lymph node. This type of lymphoma is found in 13% (n = 38/300) of cases, a much higher incidence than in humans, where neoplastic T-cells, staining strongly positive for CD3 and negative for CD20 and CD79, expand the medullary cords and paracortex without effacing the architecture of the lymph node ^{15,16}. In another study, TZL stained positive for CD4 and negative for CD8 ²⁷.

T-Zone lymphoma architecture is characteristic: peripheral sinuses are not infiltrated but compressed. Lymphoid follicles are peripheralised by the neoplastic T-lymphocytes and, therefore, appear to be pushed against the fibrovascular framework of the lymph node. The areas in between, the paracortex, consists of a homogenous population of lymphocytes ¹⁵.

There are two TZL subtypes namely small cell and intermediate cell TZL. Both types show little nuclear detail, inapparent nucleoli, relatively abundant cytoplasm compared to other lymphoma subtypes, nuclei with sharp shallow indentations and shallow or low mitoses because of its indolent nature (no mitoses in most HPFs). Despite its low grade and indolent nature, blood involvement can be found in advanced stages of TZL ¹⁵.

The peripheralisation of nodular lymphoid tissue, expansion of the paracortex and the moderate cytoplasm pertaining to the neoplastic cells helps to differentiate TZL from small lymphocytic lymphomas of B- or T-cell origin ¹⁵.

2.5.4 T-Cell Lymphoblastic Lymphoma (T-LBL)

In veterinary medicine one of the most aggressive, but rare, lymphomas are lymphoblastic lymphomas of either B- or T-cell origin, with the latter being more common (n = 12/300, 4% in the study by Valli. et al) ¹⁵.

Architecturally, there is diffuse medullary and cortical filling of densely staining CD3 positive lymphocytes (CD79 negative), perinodal colonisation focally and a thin capsule ¹⁵.

This subtype of lymphoma is characterised by a homogenous population of intermediate cells that have an intermediate-sized, irregularly indented or round to oval nuclei, dispersed dense chromatin pattern, indistinct nucleoli, and a high mitotic count (>10/HPF) ^{2,15}.

B- or T-cell LBL can only be differentiated from each other by immunophenotyping; and LBL is differentiated from acute lymphoblastic leukaemia according to the degree of topographic involvement, i.e., whether the tumour is dominated by blood and bone marrow involvement or not ⁶⁶.

2.5.5 Marginal Zone Lymphoma (MZL)

Marginal zone lymphomas are proliferations of B-cells, found in lymph nodes or the spleen, and have a distinct appearance cytologically which helps to differentiate this subtype from DLBCL, LBL, and marginal hyperplasia. In the study by Valli et al., almost 4% (n = 11/300) of the cases were MZL, with a prevalence of >10% in other studies ^{2,70}.

Marginal zone lymphomas are architecturally nodular, expressing CD20, CD79 and lacking CD3, characterised by fading germinal centres surrounded by proliferative expansion of coalescing perifollicular marginal lymphocytes that can be accompanied by paracortical atrophy ^{36,66}.

Cytologically, MZL is characterised by mostly absent mitoses (indolent lymphoma), moderate lightly stained cytoplasm, and peripheralised chromatin accentuating a prominent single central nucleolus in an intermediate sized nucleus.

This indolent lymphoma has less aggressive behaviour with patients showing no clinical signs of disease except for a single to generalised enlarged lymph node/s, however late stage MZL might behave more like aggressive lymphomas^{15,16,36}. In one study investigating the biological behaviour of MZL, 35 dogs at a referral centre diagnosed with MZL on histopathology were included. Of these 35 dogs 1/3 were showing systemic signs, almost 2/3 had bone marrow involvement and all but one had peripheral blood involvement concluding that, despite its indolent nature, it progresses to an aggressive disease³⁷. When disease is confined to the spleen, disease is usually incidentally picked up by abdominal palpation or ultrasonography and splenectomy has been described as an effective treatment^{36,38}.

Marginal zone lymphoma is distinguished from DLBCL by having smaller cells with a low mitotic count, from LBL by having a heterogenous follicular centre or an inner remnant collapsed mantle cell cuff, and from marginal zone hyperplasia by a homogenous population of intermediate size cells and cytological characteristics mentioned above.

2.5.6 Diffuse Small B-Cell Lymphoma (DSBCL)

A recent study that investigated 47 cases of small B-cell lymphomas, identified by FC, to histologically classify them according to the WHO classification system identified a group of small B-cell lymphomas (n = 27/47, 57%) that could not be correlated with any of the existing WHO classification subtypes. Only 43% (n = 20/47) of cases could be WHO classified of which 9 were MZL, 9 FL and 2 DLBCL.¹⁷ This unidentifiable group of small B-cell lymphomas, which showed consistent cellular morphology and histological features, was described as a separate and distinct group of lymphomas given the term diffuse small B-cell lymphoma (DSBCL) until further classification for these types of lymphoma exist¹⁷.

Architecturally the lymph node capsule was consistently invaded. The histological characteristics of DSBCL include round to indented, small to intermediate nuclei in a diffuse pattern (vs. MZL and FL's nodular pattern) with indistinct nucleoli and condensed chromatin^{2,15}. The cytoplasm was scant to moderate, eosinophilic, and homogenous. The mitotic count was intermediate to high (5-10 mitoses/HPF) and,

contrary to existing belief that small B cell lymphomas are indolent, appears to be an aggressive neoplasia ¹⁷.

Furthermore, eosinophilic histiocytes with a globular cytoplasm and tingible-body macrophages were regular findings in DSBCCL ¹⁷.

2.6 Epidemiology of Canine Lymphoma

B-cell lymphomas are found in the majority of cases (61-79%) but this can vary significantly between certain canine breeds ^{2,3,23,25,45,71-74}. Breeds presenting with excess B-cell lymphoproliferative disease (lymphoma as well as lymphoid leukaemia) are mostly breeds classified as recent European breeds, for example (in alphabetical order): the Basset Hound, Border Collie, Cavalier King Charles Spaniel, Doberman Pinscher and Scottish Terrier ^{72,75}.

Lymphomas diagnosed in the Airedale Terrier, Cocker Spaniel, Irish Wolfhound, Siberian Husky, Shih Tzu, and Yorkshire Terrier are of T-cell origin in most of the cases. When breeds were grouped by genetic relatedness, an increase in T-cell lymphoproliferative disease was present, particularly in the Spitz breed dogs like the Akita, Basenji, Chinese Shar-Pei, and the Siberian Husky, and the Shih Tzu group which include the Shih Tzu and other Asian toy breeds. It is hypothesised that the predisposition to develop specifically T-cell lymphoproliferative disease arose ancestrally, as these are some of the oldest domestic dog breeds ⁷².

An unrelated breed, the Boxer, has been reported in several studies to have a T-cell predilection ^{19,27,72,74,76}. However, Boxers have also been reported to develop B-cell lymphomas ⁷³.

No other breed group, other than the Spitz and Shih Tzu breed groups, shared common ancestral predilections for developing a specific lymphoid proliferative disease immunophenotype. Breeds not classified under the abovementioned two breed groups, having an excess of specific T-cell or B-cell neoplasia, developed this risk by genetic isolation during breed selection processes ⁷².

Breeds identified as having an increased risk of developing lymphoma in 5 recent studies (2010-2020) ¹⁸⁻²² with study populations in countries on 5 continents are listed in Table 2.2. The major findings and limitations of these studies are summarized in Appendix D.

It has been reported in certain breeds like the Bullmastiff, Rottweiler, and Scottish Terrier, that lymphoma has a familial occurrence ^{1,29,77}.

Prior to 2018, little information existed on breed risk factors to develop canine lymphoma in South Africa. In 2009, a South African case report was published suggesting a familial link of lymphoma in three related Rottweilers in the same household ⁷⁷. A study in 2018 by Van Rooyen et al. investigated which breeds had increased odds of developing canine lymphoma and the effects of age, sex, and neutering status on the prevalence of the disease in the South African setting ²¹. A limitation of the study was that only cases with a cytological diagnosis of canine lymphoma were included. Subsequently, not the epidemiological, nor the histological, characteristics of WHO classification subtypes could be evaluated in the study.

The following breeds were identified of having an increased risk for developing canine lymphoma in South Africa (in alphabetical order): Basset Hound, Belgian Shepherd, Boerboel, Border Collie, Boxer, Bull Terrier, Bullmastiff, English Bulldog, Kerry Blue Terrier, Labrador Retriever, Mastiff, Newfoundland, Rhodesian Ridgeback and Schipperke ²¹. This study by Van Rooyen et al. highlighted two local breeds, the Boerboel and the Rhodesian Ridgeback, that have increased odds of developing canine lymphoma, which before 2018, had not been described in the literature. Another unique South African finding was that the Maltese Poodle, an unrecognised toy breed popular in South Africa, had a significantly decreased odds of developing canine lymphoma ²¹.

Middle-aged to older dogs (mean 6.3 to 7.7 years) are mainly affected with an increasing incidence rate with age ²⁹. Dogs <1 year have an incidence rate of 1.5 cases per 100 000. This rate increased significantly to 85 per 100 000 in dogs >10 years of age ³⁰.

Table 2.2: Canine breeds recently reported (2010-2020) as having a high prevalence in the lymphoma population or having an increased risk of developing lymphoma on 5 continents.

Breed	Australia	Brazil	Europe		North America	South Africa
	OR (95% CI)	Prevalence	OR		OR (95% CI)	OR (95% CI)
Airedale Terrier	2.5 (1.6-4.0) **				1.9 (1.6-2.3) ***	
Basset Hound	2.4 (1.3-4.5) *				3.2 (2.9-3.6) ***	2.5 (1.1-5.7) *
Bernese Mountain Dog			10.2 ***	AT	3.4 (2.7-5.0) ***	
			14.4 ***	FR		
			2.6 *	IT		
			2.9 **	NL		
			5.3 ***	CH		
			3.3 *	UK		
Border Collie	1.8 (1.6-2.0) ***					2.5 (1.3-4.9) *
Boxer		7.9%	6.9 **	FR	4.0 (3.7-4.4) ***	3.0 (1.5-6.2) *
			2.9 ***	IT		
			2.7 ***	UK		
Bull Terrier	2.2 (1.7-2.9) ***					2.1 (1-4.3) *
Bullmastiff	4.8 (3.8-6.0) ***				4.8 (3.6-6.4) ***	4.4 (1.6-11.9) *
Cocker Spaniel	1.6 (1.4-1.9) ***	3.9%				
Corgi	3.1 (2.3-4.2) ***				2.0 (1.6-2.8) ***	
English Bulldog	2.8 (2.2-3.5) ***					2.8 (1.4-5.8) *
German Shepherd		3%	2.9 *	NL		
			3.3 **	CH		
Golden Retriever	2.1 (1.9-2.4) ***	9.4%	2.2 *	UK	3.1 (2.9-3.2) ***	
Gordon Setter	4.9 (2.5-9.7) ***				3.3 (2.5-4.4) ***	
Labrador Retriever	1.2 (1.1-1.4) ***		2.1 *	FR		1.8 (1.2-2.7) *
			3.3 **	CH		
Mastiff	5.3 (4.1-6.9) ***					13.5 (1.7-105.5) *
Rhodesian Ridgeback	1.8 (1.3-2.3) ***					3.1 (1.6-5.9) *
Rottweiler	2.0 (1.7-2.4) ***	7.4%	5.8 ***	AT	2.7 (2.4-3.0) ***	
			9.4 ***	FR		
			2.3 *	IT		
			5.0 ***	NL		
			3.1 *	ES		
			4.6 ***	PT		
Scottish Terrier	2.4 (1.4-3.9) **				3.4 (3.0-3.9) ***	
Saint Bernard	2.7 (1.4-5.2) *				2.3 (2.0-2.7) ***	
Vizla	1.9 (1.2-2.9) *				2.7 (2.0-3.5) ***	
Reference	Bennet et al., 2018 ¹⁸	Jark et al., 2020 ²⁰	Comazzi et al., 2018 ¹⁹		Villamil et al., 2010 ²²	Van Rooyen et al., 2018 ²¹

OR = odds ratio, CI = confidence interval, AT = Austria, CH = Switzerland, ES = Spain, FR = France, IT = Italy, NL = Netherlands, PT = Portugal, UK = United Kingdom, * p < 0.05, ** p < 0.01, *** p < 0.001

Intact females appear to have a reduced risk of developing canine lymphoma, which is similar to what is seen in humans. Pre-menopausal women have the lowest risk of developing NHL. However, this phenomenon changes when hormones are permanently altered i.e., in post-menopausal women and neutered bitches. The incidence difference between males and females, in both populations, become significantly smaller after the hormonal alteration ²².

In a recent study of 6201 dogs diagnosed with canine lymphoma, males were over presented when compared to females and neutered animals were found to have a higher risk when compared to intact animals of both sexes ¹⁸.

2.7 Epidemiological Study Design and the South African Dog Population

The goal of an epidemiological study is to obtain dependable and valid approximates of causal factors obtained from a population group to further the understanding of the disease incidence ⁷⁸.

Advantages of an observational epidemiological study include, from a welfare perspective, that studies investigate spontaneous disease occurrence, and no experimental induction of disease occurs. Furthermore, results obtained from such studies are immediately relevant to the study population under question and mimic a real-life scenario in comparison to experimental studies ⁷⁹. Because of these reasons epidemiological studies are highly valued by evidence-based medicine principles ^{80,81}. Although these mentioned characteristics of epidemiology are hugely advantageous, its limitation is also exactly that: it remains challenging to make inferences on causality of disease by observing a specie in their natural environment ⁷⁹.

A retrospective case-control study design was chosen for this study, with a histopathology laboratory population chosen to represent the wider South African dog population. Because such a study design allows investigation of multiple possible risk factors, can be performed in a relatively short period and is inexpensive to perform, it is a desirable option. However, limitations of such a

design include missing data from historic record keeping that was not collected with the intent for research use, a less distinct temporal relationship between possible risk factors and disease and difficulty in selecting an appropriate control group ⁷⁸.

Although true risk cannot be determined in a case-control study, because the prevalence of disease is not determined by spontaneous occurrence of the disease in the population' but rather by the study design, in rare diseases like cancers, inferences can be made on the odds ratio (OR) as an estimation of incidence risk and rate ^{78,82}.

The true breed composition of the South African dog population is unknown, however breed prevalence in two separate South African laboratory populations (one of which was the IDEXX Laboratory population also used for this study) and data derived from an insurance database have been published ²¹. The ten most common breeds in the IDEXX laboratory population (n = 28 523) from the aforementioned study were (in decreasing prevalence): Jack Russell Terrier (n = 2314), Labrador Retriever (n = 2289), Dachshund (n = 2213), German Shepherd Dog (n = 1792), Yorkshire Terrier (n = 1587), Maltese Poodle (n = 1237), Boerboel (n = 1001), Golden Retriever (n = 770), Rottweiler (n = 674) and Schnauzer (n = 667). Mixed breeds (n = 1303) also made up a large portion of this population. The ten most common breeds in the insurance population (n = 29 512) were (in decreasing prevalence): German Shepherd Dog (n = 2311), Dachshunds (n = 2198), Labrador Retriever (n = 1864), Yorkshire Terrier (n = 1625), Jack Russell Terrier (n = 1534), Maltese Poodle (n = 1365), Rottweiler (n = 1298), Boerboel (n = 1148), English Bulldog (n = 990) and American Staffordshire Terrier (n = 955) ²¹.

Given the large size of these populations, they are probably fairly robust representations of the population of dogs in South Africa with owners that have the socioeconomic capacity and willingness to pay for some level of advanced veterinary care for their animals. In a report by Statistics South Africa in 2017, on data gathered in 2015, it was reported that 7.4% of the country's population lived below the international poverty line of R 22,50 per day at the time of reporting. They further reported that more than half of the population in 2015 were living below the

upper bound national poverty line of R33 per day and that people living in rural areas are the biggest victims of poverty⁸³. From this information it is evident that the socioeconomic picture of South Africa is unique compared to the developed world and that routine, never mind advanced, veterinary care for animals in rural communities is highly unlikely. Information on the proportion of dogs that live in these areas and that are owned by low-income bracket families is unavailable but the fact that more than half of the South African population lives in poverty suggests that a very large proportion of the dog population is affected by these circumstances as well.

Furthermore, a recent survey by a pet food company (Mars Petcare) estimated that there were 4.1 million homeless dogs and cats in South Africa, making up 27% of the total dog and cat population⁸⁴. Data on breed distribution from this survey is not available, but this does give a further indication that around a quarter of dogs and cats in South Africa do not receive any veterinary care, as they are homeless, and are not represented in laboratory or insurance populations.

