Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado Integrado em Medicina Veterinária

Relatório de Estágio

Small Animal Clinic and Surgery

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Orientador(es) | Nuno Miguel Lourenço Alexandre

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O relatório de estágio foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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Abstract – Small Animal Clinic and Surgery

As part of the last step in concluding the Master's degree in veterinary medicine, the current report was carried out. It is divided in three parts. The first part includes statistics regarding pathologies or symptoms of animals brought to Priory Veterinary Surgeons during the traineeship, with brief detailing of a disease or procedure relevant to each clinical and surgical area of small animal medicine. The second part is a review of available literature regarding canine non-Hodgkin lymphoma addressing its aetiology, diagnosis, immunophenotypes, presentations, therapeutics and prognosis. The third and last part is a description of a canine lymphoma case in a 9-year-old border collie with data regarding the diagnosis, management and the treatment protocol.

Keywords: Medicine, Surgery, Small Animals, Diagnostic, Treatment

Resumo – Clínica e Cirurgia de Pequenos Animais

O seguinte relatório foi elaborado como parte da última etapa para completar o mestrado em medicina veterinária. Está dividido em três partes. A primeira parte inclui a estatística das patologias ou sintomas dos animais que foram levados à Priory Veterinary Surgeons durante o período de estágio curricular, com um abreve revisão de uma doença ou procedimento relevante a cada área da clínica e cirurgia de medicina de animais de companhia. A segunda parte é uma revisão da literatura disponível sobre linfoma não-Hodgkin canino abordando a sua etiologia, diagnóstico, imunofenótipo, manifestação clínica, tratamento e prognóstico. A terceira e última parte é uma descrição de um caso de linfoma não Hodgkin canino de um border collie de 9 anos com dados sobre o diagnóstico, acompanhamento e tratamento.

Palavras-chave: Clínica, Cirurgia, Animais de Companhia, Diagnóstico, Tratamento

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ACE: Angiotensin-converting enzyme	**ICC:** Immunocytochemistry
ACTH: Adrenocorticotropic hormone	**IgE:** Immunoglobulin E
ACVP: American College of Veterinary Pathologists	**IgH:** Immunoglobulin heavy chain
ALP: Alkaline phosphatase	**IHC:** Immunohistochemistry
ALT: Alanine transaminase	**ILC:** Innate lymphoid cells
ASIT: Allergen-specific immunotherapy	**IM:** Intramuscular
AST: Aspartate transaminase	**IOP:** Intraocular pressure
ATP: Adenosine triphosphate	**IV:** Intravenous
BE: Base excess	**LCPD:** Legg-Calvé-Perthes disease
BSA: Body surface area	**LLLT:** Low-level laser therapy
BSAVA: British Small Animal Veterinary Association	**MALT:** Mucosa associated lymphid tissue
BUN: Blood urea nitrogen	**MCHC:** Mean corpuscular haemoglobin concentration
CD: Cluster of designation	**MCV:** Mean corpuscular volume
CDR: Complementarity determining region	**MDA:** Maternal derived antibodies
CHF: Congestive heart failure	**MHC:** Main histocompatibility complex
CIBDAI: Canine IBD Activity Index	**MLV:** Modified live virus
CK: Creatine kinase	**MZL:** Marginal zone lymphoma
cL: canine lymphoma/lymphomas	**NFNFIHD:** Nonflea, nonfood-induced hypersensitivity dermatitis
CNS: Central nervous system	**NK cells:** Natural killer cells
cPL: Canine pancreatic lipase	**PARR:** PCR for antigen receptor rearrangements
cPLI: canine pancreatic lipase immunoreactivity	**pCO2:** Carbon dioxide partial pressure
DCU: Deep cortical unit	**PCR:** Polymerase chain reaction
DGGR: ,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester	**PCV:** Packed cell volume
DLBCL: Diffuse large B cell lymphoma	**PO:** per os
ECG: Eletrocardiogram	**pO2:** Oxigen partial pressure
EGE: eosinophilic gastroenteritis	**PTCL-NOS:** Peripheral T cell lymphoma not

	otherwise specified
ELISA: Enzyme-linked immunosorbent assay	**qPCR:** Quantitative polymerase chain reaction
FASS: Feline atopic skin syndrome	**RBC:** Red blood cells
FC: Flow cytometry	**REAL:** Revised European-American Lymphoid neoplasms classification
FCEAI: Feline Chronic Enteropathy Activity Index	**RHD:** Rabbit viral haemorrhagic disease
FCV: Feline calicivirus	**RLN:** Regional lymph nodes
FHNO: Femoral head and neck ostectomy	**SC:** Subcutaneous
FHV: Feline herpes virus	**SCC:** Squamous cell carcinoma
Fi: Absolute frequency	**SID:** once daily
FISS: Feline injection site sarcoma	**T3:** Triidothyronine
FNA: Fine needle aspiration	**T4:** Thyroxine
FPV: Feline panleukopenia virus	**TCRγ:** T cell receptor gamma
Fr: Relative frequency	**TgAA:** Thyroglobulin autoantibody
FT4: Free thyroxine	**T-LBL:** T cell lymphoblastic lymphoma
GLDH: Glutamate dehydrogenase	**TP:** Total protein
GST: Glutathione-S-transferase	**TSH:** Thyroid-stimulating hormone
GSTT: GST theta	**TT4:** Total thyroxine
Hb: Haemoglobin	**TZL:** T zone lymphoma
HCM: Hypertrophic cardiomyopathy	**WBC:** White blood cells
HCO3: Bicarbonate	**WHO:** World Health Organization
IBD: Inflammatory bowel disease	**WSAVA:** World Small Animal Veterinary Association

Part I – Casuistry

1. Introduction

The present internship report was written following a 5-month internship, beginning in mid-September 2020 and lasting until end of January 2021, at the Reigate branch of Priory Veterinary Surgeons, a mixed small animal and equine practice in the United Kingdom.

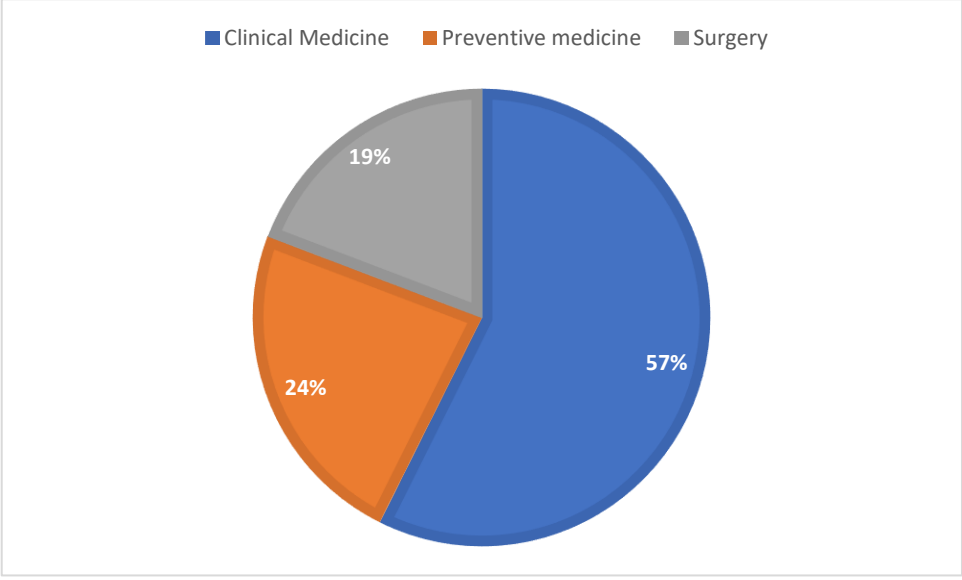
The thesis is divided in three parts. The first part is a detailing of the casuistry and activities developed during the traineeship with a brief review of a pathology or procedure of interest in each field. The second part, a review of available literature regarding canine non-Hodgkin's lymphoma. The third part is a detailing of a clinical case about a Henry, a dog with diagnosed with non-Hodgkin's lymphoma.

It should be considered this data does not reflect the normal casuistry of the practice as not every case was recorded, there were less clients due to the exceptional restrictions imposed during the Covid-19 pandemic, it was impossible to follow up every case, the same animal might have been counted multiple times and many animals had multiple conditions or owners had diverse worries. Unfortunately, due to a malfunction in the mobile app the data was being recorded a great deal of data was lost.

2. Case distribution by 3 main areas of veterinary medicine

A total of 380 cases were recorded, 57% (218) fell into the area of clinical medicine, 24% (89) represent preventive medicine and lastly 19% (73) were surgery (graphic 1). The animals recorded were dogs (*Canis lupus familiaris*), cats (*Felis catus*) and exotic were rabbits (*Oryctolagus cuniculus* domesticus), ducks (*Anas platyrhynchos* domesticus) and guinea pigs (*Cavis porcellus*).

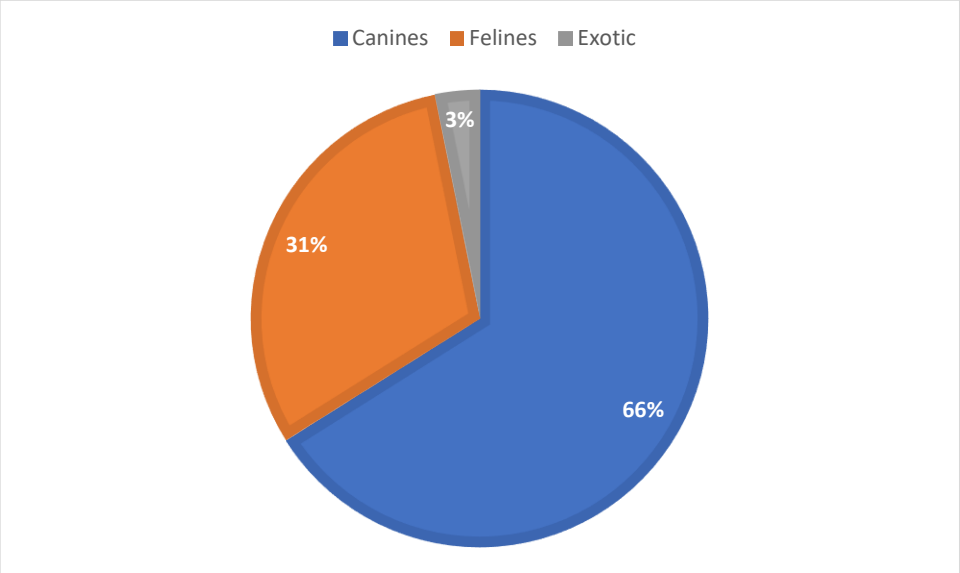
Graphic 1. Percentage of cases divided by the 3 main areas of veterinary medicine



2.1. Case distribution regarding species

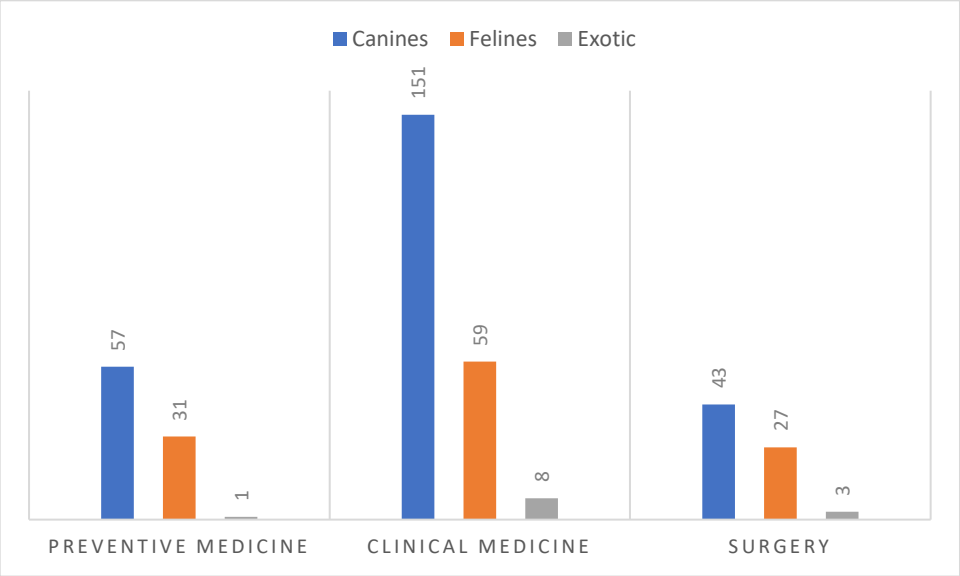
The distribution of cases is as follows: Canines made up 66% (251) of all cases, felines 31% (177) and exotic species only about 3% (12) (graphic 2).

Graphic 2. Percentage of cases by species



The area with most cases overall was clinical medicine (graphic 3). Preventive medicine has the second most cases in feline and canine patients followed by Surgery with the least cases. However, in exotic patients, preventive medicine had fewer cases than surgical medicine.

Graphic 3. Case distribution regarding species and clinical area



2.2. Case distribution regarding preventive medicine, clinical medicine and surgery

The cases were distributed among preventive medicine, 11 clinical areas with an additional section on clinical procedures, and surgery. Each section has a corresponding table with recorded diseases that required treatment. Each table has the absolute frequency (Fi) and relative frequency (Fr) of canine, feline and exotic cases.

Relative frequency is shown in percentages with a single decimal place, and it's total is corrected to 100%, as there are certain cases where values do not add up to 100% due to approximations.

In every section a relevant topic, chosen according to the author's interest, will be briefly addressed.

2.2.1. Preventive Medicine

Preventive medicine encompasses acts such as microchipping, vaccinations, worming and officialising legal documentation, mainly issuing or updating passports. These acts ensure that public health standards are not broken by preventing epidemics between animals and possible zoonosis. Since most of these acts are done in a regular schedule, they can be a great opportunity to further the relationship between clinician and owner/patient and allows the clinician to detect any early disease signs that the owners might not be aware of. The most frequent acts were vaccinations (77 cases) as seen in table 1.

Table 1. Case distribution regarding Preventive Medicine

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Vaccination	49	86%	27	87%	1	100%	
Microchipping	0	0%	2	6.5%	0	0%	
Elective check-ups	8	14%	2	6.5%	0	0%	
Total	57	100%	31	100%	1	100%	89

Due to Covid-19, to minimize contact, most 6-month check-ups were done in conjunction with booster vaccination. Microchipping was also mostly done in conjunction with other procedures. Non-core vaccines (like the kennel cough vaccine) were almost always, except for rare exceptions, administered at the time of booster vaccination. There were no passport issuances recorded most likely due to heavy travel bans during the pandemic. The vaccination protocol followed at Priory Veterinary Surgeons is similar to the one described in Tables 2 and 3.

Check-ups consisted of a general physical examination, heart and respiratory rate auscultation, temperature measurement and sometimes blood sample collection for analysis, to assess the presence of any early clinical signs of potential diseases.

Vaccines can be considered core vaccines or non-core vaccines. Core vaccines are those which cats and dogs should receive no matter the circumstances. The reason being core vaccines immunize animals against life-threatening diseases that have global distribution. For dogs, core vaccinations include immunization against canine distemper virus, canine adenovirus, and canine parvovirus type 2. As for cats, core vaccinations include feline calicivirus (FCV), feline herpesvirus-1 and feline parvovirus. In countries in which rabies is endemic, rabies vaccination is considered to be part of the core vaccination protocol. (Day *et al.* 2016). In Portugal, the rabies vaccine is mandatory for all dogs (with a tri annual booster) and optional in cats (‘Portaria 264/2013, 2013-08-16’ 2013) as opposed to the UK where it is not. The rabies vaccine is usually a requirement for dogs travelling across countries. Non-core vaccines are those which are required to be administered when animals are at risk of certain infections due to geographical location, lifestyle, and environment.

According to the World Small Animal Veterinary Association (WSAVA) guidelines vaccines can also be considered as infectious or non-infectious in nature.

Infectious vaccines or modified live virus (MLV) vaccines contain attenuated forms of certain organisms. Even with their virulence weakened, such organisms are still able to replicate and produce a small level of infection without significant clinical signs or tissue damage, thus inducing immunity. Administered parenterally or directly to mucosal sites, like intranasal or oral vaccines, infectious vaccines are very effective at inducing a robust immune response, in case of parenteral administration they induce a strong cell-mediated and anti-body mediated immunity and administered to mucosal sites they induce a strong mucosal immunity. They are especially effective in animals without maternal derived antibodies (MDA) inducing immunity with just a single dose (Day *et al.* 2016).

Non-infectious vaccines or killed core vaccines contain a dead or inactivated organism that still presents intact antigens. They can also contain a natural or synthetic antigen derived from such organisms, or even DNA that codes the antigens these organism would produce. The agents contained in these vaccines do not have the ability to replicate or induce any pathology or signs of disease. Due to their nature, non-infectious vaccines do not produce an immune response on the level of infectious vaccines, requiring adjuvants to have increased potency and even multiple doses do induce protection. They are administered parenterally and in contrast to parenteral infectious vaccines, cell-mediated and antigen-mediated immunity are less likely to be induced (Day *et al.* 2016).

Both puppies and kittens have passive immunity granted to them by maternal derived antibodies (MDA), until they reach 8-12 weeks of age, when MDA immunity will then have waned enough to begin active immunization. For both species, initial core vaccination is recommended to start at 6-8 weeks of age and then every 2-4 weeks until the puppy or kitten is 16 weeks old or older depending on the timeframes. Another integral part of the core vaccination of puppies and kittens would be the 1-year-old booster vaccine, which traditionally, has been set to make sure any animal that has not had a successful immune response up to this point will now be able to do so. Even though this "booster" has been set up to 1 year (52 weeks) as to coincide with the well-known 1 year check-up, recent re-evaluations of vaccination protocols have started to try and push this vaccine to 26 weeks of age as to reduce the previously mentioned window of susceptibility (Day *et al.* 2016).

Dogs that have responded well to infectious core vaccines will have an immunological memory that allows protections for a few years in the absence of repeat vaccination. After the 26- or 52-weeks booster, subsequent vaccinations have a 3-year interval between. This triennial vaccination does not apply to killed core vaccines (rabies being an exception) nor non-core vaccines, and vaccines containing bacterial antigens, thus requiring more frequent vaccination (yearly) for active protection. As such, adult dogs are vaccinated yearly, alternating between components, core vaccines are are given every 3 years and non-core vaccines are given annually. Core and non-core vaccines are listed in table 2. Adult dogs that missed the date of their core vaccination booster but completed their puppy vaccination course or dogs of unknown vaccination status, only need a single dose of a modified live virus vaccine to achieve full immunity (Day *et al.* 2016).

Table 2. Canine core and non-core vaccination and timings.

Vaccination		Weeks of age						Years of age
		3	8	12	16	26	52	
Core Vaccination	Canine Parvovirus-2		✓	✓	✓	✓	✓ (If not given at 26 weeks)	Triennial Booster Vaccination
	Canine Distemper Virus		✓	✓	✓	✓	✓ (If not given at 26 weeks)	
	Canine Adenovirus-2		✓	✓	✓	✓	✓ (If not given at 26 weeks)	

	Rabies (endemic countries or required by law)			✓			✓	Triennial or annual Booster (depends on availability)
Non-core Vaccination	Parainfluenza Virus	✓					✓	Annual Booster Vaccination
	Bordetella bronchiseptica	✓					✓	
	Leptospira		✓	✓			✓	
	Borrelia							
	Leishmania					✓		

Adult cats with a successful core vaccination protocol will have a strong immunity against feline panleukopenia virus (FPV) in case of any missing repeat vaccination but in contrast, immunity against feline calicivirus (FCV) and feline herpes virus type 1 (FHV-1) is only partial. The guidelines recommend a triennial FPV vaccine and annual FCV and FHV-1 vaccine for high-risk cats (cats with lifestyles that allow more contact with other cats). Regarding killed core vaccines (except for rabies) and non-core vaccines, especially containing bacterial antigens, these should be administered annually, if necessary, to guarantee minimal protection. Core and non-core vaccines are listed in table 3. Adult cats missing regular vaccination but with a completed kitten core vaccination protocol (FPV, FHV-1 and FCV), only need a single dose of MLV vaccine to achieve full immunity. Cats of unknown vaccination status, can achieve full immunity by a single MLV FPV vaccine and 2 doses of MLV FHV-1 and FCV vaccine (2-4 weeks apart) (Day *et al.* 2016).

Table 3. Feline core and non-core vaccinations and timings.

Vaccination		Weeks of age						Years of age	
		4	8	12	16	26	52	High risk	Low risk
Core Vaccination	Feline Calicivirus		✓	✓	✓	✓	✓ (If not given at 26 weeks)	Annual Booster	Triennial Booster
	Feline Herpesvirus-1		✓	✓	✓	✓	✓ (If not given at 26 weeks)		
	Feline Parvovirus		✓	✓	✓	✓	✓ (If not given at 26 weeks)	Triennial Booster	
	Rabies (endemic countries or required by law)			✓			✓		

Non-core Vaccination	Feline leukaemia virus		✓	✓			✓	Annual Booster	Not recommended
	Chlamydia felis		✓	✓			✓		
	Bordetella bronchiseptica	✓					✓		

Unlike dogs, cats can develop a disease from vaccine administration usually referred to as feline injection site sarcoma (FISS). The pathogenesis is believed to be that a localized chronic inflammation will trigger malignant transformations of mesenchymal cells, with some possible genetic basis. These tumours, infiltrative by nature, require very radical surgical removal. The consensus on administration is that vaccines should be administered distally in the hindlimb, because in case FISS develops there is enough removable tissue (Day *et al.* 2016).

