

Retrospective Analysis of 101 Canine Lymphoma Cases Diagnosed in Surgical biopsies in Latvia (2011-2020)

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Abstract. Lymphoma is a malignant tumor commonly diagnosed in dogs representing 7-24% of all canine tumors. There has been no previous studies focused on characterization of canine lymphoma cases in Latvia. The goals of this retrospective study were to determine prevalence and characteristics of lymphoma cases among biopsy submissions to a private veterinary pathology service that receives approximately 80% of all biopsy submission in Latvia and to compare this data with published canine lymphoma reviews. Data were retrieved to select records from Latvian dogs diagnosed with lymphoma between 2011 and 2020, determining characteristics of dogs and types of lymphoma based on anatomic distribution and cellular morphology. In a ten-year study period, diagnosis of lymphoma constituted 1-4% of surgical biopsy submissions each year without upwards trend. Affected dogs were middle age (median 8 years; range 2-13), with a slight male predominance (58%). The majority of dogs (19%) were mixed breed. Top 3 affected dog breeds were Rottweiler, American Staffordshire terrier and French bulldog with 6-7 cases in each breed. Multicentric form of lymphoma predominated (55%) followed by alimentary and mucocutaneous lymphoma (21% and 14%, respectively). Within multicentric form of lymphoma two thirds were intermediate to large cell lymphomas. Included in this group would be diffuse large B cell lymphomas, the most common subtype of canine lymphomas; however, lack of immunohistochemical testing precluded complete lymphoma classification according to WHO guidelines. Results of this study correlate well with the previously published results and provide important information to Latvian small animal veterinarians and pathologists.

Key words: dog, lymphoma, biopsies, histopathology, diagnosis, retrospective.

Introduction

Lymphoma (also referred to as lymphosarcoma) is a malignant tumor frequently encountered in dogs. It is tumor of lymphoid tissue derived from aberrant, clonally expanded population of lymphocytes resulting in solid masses or variably expanding tissues and interfering with their function. Among tumors, lymphoma is one of the most common ones in dogs representing 7-24% of all tumors (Zandvliet, 2016). Although canine lymphoma is often viewed as a single disease, it actually comprises a number of clinically and morphologically distinct forms of lymphoid cell neoplasia. Most frequently lymphoma in dogs presents as a single or multiple enlarged peripheral lymph nodes (multicentric lymphoma), but it can also or instead affect other tissues and organs such as skin

and mucocutaneous tissues, gastrointestinal tract, liver, spleen and others (Valli, Bienzle, & Meuten, 2016).

Based on distribution and tissues affected canine lymphomas are divided in following anatomical categories: 1) multicentric; 2) alimentary; 3) mediastinal; 4) extranodal (Vail, MacEwen, & Young, 2001). Some authors also include separate categories of mucocutaneous, hepatosplenic and CNS (central nervous system) lymphoma (Zandvliet, 2016). This underscores the fact that there is a considerable overlap between various anatomical categories of lymphoma. Additionally, regional lymph nodes may be involved in cases other than multicentric lymphoma (e. g. in cases of alimentary and mucocutaneous lymphoma) further blurring lines

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Table 1

**Characteristics of most common types of lymphomas in dogs representing >80% of cases
(adapted from Valli *et al.*, 2011; Zandvliet, 2016)**

Type of lymphoma ^a	Immuno phenotype (T vs B)	Grade	% of lymphomas	Tissues affected	Survival (months)
Diffuse large B cell lymphoma (DLBCL)	B	Low, medium, high	~50	Multicentric lymphadenopathy +/- liver, spleen, blood/BM	~9 (with large variation)
Peripheral T-cell lymphoma, NOS	T	High	~15	Multicentric lymphadenopathy +/- liver, spleen, blood/BM	~6
T zone lymphoma (TZL)	T	Low (indolent)	5-10	Regional LN or multicentric lymphadenopathy	20-33
T-lymphoblastic lymphoma	T	High	~4	Multicentric lymphadenopathy +/- liver, spleen, blood/BM, mediastinal	6-8
Marginal zone lymphoma	B	Low (indolent)	5-10	Spleen, later in LN	13-21

DLBCL – diffuse large B cell lymphoma; NOS – not otherwise specified; TZL – T zone lymphoma; BM – bone marrow; LN – lymph node

^aAccording to WHO classification (Valli *et al.*, 2011).

between various anatomical categories of lymphoma. Anatomical classification scheme is useful because it allows to correlate clinical signs with distribution of lymphoma.