Information concerning the characteristics of rural dog populations are limited but a few studies conducted in selected rural communities in South Africa exist shedding some light on the dog population characteristics in a few of these areas. Rautenbach et al. documented characteristics and compared health parameters of 220 dogs in one rural town in North West province (then Bophuthatswana) versus 101 dogs in an intensive kennel setting. They reported all rural dogs to be mixed breeds with a mean age of 2.5 years old and only 4/220 being neutered. It was further reported that >50% of dogs in the study had unrestrained movement⁸⁵. Haematological abnormalities indicating anaemia, biochemical findings including low blood iron levels, hypergammaglobulinaemia, hypoalbuminaemia and a low body condition score on clinical examination were ascribed to environmental factors: a high external and internal parasite load, chronic systemic infections, and poor nutrition^{86,87}.

Rural dog population characteristics have also been described for selected communities in the KwaZulu-Natal province. Breed was not recorded in this study

because the majority of dogs were of the local Africanis dog breed ⁸⁸. Sixty six percent of the province's dogs were 3 years or younger, out of the 1794 dogs only 7 (<1%) were neutered and only 17% of the dogs in the study were fully restricted to the household. It was further reported that a significantly higher number of households in the rural areas owned dogs when compared to the urban areas ⁸⁹.

Dogs living in rural communities in South Africa may include significantly fewer pure-bred dogs, since more dogs are free roaming and not sterilised. Also, presumably this population is younger as minimal routine healthcare are readily available for these animals, especially for senior dogs requiring more intensive veterinary care. It can further be extrapolated that homeless dogs in South Africa roughly share the same characteristics as dogs owned by households in rural communities, rather than affluent urban household dogs.

It is therefore important to note that rural and homeless dogs are hugely underrepresented in veterinary hospital, laboratory, and insurance populations in South Africa and caution should be taken to extrapolate results to the whole South African dog population from studies where populations were sourced from these institutions.

2.8 Epidemiology of Non-Hodgkin's Lymphoma in Humans

Non-Hodgkin's lymphoma is ranked, in most countries, as the 5th to 9th most common cancer in humans and the incidence rates vary markedly among national populations ⁹⁰. A recent study in Southern Africa showed that a lower proportion of low-grade B-cell NHL cases and a higher proportion of high-grade B-cell NHL are seen in this region, when compared to Western Europe and North America. A lower proportion of the population in Southern Africa had T-cell NHL than in North America and especially Western Europe. The male to female ratio in Southern Africa was 1.4:1 compared to North America (1.1:1) and Western Europe (1:1) ⁹¹.

Using companion animals, particularly the dog, as an animal model to study spontaneous developing neoplasias, like NHL, in humans has been a feasible idea over the past few decades ⁵. It has been difficult to use results from trials done with

inbred rodents to extrapolate on human tumour aetiology and treatment versus using non-inbred canine populations, which often share the same environment as humans and often survive into the cancer age group ⁹².

Diffuse large B-cell lymphoma, specifically, has been under investigation as a canine model for human DLBCL. Similarities not only include DLBCL being the most common subtypes of lymphoma seen in both species, but also chemotherapeutic treatment, poor response thereto and molecular similarities ⁹³. In both human and canine DLBCL gene expression profiles, a high expression of target genes of nuclear factor kappaB transcription factors was seen. This factor has been associated with the promotion of lymphomagenesis and resistance to chemotherapy by inhibiting cell apoptosis ⁹⁴. Treating canine DLBCL cells with nuclear factor kappaB essential modulator-binding domain peptide, induced apoptosis in vitro and reduced tumour burden when injected intralesional, pioneering studies to further investigate this as a feasible treatment in human DLBCL ⁹⁵.

2.9 Conclusion

It is evident that there is limited knowledge about breed, age, sex, and neutering status associations with canine lymphoma in the South African setting. Furthermore, histological, and phenotypical lymphoma characteristics has not yet been described in the dog population of this country. However, it will be challenging to make inferences about the entire South African dog population from a study using a laboratory study population given the diverse socioeconomic factors playing a role in the entire population characteristics. It will further be valuable to see whether the canine population follows the trends of NHL in the South African human population and whether it can possibly be used as a model for further studies in aetiology and treatment for both species.

Chapter 3

Materials & Methods

3.1 Experimental Design

3.1.1 Study Design

This study was approved by the Research Ethics Committee of the Faculty of Veterinary Science and Animal Ethics Committee of the University of Pretoria (REC115-20). In this retrospective, case-control, observational study, histopathological reports of canine lymphoma biopsy samples submitted to the private veterinary laboratory, IDEXX (Kyalami, Gauteng, South Africa), were retrieved and analysed. All cases with a definitive histopathological diagnosis of canine lymphoma, submitted to the laboratory between December 2018 to December 2020, were included in the study and matched with a control sample submitted to the same laboratory on the same date.

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., tumour origin, tumour grade, immunophenotyping and WHO classification subtype, where available, were also recorded for all cases.

3.1.2 Study Population

An unmatched case-control power analysis was performed to determine the minimum sample size using statistical freeware (OpenEpi). A previous South African study by Van Rooyen et al. ²¹, which also used data from the IDEXX Laboratory database, identified several breeds that had an increased risk of developing canine lymphoma (see Chapter 2). Of these, the Boerboel was chosen for a power analysis calculation. This breed has increased odds of developing canine lymphoma and is a popular breed in the IDEXX population ²¹.

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 (OR) 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 case inclusion criteria was any canine biopsy sample, not diagnosed as canine lymphoma, submitted to the IDEXX Laboratory on the same day as the included lymphoma case.

3.2 Experimental Procedure

3.2.1 Data Collection

Patient signalment information and histopathological findings for tissue sections with a diagnosis of canine lymphoma, that were received by IDEXX Laboratory, were retrieved by the following method:

- The Microsoft SQL Server Database (the database used by IDEXX for case record keeping) was searched using a bespoke software application by the company's information technologist. The database was interrogated with the search phrase 'lymphoma' with the search time range from December 2018 - December 2020. This query filtered all samples accessioned by IDEXX Laboratory with a diagnosis or description containing the term 'lymphoma' for the given time frame. The query results were further copied into a commercial spreadsheet program, Microsoft Excel, containing the following information: laboratory number, patient signalment and pathologist.
- All correlating 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.) via an online software service (Google Drive).
- 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 by incidence density sampling with a 1:1 ratio, and included by the following method:

- Submission dates from the included lymphoma cases were compiled into a list and sent to IDEXX's information technologist 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 sent to the primary investigator via an online software service (Google Drive). All canine lymphoma cases were removed from the control dataset.
- One control was randomly selected for each case matching on submission date. 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. Controls were selected using lookup functions and reordering of the dataset based on new random numbers when multiple cases had the same submission dates.

3.2.2 Lymphoma Classification

3.2.2.1 Report Reviewing for Inclusion

Cases suggestive of lymphoma with an inconclusive morphological diagnosis, and where a canine lymphoma diagnosis could not be extrapolated from the histopathological description by the primary investigator, 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.) from the Department of Companion Animal Clinical Studies at the Faculty of Veterinary Science of the University of Pretoria. 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 review was done by one veterinary anatomical pathologist (S.C.) from the Department of Paraclinical Studies at the Faculty of Veterinary Science of the University of Pretoria. 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.

3.2.2.2 WHO Classification Subtype Reviewing

One of the main objectives of the study was to analyse the prevalence of, and possible epidemiological associations with, specific WHO classification subtypes. Biopsies with a canine lymphoma diagnosis from a haemopoietic organ that underwent IHC, but that were not further subtyped into a specific WHO class, were reviewed by a veterinary anatomic pathologist (S.C.) to assess eligibility for WHO classification subtyping. Tissue sections from canine lymphoma cases submitted to IDEXX Laboratory, where private practitioners requested immunophenotyping, underwent IHC staining at the University of Pretoria's Immunohistochemistry Laboratory. The IHC reports and tissue sections were obtained using the following steps:

- The diagnostic laboratory database on UVIS (the software used by the Immunohistochemistry Laboratory for patient recording) was searched using the Query Tool, using the criteria 'Species = Canine' and 'Result Value Contains Lymphoma'. The search time range used was December 2018 - December 2020. This search query extracted all canine samples accessioned by the Anatomic Pathology Laboratory, which the 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 obtained from the Anatomic Pathology Laboratory were reviewed for WHO classification subtyping.
- In cases where further subtyping was not possible by report reviewing, the tissue sections were extracted from the 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. Routine CD3 Polyclonal Antibody marker (Dako, Isotype rabbit Anti-Human CD3) and unconjugated CD20 Polyclonal Antibody marker (ThermoFisher, Isotype rabbit IgG) were used on these sections, and where further T- or B-cell clarification was needed, sections were stained with purified mouse anti-PAX5 (BD Bioscience, Clone: 24/Pax-5, Isotype: mouse IgG1).

Furthermore, for completeness and accuracy, all DLBCL cases that were not further classified into immunoblastic or centroblastic, as well as cases that were diagnosed as DLBCL but were described as small cell lymphomas, were reviewed for reclassification by the veterinary anatomic pathologist given new research published during the reviewing process¹⁷. The lymphoma grade assigned by the reporting IDEXX pathologist was reviewed by the primary investigator under the criteria set out in the Valli et al., 2011¹⁵ paper to ensure uniform grading using the mitotic count reported.

3.3 Observations/Analytical Procedures

The following data were extracted from the laboratory records, histopathology reports and immunophenotyping results, where available, and recorded on a Microsoft Excel spreadsheet (Appendix A): Laboratory reference number, submission and reporting date, breed, sex, neutering status, age, geographical location of the submitting veterinarian, diagnosis, grade, anatomic location, immunophenotype, WHO classification subtype, reporting pathologist, and sample type, i.e., surgical, or post-mortem sample.

For the included control cases, submission date, reporting date, breed, sex, neutering status, age and diagnosis were recorded on a Microsoft Excel spreadsheet (Appendix B).

3.4 Methodologies

All samples submitted to the IDEXX Laboratory were stained with haematoxylin and eosin stain (H&E), histologically examined, and reported by one of three veterinary

pathologists as a diagnostic service to private referring veterinarians. These reports were extracted for retrospective analysis and possible inclusion.

Further need for the IDEXX biopsy submissions to undergo IHC staining at the Immunohistochemistry Laboratory (a section of the Anatomical Pathology Laboratory) at the Department of Paraclinical Studies at the Faculty of Veterinary Science (University of Pretoria) depended on two factors: whether a definitive morphological diagnosis could be made on the H&E stain and/or whether pet owners had the financial capacity to proceed with further staining expenses. Cases that underwent additional IHC staining were examined and reported by one of the ten veterinary anatomical pathologists or residents at the department.

All biopsy samples included as control cases were examined and diagnosed by the abovementioned IDEXX pathologists.

3.5 Statistical Data Analysis

Dog breeds were classified into groups based on the Federation 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 years, 5-9.9 years, and ≥ 10 years for statistical analysis. Cases of lymphoma were categorised based on tumour grade, tissue of origin, immunophenotype, and WHO classification subtype.

Categorical data were presented as percentages and mid-P exact 95% CIs using statistical freeware (OpenEpi). The association between lymphoma and signalment was assessed using univariate conditional logistic regression. Multivariable 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 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.

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

Chapter 4

Results

4.1 Study Population

4.1.1 Lymphoma Population

4.1.1.1. Report Reviewing for Inclusion

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. During the first review process, case reports were reviewed by veterinary clinical pathologists using the histopathological description on the report issued by the IDEXX veterinary anatomical pathologists to conclude whether lymphoma was the most likely diagnosis of the differentials listed. Of these 57 case reports, 35 cases were included, 20 excluded and 2 were inconclusive, qualifying for a second phase of review by a veterinary anatomical pathologist. Both inconclusive cases were excluded from the study during the second round of review as hyperplasia could not be discerned from neoplasia without clonality testing, which is currently unavailable in South Africa. After the report review process, the canine lymphoma data set was finalised, totalling 342 included cases.

4.1.1.2. Lymphoma Classification Reviewing

All cases that underwent immunophenotyping without a final definitive diagnosis, or where further WHO subtyping was not performed by the IDEXX veterinary anatomical pathologist, were selected for classification review. Thirty-two cases qualified for the review by a veterinary anatomical pathologist (Table 4.1). A morphological diagnosis was confirmed on 19 cases by simply reviewing the histopathological and IHC report. The remaining 13 cases were identified for slide review, as the morphological diagnoses or further classification could not be definitively made from the report alone.

The slides from 11 of these cases could be retrieved from the Immunohistochemistry Laboratory's archive. The slides from 2 cases could not be found, and the formalin blocks were requested from IDEXX Laboratory to recut and repeat the immunohistochemistry for CD3 and CD20 lymphocytes. One of the 11

cases that was selected for slide review had a complete blend of CD3 and CD20 IHC staining. The block was also requested from IDEXX to recut and stain with PAX5.

Of the 32 cases, 7 diagnoses could be confirmed from skin, subcutaneous, mucosal, and other biopsies (Table 4.1). Twenty-five haematopoietic biopsies were reviewed where 6 biopsies could not be subtyped with the WHO classification system, but a definitive diagnosis of canine lymphoma was confirmed. Nine cases, that were not originally classified with a WHO subtype, were further classified and four DLBCL cases were further classified into immunoblastic or centroblastic. Two slide review cases were newly diagnosed as DSBCL (as part of the 9 cases that was WHO subtyped) and one DLBCL was re-classified as DSBCL based on the report. Four cases were histopathologically typical for MZL and one for TZL and were classified accordingly without these samples having undergone further immunophenotyping (i.e., MZL as B-cell; TZL as T-cell).

Table 4.1: Report and slide review outcome.

		Origin	Outcome
32 Cases Reviewed -19 report reviews -13 slide reviews	Haemopoietic n=25		6 unchanged diagnoses
		19 further/ reclassified	9 newly WHO classified
			4 DLBCL further classified
			1 DLBCL reclassified as DSBCL
			5 assumed immunophenotype (MZL, TZL)
	Skin n=1	1 unchanged diagnosis	
Other n=6	3 unchanged diagnoses		
	3 reclassified		

DLBCL = diffuse large B-cell lymphoma, DSBCL = diffuse small B-cell lymphoma, MZL = marginal zone lymphoma, TZL = T-zone lymphoma, WHO = World Health Organisation

4.1.2 Control Population

Three hundred and forty-two controls were selected matching the date of submission of the lymphoma biopsy to the IDEXX Laboratory. Of these 342, 8 did not have a diagnosis assigned but signalment data was available. Diagnoses were grouped into the following 4 categories: neoplastic, inflammatory, both neoplastic and inflammatory, hyperplasia and miscellaneous (Table 4.2). Diagnoses categorised as neoplasia included all abnormal cell proliferations, whether benign

or malignant and comprised more than half ($n = 217/342$) of diagnoses in the control group. The second most common disease process were inflammatory ($n = 74/342$) accounting for $>20\%$ of the biopsies. Diagnoses included in the miscellaneous category, among others, included normal tissue, tissue calcification, haematoma, myelofibrosis, and haemorrhage.

Table 4.2: Absolute numbers and prevalence of the underlying disease process of biopsies in the control population.

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

CI = confidence interval

Tissue origins of biopsies were widely distributed and categorised according to body system (Table 4.3). The majority of biopsies submitted in the control group were from the integumentary system ($n = 224/342$, 60%) of which the skin (excluding glandular origin) had the highest prevalence within the group ($n = 161/224$, 72%). Three biopsies were from both the skin and subcutaneous tissue and 29/224 (13%) from subcutaneous tissue alone. Eighteen diagnoses were from glandular origin in the skin ($n = 18/224$, 8%). Two thirds ($n = 148/224$, 66%), of integumentary biopsies were neoplastic and a quarter inflammatory ($n = 56/224$, 25%).

Alimentary biopsies accounted for 7% ($n = 26/342$) of control biopsies and comprised biopsies from the oral cavity ($n = 17$), stomach and intestines ($n = 8$) and rectum ($n = 1$). Perianal biopsies ($n = 4$) were grouped under the integumentary system. The distribution of disease process in the alimentary group were almost split half between neoplastic ($n = 13/26$, 50%) and inflammatory ($n = 11/26$, 42%).

Biopsies from mammary gland pathology accounted for most ($n = 12/17$, 70%) of the submissions from the reproductive system ($n = 17/342$) of which all were

neoplastic. Other biopsies from this group originated from the testes (n = 3), ovary (n = 1) and vagina (n = 1).

Tissue biopsies from other body systems respectively comprised <5% of all submissions in the control group (Table 4.3).

Table 4.3: Absolute numbers and prevalence of the body system origin of biopsies in the control population.

Body system	Absolute numbers (n)	Prevalence (%) (95% CI)
Integumentary	224	60 (65;70)
Alimentary	26	7 (5;11)
Reproductive	17	5 (3;8)
Musculoskeletal	11	3 (2;6)
Haemopoietic	9	3 (1;5)
Endocrine	4	1 (0;3)
Ocular and periocular	4	1 (0;3)
Respiratory	4	1 (0;3)
Hepatobiliary and pancreas	4	1 (0;3)
Urinary	2	1 (0;2)
Aural	1	0 (0;1)
Cardiovascular	1	0 (0;1)
Multiple	2	1 (0;2)
Unknown	33	10 (7;13)
Total	342	100

CI = confidence interval

4.2 Epidemiological Factors

4.2.1 Breed

Seventy-seven different breeds, including mixed breeds, were identified in the study population. Breeds comprising $\geq 3\%$ of the entire study population (n = 684), i.e., most commonly presented breeds, included (in decreasing prevalence): Mixed (n = 109, 16%), Jack Russell Terrier (n = 52, 8%), Labrador Retriever (n = 38, 6%), Boerboel (n = 35, 5%), Dachshund (n = 33, 5%), German Shepherd Dog (n = 24, 4%), Rottweiler (n = 24, 4%), and Yorkshire Terrier (n = 23, 3%).