The recommended immunization protocol for rabbits by the British Small Animal Veterinary Association (BSAVA) is annual vaccination against myxomatosis and Rabbit Viral Haemorrhagic Disease (RHD) caused by RHDV-1 and RHDV-2, either because of environmental risks or because of individual practice's advice. Currently available vaccines immunize against myxomatosis and RHDV-1 simultaneously, RHDV-1 and RHDV-2 simultaneously and RHDV-2 solely. There is also a vaccine that covers myxomatosis and both RHDV-1 and RHDV-2. If the protocol includes the myxomatosis/RHDV-1 and RHDV-1/RHDV-2 vaccines, they should be given 2 weeks apart (Meredith 2014; BSAVA 2020).

The vaccination protocol for ferrets includes immunization against rabies if travelling to rabies endemic a country (BSAVA 2020).

2.2.2. Clinical Medicine

2.2.2.1 Dermatology

Dermatology is the area of medicine responsible for diagnosing and treating disorders of the skin and annexes. The most common disorder was atopy/allergy as seen in table 4.

Table 4. Case distribution regarding Dermatology.

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Atopy/Allergy	7	5.3%	0	0	0	0%	
Mastocytoma	1	8.3%	0	0	0	0%	
Discoid lupus erythematosus	1	8.3%	0	0	0	0%	
Seborrhea sicca	1	8.3%	0	0	0	0%	
Histiocytoma	1	8.3%	0	0	0	0%	
Feline atopic skin syndrome	0	0%	1	100%	0	0%	
Laceration	1	8.3%	0	0	0	0	
Total	12	100%	1	100%	0	0%	13

Feline atopic skin syndrome (FASS) and low-level laser therapy in the healing of cutaneous wounds

Feline atopic skin syndrome (FASS) also sometimes referred to as feline atopic dermatitis is a dermatological disease characterized by the manifestation of multiple cutaneous reaction patterns as a result of a hypersensitive reaction to a specific environmental allergen (Bajwa 2018; Santoro *et al.* 2021).

FASS appears to be more commonly reported in female cats than male cats (Hobi *et al.* 2011; Santoro *et al.* 2021) and some breeds have been reported to be predisposed, like the Abyssinian that is disproportionately over-represented in studies regarding allergy in cats which is an indicative of possible heritability factors in FASS (Ravens, Xu, and Vogelnest 2014; Vapalahti *et al.* 2016). Clinical signs can be influenced by season as some cats experience flare-ups during certain seasons and then recover on the rest of the year (Santoro *et al.* 2021).

The pathogenesis of FASS is unknown but T lymphocytes, dendritic cells and an increase of cytokines and immunoglobulin E (IgE) have all been detected in FASS cases (Bajwa 2018; Halliwell *et al.* 2021). However the roll of IgE in the disease is not as evident as in the dog for example, and as a result, the term atopic dermatitis is thought to be outdated and feline atopic skin syndrome or nonflea, nonfood-induced hypersensitivity dermatitis (NFNFIHD) has been proposed (Halliwell, Gilbert, and Lian 1998; Santoro *et al.* 2021). For the purpose of this thesis the nomenclature adapted is feline atopic skin syndrome or FASS.

The four most common cutaneous patterns found in cats with FASS are miliary dermatitis, self-induced alopecia/hypotrichosis, face, head and neck pruritus and eosinophilic granuloma complex. These patterns can manifest singularly or in combination but the most prevalent in cats seem to be self-inflicted alopecia/hypotrichosis and face, head and neck pruritus. All of these patterns cause varying degree of pruritus that can lead to self-injury and self-perpetuating wounds (excoriations, erosions and ulcerations) (Hobi *et al.* 2011; Santoro *et al.* 2021).

The diagnosis of FASS is of exclusion which means other causes for the cutaneous reaction patterns must be ruled out first before it can be attributed to FASS. Additionally there is no diagnostic tool specific for FASS, making the history of the clinical signs coupled with exclusion of other causes the most accurate method of diagnosis (Santoro *et al.* 2021).

Differential diagnoses that are important to exclude before diagnosing FASS are food allergy, flea allergy dermatitis, parasitosis like fleas, *demodex gatoi* and *notoedres cati*, fungal infections like *malassezia*, viral diseases like herpesvirus and calicivirus, neoplasia like lymphoma and mast cell tumours, pemphigus foliaceus and even reactions to medication (Bajwa 2018; Santoro *et al.* 2021).

Allergen testing either with intradermal or immunoglobulin E (IgE) serology testing is recommended when a FASS diagnosis has been reached and allergen-specific immunotherapy (ASIT) has been chosen as a treatment method (Santoro *et al.* 2021). Allergen testing should be exclusively used in confirming a diagnosis and selecting the antigens for ASIT (Rees 2001; Bajwa 2018). ASIT is the only specific therapy for FASS as it changes the pathogenic mechanisms leading to a decrease in serum IgE and reduced symptoms and seems to be very efficacious (Mueller 2019; Mueller *et al.* 2021). However, results can only be evaluated after 12 months of treatment and during the first few weeks anti-pruritic medication might need to be administered (Mueller 2019).

A systematic review of treatment modalities for cats with FASS recommends ciclosporin and systemic glucocorticoids as the most efficacious treatment methods available. Doses of 1.4-1.5mg/kg daily of methylprednisolone was found to be effective as a starting dose which needs to be tapered down once symptoms are in remission, however, multiple parameters of glucose metabolism were found to be commonly altered, for which regular monitoring is recommended. Oral and topical forms of glucocorticoids can be effective but the since these are not licensed for use in felines monitoring is advised (Mueller *et al.* 2021).

Ciclosporin at doses of 7mg/kg daily were found to be effective in inducing remission of reaction patterns (Mueller *et al.* 2021) and most cats tolerate tapering down dosing frequency to twice a week (Steffan *et al.* 2013; Roberts *et al.* 2016).

Low-level laser therapy (LLLT) is an adjunct therapy modality employed in many types of lesions with the goal to reduce pain, inflammation, scarring and healing time by making use of a laser (red light and near-infrared lights). Due to its wavelength, the laser does not produce an effect by heating the tissues but rather by the direct effect of radiation on the tissue (Kurach *et al.* 2015; Perego *et al.* 2016).

The mechanisms by which LLLT produces positive effects on target tissues are still not well studied but the strongest hypothesis is a photobiostimulation effect. Red or near-infrared lights are absorbed by cytochrome oxidase resulting in the increased production of adenosine triphosphate (ATP) which in turn will increase the production of proteins involved in cellular repair, promoting cellular growth and differentiation, and the synthesis of prostaglandins, resulting in a decrease in pain, inflammation and oedema (Kurach *et al.* 2015; Perego *et al.* 2016).

Even though *in vitro* and other studies have revealed significant positive changes like increased collagen synthesis, increased tensile strength and smaller wound sizes (Perego *et al.* 2016), many studies still cannot replicate these effects, making it very difficult to ascertain if LLLT actually takes an important role in wound healing. For example,(Michael D. Lucroy, Edwards, and Madewell 1999) reports a successful treatment of a nonhealing chronic wound, and in contrast (Schlager et al. 2000) concludes that there was no significant difference in the healing periods of the dogs treated with LLLT, conservative treatment and the control group.

At Priory Veterinarian Surgeons, the author had the opportunity to experience a case compatible with FASS and treated with LLT. The cat had history of chronic cutaneous wounds around the neck and the nose areas accompanied by intense pruritus. Reaction to an unknown environmental allergen was suspected to be the cause as food allergy trials had been done and no food allergen was detected. The wounds would heal very slowly or make no progress at all with regular medical treatment, however, noticeable progress (wounds reduced in size, less tissue inflammation and less discomfort) was made between appointments, which was confirmed by the positive response the owners communicated.

2.2.2.2 Ophthalmology

Ophthalmology is the branch of veterinary medicine that deals with diagnosis and treatment of ocular disorders. The most common disorders were suspected bacterial infections (7 cases), corneal ulceration (4 cases) and glaucoma (3 cases) as seen in table 5.

Table 5. Case distribution regarding Ophtalmology.

	Canine		Feline		Exotic	
	Fi	Fr	Fi	Fr	Fi	Fr
Glaucoma	3	30%	0	0%	0	0%
Ocular discharge (suspected bacterial infection)	4	40%	3	60%	0	0%
Uveitis	2	20%	0	0%	0	0%
Corneal Ulceration	1	10%	0	0%	3	100%
Subconjunctival	0	0%	1	20%	0	0%

haemorrhage							
Horner's syndrome	0	0%	1	20%	0	0%	
Total	10	100%	5	100%	3	100%	18

Canine glaucoma

The glaucoma is an ophthalmologic disease englobing a variety of pathological changes in the eye due to a singular or multiple ocular disorders, resulting in the elevation of the intraocular pressure (IOP), potentially leading to partial or total loss of vision (Renwick 2014). Even though there are other important risk factors that can lead to the occurrence of clinical disease, like age, breed, sex and systemic blood pressure (Pizzirani 2015), IOP is the most consistent major risk factor across all breeds of dogs, and the primary factor affecting the optic nerve (Gelatt, Gilger, and Kern 2013).

The glaucoma in dogs can be classified according to their type of cause (primary, secondary, congenital), the gonioscopic appearance of the filtration angle (width of the iridocorneal angle and opening of the ciliary cleft), and the duration of disease (early non congestive, acute/ non congestive, chronic/end stage). Glaucoma should be described using all 3 types of classification in order to present in more detail each animal's case and thus a better treatment/management protocol.(Pizzirani 2015)

IOP elevation can be due to various causes. In primary glaucoma it is only affected by defects in the drainage of the globe, either by metabolic abnormalities or physical obstruction in the outflow system. In secondary glaucoma, IOP elevates due to mechanical blockage of the outflow system by simultaneous ocular diseases. Lastly in congenital glaucoma, increases in IOP are closely tied to anomalies in the anterior chambers and begin shortly after the animal's birth. Primary and secondary glaucoma make up most of the cases of glaucoma found in dogs (Gelatt, Gilger, and Kern 2013)

There are two important tools for glaucoma diagnosing, tonometry and gonioscopy. Tonometry allows the clinician to determine the IOP by using a specific tool known as tonometer. Since increased IOP is present in almost all cases of glaucoma, tonometry should be performed every time glaucoma is suspected, and in monitoring of already diagnosed cases. Gonioscopy allows the clinician to visualize the opening of the ciliary cleft with the aid of a special contact lens (gonio lens) and detect possible abnormalities. This should be performed in case increase in IOP is not apparent and in both eyes, as the affected eye may not be suitable for gonioscopy and in these cases the contralateral eye can give information on possible causes and probability of contralateral appearance of glaucoma (Renwick 2014).

Many different clinical signs can point to a possibility of glaucoma. Pain, corneal oedema and vascularization, episcleral congestion and vision loss may be found in acute cases of glaucoma. Globe enlargement or even *phthisis bulbi* (reduction of the globe's size), corneal and lens changes, and retinal and optic nerve atrophy are common findings in chronicity (Renwick 2014).

The treatment for glaucoma is difficult because there is no way to reverse the damage done to the optic nerve nor to correct aqueous humour outflow defects in primary glaucoma and removing the pre-occurring diseases in secondary glaucoma does not rule out the possibility of glaucoma reappearing in the future. As so, glaucoma treatment consists mainly of topical and systemic medication to keep IOP below 20 mmHg. In case of failure to maintain acceptable levels of IOP, patients may be considered good candidates for surgery (Gelatt, Gilger, and Kern 2013).

Reduction of IOP can be achieved using pharmacological agents which reduce aqueous humour production, increase aqueous humour outflow, or do both (Gelatt, Gilger, and Kern 2013).

Osmotic diuretics are effective in cases of acute glaucoma with very high IOP (over 40 mmHg), intravenous mannitol (1-2 g/kg) over the course of 30 minutes is widely used. Carbonic anhydrase inhibitors reduce the aqueous humour production resulting in a very drastic lowering of the IOP (up to 50%), brinzolamide is well tolerated as a topical agent for most dogs and can be administered 2-3 times a day. Topical prostaglandin analogues, like latanoprost or travoprost, are usually used in combination with carbonic anhydrase inhibitors. In case of primary open-angle glaucoma miotics, like pilocarpine, were only used in cases where drugs like latanoprost and brinzolamide have been proved to be safer, and as such, are now rarely used. Beta-adrenergic blockers, like timolol maleate, are used in multimodal combinations with a carbonic anhydrase inhibitor (Renwick 2014).

There are several surgical options. Lens extraction can be performed in cases of primary lens luxation, laser cyclophotocoagulation (coagulative necrosis of ciliary tissue), and though less used, cyclocryotherapy (freezing of the ciliary body) can reduce aqueous production. Drainage implant surgery can help with aqueous humour outflow. Of all these techniques, drainage implant surgery and cyclophotocoagulation present the best results in IOP management for medium and long-term management. If all therapy attempts fail, both medical and surgical, enucleation may be indicated, unilaterally or bilaterally (Renwick 2014).

2.2.2.3. Cardiopulmonology

Cardiopulmonology refers to the area of medicine that is responsible for diagnosing and treating disorders of the cardiopulmonary system. The most common disorders were pulmonary effusion and feline aortic thromboembolism (both with 2 cases) as seen in table 6.

Table 6. Case distribution regarding Cardiopulmonology.

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Myxomatous Mitral Valve Disease	1	100%	0	0%	0	0%	
Feline Aortic Thromboembolism	0	0%	2	40%	0	0%	
Hypertrophic Cardiomyopathy	0	0%	1	20%	0	0%	
Pulmonary Effusion	0	0%	2	40%	0	0%	
Total	1	100%	5	100%	0	0%	6

Hypertrophic cardiomyopathy (HCM) and congestive heart failure (CHF) in felines

Hypertrophic cardiomyopathy is a disease of the myocardium characterized by increased myocardial mass (myocardial hypertrophy) of a non-dilated ventricle (Abbott 2010). Congestive heart failure arises by progression of HCM (Côté *et al.* 2011).

The genetic mutations that affect encoding of proteins related to the contractile function of myocites are believed to be the cause of HCM in felines. Other etiological factors cannot be excluded, but research points towards HCM being of genetic basis (Abbott 2010). Two genetic mutations associated with feline HCM have been found in the Maine Coon and Ragdoll (Gil-Ortuño *et al.* 2020).

The primary cause of the clinical signs shown by affected cats in early stages of the disease is considered to be diastolic disfunction. When hypertrophic processes start it becomes increasingly difficult for ventricles to fill with the rising pressure caused by hypertrophic myocardial cells. The longer these situations go untreated the higher the risk of pulmonary oedema and pulmonary venous congestion becomes. As HCM progresses both diastolic and systolic dysfunction are present (Abbott 2010).

The main clinical manifestations are heart murmurs and arrythmias which can go unnoticed depending on their severity, thus, when detected during routine examinations, especially in cats around 6 years of age, further tests should be done as these signs point towards HCM and an early diagnosis. Dyspnoea is the most common sign found in cats with heart failure in feline HCM, because of pulmonary oedema or pleural effusion. Sudden death and acute thromboembolism can be is the first clinical manifestation of HCM (Ferasin *et al.* 2003; Abbott 2010).

The diagnosis of primary HCM is one of exclusion. Before diagnosing a cat with HCM, certain disorders need to be ruled out first, as they can be secondary causes of concentric hypertrophy found in HCM. The main differential diagnostics that need to be excluded are hyperthyroidism, systemic hypertension, and acromegaly. After successful treatment for these conditions, hypertrophy reverses after a few months (Côté *et al.* 2011).

Echocardiography is the most useful tool in diagnosing feline HCM, as hearts may appear normal sized in earlier stages of disease, but concentric hypertrophy of the left ventricle wall will be visible. In turn, radiography is used to diagnose CHF in cats with HCM as pulmonary oedema, pleural effusion and cardiomegaly are easily detected in thoracic imaging. Electrocardiogram (ECG) has extremely low accuracy in diagnosing HCM but is the best choice in diagnosing the type of arrythmia present in cats with HCM (Côté *et al.* 2011).

Before treatment, comprehensive blood analysis is useful. Total serum thyroxine can point towards hyperthyroidism if the value is elevated, free thyroxine concentration can give a more accurate representation of clinical thyroid status (Ferguson 2007). A complete blood count is important in detecting anaemia which worsens cardiac function of a cat with HCM. Blood chemistry values establish a baseline that can be compared as treatment proceeds. Lastly urinalysis can indicate signs of renal dysfunction as certain medication used in treating HCM can cause abnormalities like increased blood urea nitrogen and difficulty in concentrating urine (Côté *et al.* 2011).

Unfortunately, there is no treatment that has been shown to prevent or slow down the progression of subclinical HCM. Recently, in human patients with HCM, beta blockers like atenolol have been used and recommended but such recommendations are based on empirical evidence (Abbott 2010; Côté *et al.* 2011). With lack of proven efficacy and certain concerns that atenolol can worsen CHF in cats, atenolol can be used according to clinicians' discretion and experience with the pharmacological agent (Gordon and Côté 2015).

Since there is no therapy that cures HCM, treating this disease is mainly about lifelong management of CHF and its clinical signs. The standard protocol for long-term CHF management is the use of furosemide and an angiotensin-converting enzyme (ACE) inhibitor (Côté 2017). The pros of using furosemide instead of other diuretics is the high efficacy seen and its easily tolerated by most if not all clinically ill cats. The use of ACE inhibitors like enalapril is highly recommended, even though it has not been scientifically proven to actually have

positive effects in cats, and since ACE inhibitors are a very safe drug to use and guarantee longer disease-free periods in dogs and humans they are given to feline patients as well, and lastly they counteract the overstimulation of the renin-angiotensin-aldosterone system that furosemide causes (Gordon and Côté 2015). In clinically stable cats (Gordon and Côté 2015) recommends the use of furosemide at the lowest possible dose and longest dosing interval possible that keeps cats without clinical signs and radiographic evidence in conjunction with enalapril. In the UK the licensed product to treat HCM is Hypercard (diltiazem hydrochloride) ('Hypercard® 10 Mg Coated Tablets for Cats' 2015) although there isn't much scientific evidence to support its use, (Bright *et al.* 1991) shows radiographic data that suggests diltiazem alleviates pulmonary oedema and congestion, nonetheless the drug has fallen in disuse (Gordon and Côté 2015).

Management of acute relapses of CHF is focused on managing dyspnoea, any abnormal fluid accumulations, and cardiac output. Thoracocentesis is very efficient in alleviating breathing difficulties and pain associated with plural effusion. Sedation with buprenorphine promotes relaxation of any stressed cat in conjunction with local analgesics (lidocaine being a drug of choice), pain relief for thoracocentesis procedures. Oxygen supplementation helps reduce breathing efforts. Higher doses of furosemide will reduce hydrostatic pressure in pulmonary capillaries causing a reduction of pulmonary oedema (Ferasin and DeFrancesco 2015).

Prophylactic use of anti-thrombotic drugs like clopidogrel can be helpful in preventing arterial thromboembolism which are a very common complication of heart failure in cats (Ferasin and DeFrancesco 2015).

Pimobendan has seen an increase in usage in rescue cases where it is combined with furosemide and an ACE inhibitor, as well as, in cases of ventricular systolic dysfunction where it is used in combination with furosemide, an ACE-inhibitor and an anti-thrombotic (Gordon and Côté 2015).

Other strategies can be applied to manage CHF in cats, such as, low sodium but highly palatable diets, environmental stress reduction and sleep/resting respiratory rates monitoring at home (Côté 2017).

Prognostics of cats with CHF secondary to HCM can vary widely and be from 90 to almost 600 days, but cats presenting with tachycardia and abnormal left atrial sizes have poorer prognostics (Côté *et al.* 2011).

2.2.2.4. Musculoskeletal System

Musculoskeletal medicine focuses on diagnosing and treating disorders of the musculoskeletal system. The most common disorders were pain in the limbs due to unknown injury (19 cases) and anterior cruciate ligament tears (4 cases) as seen in table 7.

Table 7. Case distribution regarding the Musculoskeletal system.

	Canine		Feline		Exotic	
	Fi	Fr	Fi	Fr	Fi	Fr
Patellar Luxation	2	7.7%	0	0%	0	0%
Legg-Calvé-Perthes disease	1	3.8%	0	0%	0	0%
Anterior Cruciate Ligament tear	4	15.4%	0	0%	0	0%
Ataxia	0	0%	2	50%	0	0%

Arthritis	2	7.7%	0	0%	0	0%	
Limb pain	17	65.4%	2	50%	0	0%	
Total	26	100%	4	100%	0	0%	30

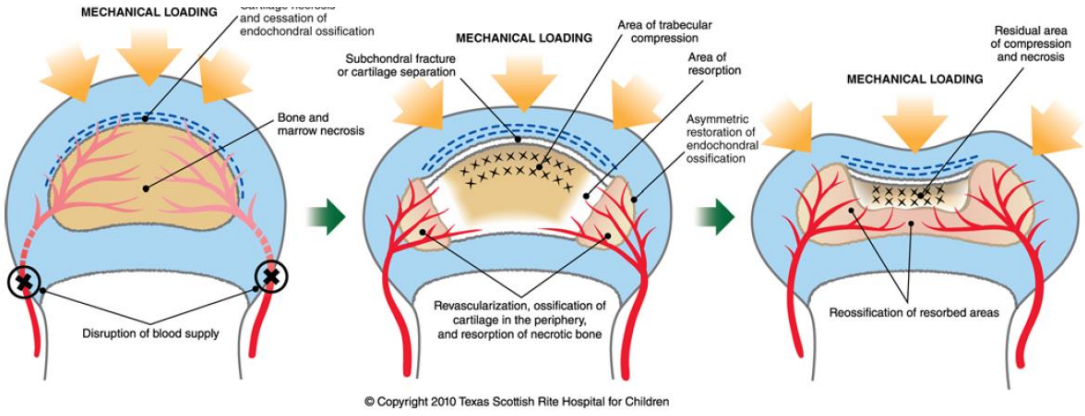
Aseptic necrosis of the femoral head/ Legg-Calvé-Perthes disease (LCPD)

Aseptic necrosis of the femoral head or Legg-Calvé-Perthes disease is a developmental disease mainly found in puppies from small breeds like the chihuahua, dachshund, miniature pinscher, miniature poodle, pug amongst others. Despite being a medium sized breed, the Australian shepherd is also at high risk of LCPD. (LaFond, Breur, and Austin 2002).