More detailed classification of canine lymphoma is based on cellular and tissue morphology and immunophenotype (T vs B cell lymphoma). Guidelines for this classification according to the World Health Organization (WHO) Classification for Neoplastic Diseases of the Lymphoid Tissues have been published more than a decade ago (Valli, Bienzle, & Meuten, 2016; Valli *et al.*, 2011). The benefit of this classification scheme is that prognosis can be more accurately predicted. For example, it is important to distinguish indolent (low grade lymphoma) from high grade lymphoma, because they have different survival times, and it is essential to treat high grade lymphoma with chemotherapy while it may not be necessary to use this treatment in case of indolent lymphomas.

According to WHO classification, there are 30 different categories of canine lymphoma; however, it is recognized that ~80% of canine lymphomas represent one of 5 WHO lymphoma subtypes (Valli *et al.*, 2011). Brief summary about 5 most common types of canine lymphoma is presented in Table 1.

Every small animal veterinarian should be familiar with and able to diagnose lymphoma. Most commonly multicentric lymphoma is diagnosed by cytology of

aspirate (fine needle aspirate, FNA) (Mortier *et al.*, 2012). However, cytology is not done in all cases, or it may be inconclusive, or the tissues suspected to have lymphoma may not be easily accessible for FNA (for example gastrointestinal tract or spleen). In all of these cases, tissues can be biopsied and submitted for histopathological examination.

There has been no published reports summarizing lymphoma cases in canine population in Latvia. This paper presents analysis of lymphoma cases in Latvian dog population based on the retrospective analysis of 10-year data from biopsy submissions to local, private veterinary pathology laboratory (Matisē Veterinary Pathology service, Riga, Latvia) which receives an estimated 80% of all biopsy submissions in Latvia. The goal was to determine prevalence of lymphoma cases among biopsy submissions from Latvian dogs, determine if there is breed predilection similar to that reported in other geographical locations and to determine characteristics of most common forms of lymphoma encountered in canine biopsy submissions in Latvia.

Materials and Methods

Data base of biopsy reports of a private veterinary pathology service (Matisē Veterinary Pathology service) was searched for diagnosis of lymphoma in Latvian dogs between 2011 and 2020 using search

terms “suns”, “limfoma” and “limfosarkoma”. Only those records that had definitive biopsy diagnosis of lymphoma were included in the data set for this study. Number of canine biopsy submissions for each year was extracted. For dogs that had repeated submissions (including diagnosis of lymphoma), only one record for particular disease entity was counted. For each record the following data were retrieved: breed, sex, age, clinical history, organs examined histologically, diagnosis and comments (where applicable). The dogs were grouped by the age group in 3 categories: 1) younger than 5 years; 2) 5 up to 10 years old; 3) older than 10 years. Anatomical location of lymphoma was recorded based on the tissues submitted and examined histologically allowing to verify presence of lymphoma in particular tissues.

Morphological types and grades of lymphoma indicated in biopsy reports were based on guidelines outlined in WHO classification scheme (Valli *et al.*, 2011). Briefly, evaluation included nodular versus diffuse growth pattern, nuclear size, the detailed nuclear morphology, and the number of mitoses per high-power field. Nuclear size was determined as small (<1.5x the size of a red blood cell), intermediate (1.5–2x the size of a red blood cell), or large (>2x the size of a red blood cell). The number of mitoses was identified in a microscopic field with a 40x objective. Lymphomas with 0 to 5 mitoses/400x field were graded low grade, those with 6 to 10 mitoses/400x field were graded medium grade, and those with greater than 10 mitoses/400x field were graded high grade.