Fifty-five different breeds, including mixed breeds, were identified in the lymphoma group of the study (n = 342). Breeds comprising $\geq 3\%$ in the lymphoma population included (in decreasing prevalence): Mixed (n = 62, 18%), 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 ($n = 13, 4\%$), Beagle ($n = 11, 3\%$), and Rhodesian Ridgeback ($n = 11, 3\%$). The absolute breed numbers per FCI group for all breeds in this group are set out in Table 4.4.

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 4.5).

The Boerboel showed a statistically significant increased risk (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 was a statistically significant lower risk of lymphoma ($P = 0.004$) with a low OR (OR = 0.17, CI = 0.05-0.57), thus, this breed is less likely to develop lymphoma in this population.

Multivariable analysis of the individual breeds, while accounting for age, sex, and neutering status, confirmed the results of the univariate analysis. Boerboels (OR = 1.63, $P = 0.002$) were more likely to develop lymphoma compared to all other breeds and Yorkshire terriers (OR = 0.59, $P = 0.05$) were less likely to.

In the univariate analysis no FCI breed group was found to have a significant positive association with canine lymphoma but 4 breed groups, namely Terriers (Group 3), Pointing Dogs (Group 7), Companion and Toy breeds (Group 9) and Sighthounds (Group 10), showed a statistically significant ($P \leq 0.05$) relative lower risk of developing lymphoma; the ORs for all 4 groups were 0.5 or less (with 95% CIs not encompassing an OR of 1.0).

Table 4.4: Breed distribution of the canine lymphoma population within each Federation Cynologique Internationale breed group.

Breed Group	N	Breed	n
1 - Sheepdogs and Cattle dogs (Except Swiss Cattle dogs)	26	Australian Shepherd	1
		Border Collie	6
		Bouvier Des Flandres	1
		Collie	1
		German Shepherd Dog	15
		Swiss Shepherd	2
2 - Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattle dogs	81	Boerboel	27
		Boxer	9
		Bulldog	8
		Bullmastiff	6
		Dobermann	2
		Great Dane	5
		Miniature Pinscher	1
		Rottweiler	15
		Schnauzer	6
		Shar-Pei	2
		3 - Terriers	64
Boston Terrier	2		
Bull Terrier	7		
Foxterrier	2		
Irish Soft-Coated Wheaten Terrier	1		
Jack Russell Terrier	23		
Kerry Blue Terrier	1		
Pitbull Terrier	10		
Scottish Terrier	5		
Staffordshire Bull Terrier	6		
Wirehair Fox Terrier	1		
Yorkshire Terrier	4		
4 - Dachshunds	14	Dachshund	14
5 - Spitz and Primitive Types	1	Siberian Husky	1
6 - Scent Hounds and Related Breeds	31	Basset Hound	8
		Beagle	11
		Bloodhound	1
		Rhodesian Ridgeback	11
7 - Pointing Dogs	5	Vizsla	1
		Weimaraner	4
8 - Retrievers - Flushing Dogs - Water Dogs	34	Cocker Spaniel	7
		English Springer Spaniel	1
		Golden Retriever	6
		Labrador Retriever	15
		Retriever	1
		Spaniel	3
9 - Companion and Toy Dogs	10	Cavalier King Charles Spaniel	1
		Chihuahua	1
		French Bulldog	1
		Maltese Poodle	2
		Papillon	1
		Pug	2
		Shih Tzu	2
10 - Sighthounds	1	Greyhound	1
11 - Mixed Breed	66	Africanis	2
		Mixed	64
No breed assigned	9		9
Total	342		342

N = absolute number of animals in FCI group, n = absolute breed numbers

Table 4.5: Univariate associations between breed and a diagnosis of lymphoma from a single veterinary histopathology laboratory.

Variable	Level	Parameter estimate ($\hat{\beta}$)	Odds ratio (95% CI)	P value
Breed				< 0.001
	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		

CI = confidence interval

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 ($P \leq 0.05$) in the multivariable analysis (Table 4.6). Terriers (Group 3), Pointing Dogs (Group 7) and Companion and Toy dogs (Group 9) had a significantly lower risk of developing lymphoma with ORs < 0.6 and $P \leq 0.05$ (Table 4.6).

Table 4.6: 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	0.489	1.63 (1.02, 2.62)	0.042
	3 Terriers	-0.521	0.59 (0.38, 0.93)	0.024
	7 Pointing dogs	-1.376	0.25 (0.08, 0.77)	0.016
	9 Companion and toy	-1.011	0.36 (0.16, 0.84)	0.018
	10 Sighthounds	-2.092	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	-0.189	0.83 (0.59, 1.17)	0.281
	Male	Referent		
Neuter status	Intact	-0.209	0.81 (0.56, 1.18)	0.277
	Neutered	Referent		

CI = confidence interval

Table 4.7: A list of breeds and their absolute numbers that comprise the control population. N = 342.

Breed	Absolute number (n)
Mixed	45
Jack Russell Terrier	29
Labrador Retriever	23
Dachshund	19
Yorkshire Terrier	19
Golden Retriever	10
Pitbull Terrier	10
Beagle	9
Border Collie	9
German Shepherd Dog	9
Rottweiler	9
Schnauzer	9
Staffordshire Bull Terrier	9
Boerboel	8
Maltese Poodle	8
Bull Terrier	7
Boxer	6
Cocker Spaniel	6
Rhodesian Ridgeback	6
Weimaraner	6
Boston Terrier	5
German Shorthaired Pointer	5
Bulldog	4
Bullmastiff	4
Chihuahua	4
Poodle	4
Whippet	4
Africanis	3
Italian Greyhound	3
Pekingese	3
Scottish Terrier	3
Basset Hound	2
Chow Chow	2
Doberman	2
French Bulldog	2
Great Dane	2
Pomeranian	2
Retriever	2
Shar-Pei	2
Shih Tzu	2
Airedale Terrier	1
American Staffordshire Terrier	1
Anatolian Shepherd	1
Australian Cattle Dog	1
Australian Shepherd	1
Bedlington Terrier	1
Bernese Mountain Dog	1
Bloodhound	1
Bouvier des Flandres	1
Cairn Terrier	1
Cane Corso	1
Collie	1
Dalmatian	1
Dutch Shepherd	1
English Bulldog	1
Fox Terrier	1
German Longhair Pointer	1
Griffin	1
Irish Setter	1
Irish Wolfhound	1
Kerry Blue Terrier	1
Miniature American Shepherd	1
Miniature Pinscher	1
Pointer	1
Swiss Shepherd	1
Wire Haired Terrier	1

Sixty-six different breeds were identified in the control population, including mixed breeds. Breeds that comprised $\geq 3\%$ of the control population ($n = 342$) included (in decreasing prevalence): Mixed breed ($n = 45, 13\%$), Jack Russell Terrier ($n = 29, 8\%$) Labrador Retriever ($n = 23, 7\%$), Dachshund ($n = 19, 6\%$) and Yorkshire Terrier ($n = 19, 6\%$). A list of all the control population breeds and their absolute numbers (n) are listed in Table 4.7.

4.2.2 Age

When age was grouped into 3 categories, 52/342 (15%) animals were < 5 years, 149/342 (44%) animals ≥ 5 and < 10 years and 99/342 (29%) animals ≥ 10 years of age. Forty-two out of 342 animals (12%) had no age recorded on the report.

The median lymphoma group age (range: 1-15) and control group age (range: 0.4-20.5) were both 8 years. Median age of the Boerboel canine lymphoma population was 6 years (range: 3-15 years). The majority of Boerboel cases (16/27, 59%) were in the ≥ 5 and < 10 years group and 8/27 (30%) of individuals were < 5 years old.

There was no significant association between age and a diagnosis of lymphoma ($P = 0.703$) (Table 4.8).

Table 4.8: Univariate associations between dog signalment and a diagnosis of lymphoma.

Variable	Level	Parameter estimate ($\hat{\beta}$)	Odds ratio (95% CI)	P value
Age	< 5 years	Referent		0.703
	5 - 9.9 years	-0.008	0.99 (0.64, 1.52)	0.969
	\geq 10 years	0.151	1.16 (0.72, 1.89)	0.544
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

4.2.3 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. The male to female ratio was 1.2:1 (i.e., males were slightly overrepresented) 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. However, neutering statuses was not consistently recorded by the submitting private veterinarians.

Sex and neutering status as a predisposing factor for developing canine lymphoma were analysed and no significant association could be found ($P = 0.113$ and $P = 0.186$, respectively) (Table 4.8).

4.3 Lymphoma Characteristics

4.3.1 Grade

Most haemopoietic organ biopsies were graded by the primary veterinary anatomical pathologist for diagnostic reporting purposes. All biopsies that were graded were reviewed by the primary investigator using the Valli et al. (2013) paper to ensure consistency. Two hundred and forty six out of 255 (97%) haemopoietic biopsies were assigned a grade. Fourteen cases from other tissue origins 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 4.10).

4.3.2 Origin

Two hundred and fifty-five out of 342 (75%) canine lymphoma cases included in this study were biopsies from haemopoietic origin with lymph nodes being the most sampled organ (n = 228, 89%) in this group. Liver and spleen biopsies were far less common (n = 17, 7%).

Seventy-two biopsies were diagnosed as skin, subcutaneous and mucosal lymphomas comprising 21% of biopsy submissions. Of these, 48 (67%) biopsies were cutaneous lymphomas, 17 (24%) mucosal/mucocutaneous lymphomas and 5 (7%) subcutaneous lymphomas (Table 4.9). Other lymphomas comprised of cerebellar (n = 1), intestinal (n = 5), mediastinal (n = 1), nasal (n = 1), rectal (n = 3), renal (n = 1) and tonsillar (n = 1) lymphoma.

In the Boerboel canine lymphoma population, 21/27 (78%) were of haemopoietic origin of which 18/27 (67%) were lymph node biopsies. The remaining biopsies constituted 5/27 (19%) from the skin (3 non-epitheliotropic and 2 epitheliotropic) and 1/27 from the subcutaneous tissue. There were no samples from other anatomical origins as mentioned here.

Table 4.9: Distribution of lymphoma biopsy submissions per anatomical origin.

Origin	n	Percentage (95% CI)	Subgroup Percentage (95% CI)
Haemopoietic	255	75 (70;79)	100
Lymph node	228	67 (62;72)	89 (85;93)
Liver/spleen	17	5 (3;8)	7 (4;10)
Other	10	3 (2;5)	4 (2;7)
Skin, subcutaneous, mucosal	72	21 (17;26)	100
Skin	48	14 (11;18)	67 (55;77)
Subcutaneous	5	2 (1;3)	7 (3;15)
Mucocutaneous/Mucosal	17	5 (3;8)	24 (15;34)
Other	2	1 (0;2)	3 (1;9)
Other	13	4 (2;6)	100

CI = confidence interval

4.3.3 Immunophenotype

One hundred and fourteen out of 342 (33%) biopsies underwent IHC, and 5 biopsies were assumed to be either B- or T-cell according to their typical histopathological appearance. Eighty-two out of 119 (69%) cases assigned an immunophenotype were of B-cell origin, and 34/119 (29%) were of T-cell origin (Table 4.10). Four of the 27 (15%) Boerboel cases were immunophenotyped and of those all stained positive for B-cell lymphoma.

Three cases (1 cutaneous, 2 haemopoietic) did not stain with either CD3 or CD20 and further staining was declined by the owners.

4.3.4 WHO Classification Subtype

The most common WHO classified lymphomas, by decreasing prevalence, were DLBCL (66/88, 75%), MZL (6/88, 6.8%), PTCL-NOS (6/88, 6.8%), TZL (5/88, 5.7%), and T-LBL (2/88, 2.3%) (Table 4.10). DSBCCL, not currently a recognised WHO classification subtype, was present in 3 cases (3/88, 3.4%). Of all the DLBCL biopsies, 52/66 (79%) were subclassified as centroblastic and the remaining 14/66 (21%) were immunoblastic. The 4 Boerboel cases that underwent immunophenotyping were also further subtyped with the WHO classification scheme and all 4 were DLBCL, of which 3 cases were subclassified as centroblastic and the remaining 1 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.

4.3.5 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, tumour origin, immunophenotype and WHO-classification, by geographical

location for these three provinces were evaluated (Table 4.10). The location of these provinces in South Africa is shown in Figure 4.1.

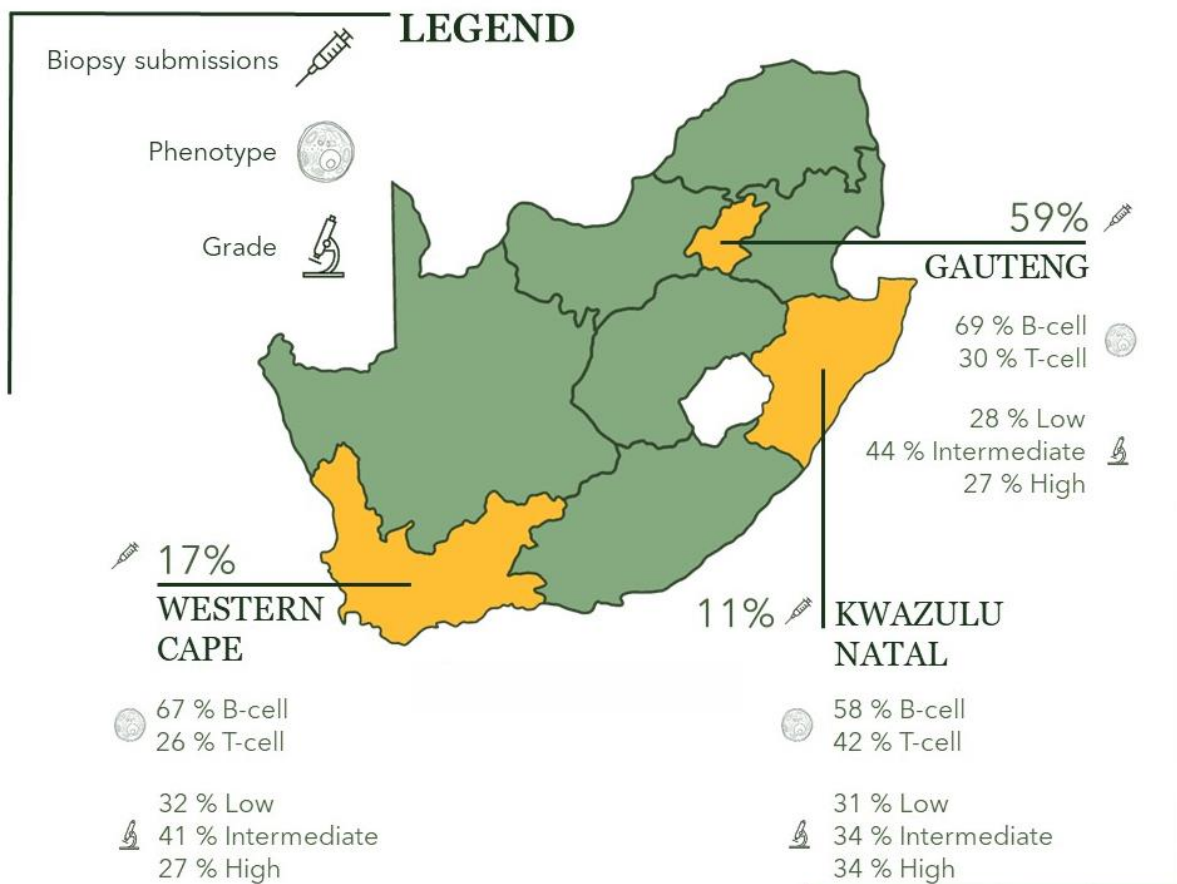


Figure 4.1: Location of the 3 provinces with the most lymphoma biopsy submissions and information about the proportion of biopsy submissions from each province with phenotype and grade distribution within each respective province.

The same trend as seen in the overall population with regards to grade were seen throughout the provinces of Gauteng (low: $n = 43/151$ (28%), intermediate: $n = 67/151$ (44%), high: $n = 41/151$ (27%)) and Western Cape (low: $n = 13/41$ (32%), intermediate: $n = 17/41$ (41%), high: $n = 11/41$ (27%)) but in KwaZulu-Natal low- ($n = 10/21$, 31%), intermediate- ($n = 11/32$, 34%), and high grade ($n = 11/32$, 34%) were split almost equally.