The biggest feature and most recognizable characteristic is the ischemic necrosis of the femoral head resulting in cartilage deformation which in turn leads to joint incongruity (figure 1) (Kim 2011). Although the pathogenesis of LCPD is known (figure 1), its initial cause is still unknown, but hypotheses like hereditary factors, hormonal imbalances, anatomical abnormalities, vascular embolisms and breed susceptibility for intracapsular tamponades have been proposed (Aguado and Goyenvalle 2020).

Before the bone growth plates fully close, vascularization of the proximal epiphysis is mainly dependant on synovial vessels along the border of the joint capsule (figure 2). In cases where there is a temporary disruption of the blood supply and no alternative blood flow to the synovial vessels is present, necrosis of the femoral epiphysis ensues which leads to cracks forming along the epiphysis. The bone becomes unable to withstand mechanical stress, microfractures begin to form and cartilage starts being deformed (Houlton and British Small Animal Veterinary Association 2006; Aguado and Goyenvalle 2020).

Figure 1. Proposed pathogenesis of LCPD. Adapted from Kim 2011

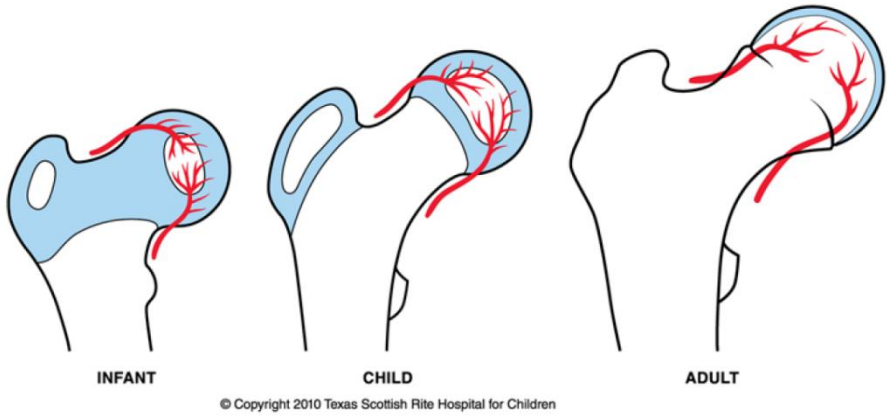


If at clinical examination, a young dog, typically between 4-12 months, from one of the high-risk breeds presents with intermittent unilateral lameness in the hindlimb, LCPD should be suspected and be one of the differential diagnosis, it should be noted that some small percentage of dogs can present non weight bearing in the affected limb instead of lameness. An in-depth examination should be done to detect any signs of gluteal atrophy, hip joint pain or crepitus (mainly during abduction). Nevertheless, hip joint and hindlimb radiography are mandatory for a correct LCPD diagnosis (Aguado and Goyenvalle 2020). A ventro-dorsal extended view radiography should be enough to confirm diagnosis. (Houlton and British Small Animal Veterinary Association 2006).

According to Ljunggren (Ljunggren 1967), 5 grades of LCPD can be visualized in radiographiy. Normal acetabulum, normal contour of femoral head and neck, clearly widened joint space and single or multiple zones of decreased density in the femoral head, as described in grade 1. In grade 2, flattening of the femoral head becomes visible, zones of decreased bone density are more numerous and are also found on the femoral neck, and the antero-lateral aspect of the acetabular rim often has a small spur. In grade 3, the acetabular spur is more pronounced as are the contour deformities. A moderate to pronounced impression of the head can be found with indentations on the articular surface. In grade 4, the normal outline of the head is no longer present. There is evident convergence of large areas of decreased density with normal density only being visible in isolated areas. The only characteristic that differentiates grade 5 from 4 is the fragmentation of the femoral head with discontinuity of the articular surface.

Treatment of LCPD can be conservative or surgical. Conservative treatment is reserved for dogs with lameness and lesser radiographical lesions although it has very low effectiveness. For cases with no success in conservative treatment or more advanced cases of LCPD, surgical treatment is recommended. The standard procedure is resection/ostectomy of the femoral head and neck (FHNO). With FHNO dogs can recover normal movement in about 50% to 80% of the cases (Kim 2011). A more in-depth description of the technique can be found further in the

Figure 2. Irrigation of the femoral head, and bone growth in multiple stages of development. Adapted from (Kim, 2011).



Surgery part of the thesis.

2.2.2.5. Toxicology

Toxicology is responsible for identifying the nature of and treating the effects of chemical substances on the organism. The most common intoxication was ingestion of toxic food items (6 cases) as seen in table 8.

Table 8. Case distribution regarding Toxicology.

	Canine		Feline		Exotic	
	Fi	Fr	Fi	Fr	Fi	Fr
Lily poisoning	0	0%	1	33%	0	0%
Toxic food	6	67%	0	0%	0	0%

ingestion							
Grape intoxication	0	0%	2	67%	0	0%	
Chocolate intoxication	3	33%	0	0%	0	0%	
Total	9	100%	3	100%	0	0%	12

Management of lily poisoning in felines

Lily poisoning in felines requires quick therapy and hospitalization. Lilies are highly toxic to cats and if not treated in the initial hours can lead to grave consequences like renal failure and death (Rumbeiha *et al.* 2004). Many owners do not know the species of plants they have in their homes and the majority do not know lilies are toxic to their pets (Fitzgerald 2010). In-door only cats are more at risk and have higher incidence of intoxication (Panziera *et al.* 2018).

Many species of plants from the *Liliaceae* family are commonly kept as ornamental house plants. The *Lilium* and *Hemerocallis* genera are the groups with devastating nephrotoxic effects for cats. Plants such as the tiger lily, white lily, easter lily and lilies which flowers last one day (“day” lilies) are all part of these 2 groups and many hybrids with other plants (also regarded as lilies even though they are not) exist leading to a variety of toxicological effects (Rumbeiha *et al.* 2004; Fitzgerald 2010) .

The lily-induced nephrotoxic effects have only been documented in cats. The mechanisms by which lilies cause their nephrotoxic effects are unknown as well as the toxic substance and even the exact amount that induces toxicity, but it is known that every part of the plant is toxic. Ingestion of extremely small quantities, like a couple of leaves, has led to death which points towards a very toxic agent or a very concentrated agent. A fast onset of clinical signs (a few hours) after ingestion suggest a rapid absorption rate. The extreme sensitivity to lilies of cats unlike other species, can indicate a production of a feline specific metabolite through a unique feline metabolic pathway, supported by laboratory experiments on direct application of plant components to cultured feline renal tubular cells, which show no toxicity on direct application.

Clinical signs start developing between 1 to 3 hours post-ingestion. Acute renal failure may develop. The first signs are excessive salivation, vomiting, loss of appetite and depressive behaviour. Vomiting will usually subside after 2 to 6 hours after ingestion while depression and anorexia will be constant throughout the whole syndrome. Polyuria will occur after 12 to 30 hours post-ingestion resulting in noticeable dehydration. Polyuria is persistent until the last and worst stage of the syndrome, the anuric phase which develops roughly 24 to 48 hours after ingestion in severe cases. The anuric phase is where most damage to tissues happens consequential to the accumulation of metabolic waste as renal shutdown progresses. In this phase vomiting reoccurs as metabolic waste further intoxicates the animal. Seizures may begin to develop when severe uraemia develops. Deaths typically occur after 3 to 7 days. In a study of six cats, 3 cats had long term complications developing chronic renal failure after months post-ingestion and the other three died or had to be euthanized due to complications (Langston 2002; Panziera *et al.* 2018).

The definitive diagnosis is not easily achievable as the clinical signs can be present in other diseases like infectious renal disease, metabolic renal disease, acute renal failure, acute flare-up of chronic renal failure and other types of intoxication like ethylene-glycol poisoning (Fitzgerald 2010). Without seeing any plant part bitten or vomited and with the misinformation of many owners it is difficult to accurately diagnose lily poisoning but it should always be considered as a differential diagnosis (Fitzgerald 2010).

Blood analysis can be performed to determine if there is a disproportionate increase in blood urea nitrogen (BUN) and serum creatine levels, usually associated to lily poisoning but they are more useful to determine which phase of the syndrome the animal is currently in thus helping in the correct treatment protocol (Langston 2002; Fitzgerald 2010).

The urinalysis can give a great insight to the renal damage that has been caused, as kidney disease can be detected in urine before serological changes can occur (as soon as 12 hours after ingestion). The urine findings clinicians should be looking for are renal casts in urine sediment due to sloughing of renal tubule epithelium (with visible nuclei and cellular detail), isosthenuria with proteinuria and glucosuria (Fitzgerald 2010).

Currently there is not any “gold standard” test to confirm lily poisoning, so suspicion should be supported by visual confirmation ingested plant parts, compatible clinical signs and clinicopathological findings and in cases of death, post-mortem lesions can corroborate the diagnosis. Despite the difficulty of making a specific diagnosis, adequate treatment should be initiated as soon as possible when the probability of lily ingestion is high enough (Fitzgerald 2010).

Treatment depends heavily on the phase of the syndrome. If caught within the initial hours of ingestion, decontamination procedures like emesis and activated charcoal should be performed as they can stop or minimize further intoxication (Fitzgerald 2010).

The fluid therapy is the most crucial part in successfully treating lily intoxication before the animal has the chance of reaching the anuric phase of the syndrome. Starting the animal on 2/3 times maintenance rates for 2 to 3 days can greatly reduce the chances of lethal renal damage. Serum chemistry panels and urinalysis should be performed to determine the current renal damage. Cats that have reached the anuric phase only have peritoneal dialysis or haemodialysis as forms of treatment. These procedures need to be performed for at least 2 weeks or longer. Unfortunately, diuretics such as furosemide and mannitol, have not be proven to be effective once anuria has settled (Langston 2002; Fitzgerald 2010).

The prognosis depends on the progression of renal damage. Cats that have had appropriate treatment in the beginning stages of disease have a better prognosis than cats which have reached anuric statuses even when being treated for anuric renal failure. Renal damage is potentially reversible if the kidneys are given appropriate time and support to heal, though in the following months there can be several complications like urinary tract infections, polyuria, acute renal failure. Prevention is the only way to avoid lily poisoning in cats. Information should be given to owners about plants considered to be lilies and owners should be encouraged to do research on such plant. Enriching the environment cats are in can avoid ingestion of plants due to boredom and planting cat safe plant like catnip or “cat-grass” are useful methods to ensure cats will not consume lilies (Langston 2002; Fitzgerald 2010; Panziera *et al.* 2018).

2.2.2.6. Endocrinology

Endocrinology is the area of medicine focused on diagnosing and treating disorders resulting from hormonal imbalances. The most common disorder was diabetes mellitus (8 cases) as seen in table 9.

Table 9. Case distribution regarding Endocrinology.

	Canine		Feline		Exotic	
	Fi	Fr	Fi	Fr	Fi	Fr
Hypoadrenocorticism	1	12.5%	0	0%	0	0%

Hypothyroidism	2	25%	0	0%	0	0%	
Diabetes Mellitus	5	62.5%	3	100%	0	0%	
Total	8	100%	3	100%	0	0%	11

Canine Hypothyroidism

Hypothyroidism is the most common endocrine pathology found in dogs. Even though it is very common, the diagnosis of the condition is very problematic, mainly due to the fact thyroid hormones have a role in almost every type of tissue, meaning the lowering of triiodothyronine (T3) and thyroxine (T4) will produce very non-specific systematic clinical signs that can progress insidiously (Ferguson 2007).

Hypothyroidism develops when there is a decrease of T3 and T4 circulating through the body. The most common form of disease in dogs is decreased production of T3 and T4 in consequence of thyroid failure (loss of functional thyroid tissue) in cases of thyroiditis or thyroid atrophy. The secondary form of this disease happens when there is a disorder of the hypothalamus or pituitary gland resulting in the decreased production of thyroid stimulating hormone (TSH) (Mooney, Peterson, and British Small Animal Veterinary Association 2012). This is referred to as central hypothyroidism and is very uncommon, accounting for only 5% of cases (Ferguson 2007).

Many non-specific clinical symptoms are present in dogs with hypothyroidism. The most common and the most recognizable to dog owners are the dermatological and metabolic effects. T3 and T4, in most tissues, are responsible for maintaining their metabolic rates and oxygen consumption, thus deficient levels of thyroid hormones will result in slower metabolic rates. Slower metabolic rates, reported in most affected dogs, can result in easily recognizable symptoms like lethargy, weakness, weight gain, exercise intolerance, longer periods of rest post exercise and mental dullness, but signs can also be intermittent and have variable onset making them subtler especially in earlier stages (Scott-Moncrieff 2007).

Since thyroid hormones play a significant role in dermatological health, dogs affected by this disorder will show dermatological changes (reported in 60 to 80% of hypothyroid dogs). Hypothyroid dogs can show signs of bilateral symmetric alopecia and hair failing to grow after clipping because thyroid hormones are thought to be crucial for the initiation of the anagen phase of hair growth. Fur may be easily epilated and have a dull and faded colour, skin can be dry and scaly, hyperkeratosis and hyperpigmentation may appear. Due to a weakening of the skin's defences, secondary infections are more prone to occur (Scott-Moncrieff 2007).

Though in small percentages, neurologic abnormalities have also been documented, mainly older large or giant breed dogs show signs of peripheral neuropathy such as generalized weakness, ataxia, or paralysis. Hypothyroidism can also put dogs at risk of developing cardiovascular abnormalities like dilated cardiomyopathy and reduced left ventricular pump function. Pre-existing cardiovascular conditions may be exacerbated with the appearance of hypothyroidism (Scott-Moncrieff 2007).

Ocular changes like corneal lipidosis and ulceration, uveitis, lipid effusion into the aqueous humour may also appear due to the hyperlipidaemia that develops in hypothyroid dogs (Scott-Moncrieff 2007).

Regarding clinicopathologic changes, 10 to 40% of hypothyroid dogs show a mild nonregenerative anaemia, 75% show fasting hypercholesterolaemia and 88% show hypertriglyceridemia (Scott-Moncrieff 2007).

Hypothyroidism testing should always be done after excluding every other differential diagnosis to make sure no other pathology is responsible for the symptoms the animal presents

with. After excluding other pathologies, the clinician should start thyroid function testing (Ferguson 2007).

First-line testing consists of measuring T4 and TSH concentrations. Low T4 concentrations coupled with increased TSH concentrations are considered diagnostic. Some dogs can present low T4 and normal TSH concentrations or normal T4 and increased TSH concentrations. These require second-line testing such as FT4 and thyroglobulin autoantibody (TgAA) testing (Mooney, Peterson, and British Small Animal Veterinary Association 2012).

FT4 concentrations provide a highly accurate representation of the animal's clinical thyroid status. Direct dialysis is the "gold standard" for measuring FT4 concentrations. It is the most accurate way (95% accuracy) to diagnose hypothyroidism being as it can distinguish animals with low TT4 due to non-thyroidal illnesses from those with hypothyroidism. It has the drawbacks of being expensive and not as fast as analog immunoassays (Ferguson 2007).

Testing for the presence of TgAA is recommended as it a positive test almost always point to an underlying thyroiditis, although dogs positive for TgAA but with no alterations to their levels of T4, FT4 and TSH does not mean the dog has hypothyroidism despite the association between hypothyroidism and lymphocytic thyroiditis and should be scheduled for repeat monitoring instead of starting treatments. A thyroid ultrasound can be useful as a confirmation of thyroid pathology and help in the diagnostic process (Ferguson 2007).

After diagnosis, hypothyroidism treatment consists in the life-long administration of synthetic levothyroxine. Starting doses based on optimal body weight are 20 µg/kg SID or 10 µg/kg BID. The animal should be re-evaluated 1 or 2 months after starting their therapy and according to the clinical response and TT4, the dose may be adjusted. The goal is to achieve euthyroidism and reversal in the clinical signs. Lethargy should be resolved after a couple of weeks, dermatological symptoms should regress after 1 to 4 months and neurological issues should regress gradually and slower than the previous ones (Daminet 2017).

The treated dog should be evaluated every 6 months with measuring of TT4. Time of sampling, medication and meals should be taken into consideration when interpreting TT4 levels. It should be considered that meals will decrease the absorption of levothyroxine and as such administering the medication 2-3 hours before a meal is recommended. Blood samples taken before medication will most likely evaluate duration of action, so it is advised to take blood samples 3-6 hours after medication thus measuring peak concentrations of TT4 (Daminet 2017).

2.2.2.7. Oncology

Oncology is the branch of medicine dedicated to the diagnosis and treatment of neoplasia. The most common neoplasia was lymphoma (3 cases) as seen in table 10.

Table 10. Case distribution regarding Oncology.

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Squamous Cell Carcinoma	0	0%	2	75%	0	0%	
Mammary gland tumour	2	50%	0	0%	0	0%	
Lymphoma	2	50%	1	25%	0	0%	
Total	4	100%	3	100%	0	0%	7

Feline squamous cell carcinoma (SCC) of the nasal planum

Squamous cell carcinoma is the most common neoplasia of the nasal planum and the second most common of the nasal cavity in cats (Lascelles and White 2011), however nasal neoplasia only account for about 8.4% of total reported neoplasia cases (Mukaratirwa, van der Linde-Sipman, and Gruys 2001). It typically affects older cats who are over 10 years of age (Mukaratirwa, van der Linde-Sipman, and Gruys 2001; S. Murphy 2013) and is heavily correlated to continued ultraviolet radiation exposure (Lascelles and White 2011; S. Murphy 2013), as such, non-pigmented tissues, areas with thinner fur and cats with white fur are at risk of developing SCC due to having less protection against ultraviolet radiation (S. Murphy 2013).

The clinical signs typically associated with SCC of the nasal planum are crusting and erythema that gradually evolve into superficial erosions and ulcers which in turn will evolve into deeper lesions (Lascelles and White 2011). Other clinical signs like, sneezing, coughing, nasal discharge, facial deformities and epistaxis can manifest (Mukaratirwa, van der Linde-Sipman, and Gruys 2001; Lascelles and White 2011). Lesions can be confined to the nasal planum, or they can appear on the ear pinnae and eyelids as well. Thickening of the edges of the ear pinnae, thinning of fur and erythema are early signs of actinic changes before cells turn neoplastic (S. Murphy 2013).

The diagnosis of SCC is best achieved with a biopsy of all areas with a lesion, but care should be taken in the biopsy placement, as it might make the margins for a future surgical resection bigger than necessary. Even though SCC is a neoplasia that does not metastasize frequently, if complete staging is required, fine needle aspiration samples from relevant lymph nodes and thoracic radiography should be performed (Lascelles and White 2011; S. Murphy 2013). Radiography and other imaging techniques like magnetic resonance and computer tomography can be used to determine the extent of disease, but SCC has a characteristically diffuse invasion in tissues that the full extent of the disease cannot always be visualized, as such, it is recommended to overestimate the extent when deciding on a treatment method (Lascelles and White 2011).

According to the world health organization guidelines for staging carcinoma of dermal and epidermal origin, complete staging can be seen in table 11 (Owen 1980).

Table 11. SCC staging guidelines. Adapted from Owen 1980

T: Primary Tumour	
Tis	Pre-invasive carcinoma/carcinoma *in-situ*
T0	No evidence of tumour
T1	Tumour under 2cm. in diameter, superficial or exophytic
T2	Tumour 2-5cm in diameter, or with minimal tissue invasion (irrespective of size)
T3	Tumours over 5 cm. in diameter, or with invasion of the subcutis (irrespective of size)
T4	Tumour invading other tissues like muscle, bone and cartilage
N: Regional Lymph Nodes (RLN)	
N0	No evidence of RLN involvement
N1	Movable ipsilateral nodes **N1a**: with no evidence of growth **N1b**: with evidence of growth
N2	Movable contralateral or bilateral nodes **N2a**: with no evidence of growth **N2b**: with evidence of growth
N3	Fixed nodes
M: Distant metastasis	

M0	No evidence of metastasis
M1	Evidence of metastasis

The treatment options available are various, but they can be divided into surgical treatment, radiotherapy and medical therapy. The current recommended treatment methods for nasal planum SCC are cryosurgery, surgical resection, intralesional chemotherapy, photodynamic therapy, radiation therapy and strontium-90 plesiotherapy (Lascelles and White 2011).