Immunophenotyping (immunohistochemistry, IHC) was done only in cases when requested by the submitting veterinarian. IHC tests for B and T cells were done in veterinary laboratories outside Latvia (Sweden and USA).

Statistical analysis of prevalence and demographics (age, sex, breed) data was done utilizing Microsoft Excel software program (version 2202; Microsoft 365).

Results and Discussion

From 2011 till 2020, there were 101 cases of lymphoma recorded in biopsy submissions from Latvian dogs. On average, lymphoma was diagnosed in 2.3% of submissions (Table 2). This prevalence of lymphoma represents only a fraction of lymphomas diagnosed in Latvian dogs since lymphoma is effectively diagnosed by cytology. For example, in the Department of Small Animal Medicine at the University of Ghent (Belgium) only 25% of lymphoma cases were diagnosed by histopathology whereas the remaining 75% cases were diagnosed by cytology (Mortier *et al.*, 2012). During the 10 years under the review, there was an overall increase in biopsy submissions; however, there was no apparent trend for an increase in lymphoma prevalence during these years. Instead, there was considerable fluctuation in the proportion of lymphoma cases each year ranging from 1 to 4%.

Majority of dogs (68.5%) with lymphoma were middle aged – 5-10 years old (Table 3). Age was not

Table 2

Prevalence of lymphoma among biopsy submissions from Latvian dogs (2011-2020)

Year	Total # of dog submissions	Lymphoma	
		n	%
2011	113	2	1.8
2012	176	4	2.3
2013	230	10	4.4
2014	367	8	2.2
2015	409	5	1.2
2016	501	15	3.0
2017	558	6	1.1
2018	635	23	3.6
2019	673	11	1.6
2020	693	17	2.5
Total	4355	101	
Avg			2.3
Range			1.1 – 4.4
SD			1.0

specified for 9 dogs and these dogs were excluded from calculating age distribution and averages. Median age of affected dogs was 8 years (range 2-13 years; SD 2.3). This matches previously published reports confirming that the number of lymphoma cases in dogs in Latvia are similar to other geographical areas (Coelho *et al.*, 2019; Mortier *et al.*, 2012; Zandvliet, 2016). There were no dogs under 2 years of age affected by lymphoma. The youngest dog was 2 years old French bulldog with intestinal transmural lymphoma in the rectum.

As regards sex of the dogs, there was a slight predominance of males (58%) (Table 3). Sex was not indicated in 1 dog. Information about castration status was available for less than 30% of cases; therefore, it was not included in the analysis. According to literature, there is no apparent sex predisposition to lymphoma, but intact female dogs appear to have a reduced risk for lymphoma (Zandvliet, 2016). Furthermore, early (<1 year) ovariohysterectomy has been suggested to increase a risk of development of lymphoma in the Golden retriever but not in Labrador retriever.

Lymphoma can affect dogs of any breed, but there seems to be preponderance of middle-sized to larger

dog breeds. In our retrospective study, the largest proportion of the dogs were reportedly mixed breed dogs (almost 20%) (Table 3). Among dogs for which a particular breed was mentioned in the submission sheet, Rottweilers were the most represented breed followed by American Staffordshire terriers and French bulldogs. These breeds together with Beagles and German Shepherd dogs represented almost 30% of lymphoma cases. In the literature, there is highly variable information about breed predisposition to lymphoma most likely because this kind of statistic is highly influenced by local popularity of particular breeds. In a recent lymphoma review article, breeds mentioned with increased risk for developing lymphoma were Bernese mountain dog, boxer, bulldog, bullmastiff, cocker spaniel, Doberman pinscher, German shepherd, Golden retriever, Labrador retriever, Rottweiler, Saint Bernard, and Scottish terrier (Zandvliet, 2016). Interestingly, in the same review Dachshunds were mentioned as having decreased risk for developing lymphomas. In our data set, there were equal (low) numbers of Labrador retrievers, Golden retrievers and Dachshunds affected by lymphoma (3 for each breed) indicating