Samples from haemopoietic origin were uniformly predominant in all provinces. KwaZulu-Natal showed an increased prevalence in submissions from this tissue origin with 84% ($n = 31/37$) compared to Gauteng ($n = 149/210$, 74%) and Western Cape ($n = 41/58$, 71%). A similar but opposite trend was seen with skin,

subcutaneous and mucosal tissue biopsies only being 14% ($n = 5/37$) when compared to Gauteng and Western Cape with 21% ($n = 43/210$) and 26% ($n = 15/58$), respectively.

Immunophenotype was also different in KwaZulu-Natal when compared to the overall and provincial specific trends. B-cell lymphomas had a 69% ($n = 46/67$) and 67% ($n = 18/27$) prevalence in Gauteng and Western Cape, respectively, and KwaZulu-Natal had a prevalence of 58% ($n = 7/12$). T-cell lymphomas had a high prevalence of 42% ($n = 5/12$) in KwaZulu-Natal when compared to 30% ($n = 20/67$) and 26% ($n = 7/27$), respectively, in Gauteng and Western Cape.

In Gauteng ($n = 40/48$, 83%), Western Cape ($n = 12/17$, 71%), and in the overall population ($n = 66/88$, 75%), the predominant WHO classification subtype was DLBCL. In KwaZulu-Natal this type of lymphoma comprised less than half ($n = 4/10$, 40%) of the province's lymphoma biopsies. Peripheral T-cell lymphoma not otherwise specified were overrepresented in this province with a prevalence of 30% ($n = 3/10$) when compared to the overall population ($n = 6/88$, 7%) and the other two major provinces, Gauteng ($n = 1/48$, 2%) and Western Cape ($n = 1/17$, 6%). Western Cape had the highest prevalence ($n = 3/17$, 18%) of MZL.

Table 4.10: Absolute numbers and prevalence of lymphoma characteristics in the entire study population, and in Gauteng, Western Cape, KwaZulu-Natal and all other provinces combined, respectively.

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
Tumour origin										
Haematopoietic	255	75 (70, 79)	149	74 (68, 80)	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	13	4 (2, 6)	9	4 (2, 8)	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 subtype										
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

CI = confidence interval, 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, TZL = T-zone lymphoma

Chapter 5

Discussion

In this study the epidemiologic features associated with canine lymphoma, lymphoma characteristics and the frequency of WHO classification subtypes, in South Africa were evaluated.

The Boerboel showed a significant increased risk of developing canine lymphoma while age, sex and neutering status showed no associations. Males were slightly overrepresented in the lymphoma population and most lymphoma biopsy submissions were from dogs between 5 and 10 years of age with a median age of 8 years. The Yorkshire Terrier had a decreased risk of developing canine lymphoma.

Most lymphoma biopsies were from lymph node origin and graded as intermediate grade. B-cell lymphoma had the highest prevalence and of the biopsies that underwent WHO classification subtyping, DLBCL was most common. More than half of the biopsies were submitted from the Gauteng province.

To the author's knowledge this is the second study to assess associations with signalment in the dog population in South Africa and the first to study the prevalence of immunophenotype and WHO classification subtypes within this population.

5.1 Limitations

The limitations of this study relate firstly to the limitations associated with a case-control study design, and, secondly, to the fact that the study population are not a true representation of the South African dog population. The limitations discussed below are not all applicable to the results of the study per se, but rather on the inferences that can be made from these results for the entire dog population of South Africa.

Although a retrospective case-control study has many advantages, limitations of such a study design have a direct limitation on the interpretation of the study results. These limitations include that data is not collected for the purpose of research and therefore results in missing data ⁷⁹. 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 may

be less precise due to this. However, missing data is most likely randomly distributed throughout the lymphoma and control population making bias of these results less likely.

Information bias, among others, is a common limitation of case-control studies, and it is also true for this study. A large proportion of dogs in South Africa are bred by informal breeders and are not registered with the Kennel Union of Southern Africa (KUSA). This results in breeds being assigned to dogs by veterinarians, breeders and owners based on phenotypic characterisation of a breed, rather than by breed registration⁹⁶ and cause non-differential misclassification. The breed variable might therefore be subjected to information bias towards the null hypothesis as the breed being reported on submission forms by the private veterinarian might in fact not be genetically true. These self-assigned dog breeds might have more genetic heterogeneity than registered pure-bred dogs and might therefore decrease the heritability of disease. In other words, although the information regarding breed might be biased, it would have caused an underestimation of the true effect of breed on lymphoma development, therefore, the information bias in this study would not have caused a false significance.

The second limitation of the study is that the study population was selected from a commercial histopathology laboratory population. This group of dogs are most likely owned by higher income bracket households that can afford advanced veterinary care. It is estimated that more than half of the dogs in this country are either homeless or residing in poverty stricken rural communities and will have minimal representation in a commercial histopathology laboratory population^{83,84,96}. Furthermore, the characteristics of this homeless and rural dog population are different compared to dogs from affluent areas, which will have a significant impact on lymphoma development in these individuals: they have a younger mean population age, they are mostly unneutered animals and have a bigger gene heterogeneity as most are non-pure bred dogs^{85-87,89}. This, however, did 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 in the study

population and risk could therefore not be evaluated for these breeds. This is also relevant for future studies evaluating environmental factors associated with disease.

Additionally, using only one commercial laboratory's population situated in Gauteng, a small and highly urbanised province ⁹⁷, from where 59% of canine lymphoma biopsy submissions originated, skewed the data to be more representative of Gauteng than for the entire South Africa.

Because of these socioeconomic and demographic factors, it is evident that the study population is not a holistic representation of the entire South African dog population. 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 minimally biased.

Lastly, because of the minimal invasive sample taking and high accuracy, many lymphoma cases are readily diagnosed by cytology ⁵⁵. Although the criteria to only include histopathology confirmed canine lymphoma cases allowed for further architectural and immunophenotypic information being available, cases readily diagnosed by cytology were not included in the study's epidemiological data. This might further account for an underestimation of the prevalence of signalment and lymphoma characteristic in South Africa, in this study.

5.2 Epidemiological Factors Associated with Canine Lymphoma

5.2.1 Breed

Several studies have identified breed, age, and sex to significantly alter the risk of developing canine lymphoma ^{18,19,21-24,26,74}. In this study, although age and sex did not show significant associations, breed was identified as an overall risk factor for developing lymphoma. There was no statistically significant association identified for the most commonly presented breeds in this study, except for the Boerboel. This finding was unexpected as associations for multiple different breeds in South Africa have previously been identified ²¹.

The following breeds were identified as being overrepresented in the entire study population ($\geq 3\%$), and each breed was used as an individual breed variable in the univariate analysis (Table 4.5): Jack Russell Terrier, Labrador Retriever, Boerboel, Dachshund, German Shepherd Dog and Rottweiler. All the above-mentioned breeds, with no exception, comprised $\geq 3\%$, respectively, of the lymphoma group too (Table 4.4). Although most of these breeds have not shown an increased risk of developing lymphoma in this study, except for the Boerboel, they are breeds commonly associated with lymphoma in the literature and will be discussed below.

The Yorkshire Terrier, a breed overrepresented in the entire population, and control group ($\geq 3\%$) (Table 4.7), and shown to have a decreased risk of developing lymphoma in this study (Table 4.5), and the Maltese Poodle, a South African toy breed dog previously described as having a decreased risk for lymphoma ²¹, will also be discussed.



Figure 5.1: The South African Boerboel.

The Boerboel (Figure 5.1), a unique South African mastiff-type breed, has only recently been identified as having an increased risk of developing canine lymphoma ^{21,98}. Although there is limited literature about this breed's association with canine lymphoma, investigation into the Boerboel's ancestry demonstrates genetic susceptibility within the breed group of dogs that it belongs to.

The timeline of the Boerboel's breed development in South Africa is vague but can be traced back to 1652, when Dutch settlers introduced the Bullenbeisser (German Bulldog) upon arrival in the Cape. It has further been documented that the English Bulldog and the Bullmastiff was brought to South Africa, in 1820 and 1928, respectively, from England ⁹⁹. These breeds, introduced by European colonists,

were over time used to crossbreed with dogs of native South African inhabitants, mostly used for the protection of people, land and livestock, and formed the origin of the Boerboel breed ¹⁰⁰. 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 ^{99,100}.

Several studies have shown a clear breed association with the above-mentioned ancestry breeds of the Boerboel and lymphoma ^{21,22,25,101-104}. Some of the first reports of associations between the Molosser group of dog breeds and canine lymphoma date back to the 1980's. Priester & McKay published an extensive multispecies report of epidemiological information in veterinary oncology in North America in 1980. In this report 523 706 dogs were included of which 1452 dogs were diagnosed with lymphoma. The English Bulldog had a significant 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; $P < 0.001$) ¹⁰³. In a prospective study by Onions in 1984, 59 Bullmastiffs from 3 households were followed over a span of 3 years to determine the case distribution and incidence of lymphoma. In this study two major findings were made for this breed: firstly, that the incidence of lymphoma in Bullmastiffs are high (5000 per 100 000 dogs) and, secondly, that the cases followed a familial distribution ¹⁰⁴. Edwards et al. studied a large set of canine data from a United Kingdom insurance company to determine the incidence of canine lymphoma in pure bred dogs. Of the 130 684 dogs included in this study 103 had a diagnosis of lymphoma. The study population were relatively young (78% of the population were ≤ 5 years). The only 3 breeds that had significantly higher incidences were the Boxer ($P = 0.002$), Bullmastiff ($P = 0.002$) and English Bulldog ($P = 0.012$) ¹⁰¹. In South Africa the Boxer, English Bulldog, Bullmastiff and Mastiff were previously shown to be at an increased risk for developing lymphoma ($P < 0.05$) ²¹.

The Boerboel was not only the most commonly presented breed in the canine lymphoma population of this study, after mixed breeds, but was the only single breed with a statistically significant association with lymphoma (Table 4.5). This study confirms the findings of Van Rooyen et al. who first described this breed's

predisposition in the literature ²¹. The Boerboel has also been shown to have a predisposition for mast cell and spindle cell tumours in another study ⁹⁶.

The Jack Russell Terrier was the most common pure-bred breed represented in the entire study population with an OR of 0.7 for developing lymphoma, but with no significant association, corresponding to the findings of Van Rooyen et. al. Risk of developing lymphoma for this breed in other studies varies. A decreased risk was reported in the Australian study by Bennet et al. where 6201 cases of lymphoma were investigated for epidemiological associations. However, in studies in the United Kingdom, Jack Russell Terriers were reported to be commonly represented in the lymphoma population ^{18,105}. In addition, Jack Russell Terriers have been previously reported to develop rare hepatosplenic T-cell lymphoma ⁴¹ and high-grade B-cell lymphoma with plasmacytoid differentiation and secondary leukaemia ¹⁰⁶.

Labrador Retrievers have been identified as having both an increased and a decreased risk for developing canine lymphoma in different studies ^{18,103}. Although this breed has been previously identified as having increased odds (OR >1.8) for developing canine lymphoma in South Africa ²¹, our findings did not support that (OR = 0.63).

Dachshunds (standard and miniature combined), although overrepresented as a breed in both South African studies using the same commercial laboratory population (IDEXX Laboratory), did not have a statistically significant odds for lymphoma in either of these studies. Conflicting findings exist in the literature with regards to this breed's association with canine lymphoma. Several case reports have been published on individual cases of canine lymphoma in this breed. These include case reports describing lymphoma from various anatomical origins including cardiac ¹⁰⁷, cutaneous ^{108,109}, gallbladder ¹¹⁰, gastrointestinal ¹¹¹ and mediastinal lymphoma ¹¹². A recent large study in Japan, where 108 cases of lymphoma in the Miniature Dachshund were investigated, reported a predisposition for this type of neoplasia in the breed ¹¹³. A higher frequency of gastrointestinal lymphoma compared to other breeds and a higher prevalence in

younger dogs (<4 years) were further reported. This is, however, in contrast to large epidemiological studies in North America, Australia and Netherland where it was reported that Dachshunds have a lower risk of developing lymphoma^{18,22,29}. These contradictory findings in the literature might indicate a geographical factor, or genetic isolation in a certain location, contributing to the breed's predisposition to certain lymphomas on one continent, but having a low association with lymphoma on another continent.

Although not statistically significant, German Shepherd Dogs and Rottweilers had a slightly high OR (OR >1.3, CI encompassing 1.0) of developing canine lymphoma (Table 4.5). Both breeds have been reported as at-risk breeds for canine lymphoma in multiple other studies worldwide^{18,19,22-24,74,114,115}. In this study and previously in the study by Van Rooyen et al. neither of these breeds were identified as at-risk breeds in the country's dog population²¹.

This study found that Yorkshire Terriers have a decreased risk of developing lymphoma (Table 4.5). This phenomenon has previously been reported in the literature from Australia, and the United Kingdom^{18,101}. In South Africa, previously, Yorkshire Terriers had an OR of <1.00 but this was not statistically significant²¹. It is, however, interesting to note that several case studies have been published in Korea reporting various different types of lymphomas in Yorkshire Terriers, namely, epitheliotropic cutaneous¹¹⁶, splenic¹¹⁷, alimentary¹¹⁸ and abdominal lymphoma¹¹⁹. All the animals in these case studies were ≥ 7 years of which 1 were female and 3 were males.

Breeds that were previously identified in South Africa as having a significant predisposition to lymphoma, which were not found in this study, include (absolute numbers (n) of breeds from this study given in brackets): Belgian Shepherd (n = 0), Bull Terrier (n = 3), Border Collie (n = 2), Boxer (n = 4), English Bulldog (n = 3), Rhodesian Ridgeback (n = 4), Basset Hound (n = 2), Bullmastiff (n = 2), Mastiff (n = 0), Newfoundland (n = 0), Kerry Blue Terrier (n = 1) and Schipperke (n = 0)²¹. Not one of the following breeds were present in this study population: Belgian Shepherd, Mastiff, Newfoundland, and Schipperke. Of the remaining breeds that

were represented in this study, all had absolute numbers of ≤ 4 and therefore were not analysed as individual breeds as they did not comprise more than 3% of the study population.

The Maltese Poodle toy breed (Figure 5.2) was previously found to have a decrease risk of developing canine lymphoma which was not confirmed in this study²¹. The Maltese Poodle had an absolute number of 2 in the lymphoma group of this study and was included in



Figure 5.2: A typical picture of a dog phenotypically classified as a Maltese Poodle.

the FCI group 9 (Companion and toy breeds) with a total number of 10 dogs included in this group (Table 4.4). This FCI group had significantly decreased odds of developing lymphoma (Table 4.6). 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 study⁹⁶. 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 a KUSA registered breed.

The differences in breed associations with lymphoma found in this study when compared to Van Rooyen et al, is most likely attributed to the selection of this study's control population. This study used a relatively small control group (1:1 case-control ratio) consisting of breeds presented in the veterinary practice for a biopsy of some disease process, mostly neoplasia. The control group breeds are similar to breeds overrepresented for lymphoma and might be the reason for a lack of associations found when compared to Van Rooyen et al. where a large population of canine cases accessioned by the respective laboratories were used, not only biopsies. In another tumour survey study in South Africa, the overrepresented breeds found in

this study's lymphoma group was also commonly diagnosed with cutaneous neoplasms further supporting their high frequency in laboratory populations⁹⁶. Too little information about breed as a risk factor for lymphoma exists in South Africa and, therefore, larger, more robust studies need to be conducted to comment on possible differences in breed associations found in this study when compared to other studies worldwide.

5.2.2 Age

The median age of the lymphoma and control group was 8 years of age and although no statistically significant association between age group and lymphoma was found (Table 4.8), almost half of the population fell into the group of ≥ 5 years and < 10 years followed by a third being older than 10 years. The lack of association is likely caused by a large proportion (63%) of the control population also being diagnosed with neoplasia and neoplasia being associated with middle-aged to older dogs¹⁰³. This might be an indication that neoplasia is a disease of senior dogs, rather than lymphoma specifically.

The mean age of the lymphoma group in this study correlates with previous epidemiological studies conducted in Brazil^{23,24}, France⁷⁴, Germany²⁵ and South Africa²¹. Some studies report slightly younger mean ages compared to reports in the aforementioned studies (6.6 – 7.3 years)^{29,120}. Nonetheless, it is evident that canine lymphoma, as seen with many other neoplasms, is a disease of middle-aged to older dogs and this is also seen in South Africa¹. A study in the United Kingdom investigating a population of insured dogs, found that dogs with lymphoma had a young median population age of 2 years but showed an increasing incidence with age, peaking at 10 years¹⁰¹.

In the Boerboel canine lymphoma population of this study the median age was 6 years which was younger than the entire canine lymphoma population age. Ninety percent of the Boerboel canine lymphoma population was < 10 years. Most of the Bullmastiff lymphoma cases in the study by Van Rooyen et al. fell into the 4 to 6-year age group but no mention of the Boerboel ages were made²¹. No further research exists to compare these findings but when compared to existing literature on canine

lymphoma age in the ancestry breeds of the Boerboel a higher incidence was seen in younger age groups for the English Bulldog (≤ 3 years) and the Bullmastiff (≥ 4 and ≤ 6 years) ¹⁰¹.