Cytotoxic agents like carboplatin can be used for intralesional chemotherapy. Intratumoral chemotherapy injection of carboplatin in a sesame oil emulsion was found to produce remission rates of 77% with over 50% of cats having a progression free period of over a year (Théon, VanVechten, and Madewell 1996). Additionally, pairing this method with superficial radiotherapy can induce complete remission in cats with advanced stages of SCC (de Vos, Burm, and Focker 2004).

Cryosurgery is recommended for superficial SCC and when safe margins cannot be guaranteed for a resection, however multiple cycles of treatment are needed for results and treatment durations can go up to 150 days (Prado *et al.* 2017). It has been reported that 81% of cats treated by this method were tumour free at 36 weeks, but reoccurrence happened to about 73% of cats (Thomson 2007; Lascelles and White 2011). The major complications of cryosurgery are scab formation, surgical site infection and nostril stenosis (Prado *et al.* 2017).

Nasal planum resection is the most effective procedure when treating advanced stage nasal planum SCC and can significantly improve the animal's quality of life, but owner can feel detracted from taking this route due to the change in their pet's cosmetic appearance even though most owners describe their pet's appearance after the procedure as satisfactory (S. Murphy 2013; Chatzimisios, Farmaki, and Papazoglou 2015). Major complications are the formation of scabs that need to be continually cleaned and nostril stenosis. Cats submitted to this procedure have disease free intervals of almost 2 years (Lascelles and White 2011; Chatzimisios, Farmaki, and Papazoglou 2015).

Strontium-90 plesiotherapy is a modality of radiation therapy where radiation is directly applied to the lesion and is effective in the earlier stages of nasal planum SCC. The treatment can be done using a single dose protocol or a fractioned protocol over few days and both induce high remission rates (Lascelles and White 2011; Berlato *et al.* 2019). Survival times and disease-free intervals are both high (around 1000 and 780 days, respectively) and reoccurrence is about 31% (Berlato *et al.* 2019).

Conventional radiotherapy gives survival times of 12 to 16 months and high disease-free intervals to animals that have lower tumour volumes. However it is very limited in terms of availability, is very time consuming and requires multiple anaesthetics (Thomson 2007; Lascelles and White 2011).

The prognosis for cats with nasal planum SCC is good, especially in early disease as later stages signify a higher level of invasion. Long survival times and disease/free intervals can be achieved with surgical treatment for later stages of SCC (Lascelles and White 2011; Chatzimisios, Farmaki, and Papazoglou 2015) and strontium-90for earlier stages (Berlato *et al.* 2019). Treatment during earlier stages does not guarantee the development of disease later in life and monitoring is essential, as well as protecting animals from ultraviolet radiation exposure (Lascelles and White 2011; S. Murphy 2013).

2.2.2.8. Nephrology

Nephrology focuses on diagnosing and treating disorders of the urinary tract. The most frequent disorder was cystitis (4 cases) as seen in table 12.

Table 12. Case distribution regarding Nephrology.

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Atopic Cystitis	0	0%	1	100%	0	0%	
Cystitis	3	75%	0	0%	1	100%	
Urolithiasis	1	25%	0	0%	0	0%	
Total	4	100%	1	100%	1	100%	6

Medical and dietary management of urolithiasis

Urolithiasis is a urological condition defined by the formation of uroliths from crystalloid precursors present in urine and can occur in any part of the urinary tract. Uroliths are formed when precursors organize and assemble around a biological matrix. Uroliths can have a single crystal precursor, multiple, or even have them arranged in layers forming a more compound stone (Bartges and Callens 2015; Defarges *et al.* 2020).

Urolith formation and composition can vary depending on the breed, sex, age, and species of the animal. Despite this , the most common uroliths found in the dog and cat are calcium-oxalate and struvite (Sturgess 2009; Defarges *et al.* 2020).

Imaging is the best tool to detect uroliths if suspicion arises. Radiography can detect uroliths that are radiopaque due to their composition. Ultrasonography and double contrast cystography are able to detect even radiolucent uroliths (Bartges and Callens 2015).

Medical dissolution aims to dilute the urine and reduce concentration of substances forming the uroliths, preventing further crystal formation and helping dissolve already formed crystalloids while also helping the flushing of crystals able to traverse the urinary tract. However medical dissolution is not a reliable approach in all types of uroliths. Struvite, urate and cystine uroliths are all amenable to dissolution in the dog while struvite is the only susceptible in cats (Defarges *et al.* 2020).

Dissolution therapy is very effective and should be attempted first when treating struvite uroliths in the canine and feline patient. In cats most struvite cases are sterile struvite uroliths thus feeding a prescribed, high moisture acidifying diet with low amounts of magnesium coupled with increased water intake should be enough to dissolve any struvite stones in 2-4 weeks. In dogs, struvite calculi are commonly secondary to a urease-producing bacterial infection. In these cases, the acidifying effect of the diet would be neutralized by the bacteria and so antibiotic therapy is obligatory. The goal is to increase urine production and decrease urea concentrations in the urine as well as the concentrations of phosphorus and magnesium. Antibiotics should be chosen according to susceptibility tests and their ability to achieve higher concentrations in urine. Antibiotic therapy should be continued after dissolution is achieved as bacteria will be released as the stones are broken down. Three months should be expected for a complete dissolution in struvite calculi secondary to a urinary tract infection (Osborne *et al.* 2009; Lulich *et al.* 2016; Queau 2019).

Urate dissolution should be attempted when an animal has a defect in urate metabolism or when no underlying condition has been determined to be the cause of urolith formation (idiopathic urate uroliths). Increased water intake (more water or higher moisture food) and a low protein alkalinizing diet that uses plant or egg whites for protein instead of organs and meat will decrease precursors in urine, increase urine volume and pH (higher pH slows down urate urolith formation). Allopurinol prevents xanthine from turning into uric acid which helps reduce urate urolith formation but in turn, if the protein intake is not sufficiently restricted, increases risk of xanthine urolith formation and compound stones of urate-xanthine. Medical dissolution of

urate stones in dogs takes 4 weeks to be successful but there is not sufficient data for cats (Bartges and Callens 2015; Lulich *et al.* 2016; Queau 2019).

Xanthine uroliths when naturally occurring can only be treated by removal. When they occur as a consequence of allopurinol administration, they can be managed with lowering the dose given and adjusting purine intake in the diet. In cats no protocol for medical dissolution is available, as such, management is done by preventing oversaturation with the appropriate high moisture alkalinizing and low protein diet (Bartges and Callens 2015; Queau 2019).

Cystine uroliths are amenable to medical dissolution in dogs whereas with cats dissolution has not been successful. As with other cases, increased water intake and a high moisture alkalinizing low protein diet is necessary. Certain specialized low protein diets already have lower amounts of sulphur-containing amino-acids. Administration of 2-mercaptopropionylglycine (2-MPG, Tiopronin) stops cystine bonding (Bartges and Callens 2015; Lulich *et al.* 2016; Queau 2019).

Calcium oxalate uroliths do not have any medical dissolution protocols available, however, dietary changes can prevent recurrence. Since calcium oxalate formation is a recurring condition, preventive measures should be taken. Maintaining urine at 6.5-7 pH helps prevent calcium oxalate crystallization. Increasing water intake and thus decreasing the time urine stays in the bladder avoids precipitation of calcium oxalate. Potassium citrate supplemented in diets can help prevent crystallization of calcium oxalate and reduces absorption and excretion of calcium. Adequate levels of phosphorus are essential to avoid activation of vitamin D which increases excretion of calcium (higher concentrations in urine). Not restricting magnesium in diets can decrease oxalic acid availability. Vitamin D and vitamin C supplementation is not advised as it increases calcium oxalate precursors in urine. In cats it is especially important to avoid acidifying diets, which are very common as they prevent struvite calculi (maintaining a urine pH of 6.5 and 7 is recommended). (Osborne *et al.* 2009; Sturgess 2009; Bartges and Callens 2015; Lulich *et al.* 2016; Queau 2019).

2.2.2.9. Gastroenterology

Gastroenterology refers to the area of medicine responsible for diagnosing and treating disorders of the digestive system. The most frequent affliction was pancreatitis (3 cases) as seen in table 13.

Table 13. Case distribution regarding Gastroenterology.

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Pancreatitis	3	100%	0	0%	0	0%	
Hypersalivation	0	0%	1	50%%	0	0%	
Inflammatory bowel disease	0	0%	1	50%%	0	0%	
Total	3	100%	2	100%	0	0%	5

Diagnosis, treatment and management of Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease, sometimes also referred to as irritable bowel syndrome, is a collective of chronic enteropathies characterized by repeated manifestation of gastrointestinal inflammation and signs, such as, diarrhoea, vomiting, urgency to defecate and mucus. The exact causes of events that lead to IBD are still unknown, however, intestinal dysbiosis, immune system abnormalities and cell barrier dysfunction are thought to be behind the appearance of IBD in cats and dogs (Steiner 2008; Burgener 2017).

IBD can be classified according to the type of infiltrative inflammatory cells in the intestinal mucosa (Malewska *et al.* 2011). Lymphocytic-plasmocytic enteritis (LPE) is the most common form found in dogs while eosinophilic gastro-enteritis is the most common form in cats (Somu *et al.* 2017).

IBD is a diagnosis of exclusion, meaning a gross amount of testing for other potential diseases and negative results are needed to reach an IBD diagnosis. The first steps are complete bloodwork including haematology and biochemistry and faecal examination. Haematology can detect cases of neutrophilia (present in about 20% of cats with IBD) and eosinophilia, which may be indicative of eosinophilic gastroenteritis (EGE), although it is not considered pathognomonic of EGE and eosinophilia in cats is extremely rare (Suchodolski 2016).

Serum biochemistry cannot identify IBD but can show profiles relating to other diseases and thus exclude or confirm them. Hypoalbuminemia and hypoglobulinaemia are present in animals with IBD, more frequently in dogs, but can also signal protein-losing enteropathy in cases of lymphoma and lymphangiectasia, and liver disease. Hypoalbuminemia is also an indicator of poorer prognosis. Hypocholesterolaemia is present in a small percentage of cats with IBD, in dogs it is an excellent malabsorption marker and is also present in metabolic abnormalities. Decreased levels of calcium and magnesium are usually associated with malabsorption. Increased liver enzymes may be a result of gastrointestinal inflammation as a result of “reactive hepatopathy” but it does not rule out the possibility of primary liver disease (Somu *et al.* 2017).

Serum cobalamin should be measured as it is a useful prognosis indicator in chronic enteropathies and can signal small intestinal abnormalities (mainly the ileum) although it can also be a sign of pancreatic disease as the pancreas produces the intrinsic absorption factor for cobalamin (Allenspach 2013). Serum folate can also indicate proximal small intestine malabsorption (Somu *et al.* 2017) Canine pancreatic lipase can be evaluated to determine the possibility of pancreatitis. Faecal examination is important to exclude any gastrointestinal parasites and determining any existing faecal microbiota alteration which can support an IBD diagnosis (Allenspach 2013; Honneffer, Minamoto, and Suchodolski 2014).

Endocrine causes must be excluded and as such, hormonal tests like adrenocorticotropic hormone (ACTH) stimulation and thyroxine concentration measurements, are necessary to exclude cases of Addison’s disease and hyperthyroidism (Burgener 2017).

Radiography and ultrasonography can reveal systemic alterations and more specifically gastrointestinal abnormalities like thickenings of the intestinal wall (common in cats with IBD) and tumours/masses. Ultrasonography also allows for cytological sampling through FNA (Washabau *et al.* 2010; Somu *et al.* 2017).

Food allergies can be excluded by doing a trial diet with novel protein or hydrolysed protein. An anthelmintic therapy course can be initiated to assess responsiveness and antibiotic therapy (like metronidazole) can be trialled to rule out antibiotic responsive diarrhoea. Animals with IBD would not respond to any of these treatments alone. A positive clinical response to immunosuppressive and anti-inflammatory treatment supports an IBD diagnosis (Washabau *et al.* 2010).

Intestinal biopsy is the gold standard of determining intestinal inflammation. Samples can be obtained either endoscopically or if larger samples are preferred, with full thickness surgical biopsies. Histological samples should be analysed using WSAVA guidelines (Washabau *et al.* 2010).

After diagnosing IBD the next step is treating the clinical symptoms and managing the condition long term. Using the canine IBD activity index (CIBDAI) and feline chronic enteropathy

activity index (FCEAI) to measure severity of inflammation helps determine the optimal treatment and management plan (Jergens *et al.* 2003; 2010).

First and foremost, stress factors should be identified, and active work be put into eliminating and accommodations should be made for the animal. Highly digestible diets and fibre supplementation can help improve hardening the stool, regulate intestinal mobility and proliferate helpful intestinal bacteria. In humans, psyllium hydrophilic mucilloid has been beneficial in managing chronic diarrhoea and since animal and human IBD have many similarities it can be of great help. Cobalamin also known as vitamin b12 can be supplemented if deemed necessary. According to the response animals have to dietary treatment and fibre supplementation they can either have the fibre doses reduced and potentially removed or they may need pharmacological treatment (Simpson 1998; Steiner 2008).

Since there might be a possibility of bacterial antigens as one of the origins of IBD, antibiotics might be an option for treating dysbiosis. Antibiotics like metronidazole, tylosin and oxytetracycline can also have some immunomodulatory effects which also help managing IBD. Another possible treatment option in cases of dysbiosis is the use of probiotics to increase populations of helpful intestinal bacteria (Malewska *et al.* 2011; Burgener 2017; Somu *et al.* 2017).

There are many options for pharmacological treatment, but the most important and mainstay treatment is immunosuppressive medication. Glucocorticoids, prednisolone being the preferred choice, are given initially (2-4 weeks) every 12 hours at a 1-2mg/kg dose and then slowly tapered to each animal's specific needs. Prolonged use of glucocorticoids comes with a variety of side effects, like polyuria and polydipsia. The use of other immunosuppressive drugs like azathioprine in dogs and chlorambucil in cats help reduce glucocorticoids dose and decrease side effects (Malewska *et al.* 2011; Burgener 2017; Somu *et al.* 2017).

Some animals experience no positive effects with immunosuppressive therapy or relapse after a few months. These cases are referred to as refractory IBD and chlorambucil and cyclosporin have been known to help (Burgener 2017).

Pharmacological agents like loperamide stimulate colonic segmentation contractions prolonging absorption of bowel content and so reduce severity and frequency of diarrhoea and can be discontinued when diarrhoea subsides. Antispasmodics like clidinium bromide can reduce intestinal pain and discomfort (Steiner 2008). Treatment is usually successful and full remission is common, however animals with hypoalbuminaemia and low cobalamin levels have a poorer prognosis (Somu *et al.* 2017).

2.2.2.10. Neurology and Behavioural Medicine

Neurology focuses on diagnosis and treatment of disorders originating in the nervous system, while behavioural medicine refers to the diagnosis and management of abnormal behaviour manifested by animals. The most frequent disorder was epilepsy and aggression (both with 3 cases) as seen in table 14.

Table 14. Case distribution regarding Neurology and Behavioural Medicine.

	Canine		Feline		Exotic	
	Fi	Fr	Fi	Fr	Fi	Fr
Epilepsy	2	28.6%	1	50%	0	0%
Vestibular Syndrome	2	28.6%	0	0%	0	0%
Seizure	0	0%	1	50%	0	0%
Aggression	3	42.9%	0	0%	0	0%

Total	7	100%	2	100%	0	0%	9

Epilepsy/Seizure management

Epilepsy and seizures are not the same condition. A seizure can be defined as an isolated occurrence of symptoms or signs as a consequence of abnormal neuronal activity, while epilepsy is regarded as a group of conditions which predispose to recurring chronic seizures. For an animal to be considered epileptic, at least two seizures over the course of a month need to occur. While epilepsy always manifests in seizures, not all seizures are associated with epilepsy, for example, seizures can occur as a reaction to a metabolic disorder or intoxication (Thomas 2010; Meland and Carrera-Justiz 2018).

Seizures can be loosely classified as generalized-onset seizures or local-onset seizures. The first group include the tonic-clonic seizures, usually recognized by the loss of consciousness and full body rigidity followed by rhythmic muscle contractions, tonic seizures (just the full body rigidity) and the clonic or atonic seizures (sudden onset of rhythmic contractions without tonic phase and the most uncommon). Local-onset seizures are restricted to one part of the body but can be followed by a generalized seizure. Cluster seizures are events where multiple seizures happen in a short timeframe with regaining of consciousness and status epilepticus refers to more than two seizures in a 24-hour period without regaining consciousness and both are life threatening (Carrera-Justiz 2017; Meland and Carrera-Justiz 2018).

Owner education and compliance are essential in on-going successful anti-epileptic drug therapy. Learning how to recognize seizures and help the animal through them, knowing potential side-effects of drugs and purpose of drugs, and understanding the need to follow strict administration schedules and rules are all needed in successfully managing seizure occurrence in pets (Thomas 2000; Meland and Carrera-Justiz 2018).

Both clinicians and owners can consult the ACVIM Small Animal Consensus statement on Seizure Management in Dogs for a compilation of information on drugs and their effects (Podell *et al.* 2016).

Benzodiazepines, such as diazepam, are very potent anti-seizure agents with a very short effect duration and resistance to the drug can occur. There is cross-tolerance documented in benzodiazepines when given long-term and it can reduce effectiveness of diazepam in case of an emergency. Diazepam is the most common pharmacological agent used to quickly stabilize a patient during status epilepticus or cluster events. In cats, it has a longer half-life and can be used as a maintenance drug, however liver parameters must be closely monitored as oral diazepam can lead to hepatotoxicity. Diazepam can be given in doses of 0.5-1mg/kg i.v or intrarectally if venous access is not possible, and can be repeated up to three times, with 10 minutes between each bolus, if anti-seizure effects are not achieved (Thomas 2000; Ramsey 2017).

Phenobarbital is the first choice for continued treatment, as it has been used for many years, is well understood and tolerated by dogs and cats and is affordable and easily administered by owners. The recommended starting dose for dogs is 2.5mg/kg orally every 12 hours while for cats is 2.5mg/kg orally every 24 hours. It is metabolized in the liver and is considered an autoinducer of hepatic microsomal enzymes which leads to the reduction of its plasma half-life, in chronic usage. Serum concentrations must be measured regularly so that the lower serum levels (troughs) and its higher levels (peaks) are always in the therapeutic interval. The doses will always be tailored to each animal and in dogs with higher metabolic tolerance, phenobarbital can be given every 8 hours with positive results in seizure control. Some adverse effects are excitability, sedation and restlessness in the first week, and chronically, animals can exhibit with polydipsia and consequent polyuria and polyphagia. Some animals can get

withdrawal seizures from physical dependence. Liver parameters should be measured every six months to check for any signs of hepatotoxicity (Thomas 2000; Podell 2013; Stabile, Barnett, and Risio 2017).

Potassium bromide can be given to dogs only, and it helps reduce the doses of other anti-epileptic drugs resulting in less hepatotoxicity. It can be given at 30mg/kg/d or in some cases where it is chosen as monotherapy the dose is 40mg/kg/d. It can provoke some gastrointestinal irritation and discomfort, polydipsia, polyphagia and increased lethargy. Kidney parameters should be monitored to detect any toxicity. The risk of allergic reactions in cats is high so its use is not recommended (Thomas 2000; Podell 2013).

Levetiracetam is a newer generation anti-seizure drug very well tolerated by dogs and cats and with minimal chances of causing side effects, as most of it is excreted unchanged. Usual dose for both dogs and cats is 20mg/kg every 8 hours. This dose can be used as monotherapy or with multimodal protocols, usually combined with bromide or phenobarbital (Thomas 2000; Podell 2013; Carrera-Justiz 2017).

Zonisamide can be used when other drugs have not been successful as it is chemically different than other drugs commonly used. Oral administration of 5mg/kg doses every 12 hours on a monotherapy basis or 10mg/kg every 12 hours if used with phenobarbital. Doses in cats vary from 5 to 10mg/kg every 24 hours (Thomas 2010; Podell 2013; Carrera-Justiz 2017).

Gabapentin can be used in patients with compromised liver function and as adjunctive therapy because, it is not metabolized in the liver so it carries no risk of worsening hepatic function since its effect is very low as an anticonvulsant it cannot be used as monotherapy but can serve as a complimentary drug (Thomas 2000; Podell 2013; Carrera-Justiz 2017).

Imepetoin can be used in dogs and its anticonvulsant effect is close to phenobarbital. With its short half-life, prolonged treatment does not result in toxic accumulation of the drug and so the risks of side effects are very low, although its short half-time might not be desirable. It does not interfere with other anti-seizure drugs. Doses vary from 10 to 30mg/kg every 12 hours (Löscher *et al.* 2013; Ramsey 2017).

When an animal continues to suffer seizures even when high therapeutical doses are being used, they are termed to be suffering from drug resistant epilepsy/seizures. The prevalence of drug resistant epilepsy and seizures in dogs is about 30-40%. To manage this condition different drugs can be used, especially newer drugs like zonisamide, imepitoin and leveritacetam (Muñana 2013). In humans the ketogenic diet has proven to be somewhat effective in seizure control, but it is extremely restrictive and in dogs ketogenic levels have not been achieved to replicate the effects. Vagal nerve stimulation has been helpful in human cases but its cost and availability in veterinarian settings makes it an unrealistic option (Podell 2013).