Table 3

Age, sex and breed characteristics of Latvian dogs diagnosed with lymphoma in surgical biopsies (2011-2020)

Characteristic	n	%
Age (years)		
unknown	9	–
younger than 5 years	14	15.2
5-10 years old	63	68.5
older than 10 years	15	16.3
Sex		
Unknown	1	–
Male	58	58
Female	42	42
Breed		
Unknown	10	–
Mixed	12	18.7
Rottweiler	7	7.7
American Staffordshire terrier, French bulldog	6 each	6.6
Beagle	5	5.5
German Shepherd dog	4	4.4
English bulldog, Labrador retriever, Shar-pei, Dachshund, Golden Retriever	3 each	3.3
Bernese Mountain dog, Miniature Schnauzer, Doberman pinscher, Maltese, Toy terrier	2 each	2.2
21 different breeds	1 each	1.1

Table 4

Case distribution based on anatomical form of lymphoma and correlation with suspected lymphoma diagnosis in the clinical history

Anatomical type	Organs involved (based on tissues submitted)	n (total n = 101)	%	Lymphoma suspected before biopsy ^a n (%)
Multicentric	One or multiple LN, +/- spleen	55	55	39 (71%)
Splenic	Only spleen	6	6	1 (17%)
Alimentary	GI tract, +/- LN, +/- spleen, +/- liver, omentum, mesentery, oral cavity	21	21	5 (24%)
Skin and mucocutaneous	Skin, mucocutaneous tissues, +/- LN	14	14	3 (21%)
Extranodal	Nasal, nasopharyngeal tissue, uterus	4	4	1 (25%)
Mediastinal	Mediastinal lymph nodes, thymus	1	1	1 (100%)

LN – lymph node; GI – gastrointestinal.

^aResults based on the information on the biopsy submission sheets.

that, according to data set from biopsies, we do not see neither significant predisposition of retrievers to lymphoma nor decreased risk for Dachshunds. These data should not be overinterpreted because, as stated in the beginning of this article, only a subset of dog lymphomas are sent in for histopathological evaluation; therefore, biopsy data do not directly correlate with patient profile in veterinary clinics.

The majority of lymphoma submissions represented multicentric lymphoma (55%) (Table 4). This is in accordance to literature stating that almost 75% of canine lymphomas are multicentric (Vail, Thamm & Liptak, 2019). In our study, we chose to separately list splenic lymphomas (occurrence limited to spleen according to history provided in biopsy report), because such lymphomas are almost always indolent B cell lymphoma – a marginal zone lymphoma (MZL) with prolonged survival after splenectomy (Table 1) (Valli *et al.*, 2013). Splenic lymphomas were outnumbered by alimentary tract lymphomas (21%) and mucocutaneous lymphomas (14%). The reason why multicentric lymphomas constitute relatively smaller proportion (55% in this study vs 75% mentioned in the literature) is that this type of lymphoma is effectively diagnosed by cytology and therefore less often submitted for histopathological evaluation. Additionally, multicentric lymphoma presents as highly characteristic peripheral lymphadenopathy readily visible in clinical examination. On the contrary, lesions in the gastrointestinal (GI) tract and skin can mimic many other diseases; therefore, diagnosis of lymphoma is more complicated. This point is further illustrated by our observation that in biopsy submissions confirmed to be multicentric lymphoma by histopathology,

clinicians had indicated lymphoma as a presumptive diagnosis in submission sheets in ~70%. In contrast, lymphoma was clinically suspected in only about 20% cases of splenic, alimentary, cutaneous and extranodal lymphoma (Table 4).