5.2.3 Sex

The male to female ratio in this study was 1.2:1 showing a slightly higher occurrence in males than females but with no statistical significance. Neuter status was evaluated in both univariate (Table 4.8) and multivariable analysis (Table 4.6) and there was no significant risk association with lymphoma. This information is contradicted by Van Rooyen et al. who did find a statistically significant association for lymphoma in the South African male and neutered female population.

In a major epidemiological study in Australia, where 6201 cases of lymphoma were analysed, a significant association was seen with regards to sex and lymphoma. Males had a significantly increased risk, as well as neutered animals of both sexes when compared to intact animals ¹⁸. These findings are supported by various other studies ^{22,26}. Although the exact mechanism for these variations in sex and neutering status as a risk factor is mostly unknown, the interaction of progesterone/oestrogen receptors and hormones on gene expression has been theorised as a feasible explanation in human studies ^{22,121}. Furthermore, low testosterone levels have been reported to increase mortality in human cancers but whether testosterone levels alter the risk of cancer development remains questionable ¹²².

In contrast, several studies also exist that show no significant associations between sex, neutering status, and lymphoma ^{2,23-25,27-29}. These factors, therefore, remain controversial, but it is interesting to note that most studies that did not find a sex/neutering predilection had relatively small sample sizes compared to studies that did find significant associations.

A study by Keller et al. showed that sex was a significant survival or prognostic variable where neutered females had the longest survival time in contrast to intact females with the shortest survival ¹⁰². This finding was not supported by other studies investigating variables associated with lymphoma survival times ^{16,28,62,123}, however a

revision of the literature (1990-2002) reported that a more favourable outcome was seen in female, small breed dogs in stage I-IV, substage *a*, compared to male, large breed dogs in stage V, substage *b* ¹⁰.

In conclusion, epidemiological factors such as breed, age, sex, and neutering status has been shown to play varying roles in the development of lymphoma in studies worldwide. Breed and sex predisposition, especially, vary greatly between studies and demographic areas. The lack of breed and age associations in this study could be attributed to the control population also constituting breeds predisposed to other neoplasias, developing in older dogs, commonly biopsied for histopathological diagnosis. Despite the lack of expected breed associations, this is the second study in South Africa to conclude the Boerboel breed as having a significantly increased risk of developing lymphoma. Although lymphoma proved to be a disease of middle-aged to older animals in South Africa, this study may indicate an earlier onset of disease in the Boerboel.

5.3 South African Canine Lymphoma Characteristics

5.3.1 Grade

In a large study in North America investigating various variables associated with survival time, 992 biopsy samples with a clinical diagnosis of canine lymphoma were included and grade was determined using the WHO classification system ¹⁶. Although the WHO classification scheme states that grade must be determined using the mitotic count per single HPF, it became evident later that the abovementioned study by Valli et al. determined grade as the mitotic count average over 10 HPFs ⁶⁵. Of the cases included in the above-mentioned study 51% were high grade, 32% low grade, 12% intermediate grade, and 5% were benign hyperplasia or a benign neoplasm (thymoma) ¹⁶. These findings differ substantially from the findings in this study where 43% cases were intermediate grade, 30% cases were assigned a low grade and 27% a high grade (Table 4.10). This appears to be a notable difference in tumour malignancy which may indicate a geographical risk factor being present in the American population. Another reason might be an inconsistent grading method used by different pathologists in South Africa.

Although all cases were retrospectively reviewed by the primary author in terms of grade, the initial grading on histopathology were inconsistently reported with regards to mitotic count per single HPF or as an average over various HPFs, which can influence the reported mitotic count substantially.

According to the author's knowledge consensus information in veterinary science on the number of fields to be counted to determine the lymphoma grade, using the WHO classification system, are not available and cause great inter-pathologist variation. As mentioned, according to the WHO classification scheme mitotic count per single high power is used to determine grade but pathologists might argue that an average over 10 HPFs increases the accuracy of the lymphoma grade. The latter method has been applied to two large cohort studies by Valli et al. ^{15,16,65}.

5.3.2 Origin

Information regarding clinical presentation of canine lymphoma in South Africa, and similarities with the findings of previous literature published in other parts of the world, has not previously been presented. According to this study, the disease demonstrates a similar clinical presentation in South Africa. Haemopoietic tissue was the most common tissue of origin in the study accounting for 75% of lymphoma cases (Table 4.10). The most frequent biopsy submissions were those obtained from lymph nodes (67%) i.e., multicentric lymphoma. This was followed by lymphoma of the skin (14%). These findings are similar to a study conducted in the United Kingdom ¹²⁴. Lymphoma of the liver, spleen, mucosa, submucosa, gastrointestinal tract, mediastinum, kidneys, tonsils, and brain remain rare in dogs in South Africa, similar as reported in other parts of the world ^{2,3}.

Clinical presentation of Boerboel cases followed the same trend as the overall population, with 78% being from haemopoietic origin and 23% of cases from the skin or subcutaneous tissue. Further studies are warranted to investigate the specific type of lymphoma predisposition in this breed. With the results from this study the conclusion can be made that this breed follows the greater South African population trend and develops both multicentric and cutaneous lymphoma proportionally to what is seen in the rest of the population.

5.3.3 Immunophenotype

This study found a higher B-cell prevalence in the South Africa canine lymphoma population (69%) with a concurrent lower T-cell prevalence (29%) (Table 4.10). All Boerboel biopsies that were immunophenotyped yielded a B-cell origin, and although case numbers were too small ($n = 4$) to make statistical inferences, this warrants investigation for a B-cell predilection in this breed in future studies.

In another study in North America that investigated B- and T-cell prevalence in 1263 dogs, B-cell immunophenotype had a prevalence of 61% and T-cell 39%⁷². Similar prevalences were seen in studies in Brazil (B-cell 62.5%, T-cell 37.5%)²³, Germany (B-cell 79.4%, T-cell 20.6%)²⁵, Italy (B-cell 68.2%, T-cell 31.8%)⁴⁵, France (B-cell 63.8%, T-cell 35.4%)⁷⁴ and in a European study (B-cell 65.7%, T-cell 34.3%)¹⁹.

It is interesting to note that an equal 50% prevalence for B- and T-cell lymphoma was seen in the canine lymphoma population in Bangkok¹²⁵. The author found limited research about immunophenotype of canine lymphoma in the eastern world and this study by Rungsipat might indicate a different picture than what is seen in the western world. A higher incidence for T-cell NHL has been documented in the human population in Japan and has been ascribed to the high incidence and endemic nature of human T-cell lymphotropic virus type-1¹²⁶. However, should the same trend of a higher T-cell immunophenotype prevalence be seen in the canine population, other factors than viral aetiology should be investigated in these countries based on the canine model.

Whether the Boerboel has a predilection for B-cell lymphoma remains uncertain at this stage, but it is noteworthy that the Boxer, also part of the Molosser group, has a T-cell predilection, as previously mentioned^{19,27,72,74,76}. This finding should be further investigated in a larger cohort of Boerboels with lymphoma.

5.3.4 WHO Classification Subtype

In the North American study by Valli et al. 285 canine lymphoma biopsy samples and 15 duplicates were reviewed by 20 pathologists to evaluate the consistency and accuracy of applying the WHO subtype classification system for canine lymphoma

¹⁵. In this study 248/300 of biopsies could be classified into 6 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. When adjusting these results to look at prevalence of WHO classification subtypes within the North American WHO classified lymphoma group and comparing this to the WHO classification subtype prevalence within the South African WHO classified lymphoma group (Table 4.10), there are large differences between B- and T-cell, and WHO classification subtype prevalence between the two studies (prevalence presented for the North American vs. this South African study, respectively): 58% (145/248) vs. 75% (66/88) DLBCL; 17% (42/248) vs. 7% (6/88) PTCL-NOS; 15% (38/248) vs. 6% (5/88) TZL; 5% (12/248) vs. 2% (2/88) T-LBL and 4% (11/248) vs. 7% (6/88) MZL. A larger percentage of biopsies were diagnosed to be DLBCL, and a much smaller prevalence was seen for PTCL-NOS and TZL in South Africa. Whether these findings are true reflections and/or significant cannot be extrapolated as larger prospective studies in South Africa need to be conducted to confirm them.

No comparisons can be made with regards to the Boerboel that had a consistent diagnosis of DLBCL in the 4 cases that were subtyped according to the WHO classification system as no literature exists in this regard, according to the author's knowledge.

Based on a recent publication three cases were diagnosed during the review as DSBCL, a separate entity not included in the WHO subtype classification ¹⁷. In this study by Hughes et al. DSBCL typically was graded as intermediate or high, however 2/3 cases were graded as low and the remaining one as intermediate in this study. The median age reported for DSBCL was 11 years (range: 7 - 14) which was slightly younger in this study (8 years, range: 4 - 9) ¹⁷. 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 missed and diagnosed under other existing classification groups.

5.3.5 Geographical Location

KwaZulu-Natal had lower numbers of intermediate grade and slightly higher numbers of high-grade lymphoma compared to other provinces and the entire canine lymphoma population of the study (Table 4.10). Lower number of B-cell lymphomas were seen, especially DLBCL, and higher number of T-cell lymphomas, especially PTCL-NOS. This may indicate a higher probability for dogs in KwaZulu-Natal to develop 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 when compared to Gauteng and the Western Cape ⁷⁴.

Limitations to further interpret these results include the lack of information regarding the exact location of cases within the province i.e., their proximity to the supposed risk factors are unknown and the low case number (37 of which only 12 were immunophenotyped) from this province makes interpretation of these results precarious and most likely insignificant.

Furthermore, geographical data is skewed by the location of the IDEXX Laboratory in the Gauteng province. Although courier services throughout South Africa routinely deliver samples to the IDEXX Laboratory, the turnaround time for private practitioners and cost for owners increase due to travel time and distance. Other private laboratories in closer proximity to or within the respective province other than Gauteng will regularly be preferred for convenience and shorter time from sample submission to results output.

5.4 Canine Lymphoma and NHL in Humans in South Africa

Limited data about NHL in South Africa exists and sustainable data keeping in regional and national cancer registries are improving but lacking, especially in rural areas ¹²⁷.

Despite human literature reporting a lower incidence for women to develop NHL ¹²⁸, an increased risk for developing NHL has been detected in South African

women. This finding is contrary to European studies ¹²⁹. Female dogs were not overrepresented in the canine lymphoma population i.e., not following the trend seen in the human female population. A significantly higher proportion of neutered females were found to be associated with canine lymphoma in another South African study, but this was also true for all males ²¹.

Canine lymphoma shows similarity with NHL in South Africa in that it affects middle-aged individuals for each species (median age for humans = 41 years). A major risk factor for the development of lymphoma in the human population in South Africa is infection with Human Immunodeficiency Virus (HIV), which has a high prevalence (up to 62%) in the NHL population in South Africa. The median age of HIV-infected individuals diagnosed with lymphoma in South Africa is 36 years of age. This is a confounding factor which decreases the age of lymphoma disease detection in humans in South Africa ¹³⁰.

The most common type of NHL in South African humans is DLBCL with a prevalence of 21% in the total study population ¹²⁷ compared to 19% in the canine study population. A higher prevalence for high grade lymphomas were seen in the Southern African (South Africa and Zimbabwe) human population when compared to North America and Western Europe ⁹¹ On the other hand, in the canine population a lower prevalence for high grade lymphomas was seen in South Africa when compared to a North American canine study ¹⁶. Furthermore, studies have documented a low prevalence for T-cell lymphoma in South African humans, in fact as low as 3% in one study (including Hodgkin lymphoma) ¹²⁷. These findings are not similar to the South African canine population and are most likely due to the viral aetiology of HIV in humans, especially in South Africa, predisposing them to more aggressive B-cell NHL ¹³⁰.

In summary, the canine population of South Africa did not show a significantly different presentation in disease epidemiology or characteristics when compared to canine populations in other parts of the western world but the same cannot be said about comparison with NHL in the South African human population when compared to the developed western world. The main reason for this is the major

influence and endemic nature of HIV in South Africa, and its aetiological role in the disease development of NHL in humans. This, and the socioeconomic cause-and-effect of HIV and NHL in humans, makes the use of the canine lymphoma model difficult in the South African setting, as the same aetiology of disease development and the socioeconomics of disease detection, monitoring and treatment cannot be appreciated in the canine population.

5.5 Conclusion

This study confirmed a breed predisposition for canine lymphoma in the Boerboel, a South African mastiff-type dog, as previously reported. Males were slightly overrepresented together with middle-aged to older dogs, however no statistical associations were found. The lack of association between age, sex, and other breeds, and canine lymphoma, was unexpected.

The study results suggest a possible predilection for B-cell lymphoma in the Boerboel, especially DLBCL, with disease onset potentially being younger in this breed. Larger prospective studies investigating associations between the Boerboel signalment and histopathological characteristics need to be conducted to establish whether a predilection to developing certain subtypes exists.

The frequency of immunophenotypes were similar to findings in other 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. Further South African studies are needed to confirm whether this is a true geographical difference and consensus on lymphoma grading in veterinary medicine needs to be reached for further uniformity in lymphoma classification worldwide and to accurately compare different study results.

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

Lastly, using canine lymphoma in South Africa as a model for human NHL is not appropriate due to HIV playing a major role in the disease development in humans which cannot be appreciated in dogs.

REFERENCES

1. Zandvliet M. Canine lymphoma: a review. *Vet Q.* 2016;36(2):76-104. doi:10.1080/01652176.2016.1152633
2. Ponce F, Marchal T, Magnol JP, et al. A morphological study of 608 cases of canine malignant lymphoma in France with a focus on comparative similarities between canine and human lymphoma morphology. *Vet Pathol.* 2010;47(3):414-433. doi:10.1177/0300985810363902
3. Vezzali E, Parodi AL, Marcato PS, Bettini G. Histopathologic classification of 171 cases of canine and feline non-Hodgkin lymphoma according to the WHO. *Vet Comp Oncol.* 2010;8(1):38-49. doi:10.1111/j.1476-5829.2009.00201.x
4. Owen LN, World Health Organization. Veterinary Public Health Unit & WHO Collaborating Center for Comparative Oncology. TNM classification of tumours in domestic animals/ edited by L.N. Owen. Geneva: World Health Organization; 1980.
5. Ito D, Frantz AM, Modiano JF. Canine lymphoma as a comparative model for human non-Hodgkin lymphoma: recent progress and applications. *Vet Immunol Immunopathol.* 2014;159(3-4):192-201. doi:10.1016/j.vetimm.2014.02.016
6. Richards KL, Suter SE. Man's best friend: what can pet dogs teach us about non-Hodgkin's lymphoma? *Immunol Rev.* 2015;263(1):173-191. doi:10.1111/imr.12238
7. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. *Cancer.* 1982;49(10):2112-2135. doi:10.1002/1097-0142(19820515)49:10<2112::AID-CNCR2820491024>3.0.CO;2-2
8. Lennert K, Feller AC. *Histopathology of non-Hodgkin Lymphomas : (based on the updated Kiel classification)*. 2nd ed. Springer-Verlag; 1992.
9. Lukes RJ, Collins RD. Immunologic characterization of human malignant lymphomas. *Cancer.* 1974;34 Suppl 4:1488-1503. doi:10.1002/1097-0142(197410)34:8+<1488::AID-CNCR2820340822>3.0.CO;2-C
10. Stansfeld AG, Diebold J, Kapanci Y, et al. Updated Kiel classification for lymphomas [published correction appears in *Lancet* 1988 Feb 13;1(8581):372]. *Lancet.* 1988;1(8580):292-293. doi:10.1016/S0140-6736(88)90367-4

11. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. *Blood*. 1997;89(11):3909-18.
12. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84(5):1361-1392.
13. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of neoplastic diseases of the haematopoietic and lymphoid tissues: Report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Histopathology*. 2000;36(1):69-86. doi:10.1046/j.1365-2559.2000.00895.x
14. Teske E, Heerde P. Diagnostic value and reproducibility of fine-needle aspiration cytology in canine malignant lymphoma. *Vet Q*. 10/01 1996;18(3):112-115. doi:10.1080/01652176.1996.9694630
15. Valli VE, San Myint M, Barthel A, et al. Classification of canine malignant lymphomas according to the World Health Organization criteria. *Vet Pathol*. 2011;48(1):198-211. doi:10.1177/0300985810379428
16. Valli VE, Kass PH, San Myint M, Scott F. Canine lymphomas: association of classification type, disease stage, tumor subtype, mitotic rate, and treatment with survival. *Vet Pathol*. 2013;50(5):738-748. doi:10.1177/0300985813478210
17. Hughes KL, Ehrhart EJ, Rout ED, et al. Diffuse small B-cell lymphoma: a high-grade malignancy. *Vet Pathol*. 2021;58(5):912-922. doi:10.1177/0300985820985221
18. Bennett PF, Taylor R, Williamson P. Demographic risk factors for lymphoma in Australian dogs: 6201 cases. *J Vet Intern Med*. 2018;32(6):2054-2060. doi:10.1111/jvim.15306
19. Comazzi S, Marelli S, Cozzi M, et al. Breed-associated risks for developing canine lymphoma differ among countries: an European canine lymphoma network study. *BMC Vet Res*. 2018;14(1):232. doi:10.1186/s12917-018-1557-2
20. Jark PC, Fracacio CP, Anai LA, et al. Caracterização histopatológica e imunofenotípica do linfoma multicêntrico canino no Brasil: um estudo de 203 casos [Histopathological and immunophenotypical characterization of canine multicentric lymphoma in Brazil: a study of 203 cases]. *Arq Bras Med Vet Zootec*. 2020;72(3):787-793. doi:10.1590/1678-4162-11484
21. Van Rooyen LJ, Hooijberg E, Reyers F. Breed prevalence of canine lymphoma in South Africa. *J S Afr Vet Assoc*. 2018;89(1):1-11. doi:10.4102/jsava.v89i0.1530