The most important aspect of managing epilepsy and seizures in domestic animals is making sure their quality of life improves and remains good enough for a comfortable living. Informing the owners that even with therapy seizures will happen and adjusting their expectations can emotionally prepare them for the difficult journey of managing an epileptic pet and avoid any premature cases of euthanasia. Using cheaper medication and teaching how to deal with side-effects helps owners continue therapy (Podell *et al.* 2016; Carrera-Justiz 2017).

2.2.2.11. Parasitology and Infectiology

Parasitology and infectiology are responsible for diagnosing and treating protozoa, helminths and arthropod infections and infections of bacterial and viral nature. Only 4 cases of infection were recorded, sarcoptic mange, *staphylococcus*, tapeworm and giardiasis as seen in table 15.

Table 15. Case distribution regarding Parasitology and Infectiology.

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Sarcoptic Mange	0	0%	0	0%	1	50%	
Suspected *Staphylococcus spp.* infection	0	0%	0	0%	1	50%	
Tapeworm	0	0%	1	100%	0	0%	
Giardiasis	1	100%	0	0%	0	0%	
Total	1	0%	1	100%	2	100%	4

Giardiasis

Giardia sp. are a protozoan intestinal parasite commonly found in domestic animals and many other species. It exists worldwide and can become a zoonotic risk in some cases. *Giardia* infection is common worldwide and it mainly causes acute, chronic, or intermittent diarrhoea with a higher frequency in younger dogs and cats. It can also cause dysbiosis which can lead to malabsorption and in worse cases it can cause extreme abdominal pain (Zajac and Conboy 2012).

Infection happens when animals drink water contaminated with *Giardia spp.* cysts. When given the right conditions inside the host, the trophozoites will mature and invade the intestinal epithelium resulting in many pathological changes like erasure of the brush border and increased permeability. Later they will encyst and be released into the environment with the host's faeces (Ivanov 2010; Tangtrongsup and Scorza 2010; Zajac and Conboy 2012).

Infection does not always manifest itself and many dogs are sub-clinical, which poses a problem, as *Giardia spp.* can infect humans who coexist with asymptomatic animals, although the risk is low. Variable degrees of response top infection in mammals may be related to differences in virulence and host immunosusceptibility (Thompson, Palmer, and O'Handley 2008) .

Diagnosis can be achieved through a variety of faecal tests that can be done individually or combined. Smears, faecal floatation (passive or centrifugal) followed by microscopic examination can detect cysts, enzyme-linked immunosorbent assay (ELISA) can detect antigens and polymerase chain reaction and quantitative polymerase chain reaction (PCR and qPCR) tests can detect *Giardia spp.* DNA (Tangtrongsup and Scorza 2010; Zajac and Conboy 2012).

Prevention is crucial, as animals who receive regular anthelmintic treatment (fenbendazole has been observed to be highly effective) have substantially reduced rates of infection. A study done in Colorado shows dogs that regularly attend dog-parks and dogs which do not, have the same extremely low number of infections while on anthelmintic treatment (in this study about 80% of dogs in each group were receiving regular anthelmintic treatment) (Barr, Bowman, and Heller 1994; Wang *et al.* 2012). These studies reinforced the need of avoiding letting animals access stagnant water or water that is frequently accessed by many other animals, in reducing chances of initial infection.

Treatment aims to control the symptoms and infection. The animal's condition should be assessed and depending on the severity of the symptoms, appropriate measures should be taken as animals with mild symptoms might only need managing of diarrhoea while more severe cases need pain relief. Successful elimination of infection might be difficult to confirm as cysts are excreted intermittently over the course of many weeks, as such, repeated faeces testing is needed. Drugs such as metronidazole and fenbendazole or a combination of praziquantel/pyrantel/febantel can be used as prevention. Disinfecting living spaces regularly can eliminate re-infections (Tangtrongsup and Scorza 2010).

2.2.2.12. Other clinical medicine procedures

This section contains many procedures that were the main reason for visiting the practice but as they are neither diseases nor clinical signs needing treatment are thus put in this section. The most frequent procedures were routine procedures regarding dermatological care, like, ear cleaning, removing sutures that do not need sedation/anaesthesia, anal gland expression (54 cases) as seen in table 16.

Table 16. Frequency of clinical medicine procedures

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Sample collection for haematology and urinalysis	4	6.1%	5	17.2%	0	0%	
IOP measurement	1	1.5%	1	3.4%	0	0%	
Ophthalmic examination	2	3%	0	0%	0	0%	
Post-enucleation consult	0	0%	1	3.4%	1	50%	
Blood pressure measurement	4	6.1%	3	10.3%	0	%	
FNA	7	10.6%	0	0%	0	0%	
Dermatological routine procedures	41	62.1%	13	44.8%	0	0%	
Bladder echography	0	0%	1	3.4%	1	50%	
Endoscopy	1	1.5%	0	0%	0	0%	
Euthanasia	6	9.1%	5	17.2%	0	0%	
Total	66	100%	29	100%	2	100%	97

Euthanasia was always performed as a last resort procedure. Animals that were euthanized were already living long periods of time with debilitating conditions and had significantly decreased quality of life, were diagnosed with fast progressing terminal diseases or treatment was not an option, either due to the nature of the pathology or treatment was too costly.

2.2.3. Surgical Medicine

2.2.3.1. Soft tissue surgery and dentistry

Soft tissue surgery refers to all the surgical procedures on varied tissues that do not classify as orthopaedic, while dentistry refers to the procedures done specifically to the teeth. The most frequent procedure was gonadectomy (castration/ovariohysterectomy) (25 cases) and dentistry procedures like teeth cleaning (removal of tartar and plaque) and teeth extractions (11 cases) as seen in table 17.

Table 17. Case distribution regarding Soft Tissue Surgery and dentistry.

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Splenectomy	2	4.88%	0	0%	0	0%	
Castration/Ovariohysterectomy	22	53.7%	12	46.2%	2	75%	
Cryptorchidectomy	1	2.44%	0	0%	0	0%	
Enucleation	0	0%	1	3.85%	1	25%	
Melanoma excision + Neck Bilateral Lymphadenectomy	1	2.44%	0	0%	0	0%	
Mastectomy for sarcoma	1	2.44%	0	0%	0	0%	
Mast Cell Tumour + Basal Cell Carcinoma Removal	1	2.44%	0	0%	0	0%	
Lipoma excision	4	9.76%	0	0%	0	0%	
Unilateral Thyroidectomy	0	0%	1	3.85%	0	0%	
Nail Removal	2	4.88%	0	0%	0	0%	
Saculectomy	1	2.44%	0	0%	0	0%	
Anal Fistula Flushing	0	0%	1	3.85%	0	0%	
Nasal Biopsy	0	0%	1	3.8%	0	0%	
Large Wound Cleaning/Suturing	0	0%	3	11.54%	0	0%	
Exploratory laparoscopy	0	0%	1	3.85%	0	0%	
Bladder Stones Flushing	0	0%	1	3.85%	0	0%	
Teeth cleaning/extractions	6	14.63%	5	19.23%	0	0%	
Total	41	100%	26	100%	3	100%	70

Splenectomy

Splenectomy can be required in a multitude of situations ranging from tumours (haemangiosarcoma, lymphoma and mast cell tumours), trauma and torsion, autoimmune diseases, to haemoabdomen (with splenic origin like splenic rupture). Although the spleen has important functions to perform in the body, such as being an emergency blood supply, splenectomy rarely results in long-term sequelae (Culp 2012; Charlesworth 2014).

A few techniques have been performed in splenectomies. Partial splenectomy is rarely indicated, and it comes with various unwanted risks (post-operative haemorrhage, leaving behind neoplastic cells and longer operative time) and so is rarely performed if ever. Complete splenectomy can be achieved via two techniques, hilar ligation and major vessel ligation (Charlesworth 2014).

Hilar ligation entails ligation of the small blood vessels entering and leaving the spleen and can be advantageous in cases where tumours hide the major vascular anatomy leading to the spleen (Charlesworth 2014).

Major vessel ligation is the preferred technique in most splenectomies. In this procedure, the surgeon will open the omental bursa and then the splenic artery, the short

gastric and left gastroepiploic arteries as well as all associated veins are ligated. This procedure has reduced operative time compared to hilar ligation (Culp 2012).

To access the spleen a regular midline incision is performed. The size of incision correlates to the size of the spleen, for example, in cases with larger splenic masses, an incision from the xyphoid process to the pubis may be required so that the spleen can be properly exteriorized. After exteriorizing the spleen, vessels are blunt dissected, and ligation performed. Double ligatures are placed proximally onto a crushed area created by artery forceps. A single ligature is placed on the distal end. It is advised to place a transfix ligature on the splenic artery to avoid suture migration. The vessels are sectioned between the second and third ligature from proximal to distal (Culp 2012; Fossum 2013; Charlesworth 2014). Sutures used are absorbable synthetic and some clinician's might prefer chromic catgut as it ties very well in fatty tissue (Charlesworth 2014; Petroianu 2017).

Alternatively, vessel sealers can be used in vessels with less than 7mm diameter. Vessel sealers have high efficacy and according to (Monarski, Jaffe, and Kass 2014) actually reduce operating time.

Prognosis and recovery vary case by case. Animals presenting with complications before splenectomy typically recover more slowly and have a poorer prognosis. Conditions like lymphoma and haemangiosarcoma with metastasis also have a poorer prognosis and animals typically require adjuvant therapy to improve quality of life and life expectancy (Stee *et al.* 2015; Story *et al.* 2020).

2.2.3.2. Orthopaedic Surgery

Orthopaedic surgery focuses on surgical procedures of the musculoskeletal system. Only 3 cases were recorded, a femoral head a neck recession in a dog with LCPD and 2 posterior limb amputations in a dog and cat with malignant bone neoplasia as seen in table 18.

Table 18. Case distribution regarding Orthopaedic Surgery.

	Canine		Feline		Exotic		
	Fi	Fr	Fi	Fr	Fi	Fr	
Femoral Head and Neck Ostectomy	1	50%%	0	0%	0	0%	
Posterior Limb Amputation	1	50%	1	100%	0	0%	
Total	2	100%	1	100%	0	0%	3

Femoral head and neck ostectomy (FHNO) technique

Femoral head and neck ostectomy or femoral head and neck excision is a surgical procedure commonly recommended for patients suffering from diseases like, hip dysplasia, Legg-Calvé-Perthes disease, femoral head and neck fractures, acetabular fractures and coxofemoral hip luxation. As the name implies, the goal of the procedure is to remove the femoral head and neck, and allowing the body to form a pseudo-joint to regain pain-free mobility in the affected hindlimb (Peycke 2011).

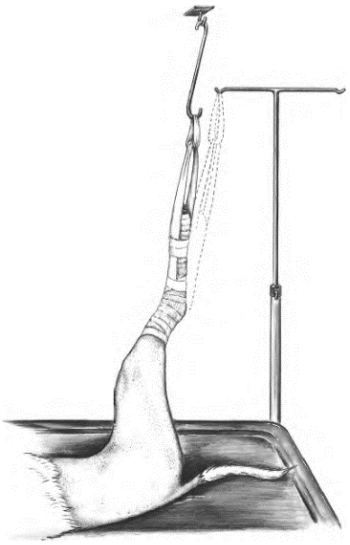
Proper aseptic preparation is important to reduce post-operative complications. The animal should be clipped from dorsal midline to the stifle, a hanging-hind limb technique is optimal for limb preparation and draping should be done in order to easily manipulate the limb without touching any contaminated surface (figures 3 and 4) (Piermattei and Johnson 2004).

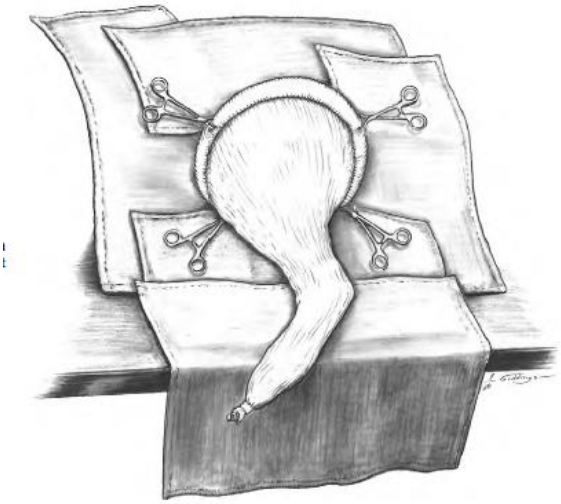
Figure 4. Hanging hind limb after proper aseptic technique. Adapted from (Piermattei and Johnson, 2004).

Figure 3. Correct draping placement to avoid surgical area contamination coupled with sterilized stockinette. Adapted from (Piermattei and Johnson, 2004).

Before describing the FHNO technique itself, it is important to first understand the approach to the hip joint. The approach that allows for a better visualization is the caudolateral approach to the hip joint (Harper 2017).

The approach, as described in (Piermattei and Johnson 2004), is as follows. The animal should be in lateral recumbency with the affected limb uppermost. The incision is centred around the greater trochanter and along





the cranial border of the femur. The incision extends distally up to mid length of the femur and proximally it curves slightly cranially ending close to the dorsal midline (Piermattei and Johnson 2004).

The next step is to undermine the skin and proceed with an incision in the fascia lata alongside the cranial border of the biceps femoris muscle. The biceps femoris is retracted caudally and another incision, now in the deep leaf of the fascia lata, is made to free the tensor fasciae latae muscle, then continuing through the septum between the superficial gluteal muscle and the tensor fasciae latae muscle (Piermattei and Johnson 2004).

Fascia lata and the tensor fasciae latae muscle are both retracted cranially with the biceps femoris being retracted caudally. The area which we want to access can be seen by blunt dissecting or separating the tissue around the femoral neck. This area is contained in between the middle and deep gluteal muscles (cranially), the vastus lateralis muscle (laterally) and the rectus femoris muscle (medially). Further exposure for better visualization can be achieved by partial tenotomy of the deep gluteal tendon close to the trochanter (Piermattei and Johnson 2004).

Areolar connective tissue surrounds the joint capsule and needs to be cleared by blunt dissection before incising the joint capsule. The incision is made and continues laterally along the femoral neck passing the origin of the vastus lateralis muscle situated on the neck and lesser trochanter. The origin of the vastus lateralis is then reflected distally (Piermattei and Johnson 2004).

Lastly, Hohmann retractors are placed intracapsular ventrally and caudally to the femoral neck to achieve visualization of the femoral head. Extra care should be given in placing the retractors as to refrain from accidentally trapping the sciatic nerve (Piermattei and Johnson 2004).

Making use of stay sutures along these steps, especially in the partial tenotomy of the deep gluteal and the opening of the joint capsule, is recommended for guidance, easier manipulation, and after the FHNO, easier and faster closure (Piermattei and Johnson 2004).

After successfully accessing the joint capsule and visualizing the femoral head, luxation of the joint outwards (the knee should be rotated 90º in relation to the table) is needed for a correct angle to perform the ostectomy (both the head and the femoral neck should be clearly visualized). To free the femur and luxate the joint, the round ligament needs to be severed with the help of curved scissors or a bone curette small enough to fit between the acetabulum and the femoral head (Peycke 2011).

Next, the ostectomy line should be visualised from the greater trochanter to the lesser trochanter. Ideally the insertion of the iliopsoas muscle should be preserved unless, leaving it intact presents risk of bony contact with the pelvis. To excise the bone, an osteotome or a power/oscillating saw can be used, making sure the instrument chosen is carefully placed preferably with a slight distal to proximal orientation so as to prevent damage to tissues around the femur and the sciatic nerve (Peycke 2011).

Bone holding forceps are used to secure the excised bone and safely remove it. The femur needs to be carefully palpated, noting any sharp edges or prominences that must be corrected. Moving the limb to its full range of motion is advised to detect any grinding noises that signal bony contact. Flushing the area with sterile saline may help remove any small debris left unnoticed (Peycke 2011; Harper 2017).

The joint capsule is closed either with a simple interrupted or mattress suture using absorbable suture materials. Mattress sutures are also placed in the deep gluteal tendon to repair the tenotomy, and the vastus lateralis is sutured to the deep gluteal muscle with a simple interrupted pattern. Continuous sutures are placed in the insertion of the tensor fasciae latae and along the superficial gluteal muscle. The leaves of the fascia lata are sutured to the biceps femoris muscle with a continuous pattern. Routine closure of the remaining layers closes the initial incision (Piermattei and Johnson 2004; Peycke 2011).

Post-operative care includes good multimodal analgesia and aggressive physiotherapy regimens. Restricting movement is highly discouraged as it will not promote the formation of the pseudo-joint and pain management will help ease pain and discomfort during exercise. Passive range of motion manipulations and light walking is appropriate in the first two to three weeks and then more active exercise can be initiated. Case by case physiotherapy protocols are preferred (Harper 2017).

A retrospective study of 169 dogs reports that animals subjected to FHNO start weight bearing on the affected leg after 3 to 4 weeks with smaller dogs being reported to have less cases of post-operative lameness and milder in intensity compared to larger dogs after being subjected to the FHNO technique, though all dogs benefitted greatly from having the procedure (Montgomery *et al.* 1987).

Part II – Monograph: Canine Non-Hodgkin's Lymphomas

1. Introduction

Canine lymphomas (cL) are the most common haematopoietic neoplasia found in the dog, with annual incidence rates between 20-100 cases in every 100,000 dogs, but annual incidence increase has been observed. CL originate from lymphoid tissue and lymphocytes and develop as various entities with different clinical presentations. Multicentric nodal lymphoma is the most common clinical presentation of cL with roughly 80% of cases fitting this anatomical classification (M. Zandvliet 2016) and diffuse large B cell lymphoma appears to be the most common entity found in the dog (Valli *et al.* 2011).

By definition, cL is a clonal tumour, as such it typically only has origin in one type of cell which in this case would be B, T or NK cells be they immature or mature cells and any stage of differentiation (Valli *et al.* 2011).

CL affects any dog breed at any age, but it is most commonly diagnosed in medium to larger dog breeds and older animals. Breeds like the cocker spaniel, boxer, bulldog, bullmastiff, German shepherd and golden retriever are all considered to be at increased risk, while breeds like the chihuahua, dachshund and miniature and toy poodle are considered low risk (Villamil *et al.* 2010).

Due to characteristics like clinical presentation, molecular biology and treatment, it is considered similar to and even regarded as a large-scale animal model for human non-Hodgkin lymphoma (Teske *et al.* 1994; Laura Marconato, Gelain, and Comazzi 2013). Furthermore, since cL has faster onset and progress than human non-Hodgkin's lymphoma, it can itself serve as an indicator for high risk locations regarding human non-Hodgkin's lymphoma (Pastor *et al.* 2009; Pinello *et al.* 2019).

CL is generally chemosensitive meaning chemotherapy protocols are the preferred treatment. Protocols have produced high remission rates when implemented and overall survival can go up to over a year (Ettinger 2003).

Due to the nature of the cells found in lymphomas and importance of changes in the normal structure of lymph nodes, an introductory section regarding lymphoid tissue and cells is helpful in better understanding the possible aetiology, diagnosing and prognosis of cL.

2. The lymphoid tissue and cells

All the cells that circulate throughout the lymphatic system originate from pluripotent haematopoietic stem cells found in bone marrow. These pluripotent stem cells differentiate into two different lineages, the lymphoid and myeloid lineage, both with respective progenitor cells. The myeloid progenitor cells differentiate into erythrocytes, megakeratocytes which produce platelets, granulocytes (basophils, neutrophils and eosinophils), mast cells and macrophages, while the lymphoid progenitor cells differentiate into B and T lymphocytes (also known as B and T cells), the natural killer (NK) cells and innate lymphoid cells (ILC). Both progenitor cells can differentiate into dendritic cells (K. Murphy and Weaver 2016).

The B and T lymphocytes are part of the adaptive immune system and thus possess specific antigen receptors which distinguishes them from other immune cells. Although they are similar in this way, they mature in different tissues. T cells mature in the thymus and B cells mature in the bone marrow. They circulate through the body between blood, lymph and peripheral lymphoid tissue, in their inactive form. Upon making contact with an antigen, they rapidly undergo multiple divisions creating a vast quantity of effector (active form) B and T cells specific to the situation (K. Murphy and Weaver 2016).

The B cells originate from a common lymphoid cell and then mature mostly in the bone marrow as their initial development is dependent on bone marrow stromal cells. The last stages of maturation occur in the spleen. During the process from stem cell to B cell various gene rearrangements are performed and B cells that fail tests do determine successful gene rearrangement are discarded but some will continue to rearrange until success is verified. Pax5 is an important isoform of the B cell activator protein and in its absence further development is impossible but cells in this situation may be used for T cell production. Before leaving the bone marrow, B cells, still in their immature form, are subjected to an autoreactivity test to make sure that autoreactive cells are not sent into circulation. Cells that are autoreactive can be subjected to various forms of control, which are, apoptosis (cell death), receptor editing creating new non autoreactive receptors and induction of permanent unresponsiveness to antigens (K. Murphy and Weaver 2016).

The B cells can be divided in 3 groups. The follicular B cells or B-2 B cells which reside in the spleen and peripheral lymphoid tissue, the marginal zone B cells which are exclusive to certain tissues like the spleen, specifically in the margins between the red and white pulp, and B-1 B cells which reside predominantly in the pleural and peritoneal cavities. While B-2 B cells and marginal zone B cells are considered part of the adaptive immune response playing a big role in humoral immunity, B-1 B cells are part of the innate immune response and are responsible for large scale production of circulating pre-infection antibodies. B-1 B cells also differ in their development, as they are predominantly produced in the fetus. (K. Murphy and Weaver 2016).