In Matisē Veterinary Pathology service which is run by a single board-certified veterinary pathologist (IMVH) all lymphomas (except for epitheliotropic) were subtyped based on the tissue and cellular morphology and grade according to recommendations of WHO. However, because of lack of routine access to IHC, immunophenotyping for B and T cells was only done on a few cases when requested by a clinician. Therefore, WHO canine lymphoma classification scheme could not be completely applied. In cases when morphology was very typical of a particular subtype, e. g. DLBCL or MZL, it was indicated in comments. Table 5 presents a summary of distribution of multicentric lymphoma cases based on cellular features and grade. It is evident that majority of multicentric lymphoma cases were diffuse, intermediate to large cell lymphomas. DLBCL which, according to literature, represents around 50% of all canine lymphoma cases (Aresu, 2016) is included in these groups. In this regard, our analysis tends to support data in literature. It needs to be mentioned that within DLBCL group there was no uniformity, because it can present as low, medium or high grade. It was recently reported that median survival in dogs with DLBCL with fewer than 20 mitoses per 400x field was found to be 188 days; in contrast, the median survival of dogs with 21 and more mitoses per 400x field was 31 days (Valli *et al.*, 2013). Our analysis also shows that histologically it is possible to identify some indolent lymphomas (e. g. T zone lymphoma,

Table 5

Characteristics of cellular features of multicentric lymphoma diagnosed in surgical submissions from Latvian dogs (2011-2020)

Neoplastic cell distribution	Nuclear size of lymphocytes (Sm, In, Lg)	Grade based on mitotic rate (L, M, H)	# of cases	IHC results available	Presumptive lymphoma subtype noted in the report ^a	Comments
Diffuse	In, Lg	M, H	17	0	6 DLBCL 1 possibly T cell	Heterogeneous group – some aggressive
Diffuse	In, Lg	L	20	1 (B cell)	1 DLBCL 1 B cell lymphoma	Low grade B and T cell lymphomas
Diffuse	Sm	L (n=8) M (n=1)	9	2 (T cell)	1 TZL 1 peripheral T cell NOS	TZL = indolent lymphoma
Nodular	In, Lg	L (n=6) M (n=2) H (n=1)	9	0	2 FL 2 MZL	FL and MZL = indolent lymphomas

Sm – small nuclear size (<1.5x RBC); In – intermediate nuclear size (1.5 – 2x RBC); Lg – large nuclear size (>2x RBC); L – low grade (0-5 mitoses in 400x field); M – medium grade (6-10 mitoses in 400x field); H – high grade (>10 mitoses in 400x field); IHC – immunohistochemistry; DLBCL – diffuse large B cell lymphoma; TZL – T zone lymphoma; NOS – not otherwise specified; FL – follicular lymphoma; MZL – marginal zone lymphoma.

^aSpecific subtype of lymphoma was suggested in comments sections of biopsy report only in those cases that had very characteristic morphology; however, for confirmation IHC was recommended.

follicular lymphoma and marginal zone lymphoma) and that their prevalence in our study may be similar to that reported in literature (Table 1).

For lymphoma in the alimentary tract (n=21) the majority of the lymphomas were in the intestine (n=15). There were no cases of lymphoma in the stomach. Small intestinal segments were affected more commonly than large intestine and transmural lymphoma was more common than lymphoma limited to mucosa (Figure 1). Epitheliotropism was noted in 3 dogs. In 4 cases of intestinal lymphoma neoplastic cells were also in mesenteric lymph nodes with further involvement of omentum in 1 case and liver and spleen

in another case. In 6 dogs in which lymphoma was not detected in the intestine, there were mass-like lesions in the oral cavity (2 dogs), involvement of the liver and spleen (2 dogs) and nodular lesions in the mesentery and mesenteric lymph node (1 dog each). Intestinal lymphoma in dogs reportedly is an aggressive cancer with short median survival time of only 2-10 weeks. Colorectal site may be more favorable than small intestinal site (Gieger, 2011; Valli, Bienzle & Meuten, 2016).