22. Villamil AJ, Henry CJ, Hahn AW, Bryan JN, Tyler JW, Caldwell CW. Hormonal and sex impact on the epidemiology of canine lymphoma. *J Cancer Epidemiol*. 2010;2009:591753. doi:10.1155/2009/591753
23. Coelho ACB, Echenique JVZ, Lemos BS, et al. Linfomas caninos diagnosticados na região Sul do Rio Grande do Sul de 2000 a 2017: epidemiologia e imunofenótipo [Canine lymphomas diagnosed in southern Brazil from 2000 to 2017: epidemiology and immunophenotype]. *Pesq Vet Bras*. 2019;39(7):492-498. doi:10.1590/1678-5150-pvb-6027
24. Cunha FM, Silveira LMG, Xavier JG, Allegretti L. Linfoma multicêntrico em *Canis familiaris* (cão doméstico): estudo retrospectivo de 60 casos, entre agosto de 2009 e dezembro de 2010, no Município de São Paulo-SP [Multicentric lymphoma in *Canis Familiaris* (domestic dog): retrospective study of 60 cases between August 2009 and December 2010 in São Paulo-SP]. *J Health Sci Inst*. 2011/12 2011;29(4):209-301.
25. Ernst T, Kessler M, Lautscham E, Willimzig L, Neiger R. Das multizentrische Lymphom bei 411 Hunden - eine epidemiologische Studie [Multicentric lymphoma in 411 dogs - an epidemiological study]. *Tierarztl Prax Ausg K Kleintiere Heimtiere*. 2016;44(4):245-251. doi:10.15654/TPK-150338
26. Grüntzig K, Graf R, Boo G, et al. Swiss Canine Cancer Registry 1955-2008: Occurrence of the most common tumour diagnoses and influence of age, breed, body size, sex and neutering status on tumour development. *J Comp Pathol*. 2016;155(2-3):156-170. doi:10.1016/j.jcpa.2016.05.011
27. Fournel-Fleury C, Ponce F, Felman P, et al. Canine T-cell lymphomas: a morphological, immunological, and clinical study of 46 new cases. *Vet Pathol*. 2002;39(1):92-109. doi:10.1354/vp.39-1-92
28. Greenlee PG, Filippa DA, Quimby FW, et al. Lymphomas in dogs. A morphologic, immunologic, and clinical study. *Cancer*. 1990;66(3):480-490. doi:10.1002/1097-0142(19900801)66:3<480::aid-cnrcr2820660314>3.0.co;2-x
29. Teske E. Canine malignant lymphoma: a review and comparison with human non-Hodgkin's lymphoma. *Vet Q*. 1994;16(4):209-219. doi:10.1080/01652176.1994.9694451
30. Dorn CR, Taylor DO, Hibbard HH. Epizootiologic characteristics of canine and feline leukemia and lymphoma. *Am J Vet Res*. 1967;28(125):993-1001.
31. Moore PF, Affolter VK, Graham PS, Hirt B. Canine epitheliotropic cutaneous T-cell lymphoma: an investigation of T-cell receptor immunophenotype, lesion topography and molecular clonality. *Vet Dermatol*. 2009;20(5-6):569-576. doi:10.1111/j.1365-3164.2009.00814.x

32. Couto CG, Rutgers HC, Sherding RG, Rojko J. Gastrointestinal lymphoma in 20 dogs. A retrospective study. *J Vet Intern Med.* 1989;3(2):73-78. doi:10.1111/j.1939-1676.1989.tb03082.x
33. Coyle KA, Steinberg H. Characterization of lymphocytes in canine gastrointestinal lymphoma. *Vet Pathol.* 2004;41(2):141-146. doi:10.1354/vp.41-2-141
34. Vail DM, Young KM. Hematopoietic tumors. *Withrow & MacEwen's Small Animal Clinical Oncology.* 4th ed. W B Saunders; 2007:699-733.
35. Stefanello D, Valenti P, Zini E, et al. Splenic marginal zone lymphoma in 5 dogs (2001-2008). *J Vet Intern Med.* 2011;25(1):90-93. doi:10.1111/j.1939-1676.2010.0639.x
36. Valli VE, Vernau W, de Lorimier LP, Graham PS, Moore PF. Canine indolent nodular lymphoma. *Vet Pathol.* 2006;43(3):241-256. doi:10.1354/vp.43-3-241
37. Cozzi M, Marconato L, Martini V, et al. Canine nodal marginal zone lymphoma: Descriptive insight into the biological behaviour. *Vet Comp Oncol.* 2018;16(2):246-252. doi:10.1111/vco.12374
38. O'Brien D, Moore PF, Vernau W, et al. Clinical characteristics and outcome in dogs with splenic marginal zone lymphoma. *J Vet Intern Med.* 2013;27(4):949-954. doi:10.1111/jvim.12116
39. van Stee LL, Boston SE, Singh A, Romanelli G, Rubio-Guzman A, Scase TJ. Outcome and prognostic factors for canine splenic lymphoma treated by splenectomy (1995-2011). *Vet Surg.* 2015;44(8):976-982. doi:10.1111/vsu.12405
40. Fry MM, Vernau W, Pesavento PA, Brömel C, Moore PF. Hepatosplenic lymphoma in a dog. *Vet Pathol.* 2003;40(5):556-562. doi:10.1354/vp.40-5-556
41. Cienava EA, Barnhart KF, Brown R, Mansell J, Dunstan R, Credille K. Morphologic, immunohistochemical, and molecular characterization of hepatosplenic T-cell lymphoma in a dog. *Vet Clin Pathol.* 2004;33(2):105-110. doi:10.1111/j.1939-165X.2004.tb00357.x
42. Keller SM, Vernau W, Hodges J, et al. Hepatosplenic and hepatocytotropic T-cell lymphoma: two distinct types of T-cell lymphoma in dogs. *Vet Pathol.* 2013;50(2):281-290. doi:10.1177/0300985812451625
43. Geyer NE, Reichle JK, Valdés-Martínez A, et al. Radiographic appearance of confirmed pulmonary lymphoma in cats and dogs. *Vet Radiol Ultrasound.* 2010;51(4):386-390. doi:10.1111/j.1740-8261.2010.01683.x

44. Hawkins EC, Morrison WB, DeNicola DB, Blevins WE. Cytologic analysis of bronchoalveolar lavage fluid from 47 dogs with multicentric malignant lymphoma. *J Am Vet Med Assoc.* 1993;203(10):1418-1425.
45. Gavazza A, Lubas G, Valori E, Gugliucci B. Retrospective survey of malignant lymphoma cases in the dog: clinical, therapeutical and prognostic features. *Vet Res Commun.* 2008;32 Suppl 1:S291-S293. doi:10.1007/s11259-008-9131-1
46. Grindem CB, Breitschwerdt EB, Corbett WT, Page RL, Jans HE. Thrombocytopenia associated with neoplasia in dogs. *J Vet Intern Med.* 1994;8(6):400-405. doi:10.1111/j.1939-1676.1994.tb03258.x
47. Messinger JS, Windham WR, Ward CR. Ionized hypercalcemia in dogs: a retrospective study of 109 cases (1998-2003). *J Vet Intern Med.* 2009;23(3):514-519. doi:10.1111/j.1939-1676.2009.0288.x
48. Ruslander DA, Gebhard DH, Tompkins MB, Grindem CB, Page RL. Immunophenotypic characterization of canine lymphoproliferative disorders. *In Vivo.* 1997;11(2):169-172.
49. Di Bella A, Maurella C, Cauvin A, Schmidt JM, Tapia BB, North SM. Proteinuria in canine patients with lymphoma. *J Small Anim Pract.* 2013;54(1):28-32. doi:10.1111/jsap.12004.x
50. Aubry OA, Spangler EA, Schleis SE, Smith AN. Evaluation of bone marrow aspirates from multiple sites for staging of canine lymphoma and mast cell tumours. *Vet Comp Oncol.* 2014;12(1):58-66. doi:10.1111/j.1476-5829.2012.00331.x
51. Raskin RE, Krehbiel JD. Prevalence of leukemic blood and bone marrow in dogs with multicentric lymphoma. *J Am Vet Med Assoc.* 1989;194(10):1427-1429.
52. Blackwood L, Sullivan M, Lawson H. Radiographic abnormalities in canine multicentric lymphoma: a review of 84 cases. *J Small Anim Pract.* 1997;38(2):62-69. doi:10.1111/j.1748-5827.1997.tb02989.x
53. Crabtree AC, Spangler EA, Beard D, Smith AN. Diagnostic accuracy of gray-scale ultrasonography for the detection of hepatic and splenic lymphoma in dogs. *Vet Radiol Ultrasound.* 2010;51(6):661-664. doi:10.1111/j.1740-8261.2010.01725.x
54. Yoon JH, Feeney DA, Cronk DE, Anderson KL, Ziegler LE. Computed tomographic evaluation of canine and feline mediastinal masses in 14 patients. *Vet Radiol Ultrasound.* 2004;45(6):542-546. doi:10.1111/j.1740-8261.2004.04093.x

55. Martini V, Marano G, Aresu L, et al. Performance of lymph node cytopathology in diagnosis and characterization of lymphoma in dogs. *J Vet Intern Med.* 2022;36(1):204-214. doi:10.1111/jvim.16326
56. Sözmen M, Tasca S, Carli E, De Lorenzi D, Furlanello T, Caldin M. Use of fine needle aspirates and flow cytometry for the diagnosis, classification, and immunophenotyping of canine lymphomas. *J Vet Diagn Invest.* 2005;17(4):323-330. doi:10.1177/104063870501700404
57. Comazzi S, Gelain ME. Use of flow cytometric immunophenotyping to refine the cytological diagnosis of canine lymphoma. *Vet J.* 2011;188(2):149-155. doi:10.1016/j.tvjl.2010.03.011
58. Thalheim L, Williams LE, Borst LB, Fogle JE, Suter SE. Lymphoma immunophenotype of dogs determined by immunohistochemistry, flow cytometry, and polymerase chain reaction for antigen receptor rearrangements. *J Vet Intern Med.* 2013;27(6):1509-1516. doi:10.1111/jvim.12185
59. Lana SE, Jackson TL, Burnett RC, Morley PS, Avery AC. Utility of polymerase chain reaction for analysis of antigen receptor rearrangement in staging and predicting prognosis in dogs with lymphoma. *J Vet Intern Med.* 2006;20(2):329-334. doi:10.1111/j.1939-1676.2006.tb02864.x
60. Burnett RC, Vernau W, Modiano JF, Olver CS, Moore PF, Avery AC. Diagnosis of canine lymphoid neoplasia using clonal rearrangements of antigen receptor genes. *Vet Pathol.* 2003;40(1):32-41. doi:10.1354/vp.40-1-32
61. Flory AB, Rassnick KM, Stokol T, Scrivani PV, Erb HN. Stage migration in dogs with lymphoma. *J Vet Intern Med.* 2007;21(5):1041-1047. doi:10.1111/j.1939-1676.2007.tb03062.x
62. Dobson JM, Blackwood LB, McInnes EF, et al. Prognostic variables in canine multicentric lymphosarcoma. *J Small Anim Pract.* 2001;42(8):377-84. doi:10.1111/j.1748-5827.2001.tb02485.x
63. Rosenberg MP, Matus RE, Patnaik AK. Prognostic factors in dogs with lymphoma and associated hypercalcemia. *J Vet Intern Med.* 1991;5(5):268-271. doi:10.1111/j.1939-1676.1991.tb03133.x
64. Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. *J Clin Oncol.* Dec 1999;17(12):3835-3849. doi:10.1200/jco.1999.17.12.3835
65. Avallone G, Rasotto R, Chambers JK, et al. Review of histological grading systems in veterinary medicine. *Vet Pathol.* 2021;58(5):809-828. doi:10.1177/0300985821999831

66. Seelig DM, Avery AC, Ehrhart EJ, Linden MA. The comparative diagnostic features of canine and human lymphoma. *Vet Sci*. 2016;3(2):11. doi:10.3390/vetsci3020011
67. Baars JW, de Jong D, Willems EM, et al. Diffuse large B-cell non-Hodgkin lymphomas: the clinical relevance of histological subclassification. *Br J Cancer*. 1999;79(11-12):1770-1776. doi:10.1038/sj.bjc.6690282
68. Engelhard M, Brittinger G, Huhn D, et al. Subclassification of diffuse large B-cell lymphomas according to the Kiel classification: distinction of centroblastic and immunoblastic lymphomas is a significant prognostic risk factor. *Blood*. 1997;89(7):2291-2297.
69. Ponce F, Magnol JP, Marchal T, et al. High-grade canine T-cell lymphoma/leukemia with plasmacytoid morphology: a clinical pathological study of nine cases. *J Vet Diagn Invest*. 2003;15(4):330-337. doi:10.1177/104063870301500405
70. Fournel-Fleury C, Magnol JP, Bricaire P, et al. Cytohistological and immunological classification of canine malignant lymphomas: comparison with human non-Hodgkin's lymphomas. *J Comp Pathol*. 1997;117(1):35-59. doi:10.1016/s0021-9975(97)80065-5
71. Comazzi S, Avery PR, Garden OA, Riondato F, Rütgen B, Vernau W. European canine lymphoma network consensus recommendations for reporting flow cytometry in canine hematopoietic neoplasms. *Cytometry B Clin Cytom*. 2017;92(5):411-419. doi:10.1002/cyto.b.21382
72. Modiano JF, Breen M, Burnett RC, et al. Distinct B-cell and T-cell lymphoproliferative disease prevalence among dog breeds indicates heritable risk. *Cancer Res*. 2005;65(13):5654-5661.
73. Neuwald EB, Teixeira LB, Conrado FO, da Silva MOD, Hlavac NRC, González FHD. Aspectos epidemiológicos, clínicos e imuno-histoquímicos do linfoma canino na região de Porto Alegre [Epidemiological, clinical and immunohistochemical aspects of canine lymphoma in the region of Porto Alegre, Brazil]. *Pesq Vet Bras*. 2014;34(4):349-354. doi:10.1590/S0100-736X2014000400009
74. Pastor M, Chalvet-Monfray K, Marchal T, et al. Genetic and environmental risk indicators in canine non-Hodgkin's lymphomas: breed associations and geographic distribution of 608 cases diagnosed throughout France over 1 year. *J Vet Intern Med*. 2009;23(2):301-310. doi:10.1111/j.1939-1676.2008.0255.x
75. Parker HG, Kim LV, Sutter NB, et al. Genetic structure of the purebred domestic dog. *Science*. 2004;304(5674):1160-1164. doi:10.1126/science.1097406
76. Lurie DM, Lucroy MD, Griffey SM, Simonson E, Madewell BR. T-cell-derived malignant lymphoma in the boxer breed. *Vet Comp Oncol*. 2004;2(3):171-175. doi:10.1111/j.1476-5810.2004.00047.x

77. Lobetti RG. Lymphoma in 3 related Rottweilers from a single household. *J S Afr Vet Assoc.* 2009;80(2):103-105. doi:10.4102/jsava.v80i2.180
78. Fosgate GT, Cohen ND. Epidemiological study design and the advancement of equine health. *Equine Vet J.* 2008;40(7):693-700. doi:10.2746/042516408X363323
79. Cohen ND. Equine epidemiology: counting for something in equine practice. 2011:85-98.
80. Evidence-Based Medicine Working Group. Evidence-based medicine. A new approach to teaching the practice of medicine. *JAMA.* 1992;268(17):2420-2425. doi:10.1001/jama.1992.03490170092032
81. Straus SE, Glasziou P, Richardson WS, Haynes RB. *Evidence-based medicine : how to practice and teach EBM.* Fifth edition. ed. Elsevier; 2019.
82. Rodrigues L, Kirkwood BR. Case-control designs in the study of common diseases: updates on the demise of the rare disease assumption and the choice of sampling scheme for controls. *Int J Epidemiol.* 1990;19(1):205-213. doi:10.1093/ije/19.1.205
83. Stats SA. Sustainable Development Goals (SDGs): Indicator Baseline Report. *Statistics South Africa.* 2017;
84. Mars Petcare. State of pet homelessness index. Accessed 09 October, 2022. <https://endpethomelessness.com/the-index/>
85. Rautenbach GH, Boomker J, de Villiers IL. A descriptive study of the canine population in a rural town in southern Africa. *J S Afr Vet Assoc.* 1991;62(4):158-162. doi:10.4102/jsava.v62i4.1778
86. Rautenbach GH, Booth C, Höhn EW. A comparison of health parameters in two different canine populations. Part 1: Haematological data. *J S Afr Vet Assoc.* 1987;58(4):179-182.
87. Rautenbach GH, Joubert HF. A comparison of health parameters in two different canine populations. Part II: Chemical pathology data. *J S Afr Vet Assoc.* 1988;59(3):135-138.
88. Greyling LM, Grobler PJ, Van der Bank HF, Kotze A. Genetic characterisation of a domestic dog *Canis familiaris* breed endemic to South African rural areas. *Acta Theriol.* 2004;49:369-382. doi:10.1007/BF03192535
89. Hergert M, Le Roux K, Nel LH. Characteristics of owned dogs in rabies endemic KwaZulu-Natal province, South Africa. *BMC Vet Res.* 2018;14(1):278. doi:10.1186/s12917-018-1604-z