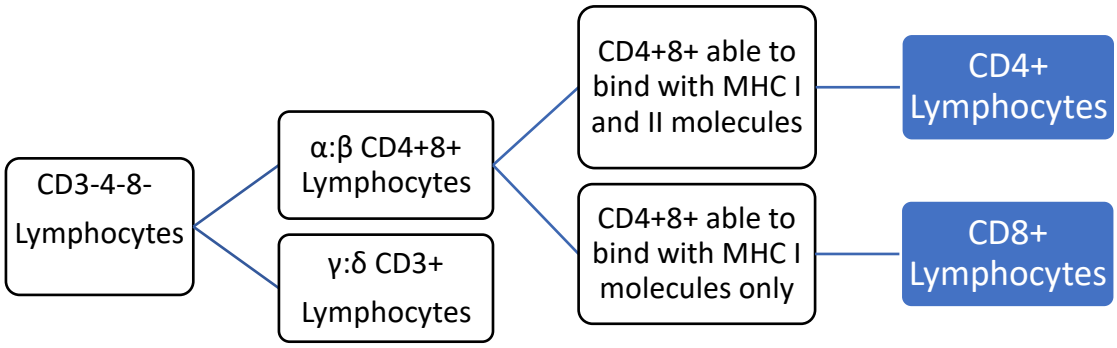
The circulating B cells can be activated in two ways. First, B cells can process antigens intracellularly and then present major histocompatibility complex (MHC) II molecules on their surface which are recognized by effector T cells that have previously contacted with the same pathogen. Effector T cells that are responsible for helping B cells proliferate are also known as helper T cells. This interaction stimulates B cells to differentiate into antibody-secreting cells and memory B cells. Secondly, B cells can directly recognize a variety of antigens and thus do not require helper T cells to multiply and differentiate (K. Murphy and Weaver 2016).

Contrary to B cells, T cell progenitors migrate to the thymus before starting their development into mature T cells. Not unlike B cells, T cells rely on being among a network of thymic stromal cells for an adequate development environment. In the thymus, T cell progenitors are subjected to a specific signal called the Notch signal, which conditions the development of progenitor cells into the T cell lineage. During development T cells are also

subjected to several surface receptor changes and screening processes, so as to avoid possible faulty cells (K. Murphy and Weaver 2016).

There are two lineages of T cells, the α:β lineage and the γ:δ lineage, but both originate from cluster of designation (CD) 3,4,8 negative (CD3-4-8-) T lymphocytes. The α:β lineage is further divided into two subtypes the CD4 positive (CD4+) and CD8 positive (CD8+) T cells which express CD4 and CD8 co-receptors respectively. These two subsets of T cells are formed when developing α:β are subjected to major histocompatibility complex (MHC) I or II molecules. While MHC I molecules are required for both subsets, MHC II is specific to the proper development of CD4 T cells, which become fewer and abnormal in cases where MHC II molecules are missing (K. Murphy and Weaver 2016). The two lineages can be seen in figure 5.

Figure 5. The two lineages of lymphocytes that develop in the thymus



After T cells complete the final steps of development in the thymic medulla, they are put into circulation in the blood stream where they become part of the adaptive immune system. Upon contact with an MHC molecule of an antigen-presenting cell they are induced into a state of fast proliferation and differentiation into effector T cells ready to eliminate any cell that presents the specific MHC complex that activated them. T cells only act on host cells and not the pathogens themselves, due to only recognizing peptide antigens presented on MHC molecules on the surface of other cells. CD8 T cells recognize pathogen peptides on MHC I molecules and differentiate into cytotoxic effector T cells which are responsible for recognizing and eliminating infected cells by activating the cells' apoptosis pathways. CD4 T cells after coming into contact with MHC II molecules can differentiate into effector subtypes which eliminate target cells, or differentiate into regulatory cells which inhibit the extent of the CD4 and CD8 effector T cells and antigen presenting cells, thus regulating the level of immune response (O'Neill *et al.* 2009; K. Murphy and Weaver 2016).

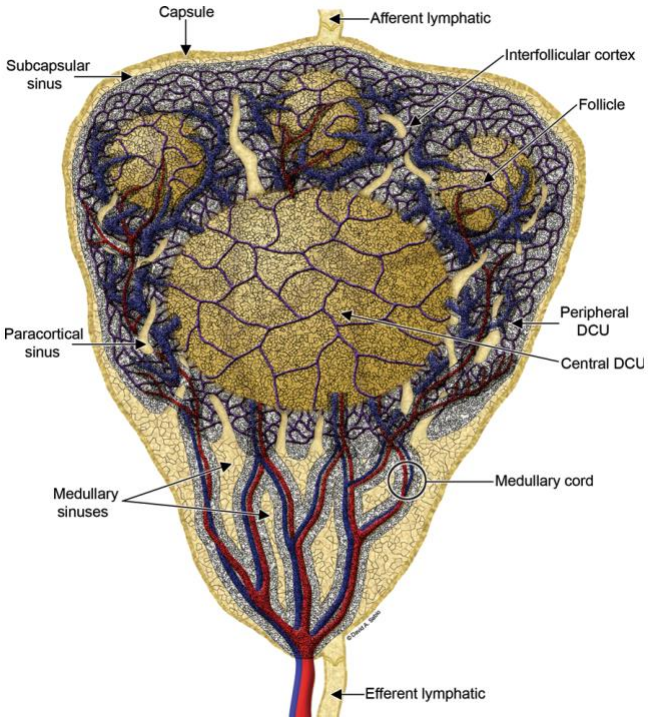
NK cells and ILC originate from the same progenitor cells as B and T cells, the common lymphoid cells and are part of the innate immune system. Both NK cells and ILC lack the specific antigen receptors B and T cells possess, although NK cells have receptors that allow them to perceive cellular stress and infection, like in the case of tumour cells and cells infected with particular viruses like the herpesvirus for example (K. Murphy and Weaver 2016).

NK cells contain cytoplasmic granules that contain proteins with cytotoxic effects. They exert their function by releasing their granules when presented with their target cells. Unlike cytotoxic T cells, NK cells do not need previous contact with the specific target in order to perform their function. Cell death can be induced consequential to cell surface perforation by cytotoxic granules, although NK cells have two additional apoptosis inducing mechanisms. One is the interaction between a ligand of the tumour necrosis factor (TNF) family and certain receptors expressed by cells, and the other, is the interaction between antibodies and specific receptors found in NK cells resulting in the release of granules and is called antibody-dependent cellular toxicity (K. Murphy and Weaver 2016).

The mucosa associated lymphoid tissue (MALT) is a specialized lymphoid tissue associated with epithelial surfaces of the body. One example are the Payer's patches which are located throughout the gut epithelium. MALT is one of the first lines of defence the body has against infections and it has cells, the M cells, that specialize in presenting antigens to the B and T cell population found in these tissues (K. Murphy and Weaver 2016).

The lymph nodes are composed of a single or many lymphoid lobules, which are the basic anatomical unit (figure 6). A lobule can be divided into 3 important areas, the cortex, the paracortex and the medulla. Any changes to these structures reflect on changes in lymph node function. Such changes can be seen in many lymphomas, where normal arrangement of cells and structures is disrupted by neoplastic cells (Willard-Mack 2006)

Figure 6. Anatomical structure of a lymphoid lobule. Adapted from (Willard-Mack, 2006).



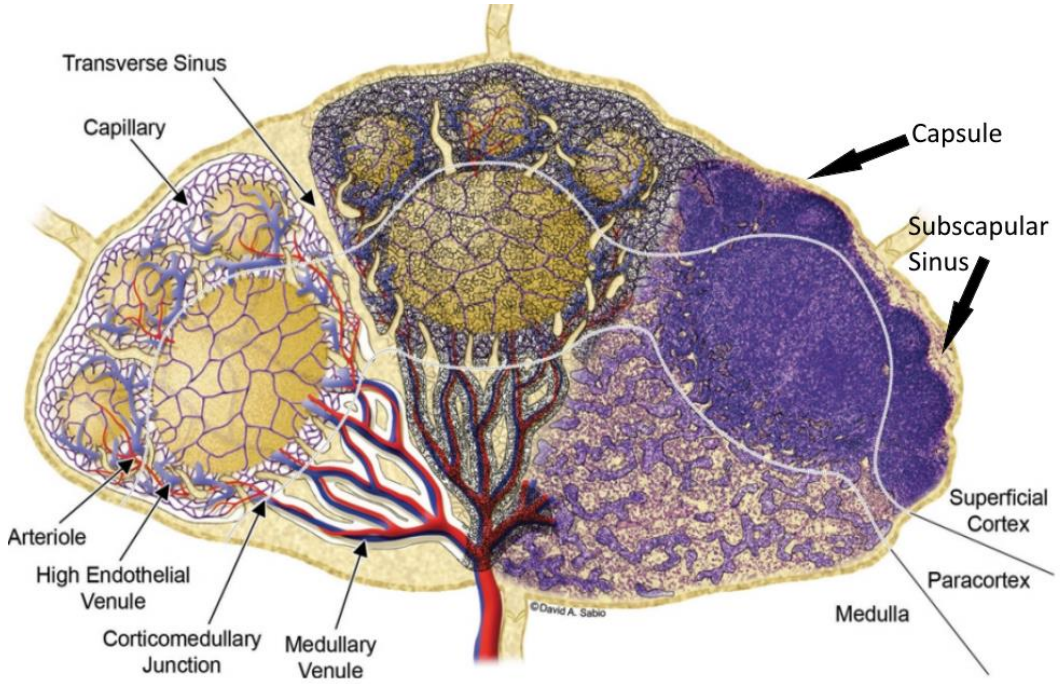
The outermost of these 3 structures, the cortex, also referred to as the outer cortex, is located beneath the capsule that covers the lymph node. Between the capsule and the cortex, a layer of separation designated the subscapular sinus, allows for the flow of lymphatic fluid and continues through the transverse sinuses which ends in the medullar sinuses in the medullar region of the node (figure 7). The cortex region is also referred to as the B cell layer/zone due to

the high concentration of these cells in this particular region. Most B cells are present in the structures known as follicles, where they form the germinal centres (temporary structures formed in B cell zones where B cells mature and develop) (Willard-Mack 2006; Bujoreanu and Gupta 2022).

The middle layer, the paracortex or inner cortex is where most T cells reside (T zone). T cells do not have follicles where they form germinal centres like B cells but instead are stimulated in areas known as deep cortical units (DCUs). Each lobule has a single DCU that can vary in size due to different lobules receiving immunological stimulation via lymphoid afferent vessels from different parts, which can lead to unevenness of the paracortex of a lymph node (Willard-Mack 2006; Bujoreanu and Gupta 2022).

The innermost region of a lymph node is the medulla. The medullary region is where large blood vessels sit and where the medullary sinuses drain the lymph into the efferent lymphatic vessels. Medullary cords contain many plasma cell precursors that when mature release antibodies, B cells and macrophages (Willard-Mack 2006; Bujoreanu and Gupta 2022).

Figure 7. Anatomy of a lymph node. Adapted from (Willard-Mack, 2006).



Left lobule shows the blood flow of the lymph node. Centre lobule has the reticular meshwork superimposed. The lobule on the right is a histological section of a rat mesenteric lymph node.

3. Aetiology

The concrete aetiology of cL has not been completely characterized, but it is multifactorial. The genetic, environmental, and immunologic factors can all increase the risk of developing cL. Some studies have pointed to increased risk of cL development in dogs close to rural and industrial areas, as well as dogs exposed to the use of chemicals like paints and

solvents. This association is further reinforced by the shorter ages of cL onset between exposed dogs and non-exposed dogs (Gavazza *et al.* 2001; Takashima-Uebelhoer *et al.* 2012). Even magnetic fields can increase risk of developing cL. A study regarding residential exposure to magnetic fields recorded an increase in neoplasia risk of 60% in dogs exposed to higher magnetic fields (Reif, Lower, and Ogilvie 1995).

Errors in B cell and T cell development, caused by non-productive rearrangement of genes and failure to recombine these, can give rise to mutations and oncogenes causing abnormal cells to develop (K. Murphy and Weaver 2016). Combined with failure of the innate immune system in detecting and deleting abnormal cells and controlling tumour cells can lead to lymphoma development (M. Zandvliet 2016; Seifert, Scholtysik, and Küppers 2019). A study done with golden retriever dogs found that dogs with cL had fewer capacities to repair DNA than dogs without cL (Thamm *et al.* 2013).

Although there is lack of data regarding association between immune-mediated diseases and higher risk of cL, human data reveals that higher risk for non-Hodgkin's lymphoma development exists in patients with HIV or are receiving immunosuppressive therapy. One retrospective study found a significant association between immune-mediated thrombocytopaenia and cL (E. T. Keller 1992; M. Zandvliet 2016).

Some factors interact with one another, for example, genetic polymorphism of the glutathione-S-transferase (GST) enzymes can lead to failure in detoxification of environmental chemicals with carcinogenic properties. Polymorphism of the GST theta 1 (GSTT1) variant was found to be overrepresented in dogs with cL (Ginn *et al.* 2014; M. Zandvliet 2016).

4. Clinical signs and Diagnosis

Clinical signs of cL are typically non-specific other than generalized lymphadenopathy. Dogs can show signs of fatigue, diminished appetite, fever, polydipsia and polyuria, diarrhoea and vomiting, weight loss, dyspnoea and even seizures (Ettinger 2003). Clinical signs typically vary according to the immunophenotype and location of the lymphoma. The most common paraneoplastic syndrome found in dogs with cL is hypercalcaemia followed by thrombocytopaenia but, abnormal blood proteins, neuropathies and lymphocytosis can also be found (Vail 2011).

The diagnosis of cL uses methods like cytology, histology, flow cytometry, immunocytochemistry and immunohistochemistry of samples obtained by fine needle aspiration (FNA) and biopsies to determine the presence and characteristics of neoplastic cells and changes in normal tissue architecture. Additionally, radiography and echography detect internal masses and organ involvement which can be characteristic of specific lymphoma presentations (M. Zandvliet 2016).

Haematological profile parameters like platelet count, complete blood count, differential cell count for quantification of leukocytes and serum biochemistry, especially total and ionized calcium are important aspects for accurate diagnosis and general wellbeing of the patient (Vail 2011). Although, these parameters can be indicative of prognosis they are not predictive of treatment response (M. Zandvliet 2016).

In order to obtain cell samples for cytology, FNA is the quickest and safest method available. FNA is a fairly quick procedure, it is relatively painless and is very cost effective. It does not require any specialized skill set, as such, any veterinarian should be able to perform it. FNA can be repeated on multiple lesions and carries almost no risks of complications making it safe even for the more debilitated patients (Ansari and Derias 1997). Cytology of lymph node FNA samples can be used to choose an appropriate lymph node for future biopsy (Sapierzyński *et al.* 2010).

Although FNA can be used as the first method to detect neoplastic cells, cytology does not provide much information beyond cell presence and morphology (M. Zandvliet 2016).

Although certain lymphoma presentations can have a very specific cytological appearance, others are difficult to differentiate and in order to further classify these cases, immunophenotyping and tissue architecture is needed due to specific architectural changes (Sapierzyński *et al.* 2016).

Histology and immunohistochemistry reveal information of tissue architecture and cellular nature (mainly receptors that identify B and T cells), respectively, specific to certain types of lymphoma, which combined with cytological evidence makes it possible to classify each lymphoma and get a better understanding of the lymphoma's clinical progression and prognosis (Vail 2011). Lymph node excision is needed to produce a large enough sample for histology which makes it a more invasive procedure than FNA (M. Zandvliet 2016).

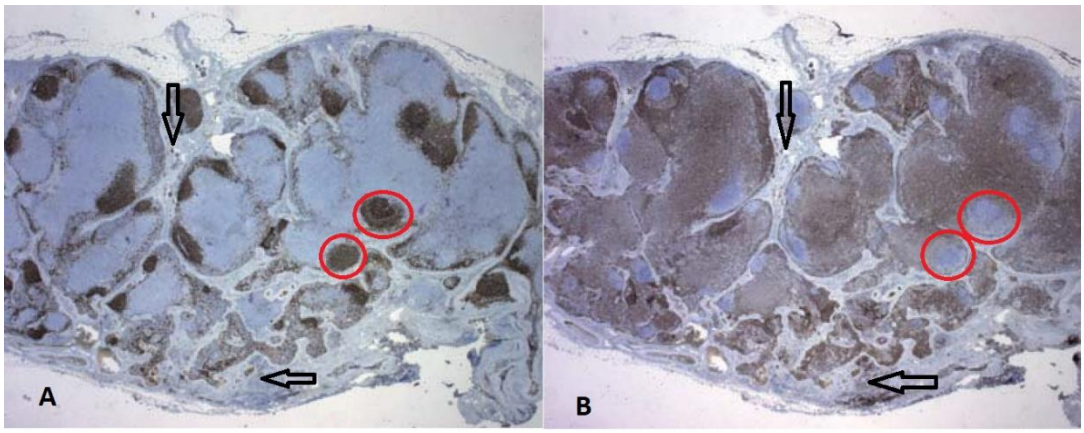
Immunophenotyping is the identification of molecules expressed on the surface of cells. In the case of cL, the target molecules are known as clusters of designation and are specific molecules expressed on the surface of lymphocytes (Herold and Mitra 2022; McKinnon 2018). To determine the presence of such molecules, antigens are used among which the most commonly used are CD20, CD21 and CD79-α antigens to detect B cell lymphomas and CD3, CD4 and CD8 to detect T cell lymphomas. When a cell expresses a CD molecule they are marked as CD positive (+), for example CD8+, and when they do not express a CD molecule they are considered negative (-), for example CD8- (K. Murphy and Weaver 2016; M. Zandvliet 2016).

Flow cytometry (FC), immunohistochemistry (IHC) and immunocytochemistry (ICC) are the preferential techniques available for immunophenotyping cytological and histological samples. Although PCR based techniques are available for immunophenotyping cells, they aren't as accurate as FC or staining based methods like IHC and ICC, as such, they are mainly used to determine cell clonality (M. Zandvliet 2016).

4.1.1. Immunostaining

Immunostaining processes like ICC and IHC aim to detect specific proteins, in this case CD molecules, by making use of target specific antibodies. Visualization through a microscope is possible due to immunofluorescence. The only difference between ICC and IHC is that ICC is the staining of cytological samples while IHC refers to the staining of tissue samples (Maity, Sheff, and Fisher 2013). An example of immunostaining can be seen in figure 8.

Figure 8. Immunostaining of canine lymph node with T zone lymphoma. Adapted from (Valli, 2007b).



A- CD20 B cell staining. Red circles show B cell clusters CD20+. Black arrows point to collagenous connective tissue.

B- CD3 staining. The heavy coloured areas are T cells CD3+ and show marked proliferation. Black arrows point to collagenous connective tissue. In B the red circles now show the B cells unstained with the CD3 staining.

4.1.2. Flow cytometry (FC)

FC is a technique that allows the analysis of varied cell characteristics based on light scattering and fluorescence patterns. Fluorescently conjugated antibodies give CD surface and internal molecules fluorescent properties which are picked up by the optics system of FC equipment which then identify the presence of B cells, T cells or both. FC can be used on cytological samples obtained by FNA which combined with the speed of the equipment can produce accurate information quickly after sample collection and cells only need be put in a saline solution for them to be able to be subjected to FC. However, high costs of equipment maintenance makes it a less common service offered by veterinary diagnostic laboratories and cells need to be labelled (addition of the fluorescently conjugated antibodies to the sample) in the first 24h to ensure the best conditions for immunophenotyping (Comazzi and Gelain 2011; McKinnon 2018).

FC typically employs antigens anti-CD4/CD8 for surface molecules and anti CD3/CD79-β for intracellular molecules. After being subjected to analysis, cells are classified depending on their staining and their relative difference to the normal percentage references is calculated and a diagnosis of lymphoma can be achieved. Cells that have dual staining like CD3+CD79-β+ and CD4+/CD8+ or do not have a regular staining pattern like cells that do not have surface marker staining (CD79-β+ or CD3+ only) are indicative of lymphoma. An increase in an intracellular marker of over 1.5 times relatively to the reference values (60% for CD3 and 30% for CD79-β) coupled with a decrease or absence of the other is also indicative of lymphoma. Since CD79-β is specific to B cells an increase in such indicates B cell lymphoma and increase of CD3 indicates T cell lymphoma (Thalheim *et al.* 2013).

FC sensitivity for confirming a lymphoma diagnosis is around 98% making it an excellent first line diagnostic tool (Thalheim *et al.* 2013).

4.1.3. Polymerase chain reaction (PCR) for antigen receptor rearrangements (PARR)

PARR is a molecular diagnostic method that assesses the presence of lymphoid cell clonality in a certain sample by amplifying DNA that encodes receptors specific to B and T cells (Thalheim *et al.* 2013). For B and T cells the DNA section targeted for amplification is the complementarity determining region 3 (CDR3) responsible for encoding the binding part of immunoglobulin heavy chain (IgH) and encoding T cell receptor gamma (TCRγ), in B and T cells respectively (Lana *et al.* 2006; Waugh *et al.* 2016). CDR3 is originated by recombination of V, D and J genes in B cells and V and J genes in T cells, and in a healthy cellular population of cells these segments vary as they have between 5 to 20 random nucleotides between each gene segment, however in clonal cells the same sequence is repeated without variation which is detected and amplified by PARR by using primers specific to V and J genes conserved regions (Burnett *et al.* 2003).