In the study period, there were 14 cases of lymphoma involving mucocutaneous tissues. Consistently with reports in literature, most cases

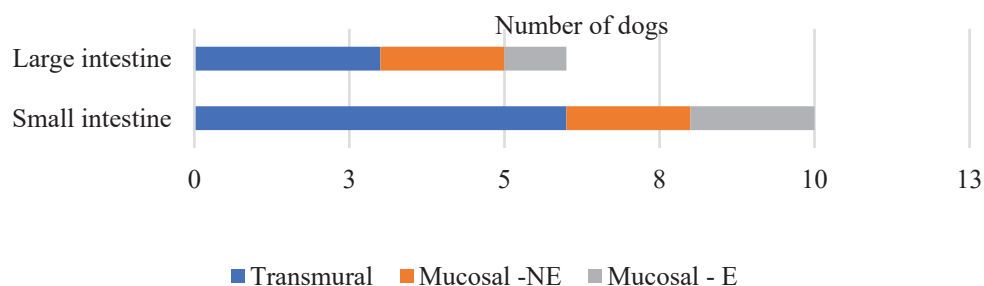


Figure 1. Characteristics of intestinal lymphoma (n=15) in surgical biopsy submissions from Latvian dogs (2011-2020). In one case epitheliotropic lymphoma was located in small and large intestine of the same dog.

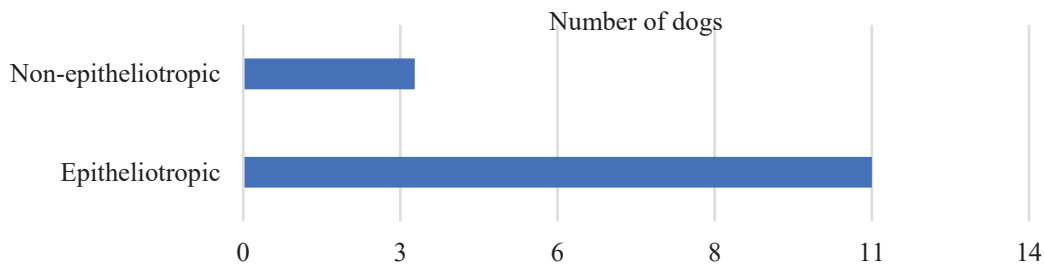


Figure 2. Characteristics of mucocutaneous lymphoma (n=14) in surgical biopsy submissions from Latvian dogs (2011-2020).

were epitheliotropic lymphoma (n=11) (Figure 2). Mucocutaneous epitheliotropic lymphoma is typically a T-cell lymphoma presenting as chronic multifocal skin and mucocutaneous tissue disease with papules, nodules, erythema, erosions and ulcers (Chan, Frimberger, & Moore, 2018; Fontaine *et al.*, 2009). Epitheliotropic lymphoma can mimic autoimmune diseases, dermatophytosis and other inflammatory skin diseases. Initially, the disease is limited to the skin, but later in the disease, lymphadenopathy, leukemia and involvement of internal organs can occur. The disease has waxing and waning course, but it is always progressive over the period of months or years.

Conclusions

This retrospective study of lymphoma in dogs in Latvia confirmed that it is a heterogenous disease similar to that reported elsewhere. Increase in the prevalence of lymphoma in surgical biopsy submissions over the ten year period was not observed. Each year diagnosis of lymphoma constituted on average 2.3% of cases fluctuating from 1.1 to 4.4% of surgical biopsy submissions. The age and sex distribution were similar to previously reported with most affected dogs being middle aged (median 8 years; range 2-13 years), with a slight male predominance (58%). Among pure breed dogs, Rottweilers, American Staffordshire terriers and French bulldogs were top 3 breeds representing together almost 20% of cases. Multicentric lymphoma was predominant anatomical type of lymphoma with alimentary and mucocutaneous lymphoma together forming about third of the cases. Our study showed that even without routine use of IHC, histopathological examination can provide useful information to the clinicians especially in regards to distinguishing indolent lymphomas with a slow disease course from aggressive lymphomas with a short disease course. We recommend that all large to intermediate type of lymphomas should be further characterized by IHC, so more accurate prognosis could be provided to the animal owners. We conclude that diagnostic investigation of potential lymphoma

cases is very important for practice of evidence-based medicine and for demystification of lymphoma in dogs, especially since this disease can be treated with chemotherapy.

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