90. Miranda-Filho A, Piñeros M, Znaor A, Marcos-Gragera R, Steliarova-Foucher E, Bray F. Global patterns and trends in the incidence of non-Hodgkin lymphoma. *Cancer Causes Control*. 2019;30(5):489-499. doi:10.1007/s10552-019-01155-5
91. Perry AM, Perner Y, Diebold J, et al. Non-Hodgkin lymphoma in Southern Africa: review of 487 cases from The International Non-Hodgkin Lymphoma Classification Project. *Br J Haematol*. 2016;172(5):716-723. doi:10.1111/bjh.13885
92. Owen LN, Bostock DE, Betton GR, et al. The role of spontaneous canine tumours in the evaluation of the aetiology and therapy of human cancer. *J Small Anim Pract*. 1975;16(3):155-162. doi:10.1111/j.1748-5827.1975.tb05729.x
93. Richards KL, Motsinger-Reif AA, Chen H-W, et al. Gene profiling of canine B-cell lymphoma reveals germinal center and postgerminal center subtypes with different survival times, modeling human DLBCL. *Cancer research*. 2013;73(16):5029-39. doi:10.1158/0008-5472.CAN-12-3546
94. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med*. 2001;194(12):1861-1874. doi:10.1084/jem.194.12.1861
95. Gaurnier-Hausser A, Patel R, Baldwin AS, May MJ, Mason NJ. NEMO-binding domain peptide inhibits constitutive NF- κ B activity and reduces tumor burden in a canine model of relapsed, refractory diffuse large B-cell lymphoma. *Clin Cancer Res*. 2011;17(14):4661-4671. doi:10.1158/1078-0432.CCR-10-3310
96. Tompkins S, Fosgate GT, Williams J, Clift S. Breed and anatomical predisposition for canine cutaneous neoplasia in South Africa during 2013. *Vet Rec*. 2020;186(7):218. doi:10.1136/vr.105277
97. Kok P, Collinson M. *Migration and urbanization in South Africa*. 2006.
98. Emikpe BO, Omobowale TO, Jarikre TA, Otuh PI, Oyebanji VO, Antia RE. High grade lymphoma in a six-year old Boerboel: A case report. *Bulg J Vet Med*. 2016;21(2):242-249. doi:10.15547/bjym.1021
99. Grabe B. *Die boerboele van Suidelike-Afrika*. Suid-Afrikaanse Boerboeltelersvereniging; 1995.
100. Pretorius A. History of the Boerboel. Accessed 26 July, 2022. <https://sabbs.org/the-boerboel/history>

101. Edwards DS, Henley WE, Harding EF, Dobson JM, Wood JLN. Breed incidence of lymphoma in a UK population of insured dogs. *Vet Comp Oncol*. 2003;1(4):200-206. doi:10.1111/j.1476-5810.2003.00025.x
102. Keller ET, MacEwen EG, Rosenthal RC, Helfand SC, Fox LE. Evaluation of prognostic factors and sequential combination chemotherapy with doxorubicin for canine lymphoma. *J Vet Intern Med*. 1993;7(5):289-295. doi:10.1111/j.1939-1676.1993.tb01021.x
103. Priester WA, McKay FW. The occurrence of tumors in domestic animals. *Natl Cancer Inst Monogr*. 1980;54:1-210.
104. Onions DE. A prospective survey of familial canine lymphosarcoma. *J Natl Cancer Inst*. 1984;72(4):909-912.
105. Day MJ, Whitbread TJ. Pathological diagnoses in dogs with lymph node enlargement. *Vet Rec*. 1995;136(3):72. doi:10.1136/vr.136.3.72
106. Kol A, Christopher MM, Skorupski KA, Tokarz D, Vernau W. B-cell lymphoma with plasmacytoid differentiation, atypical cytoplasmic inclusions, and secondary leukemia in a dog. *Vet Clin Pathol*. 2013;42(1):40-46. doi:10.1111/vcp.12003
107. Kimura Y, Harada T, Sasaki T, Imai T, Machida N. Primary cardiac lymphoma in a 10-week-old dog. *J Vet Med Sci*. 2018;80(11):1716-1719. doi:10.1292/jvms.18-0272
108. Brachelente C, Affolter VK, Fondati A, et al. CD3 and CD20 coexpression in a case of canine cutaneous epitheliotropic T-cell lymphoma (Mycosis Fungoides). *Vet Pathol*. 2016;53(3):563-566. doi:10.1177/0300985815604724
109. Lam A. Cutaneous lymphoma in a Dachshund. *Can Vet J*. 2021;62(7):771-774.
110. Nagata N, Shibata S, Sakai H, et al. Gallbladder lymphoma in a miniature dachshund. *J Vet Med Sci*. 2015;77(1):117-121. doi:10.1292/jvms.14-0300
111. Kodama A, Sakai H, Kobayashi K, et al. B-cell intestinal lymphoma with Mott cell differentiation in a 1-year-old miniature Dachshund. *Vet Clin Pathol*. 2008;37(4):409-415. doi:10.1111/j.1939-165X.2008.00067.x
112. Borska P, Faldyna M, Blatny J, et al. Gamma/delta T-cell lymphoma in a dog. *Can Vet J*. 2009;50(4):411-416.

113. Rimpo K, Hirabayashi M, Tanaka A. Lymphoma in Miniature Dachshunds: A retrospective multicenter study of 108 cases (2006-2018) in Japan. *J Vet Intern Med.* 2022;36(4):1390-1397. doi:10.1111/jvim.16455
114. Weiss DJ. A retrospective study of the incidence and the classification of bone marrow disorders in the dog at a veterinary teaching hospital (1996-2004). *J Vet Intern Med.* 2006;20(4):955-961. doi:10.1111/j.1939-1676.2006.tb01811.x
115. Yau PPY, Dhand NK, Thomson PC, Taylor RM. Retrospective study on the occurrence of canine lymphoma and associated breed risks in a population of dogs in NSW (2001-2009). *Aust Vet J.* 2017;95(5):149-155. doi:10.1111/avj.12576
116. Bhang DH, Choi US, Kim MK, et al. Epitheliotropic cutaneous lymphoma (mycosis fungoides) in a dog. *J Vet Sci.* 2006;7(1):97-99. doi:10.4142/jvs.2006.7.1.97
117. Han JI, Jang HJ, Chang DW, Kim GH, Ahn B, Na KJ. What is your diagnosis? Ascites fluid from an 11-year-old dog with epigastric bulging. *Vet Clin Pathol.* 2009;38(4):541-544. doi:10.1111/j.1939-165X.2009.00149.x
118. Choi JH, Kim HW, Jang JY, Kim HJ, Kim JY, Yoon JH. Diagnostic imaging for solitary nodular form of alimentary lymphoma in a dog. *J Vet Clin.* 2007;24(3):467-472.
119. Bae BK, Kim CW, Choi ES, Chang-Woo L. 누출액성 복수의 세포학적 검사로 진단된 림프종 1예 [A Canine Lymphoma Patient Diagnosed by the Cytologic Examination of Transudative Ascites]. *J Vet Clin.* 2004;21(2):194-196.
120. Jagielski D, Lechowski R, Hoffmann-Jagielska M, Winiarczyk S. A retrospective study of the incidence and prognostic factors of multicentric lymphoma in dogs (1998-2000). *J Vet Med A Physiol Pathol Clin Med.* 2002;49(8):419-424. doi:10.1046/j.1439-0442.2002.00458.x
121. Ladikou EE, Kassi E. The emerging role of estrogen in B cell malignancies. *Leuk Lymphoma.* 2017;58(3):528-539. doi:10.1080/10428194.2016.1213828
122. Khaw KT, Dowsett M, Folkard E, et al. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: European prospective investigation into cancer in Norfolk (EPIC-Norfolk) Prospective Population Study. *Circulation.* 2007;116(23):2694-2701. doi:10.1161/CIRCULATIONAHA.107.719005
123. Hahn KA, Richardson RC, Teclaw RF, et al. Is maintenance chemotherapy appropriate for the management of canine malignant lymphoma? *J Vet Intern Med.* 1992;6(1):3-10. doi:10.1111/j.1939-1676.1992.tb00979.x

124. Pittaway C, Schofield I, Dobson J, O'Neill DG, Brodbelt DC. Incidence and risk factors for the diagnosis of lymphoma in dogs in UK primary-care practice. *J Small Anim Pract.* 2019;60(10):581-588. doi:10.1111/jsap.13054
125. Rungsipipat A, Chayapong J, Jongchalermschai J, et al. Histopathological classification and immunophenotyping of spontaneous canine lymphoma in Bangkok metropolitan. *Comp Clin Pathol.* 2014;23(1):213-222. doi:10.1007/s00580-012-1600-9
126. Arisawa K, Soda M, Endo S, et al. Evaluation of adult T-cell leukemia/lymphoma incidence and its impact on non-Hodgkin lymphoma incidence in southwestern Japan. *Int J Cancer.* 2000;85(3):319-324. doi:10.1002/(sici)1097-0215(20000201)85:3<319::aid-ijc4>3.0.co;2-b
127. Mantina H, Wiggill TM, Carmona S, Perner Y, Stevens WS. Characterization of lymphomas in a high prevalence HIV setting. *J Acquir Immune Defic Syndr.* 2010;53(5):656-660. doi:10.1097/QAI.0b013e3181bf5544
128. Alexander DD, Mink PJ, Adami HO, et al. The non-Hodgkin lymphomas: a review of the epidemiologic literature. *Int J Cancer.* 2007;120 Suppl 12:1-39. doi:10.1002/ijc.22719
129. AIDS-defining Cancer Project Working Group of IeDEA, COHERE in EuroCoord. Non-Hodgkin lymphoma risk in adults living with HIV across five continents. *AIDS.* 2018;32(18):2777-2786. doi:10.1097/QAD.0000000000002003
130. Wiggill TM, Mantina H, Willem P, Perner Y, Stevens WS. Changing pattern of lymphoma subgroups at a tertiary academic complex in a high-prevalence HIV setting: a South African perspective. *J Acquir Immune Defic Syndr.* 2011;56(5):460-466. doi:10.1097/QAI.0b013e31820bb06a

Figures:

Figure 5.1: Leoniek. Young boerboel or South African mastiff seen from the front in a forrest setting [Online]. Adobe Stock.

Figure 5.2: Vallee J. Maltese toy poodle mixed puppy sitting in grass [Online]. Adobe Stock.

Appendix A: Lymphoma Population Data Capturing Sheet

AutoSave MSc Lymphoma_Data Collection Sheet FINAL_25_01_22 • Saved Search (Alt+Q) Sybrand Harris SH

File Home Insert Page Layout Formulas Data Review View Help

Clipboard Font Alignment Number Styles Cells Editing Analysis

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	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Index	Lab_nr	Lab	Submission	Reporting dat	Region_SA	Breed	Breed_gr	Sex	Neut_status	Age_yrs	Diagnosis_1	Diagnosis_2	Neoplastic/In	Grade
2	1	JB613592	IDEXX	2020/09/22	2020/09/28	Gauteng	Minature Pinsch		2 Male	Neutered	14	Lymphoma, Diffuse	Large cell	Neoplastic	Intermed
3	2	JB613567	IDEXX	2020/09/22	2020/09/28	Gauteng	Mixed		11 Male	Unsterilized	12	Lymphoma, Diffuse	Small cell	Neoplastic	Low
4	3	JB613551	IDEXX	2020/09/21	2020/09/25	Gauteng	German Shepher		1 Female	Spayed	10	Lymphoma, Diffuse	Large cell	Neoplastic	Intermed
5	4	JB611246	IDEXX	2020/08/26	2020/08/31	Gauteng	German Shepher		1 Female	Spayed	11	DLBCL	Centroblastic	Neoplastic	Intermed
6	5	JB612592	IDEXX	2020/09/10	2020/09/14	Gauteng	Retriever		8 Female	Spayed	4	Lymphoma, Diffuse	Intermediate cell	Neoplastic	Low
7	6	JB612371	IDEXX	2020/09/08	2020/09/14	Gauteng	Bulldog		2 Male	Neutered	11,42	Lymphoma, Diffuse	Large cell	Neoplastic	High
8	7	JB612329	IDEXX	2020/09/08	2020/09/11	KwaZulu Natal	Boerboel		2 Male	Unsterilized	5	Lymphoma, Diffuse	Intermediate cell	Neoplastic	High
9	8	JB611255	IDEXX	2020/08/26	2020/08/31	Gauteng	Jack Russel Terri		3 Male	Unsterilized	11	Mucocutaneous/mucousal lymphoma	Epitheliotropic	Neoplastic	
10	9	JB611326	IDEXX	2020/08/27	2020/08/31	Gauteng	Labrador Retriev		8 Female	Unsterilized	4	Lymphoma, Diffuse	Intermediate cell	Neoplastic	Intermed
11	10	JB608820	IDEXX	2020/07/24	2020/08/20	Mpumalanga	Scottish Terrier		3 Female	Unsterilized	2	DLBCL	Centroblastic	Neoplastic	High
12	11	JB610211	IDEXX	2020/08/13	2020/08/17	Gauteng	Mixed		11 Male	Unsterilized	8	Mucocutaneous/mucousal lymphoma	Epitheliotropic	Neoplastic	
13	12	JB609235	IDEXX	2020/07/30	2020/08/03	Gauteng	Mixed		11 Female	Unsterilized	0	Cutaneous lymphoma	Non-epitheliotropic	Neoplastic	
14	13	JB609147	IDEXX	2020/07/29	2020/08/03	Gauteng	Jack Russel Terri		3 Male	Neutered	14	Mucocutaneous/mucousal lymphoma	Epitheliotropic	Neoplastic	
15	14	JB608970	IDEXX	2020/07/27	2020/07/30	Gauteng	Rottweiler		2 Male	Unsterilized	10	Lymphoma, Diffuse	Large cell	Neoplastic	High
16	15	JB608488	IDEXX	2020/07/21	2020/07/24	Western Cape	Jack Russel Terri		3 Female	Unsterilized	14	Lymphoma, Diffuse	Small cell	Neoplastic	Low
17	16	JB608225	IDEXX	2020/07/17	2020/07/22	Gauteng	Jack Russel Terri		3 Female	Spayed	3	Lymphoma, follicular	0	Neoplastic	Low
18	17	JB608382	IDEXX	2020/07/18	2020/07/21	Western Cape	Mixed		11 Male	Neutered	12	Lymphoma, Diffuse	Intermediate cell	Neoplastic	Intermed
19	18	JB607871	IDEXX	2020/07/13	2020/07/21	Gauteng	Labrador Retriev		8 Male	Neutered	8	DLBCL	Centroblastic	Neoplastic	High
20	19	JB607114	IDEXX	2020/07/03	2020/07/21	Western Cape	Kerry Blue Terrie		3 Male	Neutered	11	Cutaneous lymphoma	Epitheliotropic	Neoplastic	
21	20	JB608094	IDEXX	2020/07/16	2020/07/20	KwaZulu Natal	Bullmastiff		2 Male	Neutered	4	Lymphoma, Diffuse	Large cell	Neoplastic	Intermed
22	21	JB608006	IDEXX	2020/07/15	2020/07/17	Gauteng	Jack Russel Terri		3 Male	Unsterilized	6	Lymphoma, Diffuse	Large cell	Neoplastic	Intermed
23	22	JB607633	IDEXX	2020/07/09	2020/07/15	Gauteng	Dachshund		4 Male	Unsterilized	12	Mucocutaneous/mucousal lymphoma	Epitheliotropic	Neoplastic	