The samples for PARR are the same as the previous methods (IHC and FC) (Lana *et al.* 2006; Thalheim *et al.* 2013).

When compared to FC, PARR has lower sensitivity (around 74%) and have similar specificity (95-100%) (Thalheim *et al.* 2013; M. Zandvliet 2016).

5. Lymphoma classification

Canine lymphoma can be classified according to location, cytological, histological and immunophenotypic evidence. They can also be assigned a grade (low, intermediate, high) according to cell mitotic rate (also known as mitotic index) which is indicative of progression speed and responsiveness to chemotherapy, with higher mitotic rate lymphomas progressing faster but also responding better to chemotherapy protocols (Ettinger 2003).

Anatomical classification refers to the location (anatomical sites) of the lymphoma. In this classification scheme, lymphomas can be multicentric lymphomas, alimentary tract or gastrointestinal lymphomas, mediastinal lymphomas, cutaneous lymphomas, nervous system lymphoma among other locations (Vail 2011; M. Zandvliet 2016). The most common presentations are multicentric, alimentary tract, cutaneous and mediastinal (Vail 2011).

Histological systems for in depth classification of human lymphoma are used to properly identify the different subtypes, also known as entities, among the many presentations lymphoma can take. The first system that took in account multiple parameters, from nodal location to histogenetic and molecular data, to identify the multiple possible entities were the Revised European-American Lymphoid neoplasms (REAL) classification proposed by the International Lymphoma Study Group and published in 1994. Later the World Health Organization (WHO) adopted the REAL classification guidelines and since 2001 has done several revisions (de Leval and Jaffe 2020) making it the most up to date classification system.

Although older and only taking into account cytohistological and immunologic data (Fournel-Fleury *et al.* 1997), the updated Kiel classification system is still widely used, although adjustments have been made for classification of cL. It is most useful for diagnosing high-grade lymphomas off cytological and histological samples, struggling to diagnose low-grade lymphomas (M. Zandvliet 2016).

A standardized in-depth classification system specially formulated for cL has not been published, but the Kiel classification has been successfully adapted to cL (Ponce *et al.* 2010; M. Zandvliet 2016). Additionally, a study was carried out in order to find the degree of accuracy and consistency of pathologists not specialized in haematopathology in applying the WHO classification to cL. The study was proposed by the American College of Veterinary Pathologists (ACVP) Oncology Committee and was endorsed by the World Small Animal Veterinary Association (WSAVA) (Valli *et al.* 2011).

This study of 300 cases, 17 pathologists with no specialization in haematopathology and 3 haemopathologists (to determine the consensus in diagnosis), demonstrated that the overall accuracy of the 17 pathologists using the WHO classification was 83% when applying it to cL (Valli *et al.* 2011).

The WHO classification system has described a great number of distinct entities that compose human lymphoma, but not many large-scale studies have been performed in small animals to the extent it has been done in humans. The most common neoplasms found in larger-scale studies of 300 cases (Valli *et al.* 2011), 608 cases (Ponce *et al.* 2010) and 992 cases (Valli *et al.* 2013) of cL were: diffuse large B cell lymphoma, marginal zone lymphoma, T zone lymphoma, peripheral T cell lymphoma and lymphoblastic lymphoma and are the ones addressed in this thesis.

5.1. Anatomical location classification

This classification focuses on the location the lymphoma originates on and the clinical presentation associated with such.

5.1.1. Multicentric lymphoma

Multicentric lymphomas also known as nodal lymphomas are lymphomas that develop in the lymph nodes. They are the most common presentation of lymphoma with 75-80% of cases being multicentric (Vail 2011; M. Zandvliet 2016).

Multicentric lymphoma is staged according to the degree of lymph node involvement and substages according to presence or absence of systemic signs (M. Zandvliet 2016). According to the World Health Organization there are 5 stages, with the first stage being a single lymph node involved and fifth stage being generalized lymph node involvement and multiple organs affected. There are only two substages, no systemic signs present and systemic

signs present, and are given the letter a and b respectively. This staging can be applied to (Owen 1980). The stages and substages can be seen in table 19.

Multicentric cL are predominantly B cell lymphomas (Jark *et al.* 2020; Neuwald *et al.* 2014) which is the most common immunophenotype found in cL (Ponce *et al.* 2010; Valli *et al.* 2011; 2013).

The main clinical sign of multicentric lymphoma is non-painful generalized lymphadenopathy. Some non-specific signs like vomiting, anorexia, lethargy and weight loss can be present (Ettinger 2003; M. Zandvliet 2016).

Table 19. The World Health Organization staging for multicentric cL. Adapted from (Owen, 1980).

STAGE	
I	Single lymph node or lymphoid tissue in single organ (excluding bone marrow)
II	Involvement of many lymph nodes in a regional area (± tonsils)
III	Generalized lymph node involvement
IV	Liver and/or spleen involvement (± Stage III)
V	Involvement of blood and bone marrow (± Stages I-IV)
SUBSTAGE	
a	No systemic signs
b	Systemic signs present

5.1.2. Alimentary tract lymphoma

Unlike human alimentary tract lymphoma, alimentary tract cL mainly exhibits T cell origin ranging from 60% to 70% of alimentary tract cL cases (Coyle and Steinberg 2004; Frank *et al.* 2007; Kojima *et al.* 2021). Alimentary tract lymphoma accounts for about 7% of cL cases (Ettinger 2003; Vail 2011)

Although there isn't any known breed predisposition, the boxer and sharpei are the most represented (Coyle and Steinberg 2004). Breed representation can depend on multiple factors like rising trendiness in certain breeds like the sharpei (Coyle and Steinberg 2004) or country, as in Japan the pug and shiba seem to be most affected (Kojima *et al.* 2021).

Clinical signs are diarrhoea, vomiting, lethargy, inappetence, weight loss and distended abdomen, but during physical exams, muscle wasting, thickened intestines and painful abdomen can be observed (Couto *et al.* 2018).

Lesions are commonly focal but can affect multiple gastrointestinal layers and tissues (Ettinger 2003; Frank *et al.* 2007) and during echography loss of wall structuring is indicative of neoplasia (Penninck *et al.* 2003).

5.1.3. Cutaneous lymphoma

Cutaneous lymphoma is predominantly a T cell lymphoma with epitheliotropic and non-epitheliotropic natures, with the first being the most prevalent in canine patients (Fontaine *et al.* 2009; M. Zandvliet 2016). Three forms of cutaneous lymphoma have been described, pagetoid reticulosis, Sézary syndrome and the most common clinical manifestation , mycosis fungoides (P. F. Moore, Olivry, and Naydan 1994; Fontaine *et al.* 2009; M. Zandvliet 2016). No breed or sex disposition has been found but median age of onset was 11 years (P. F. Moore, Olivry, and Naydan 1994).

Although cutaneous lymphoma primarily affects the skin, it can spread to mucocutaneous junctions like the eyelids, nose and lips, but also the oral mucosa like the

gingiva, tongue and palate (Ettinger 2003; Fontaine *et al.* 2009). The most common lesion found in cutaneous lymphoma cases is erythema. Prevalence of lesions can be seen in table 20.

Table 20. Prevalence of lesions found in dogs with cutaneous lymphoma. Adapted from (Fontaine et al., 2009).

Lesion	Prevalence
Erythema	80.8%
Scaling	61.5%
Plaques	61.5%
Nodules	57.7%
Ulceration	42.3%
Crusts	38.5%
Pruritus	38.5%
Mucosal lesioning	38.5%
Papules	15.4%

5.1.4. Mediastinal lymphoma

Mediastinal lymphomas make up 3-5% of cL cases (Vail 2011; Ettinger 2003) and is almost exclusively of T cell origin, with almost 97% prevalence among mediastinal lymphoma cases (M. Zandvliet 2016; E. L. Moore *et al.* 2018).

Mediastinal lymphomas manifest as thoracic masses that due to location and size can result in dyspnoea and cranial vena cava syndrome (figures 9 and 10). Hypercalcaemia is very common in dogs with mediastinal lymphoma (43-67.5%) and leads to polyuria/polydipsia (Ettinger 2003; E. L. Moore *et al.* 2018).Cranial vena cava syndrome occurs when a mediastinal mass compromises the venous return to the heart, which results, in large oedema of the head and neck (Figure 8) and can be reversed after mass size is reduced (Figure 9), typically with chemotherapy (M. Zandvliet 2016).

Figure 9. Oedema of face and neck in a dog with cranial vena cava syndrome. Adapted from (Zandvliet, 2016).

Figure 10. Same dog as figure 9 after chemotherapy. Adapted from (Zandvliet, 2016).

5.1.5. Central nervous system (CNS) lymphoma

Central nervous system involvement occurs very rarely. It's estimated that 5% of canine lymphomas are primary to the CNS and about 5-12% are secondary (Sisó *et al.* 2017). Primary CNS lymphoma means that only the brain and meninges are affected while secondary CNS lymphoma affects other tissues as well (M. Zandvliet 2016). Animals affected by lymphomas located in the CNS typically present with seizures, alterations in their mental state and function, vestibular syndrome and circling, but animals could also have blindness, neck pain, anisocoria and tremors of the limbs (Snyder *et al.* 2006; 2008).

5.1.6. Ocular lymphoma

Primary ocular lymphoma is very rare and is confined to the ocular globe and conjunctiva, but extraocular structures like the third eyelid and palpebral conjunctiva may be affected while also showing signs of systemic involvement like enlarged lymph nodes and biochemistry results that show other organs might be affected (M. Zandvliet 2016; Lanza *et al.* 2018).

The most common clinical signs found in dogs with ocular lymphoma are glaucoma and uveitis. Of the cases that presented with uveitis, unilateral anterior uveitis was more common that bilateral anterior uveitis (Lanza *et al.* 2018). B-cell origin is the most common cell origin for ocular lymphomas (M. Zandvliet 2016).

Secondary ocular lymphoma is heavily associated with multicentric lymphoma as 37% of multicentric lymphoma cases might show ocular involvement (Krohne *et al.* 1995; Ota-Kuroki *et al.* 2014).

5.1.7. Hepatic lymphoma

Primary hepatic lymphomas are very rare in dogs (M. Zandvliet 2016) as most lymphomas that do affect the liver are part of the multicentric presentation (Dank *et al.* 2011).

Dogs with hepatic lymphoma can appear to be lethargic, anorexic, have vomiting episodes and present with polydipsia and polyuria. Upon physical examination the most common findings are hepatomegaly, ascites, cranial abdominal organomegaly and a small portion of dogs can show signs of jaundice (Dank *et al.* 2011).

Liver function tests commonly show increased activity of serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase consistent with liver damage. Other common findings are hyperbilirubinaemia, hypoalbuminaemia, leucocytosis, neutrophilia and anaemia (Dank *et al.* 2011).

Two distinct entities of primary hepatic lymphoma are recognized in the dog, and both are of T-cell origin: Hepatosplenic T-cell lymphoma and hepatocytotropic T-cell lymphoma. Both of these lymphomas have very poor prognosis (S. M. Keller *et al.* 2013).

5.1.8. Splenic lymphoma

Primary splenic lymphomas are relatively rare. They are mainly of B-cell origin and marginal zone lymphoma is the most common entity diagnosed. Typically, splenic lymphomas are of indolent nature, which means they have very slow progression, as such, they are typically detected as a singular mass on the spleen during routine echographic examinations (Fracácio *et al.* 2018).

5.2. Immunophenotypical classification/ Lymphoma entities

This classification focuses on the histological and cellular characteristics of the lymphoma and associated clinical presentation

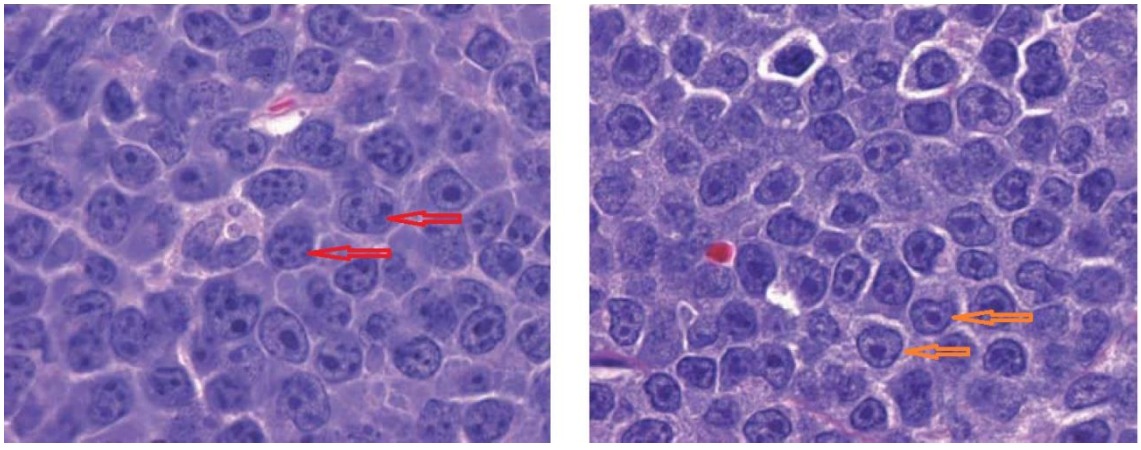
5.2.1. Diffuse Large B Cell Lymphoma (DLBCL)

DLBCL is the most common B cell lymphoma found in dogs. It can present itself as low grade and intermediate grade, but most commonly manifests as a high grade lymphoma. (Valli *et al.* 2013).

DLBCL can be further described as centroblastic and immunoblastic, depending on histological data. Centroblastic DLBCL is characterized by uniform diffuse proliferation of large, not cleaved B cells with multiple peripheral nucleoli, typically twice the size of erythrocytes, and minimal cytoplasm (figure 11). The immunoblastic variant of DLBCL, although more aggressive in nature, is less common and contrary to the previous variant, cells typically only manifest a single large sized central nucleolus (figure 11) (Valli *et al.* 2011; Luca Aresu 2016; SH *et al.* 2017). It is possible for both cellular changes to be present in the neoplasia which can lead to doubts in classification (Valli *et al.* 2011). DLBCL cells are typically CD79-α+, CD20+ and CD3- (Valli 2007a; Valli *et al.* 2011).

Clinically, patients present with a single or multiple enlarged lymph nodes (lymphadenopathy), but in later stages, extranodal location is possible and depending on location, various signs can be observed typically due to the mass effect of tumours, like cranial vena cava syndrome and intestinal obstruction. These masses usually grow rapidly due to high mitotic rates of DLBCL (Valli *et al.* 2011; SH *et al.* 2017).

Figure 11. Centroblastic and immunoblastic DLBCL. Adapted from (Valli, 2007a).



Left: Centroblastic DLBCL, red arrows show the characteristic peripheral nucleoli.

Right: Immunoblastic DLBCL, orange arrows show a more central singular nucleolus.

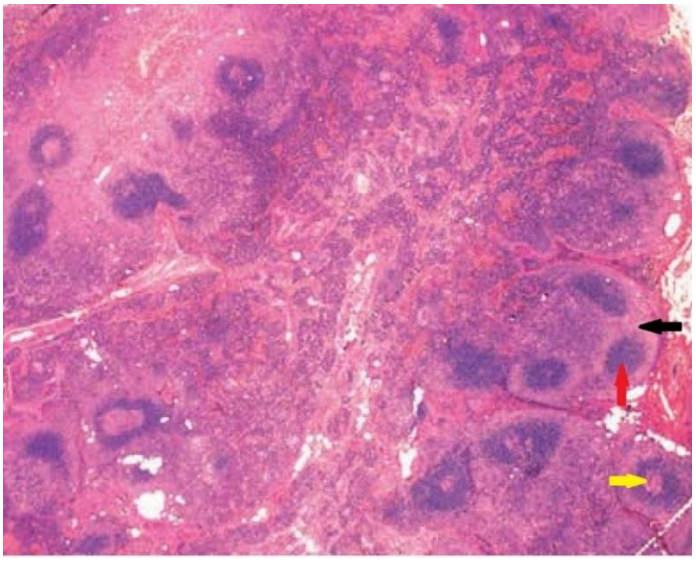
5.2.2. Marginal zone lymphoma (MZL)

MZL can develop in the spleen, in the lymph nodes or in mucosal associated lymphoid tissue (MALT) and is considered an indolent lymphoma, as such, it progresses very slowly and symptoms mainly manifest in more advanced stages (Valli *et al.* 2011; SH *et al.* 2017). In humans, nodal MZL appears to be the least common form of MZL (Angelopoulou *et al.* 2014), however, in a retrospective study, 56 out of 66 dogs diagnosed with MZL were diagnosed with the nodal form (Ponce *et al.* 2010). This higher frequency of nodal MZL compared to splenic MZL in canine patients was also seen in (Valli *et al.* 2011). MALT MZL is the least common form found in the dog (Cozzi *et al.* 2018).

MZL has a very characteristic cell organization, in which, neoplastic B cells form groups around remnants of germinal centres (figure 12) resembling the marginal zone of the lymph node follicles (Valli 2007b; Ponce *et al.* 2010; Valli *et al.* 2011). Neoplastic cells are medium sized and have medium sized nuclei with a visible single central nucleolus. The presence of the prominent nucleoli might be confused with the same nucleoli found in immunoblastic DLBCL, however, in MZL, cells have a moderate amount of cytoplasm contrasting with the scant cytoplasm of DLBCL cells (Cozzi *et al.* 2018). Immunostaining reveals positive staining for CD79-α and CD20 and negative for all T cell markers (Valli 2007b).

The characteristic clinical presentation is of a single enlarged lymph node (Valli 2007b), but in more advanced stages, generalized lymphadenectomy develops (Cozzi *et al.* 2018).

Figure 12. Dog lymph node with MZL. Adapted from (Valli, 2007b).



Lighter stained neoplastic mantle cells form a cuff (black arrow) around healthy mantle cells (red arrow) which is the characteristic lesion of MZL. Yellow arrow shows a follicle centre that still retains some function.

5.2.3. T zone lymphoma (TZL)

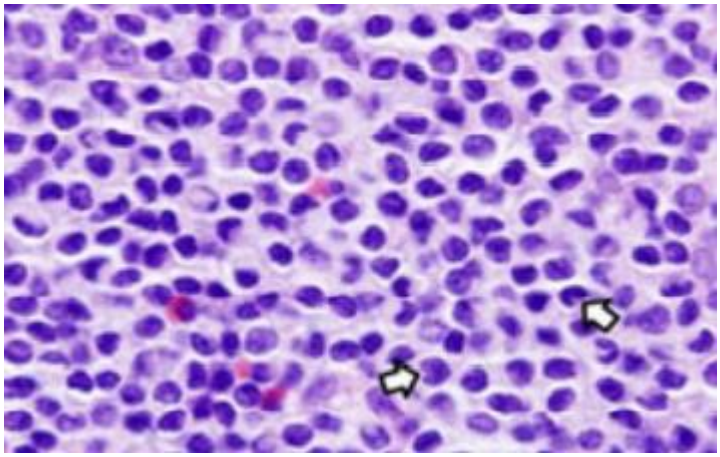
TZL is a nodal T cell lymphoma characterized by small to medium sized neoplastic cells with scant nuclear detailing but visible nuclear indentation (figure 13) and clear cytoplasm. Cells appear to have no mitotic rate in earlier phases but in later stages low mitotic rates can be

perceived, classifying TZL as an indolent lymphoma (Valli *et al.* 2011; Seelig *et al.* 2014; Stein, Bacmeister, and Kiupel 2021).

TZL has a very characteristic histological appearance. Lymph nodes present with paracortex expansion and compression of follicles against the lymph node capsule (figure 14) due to clonal growth of neoplastic T cells, however no erasure of nodal architecture can be observed (Seelig *et al.* 2014).

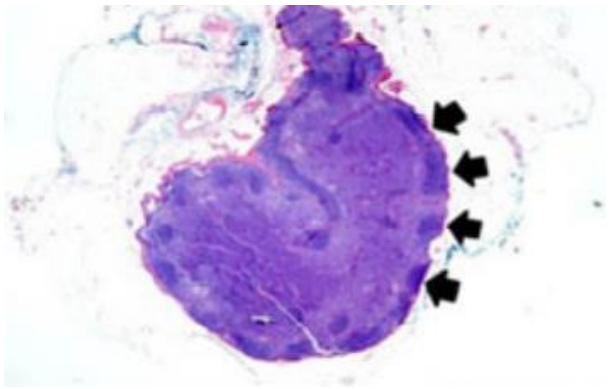
TZL typically manifests as a single non-painful enlarged peripheral lymph node, most commonly the submandibular lymph node. Being an indolent lymphoma, TZL has a very slow progression meaning the enlarged lymph node is not visible in earlier stages and patients do not show any changes like loss of appetite. Due to these characteristics, TZL is many times detected during routine procedures, like physical exams and vaccinations (Valli 2007c; Valli *et al.* 2011).

Figure 13. Neoplastic T cells in canine TZL. Adapted from (Seelig et al, 2014).



Arrows point to visible nuclear indentations.

Figure 14. Smaller amplification of figure 12. Adapted from (Seelig et al, 2014).



Arrows point to a thinned capsule and follicles visibly compressed against it.