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AH23

	O	P	Q	R	S	T	U	V	W	X	AA	AD	AE	AF	AG	AH
1	Grade	Benign/Malignant	Origin	Topography	Tumour type	Topography	Immunophen	WHO_class	Surgery/PM	Patholog	Mitotic Count					
2	Intermediate	Malignant	Haematopoietic	Multiple Inn	Lymphoma	Popliteal, Mandi		0	0 Surgery	Liza	7					
3	Low	Malignant	Haematopoietic	Single Inn	Lymphoma	Popliteal		0	0 Surgery	Liza						
4	Intermediate	Malignant	Haematopoietic	Single Inn	Lymphoma	Unknown		0	0 Surgery	Liza	10					
5	Intermediate	Malignant	Haematopoietic	Multiple Inn	Lymphoma	Unknown	B-cell	DLBCL	Surgery	Liza	6					
6	Low	Malignant	Haematopoietic	Single Inn	Lymphoma	Prescapular, R		0	0 Surgery	Liza	2 to 3					
7	High	Malignant		0 Mass	Lymphoma	Intra-abdominal		0	0 Surgery	Liza	13					
8	High	Malignant	Haematopoietic	Mass	Lymphoma	Gastric, tonsils		0	0 Surgery	Liza	12					
9		0 Malignant	Skin/mucosal/subcuta	Mass & Multiple	Lymphoma	Oral, Mandibula		0	0 Surgery	Liza						
10	Intermediate	Malignant	Haematopoietic	Single Inn	Lymphoma	Popliteal		0	0 Surgery	Liza	6					
11	High	Malignant	Haematopoietic	Single Inn & Live	Lymphoma	Unknown, Liver	B-cell	DLBCL	Surgery	Liza	15					
12		0 Malignant	Skin/mucosal/subcuta	Multiple Masses	Lymphoma	Oral		0	0 Surgery	Liza						
13		0 Malignant	Skin/mucosal/subcuta	Multiple Masses	Lymphoma	Subcutaneous		0	0 Surgery	Liza	10					
14		0 Malignant	Skin/mucosal/subcuta	Lesions	Lymphoma	Oral		0	0 Surgery	Liza						
15	High	Malignant	Haematopoietic	Multiple Inn	Lymphoma	Prescapular, Sut		0	0 Surgery	Liza	12					
16	Low	Malignant	Haematopoietic	Single Inn	Lymphoma	Mandibular		0	0 Surgery	Liza	0 to 1					
17	Low	Malignant	Haematopoietic	Single Inn	Lymphoma	Unknown		0	0 Surgery	Liza	Up to 5					
18	Intermediate	Malignant	Haematopoietic	Multiple Inn	Lymphoma	Unknown		0	0 Surgery	Liza	6					
19	High	Malignant	Haematopoietic	Multiple Inn	Lymphoma	Unknown	B-cell	DLBCL	Surgery	Liza	16					
20		0 Malignant	Skin/mucosal/subcuta	Lesions	Lymphoma	Cutis	T-cell		0 Surgery	Liza						
21	Intermediate	Malignant	Haematopoietic	Multiple Inn	Lymphoma	Unknown		0	0 Surgery	Liza	6					
22	Intermediate	Malignant	Haematopoietic	Multiple Inn	Lymphoma	Popliteal, Presca		0	0 Surgery	Liza	7					
23		0 Malignant	Skin/mucosal/subcuta	Lesions	Lymphoma	Oral		0	0 Surgery	Liza						

Appendix B: Control Population Data Capturing Sheet

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BT24

	A	D	G	P	Q	AK	AM	AO	AR	AS	AT	AU	BS	BT
1	Inde	Case	Submission_dt	Breed	Breed_gr	Sex	Leut_st	Age_y	Diagnosis_1	lammatory/neo	Origin 2	Origin		
2	343	Case_001	2018/12/13	Shih Tzu		9	Male	Intact	3 INFUNDIBULAR KERATINIZING ACANTHOMA	Neoplastic	Integumentary	Skin		
3	344	Case_002	2018/12/19	Mixed		11	Male	Intact	10 THIRD EYELID HAEMANGIOSARCOMA	Neoplastic	Occular and periocular	Mucousa		
4	345	Case_003	2018/12/21	Labrador Retriever		8	Female	Intact	6 Cutaneous melanocytic neoplasm	Neoplastic	Integumentary	Skin		
5	346	Case_004	2019/01/04	Bloodhound		6	Male	Intact	2 Pyotraumatic dermatitis with multifocal su	Inflammatory	Integumentary	Skin		
6	347	Case_005	2019/01/11	Jack Russel Terrier		3	Male	Intact	14 Splenic stromal sarcoma	Neoplastic	Haemopoeitic	Spleen		
7	348	Case_006	2019/01/11	Cocker Spaniel		8	Female	Intact	9 CUTANEOUS MELANOCYTOMA	Neoplastic	Integumentary	Skin		
8	349	Case_007	2019/01/11	Mixed		11	Male	Intact	11 Solid – cystic apocrine ductular carcinoma	Neoplastic	Integumentary	Gland (skin)		
9	350	Case_008	2019/01/15	Cairn Terrier		3	Male	Intact	7 Mild eosinophilic rhinitis	Inflammatory	Respiratory	Nose		
10	351	Case_009	2019/01/18	Rhodesian Ridgeback		6	Female	Intact	5,5 CUTANEOUS MAST CELL TUMOUR	Neoplastic	Integumentary	Skin		
11	352	Case_010	2019/01/23	Schnauzer		2	Male	Neutered	10 LOW-GRADE CUTANEOUS MAST CELL TUMOU	Neoplastic	Integumentary	Skin		
12	353	Case_011	2019/01/23	Jack Russel Terrier		3	Male	Neutered	9,21 PERIANAL GLAND ADENOMA INFILTRATIVE LI	Neoplastic	Integumentary	Perianal		
13	354	Case_012	2019/01/24	Boxer		2	Female	Neutered	8 A+B: GRADE II / LOW GRADE MAST CELL TUM	Neoplastic	Integumentary	Skin		
14	355	Case_013	2019/01/28	Jack Russel Terrier		3	Female	Intact	10,5 TRAUMATIC PANNICULITIS	Inflammatory	Integumentary	Subcutaneous		
15	356	Case_014	2019/01/29	Schnauzer		2	Female	Intact	4 LOW GRADE CUTANEOUS MAST CELL TUMOU	Neoplastic	Integumentary	Skin		
16	357	Case_015	2019/02/05	Labrador Retriever		8	Female	Intact	11 Subungual keratoacanthoma	Neoplastic	Integumentary	Nail		
17	358	Case_016	2019/02/12	Airedale Terrier		3	Male	Intact	9 CUTANEOUS MELANOCYTOMA	Neoplastic	Integumentary	Skin		
18	359	Case_017	2019/02/13	Rottweiler		2	Male	Intact	7 GRADE II / HIGH GRADE MAST CELL TUMOUR	Neoplastic	Integumentary	Skin		
19	360	Case_018	2019/02/22	Mixed		11	Male	Neutered	12 COLLAGENOUS HAMARTOMA NODULAR SEBA	Neoplastic	Integumentary	Gland (sebaceous)		
20	361	Case_019	2019/02/22	Staffordshire Bull Terrier		3	Male	Intact	0,42 CUTANEOUS HISTIOCYTOMA	Neoplastic	Integumentary	Skin		
21	362	Case_020	2019/02/27	Mixed		11	Male	Intact	1 ULCERATIVE DERMATITIS AND FOLLICULITIS –	Inflammatory	Integumentary	Skin		
22	363	Case_021	2019/03/01	Mixed		11	Female	Intact	6 SOLID AND MICROFOLLICULAR THYROID CAR	Neoplastic	Endocrine	Thyroid		
23	364	Case_022	2019/03/06	Bull Terrier		3	Female	Intact	9 Severe chronic pyogranulomatous dermatit	Inflammatory	Integumentary	Skin		

Appendix C: Research Poster

- Presented at the European College/Society of Veterinary Clinical Pathology's 24th Annual Congress in Belgrade, Serbia (5-8 October 2022).
- Presented at the University of Pretoria, Faculty of Veterinary Science's Annual Faculty Day at the Onderstepoort Campus, South Africa (20 October 2022).



An Epidemiological Study of Canine Lymphoma in South Africa

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Background
Breed, age, and sex predispositions for canine lymphoma (cL) have been reported for various locations¹⁻⁵. However, epidemiological information concerning cL in South Africa is scarce.

Objectives
To describe the epidemiologic features of cL and the frequency of World Health Organisation (WHO) subtypes in South Africa⁶.

Material and Methods
A retrospective case-controlled study was performed that included 342 cases with a histopathological diagnosis of cL matched with non-cL control cases. Associations between cL and breed, sex and age were assessed using univariate and multivariable conditional logistic regression. Associations were reported as odds ratios (ORs) and significance set as $P < 0.05$.

Results
Breed, in general, was significantly associated with cL, but not age, sex, or neutering status. Median population age was 8 years, with a male to female ratio of 1.2:1. The Boerboel had an increased (OR = 3.25, $P = 0.004$) and the Yorkshire Terrier a decreased risk (OR = 0.17, $P = 0.004$) of developing cL. The Boerboel cL group (n = 27) had a younger median age of 6 years and a higher male to female ratio of 1.5:1.

Most biopsy submissions were from haemopoietic origin (n = 255/342, 75%). Immunophenotyping was performed on 119 (35%) cases, of which 82 (69%) were B-cell, 34 (29%) T-cell and 3 (2%) neither. World Health Organisation subtype was available for 88 cases; of these 66 (75%) were diffuse large B-cell lymphoma (DLBCL) with remaining subtypes each $\leq 7\%$ (Table 1). All Boerboel cases (n=4) that were WHO subtyped were DLBCL.




Table 1: Absolute numbers and prevalence of lymphoma characteristics. Total n = 342.

Variable	n	Prevalence(%) (95% CI)
Tumour origin		
Haemopoietic		
Lymph node	255	75 (70,79)
Liver/spleen	228	67 (62,72)
Other	17	5 (3,8)
Skin/subcutis/mucosal		
Skin	10	3 (2,5)
Subcutaneous	72	21 (17,26)
Mucocutaneous/	48	14 (11,18)
Mucosal	5	2 (1,3)
Other	17	5 (3,8)
Other		
	2	1 (0,2)
	13	4 (2,6)
Total	340	100
Tumour grade		
Low	78	30 (25, 36)
Intermediate	111	43 (37, 49)
High	71	27 (22, 33)
Total	260	100
Immunophenotype		
B-cell	82	69 (60, 77)
T-cell	34	29 (21, 37)
Neither	3	3 (1, 7)
Total	119	100
WHO classification		
DLBCL	66	75 (65, 83)
DSBCL	3	3 (1, 9)
MZL	6	7 (3, 14)
PTCL-NOS	6	7 (3, 14)
T-LBL	2	2 (0,4, 7)
TZL	5	6 (2, 12)
Total	88	100

CI = Confidence Interval, WHO = World Health Organisation, 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, TZL = T-zone lymphoma

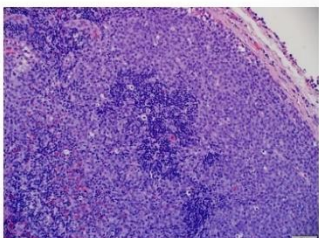


Figure 1: Centrioblastic diffuse large B-cell lymphoma with large neoplastic cells (> 2 times the diameter of a red blood cell) and prominent central and peripheral nucleoli. Haematoxylin and eosin (H&E) stain.

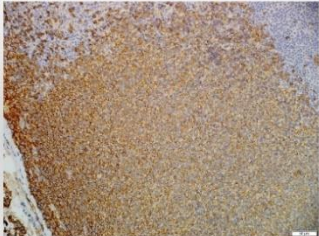



Figure 2: Centrioblastic diffuse large B-cell lymphoma with neoplastic cells exhibiting strong membranous CD20 immunoreactivity. Haematoxylin counterstain.

Conclusion
This study confirmed a breed predisposition for cL in the Boerboel, a South African mastiff-type dog, as previously reported⁴. However, the lack of association between age, sex, and other breeds, and cL, was unexpected. The frequency of immunophenotypes was similar to findings in other locations⁷. A larger percentage of biopsies were diagnosed to be DLBCL, and a much smaller prevalence was seen for peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) and T-zone lymphoma (TZL) in South Africa when compared to an American study⁸. This study might further indicate a predilection for B-cell lymphoma in the Boerboel.

References:

- Bennett PF, Taylor R, Williamson P. Demographic risk factors for lymphoma in Australian dogs: 6201 cases. *Journal of Veterinary Internal Medicine*. 2018;32:2054-2060.
- Comazzi S, Marelli S, Cozzi M, et al. Breed-associated risks for developing canine lymphoma differ among countries: an European canine lymphoma network study. *BMC Veterinary Research*. 2016;14:232.
- Jark PC, Fracacio CP, Anai LA, et al. Histopathological and immunophenotypical characterization of canine multicentric lymphoma in Brazil: a study of 203 cases. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 2020;78:793.
- van Rooyen LJ, Hooijberg E, Feyers F. Breed prevalence of canine lymphoma in South Africa. *Journal of the South African Veterinary Association*. 2018;1-11.
- Villanil AJ, Henry CJ, Hahn AW, et al. Hormonal and sex impact on the epidemiology of canine lymphoma. *Journal of Cancer Epidemiology*. 2010.
- Vail VE, San Myint M, Bartheil A, et al. Classification of canine malignant lymphomas according to the World Health Organization criteria. *Veterinary Pathology*. 2011;48:198-211.
- Mogliano JF, Breen M, Burnett RC, et al. Distinct B-cell and T-cell lymphoproliferative disease prevalence among dog breeds indicates heritable risk. *Cancer Research*. 2005;65:5654-5661.



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Appendix C: Table summary of the most important findings of 5 canine lymphoma studies from 2010-2020.

Paper Citation	Bennet et al., 2018 ¹⁸	Jark et al., 2020 ²⁰	Comazzi et al., 2018 ¹⁹	Villamil et al., 2010 ²²	Van Rooyen et al., 2018 ²¹
Location	Australia	Brazil	Europe	North America	South Africa
Study design	Retrospective case-control	Retrospective case study	Retrospective case-control	Retrospective case-control	Retrospective case-control
Type					
Case population origin	<ul style="list-style-type: none"> 1 Commercial veterinary laboratory General practice group 2 Referral centres 	<ul style="list-style-type: none"> Multiple immunohistochemistry laboratories 	<ul style="list-style-type: none"> Oncology referral centres in 8 different countries (Austria, France, Italy, Netherlands, Portugal, Spain, Switzerland, and United Kingdom) 	<ul style="list-style-type: none"> Multiple veterinary academic hospitals 	<ul style="list-style-type: none"> 1 Commercial veterinary laboratory (A) 1 Academic veterinary laboratory (B)
Case population (n)	6 201	203	1 529	14 573	213 (A), 39 (B)
Control population (n)	640 105	No control population	55 529	1 157 342	28 523 (A), 5 569 (B)
Main findings					
Breed (See table 2.2)	<ul style="list-style-type: none"> Increased risk breeds identified (n): 30 (15 new) Decrease risk breeds identified (n): 26 (18 new) 	<ul style="list-style-type: none"> Golden Retriever, Boxer, Rottweiler, Poodle and English Cocker Spaniel had a prevalence of > 3%, respectively. 	<ul style="list-style-type: none"> Increased risk breeds identified (n): 7 Golden Retriever and Labrador showed no predisposition. 	<ul style="list-style-type: none"> Increased risk breeds identified (n): 15 Decrease risk breeds identified (n): 11 	<ul style="list-style-type: none"> Increased risk breeds identified (n): 14 Decrease risk breeds identified (n): 1
Age	Not assessed	<ul style="list-style-type: none"> Mean age 7.8 years. 	Not assessed	<ul style="list-style-type: none"> Age group < 4 years less likely to develop disease. 	<ul style="list-style-type: none"> Median age 8 (A) and 6.5 (B) years. Age group 6.1 – 9 years increased odds (A).
Sex	<ul style="list-style-type: none"> Males increased risk. Neutered animals increased risk (both sexes). 	Not assessed	Not assessed	<ul style="list-style-type: none"> Intact females decreased risk. 	<ul style="list-style-type: none"> Increased number of males and neutered females (A).
Other		<ul style="list-style-type: none"> 71.4 % B-cell, 27.1 % T-cell Boxers mostly associated with B-cell. Golden Retrievers mostly associated with T-cell. 	<ul style="list-style-type: none"> Rottweilers mostly associated with B-cell. Boxers mostly associated with T-cell. 	<ul style="list-style-type: none"> Sex effect on development of lymphoma confirmed in dogs. 	<ul style="list-style-type: none"> Boerboel identified as having an increased risk. Maltese identified as having a decreased risk.
Main limitations	<ul style="list-style-type: none"> Possible double entries in case and control populations. Limited information on lymphoma subtype classification and samples not available for review. Discrepancy of time periods of data collection. Missing data on records. 	<ul style="list-style-type: none"> None listed. Author identified: <ul style="list-style-type: none"> No risk factors analysed. No information regarding sex and neutering status. Relatively small sample size. 	<ul style="list-style-type: none"> No standard lymphoma classification. Control group not representative of entire country populations. 	<ul style="list-style-type: none"> Limited information on lymphoma subtype classification. 	<ul style="list-style-type: none"> Relatively small sample size. Control group not representative of entire country's population. Missing data on records. No information of lymphoma subtype classification.