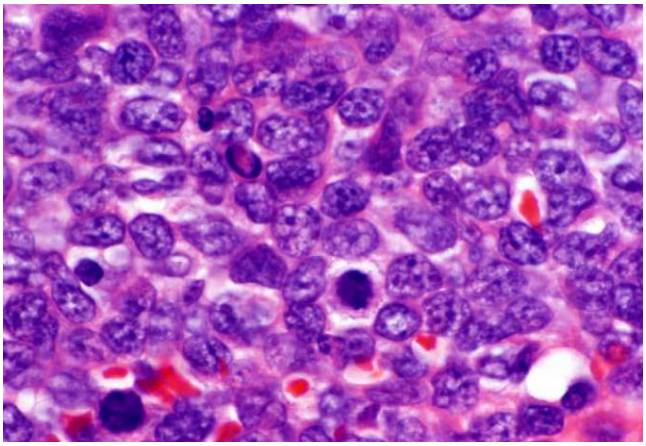
5.2.4. Peripheral T cell lymphoma not otherwise specified (PTCL-NOS)

PTCL-NOS is a group of T cell lymphomas that have not yet been fully specified. Although in humans the category has been diminishing, the unavailability of specific canine antibodies used in diagnosis impedes further differentiation of neoplastic entities in canine patients. As such, all peripheral T cell lymphomas that cannot be further described end up in this general category (Valli 2007e).

Characteristics of PTCL-NOS can only be described in general terms due to the varied behaviours these neoplasms can exhibit. Lymphomas included in this group can be contained to the lymph nodes or originate in the dermis and other tissues, the lesions can be focal and systemic, they can develop around blood vessels (angiocentric) or they can later invade them (angioinvasive) (Valli 2007e).

PTCL-NOS has a morphological heterogenous cell population with a spectrum of neoplastic lymphoid cells present. Cells may have abundant clear cytoplasm, and many nuclear shapes can be observed (figure 15). Neoplastic cells can sometimes appear to resemble Reed-Sternberg cells, meaning, neoplastic T cells exhibit more than one nucleus. A paracortical pattern like in TZL may be present, but the normal architecture of the lymph node is partially destroyed. Cells have varied mitotic rates (Valli 2007e; Feldman and Dogan 2014).

Figure 15. Neoplastic cells in PTCL-NOS. Adapted from (Valli et al, 2011)

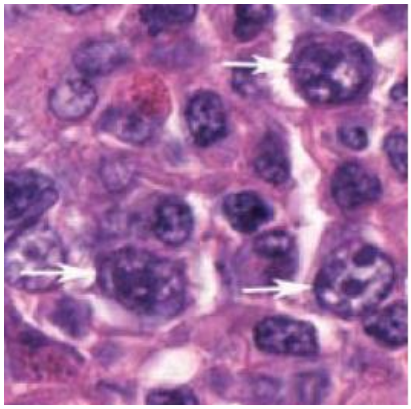


Cells have large nuclei with 2-4 nucleoli.

There are no characteristic clinical signs of PTCL-NOS with many patients having only lymphadenopathy. However, two rare types of presentation have been recorded, one in young larger breed dogs and a second found in mature dogs with a singular lesion. Dogs with the first presentation, have peripheral lymphadenopathy, generalized skin disease with dependent oedema and are usually acutely ill. Histologically, this presentation is characterized plugging of small vessels with congestion of the surrounding tissues, infiltration by large cells with an elongated oval nuclei and prominent nucleoli, and presence of varied inflammatory cells. Dogs

with the second presentation are typically in good health and have a singular mass in the panniculus. Neoplastic cells can vary between medium and large in size with parachromatin clearing (figure 16) and are found surrounding smaller vessels. These cells make up a small percentage cell population (10-20%) and are only clearly visible after immunohistochemical staining (Valli 2007e; Valli *et al.* 2011).

Figure 16. Neoplastic cells with parachromatin clearing. Adapted from (Valli, 2007e).



Parachromatin clearing (clear areas in the nuclei) visible in neoplastic cells (arrows).

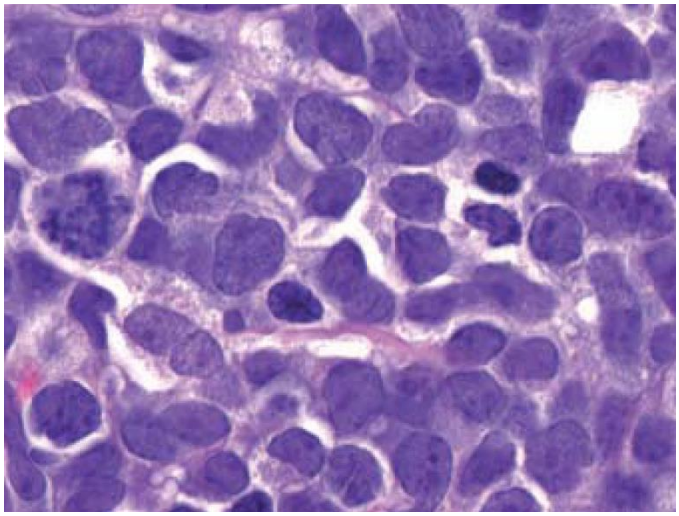
5.2.5. T cell lymphoblastic lymphoma (T-LBL)

T-LBL is a neoplasm originating in peripheral tissues involving the peripheral lymph nodes and rapid spreading to the liver, spleen, bone marrow and all other tissues in later stages (only after bone marrow has been involved). The peripheral tissue origin is what differentiates T-LBL from acute lymphoblastic leukaemia, which has its origins in the thymus and bone marrow (Valli 2007d; Valli *et al.* 2011). T-LBL is considered to be the most aggressive lymphoma and has a very small remission window (Valli *et al.* 2011).

Cytologically, neoplastic cells are medium sized with dispersed chromatin obscuring nucleoli, giving the cells their heavily stained appearance. Nuclei can have shallow indentations which are a characteristic of the T-LBL convoluted type (figure 17). High mitotic rates can be observed. Histologically, a few architectural changes like medullary and cortical filling, thinned capsule and lack of tingible body macrophages (macrophages, containing chromatin from degraded cells, commonly observed in normal germinal centres). T-LBL can be distinguished from PTCL that have similar cell size by the nucleoli visibility, as PTCL has more prominent nucleoli than T-LBL (Valli 2007d; Valli *et al.* 2011).

Clinically dogs present with acute onset of loss of appetite and reduced activity but with good body condition due to the acute nature of this lymphoma. A mediastinal mass is typical in younger dogs, but peripheral lymphadenopathy is present in all ages. In older animals generalized lymphadenopathy can develop (Valli 2007d; Valli *et al.* 2011).

Figure 17. convoluted lymphoblastic lymphoma. Adapted from (Valli, 2007d).



Neoplastic cells with variable nuclear shapes, dispersed chromatin and obscured nucleoli. Shallow nuclear indentations can be seen and are characteristic of convoluted lymphoblastic lymphoma.

6. Therapeutics

Chemotherapy is the most successful treatment available for cL and combination/multi-agent protocols can reach 90% success rates on initial treatment (Vail 2011). Although multi-agent protocols are considered the most successful in terms of remission periods, they are also the most expensive and most intensive and might not be suitable for every case, as such, single agent protocols may be considered for this reason. Every owner's financial situation and availability to follow time intensive protocols is different, and so, owner's and veterinarians should work together to reach a consensus in what type of protocol they should implement seeing as dogs without treatment will have a survival time of 4-6 months (Ettinger 2003; M. Zandvliet 2016).

Chemotherapy may be considered the best treatment for cL however, it does not cure lymphoma. Considering this fact, the fundamental goals of chemotherapy are achieving a complete remission and the longest remission times possible while reinducing remission whenever neoplasms develop again, reversal of clinical signs and preserving quality of life for the patient for as long as possible (Ettinger 2003; Lascelles and White 2011).

Canine lymphoma is a systemic pathology and benefits from systemic treatment modalities like chemotherapy, however, lymphoma presentations that manifest as local lesions like oral mycosis fungoides or focal lesions in the nasal cavity can benefit from radiotherapy. Radiotherapy is also recommended as a form of palliative treatment when chemotherapy is no longer successful, especially when targeted on enlarged lymph nodes that can compromise quality of life by causing dyspnoea and difficulties in ingesting food and defecation or skin lesions that cause persistent discomfort (Meleo 1997).

A few studies have been published regarding half-body radiation therapy coupled with a CHOP chemotherapy protocol and although commendable results were obtained, with reported median remission intervals and median survival times between 427-1126 and 456-1131 days

respectively (Gustafson *et al.* 2004; Lurie *et al.* 2008), these studies were done in very small scales (8 and 13 dogs) and larger scale studies need to be done in order to confirm the results.

6.1. Multi-agent chemotherapy protocols

Multi-agent protocols produce the greatest remission rates (80-90%) and survival times (median survival times of 1 year) (Ettinger 2003; Vail 2011). Depending on the author of the protocols they may have different names, for example VELCAP, CHOP and L-CHOP, but they use the same 4-5 chemotherapy agents (Zemann *et al.* 1998; M. Zandvliet 2016): cyclophosphamide, hydroxydaunorubicin or doxorubicin hydrochloride, oncovin or vincristine sulfate, prednisolone and L-asparaginase. Interestingly (M. Zandvliet, Rutteman, and Teske 2013) reports that prednisolone inclusion in protocols does not have any benefits regarding remission rates and survival times and is not essential in multi-agent protocols.

Multi-agent protocols are preferably used as a first-line treatment because they are the most effective when drug resistance isn't present (Vail 2011).

Being an aggressive systemic treatment means that drug side-effects will occur either immediately or after a few weeks of treatment and should be made known to the owner before starting any treatment so that an informed decision can be made (Ettinger 2003).

Cyclophosphamide's mechanism of action is inhibition of DNA synthesis and function of tumour cells caused by cyclophosphamide metabolites connecting two nucleotides residues originating from a common DNA strand (DNA cross-link) and interrupting DNA strand separation putting a stop to DNA replication and transcription (Thamm *et al.* 2013; Ramsey 2017). Cyclophospamide induces myelosuppression and white blood cells typically reach their lowest concentration 5-14 days after drug administration, as such, neutrophil concentration needs to be monitored regularly (Ramsey 2017) and should their concentrations reach lower levels than recommended (<1500 cells/µl) treatment should be postponed until their concentration normalizes (Vail 2011). A metabolite of cyclophosphamide known as acrolein can cause the development of sterile haemorrhagic cystitis which can lead to fibrosis and carcinoma of the bladder in chronic situations. For this reason, adequate water intake needs to be maintained and furosemide may be given as part of the protocol (Ramsey 2017).

Doxorubicin affects DNA transcription and replication, effectively preventing tumour cell proliferation. Doxorubicin achieves this effect by binding to DNA-associated enzymes like topoisomerase I and II and disrupting their normal function resulting in DNA damage. Histone eviction can result in deregulation of DNA repair and doxorubicin can also activate signals that lead to apoptosis (Pang *et al.* 2013; Tacar, Sriamornsak, and Dass 2013) . Doxorubicin can cause acute anaphylactic reactions and it is recommended to have adrenaline, steroids and fluids ready if a dog happens to have an allergic reaction to the drug. Cardiotoxicity is another side-effect which is dose-dependent and can lead to the development of cardiomyopathy and congestive heart disease, as such, it is not recommended in patients with heart disease. Auscultation during administration is also recommended as tachycardia and arrythmias can be caused by administration. Anorexia, vomiting, haemorrhagic gastroenteritis, and nephrotoxicity are common side-effects. Complete blood count and platelet count (normally done before and after each chemotherapy session) can detect low concentrations of leukocytes and platelets. A great deal of care needs to be present when administrating doxorubicin because any extravasation resulting from perivascular administration of the drug can lead to severe tissue necrosis. Dexrazoxane is recommended in these cases (Ramsey 2017).

Vincristine targets and bind to tubulin which results in failure of microtubule arrest and metaphase arrest triggering apoptosis of tumour cells (Pasquier and Kavallaris 2008; Ramsey 2017). Vincristine may cause gastrointestinal problems like ileus and constipation due to toxicity, peripheral neuropathy (rarely reported) and severe tissue irritation when not correctly administered intravenously (Ramsey 2017).

Prednisolone targets specific glucocorticoid receptors in cells and alters DNA transcription resulting in immunosuppressive and lympholytic effects (Bansal and Acharya 2014; Ramsey 2017). Prednisolone can cause vomiting, diarrhoea and gastrointestinal ulceration and some animals can have hyperglycaemia episodes. Adrenal atrophy and glomerular changes can develop but is almost exclusively on chronic usage of the drug (Ramsey 2017).

Asparaginase or L-asparaginase depletes serum asparagine levels and deprives tumour cells, which are unable to produce their own, from acquiring this amino acid that is required for protein synthesis resulting in cell death (Egler, Ahuja, and Matloub 2016; Ramsey 2017). Like doxorubicin can induce anaphylactic reactions. It may cause gastrointestinal upset, hepatotoxicity and coagulation deficits. Bone marrow depression and haemorrhagic pancreatitis have both been reported in dogs. Asparaginase is not recommended for dogs with liver disease or active pancreatitis and should be given after vincristine, to avoid vincristine clearance problems, and to avoid anaphylactic reactions a single dose of an antihistamine can be given before asparaginase administration (Ramsey 2017).

The British Small Animal Veterinary Association (BSAVA) recommends the University of Wisconsin-Madison-Short CHOP protocol, which is a 19 week protocol (Vail 2011; Ramsey 2017). Longer protocols, known as continuous protocols, that last over 78 weeks have the same remission rates and survival times making shorter protocols the preferred recommendation when it comes to cL treatment (Chun 2009).

Less aggressive multi-agent protocols have lower complete remission rates (60-75%) and survival times (6-7 months) (Ettinger 2003). COP is the recommended protocol and has 2 variations, a high dose and a low dose protocol (figure 18) (Lascelles and White 2011; Vail 2011). Another disadvantage is the fact these protocols are very long due to having maintenance periods (Chun 2009).

Protocols that combine doxorubicin with prednisolone and optionally l-asparaginase are an affordable alternative to CHOP protocols, but remission rates and survival times are lower (Al-Nadaf *et al.* 2018).

Figure 18. COP high and low dose chemotherapy protocols for canine lymphoma recommended by the BSAVA. Adapted from (Dobson and Lascelles, 2011).

COP high dose for dogs		*COP low dose for dogs*	
Cyclophosphamide (250–300 mg/m² i.v. or orally)	Give once every 3 weeks for 1 year. If dog is in complete remission at 1 year, decrease to once every 4 weeks for 6 additional months. Discontinue if animal is in complete remission at 1.5 years	Cyclophosphamide (50 mg/m² orally)	Give q48h or for the first 4 days of each week
		Vincristine (0.5 mg/m² i.v.)	Give q7d
Vincristine (0.75 mg/m² i.v.)	Give once a week for 4 doses, then once every 3 weeks for a year on the same day as cyclophosphamide. If dog is in complete remission at 1 year, decrease to once every 4 weeks for 6 additional months. Discontinue if animal is in complete remission at 1.5 years	Prednisone/prednisolone	Give at 40 mg/m² orally q24h for 7 days, then at 20 mg/m² orally q48h
		Maintenance	After 8 weeks of induction, continue COP alternate-week treatment for 4 months, then 1 week in 3 for 6 months, and reduce to 1 week in 4 after 1 year
Prednisone/prednisolone (1 mg/kg orally)	Give daily for 22 days, then every other day for 1.5 years. Discontinue by gradual tapering over 3 weeks if animal is in complete remission at 1.5 years		

6.2. Single-agent chemotherapy protocols

Single-agent protocols are not as successful as multiple-agent CHOP based protocols, with remission rate and median survival time of single-agent protocols being 75-85% and 6-9 months compared to 80-90% remission rate and 12 months of median survival time of CHOP protocols. However, they have higher remission rate than COP protocols (60-75%) and similar survival times to COP based protocols (6-7) (Ettinger 2003; Simon *et al.* 2008; Chun 2009).

Single-agent prednisone protocols should be considered as an option for owners that cannot afford other type of chemotherapy, but disease-free times are very short (1-2 months) and all the side-effects previously mentioned may appear (Ettinger 2003; Chun 2009).

Lomustine has been reported to not have a very successful therapeutic effect on cL (Sauerbrey *et al.* 2007) and the same happens for mitoxantrone (Michael D. Lucroy *et al.* 1998) and these drugs are recommended for relapse of cL when multi drug resistance has developed (Michael D. Lucroy *et al.* 1998; Sauerbrey *et al.* 2007; M. Zandvliet 2016).

Side effects for lomustine include myelosuppression with neutropenia that can be life threatening in some cases, thrombocytopaenia and gastrointestinal and hepatic toxicity. Mitoxantrone can cause vomiting, diarrhoea, anorexia, myelosuppression and can be cardiotoxic but to a lesser degree than doxorubicin. Both these drugs cause myelosuppression and should be avoided when dogs already have a compromised bone marrow (Ramsey 2017).

The most successful single-agent chemotherapy protocol is monotherapy doxorubicin. This method of treatment is more affordable and less time consuming than multi-agent protocols and has a relatively high success rate regarding remission rates (75-85%) and survival times (6-9 months) (Ettinger 2003; M. Zandvliet 2016). An intermittent monotherapy protocol using doxorubicin is very well tolerated by dogs and is an affordable option for owners with lesser financial power and is also a valid option for owners that cannot commit to weekly visits for extended periods of time (Higginbotham *et al.* 2013). The induction phase of this protocol consisted of one doxorubicin administration every two weeks with 30mg/m^2 doses or 1mg/kg doses for dogs under 15kg. After remission was achieved, bimonthly evaluation was performed to assess state of remission and if signs of progressive disease (lymph node enlargement) were shown a new dose of doxorubicin was administered. This process was repeated until dogs showed signs of drug resistance (lymph nodes keep enlarging).

6.3. Radiotherapy

Radiotherapy as the sole treatment for cL has not shown results that encourage its use as a single first line treatment. In a study of 14 dogs treated with radiotherapy as sole therapeutic method the overall response was low (34%), but out of 14 dogs only 9 completed the full radiotherapy protocol (Laing *et al.* 1989; Meleo 1997).

Radiotherapy coupled with a CHOP protocol can be an effective way to obtain longer remission and survival times. A study of 8 dogs that were subjected to a 25 week CHOP protocol with 2 sessions of half-body radiotherapy reported remission and survival times of 451 and 532 days, respectively. The small number of dogs means that results are not an accurate estimate, however the lack of more severe toxicity side-effects means that it can be further studied in the future (Gustafson *et al.* 2004).

Radiotherapy doses are typically between 6-10 Grays (Gy) and given on one half of the body first and then 4 weeks later on the other half to minimize radiation toxicity. Side-effects from radiotherapy include alopecia, faster shedding and lighter coloured fur, myelosuppression resulting in thrombocytopaenia and neutropoenia, vomiting, nausea and diarrhoea (Laing *et al.* 1989; Meleo 1997; Gustafson *et al.* 2004). Radiation pneumonitis can develop and cause non-productive coughing, fever and dyspnoea. It is managed with a long course of glucocorticoids, but when tapering down doses relapse of symptoms can happen (Bledsoe, Nath, and Decker 2017).

Additionally, tumour lysis syndrome has been reported in dogs (Laing *et al.* 1989). The typical signs of this syndrome are metabolic acidosis and hyperuricemia which can lead to acute kidney injury due to uric acid precipitates and in turn compromise the kidneys' filtration capabilities. Kidney injury and the lysis of tumour cells leads to hyperphosphatemia, hyperkalaemia and hypocalcaemia that can provoke cardiac arrhythmias and sudden death (Laing *et al.* 1989; Howard, Jones, and Pui 2011; M. Zandvliet, Rutteman, and Teske 2013).

6.4. Re-induction and rescue protocols

As previously stated cL cannot be cured, as such, at some point clinically significant lymphoma will reappear and re-induction using previous chemotherapy protocol may be attempted. The closer to the discontinuation of the initial protocol the more successful re-induction is, but remission times most likely will lost only half the time (Ramsey 2017).

If induction or reinduction is not successful owners may resort to rescue protocols. These protocols use a variety of novel drugs that were not used on the initial chemotherapy and may be used in combination with each other (Ramsey 2017).

Rescue protocols vary in response rate (15-83%) and response duration is typically 1.5 to 2.5 months. The protocols that appear to have the best response rates are single-agent doxorubicin and actinomycin-D, a multi-agent protocol known as MOPP (Lascelles and White 2011) and a combination of lomustine, l-asparaginase and prednisone (Saba, Thamm, and Vail 2007; Ramsey 2017). Although with an apparent lower response rate, single-agent lomustine has a relatively high response duration (Lascelles and White 2011).

Actinomycin-D monotherapy has achieved an overall response rate of 83% in dogs with lymphoma (Hammer *et al.* 1994). Administration is every 3 weeks until effects are no longer noticeable. the protocol begins with doses of 0.5 mg/m^2 and increases 0.1 mg/m^2 every administration until toxicity is noted and treatment continues with the highest dose before toxicity was noted (Hammer *et al.* 1994). Actinomycin-D administration may cause myelosuppression, hepatotoxicity and gastrointestinal toxicity (Ramsey 2017). Gastro intestinal toxicity is the most common side-effect of actinomycin-D rescue chemotherapy and about 33% show signs like nausea, anorexia, vomiting, diarrhoea and weight loss, however gastrointestinal toxicity is not dose related (Hammer *et al.* 1994).

MOPP has been successful in dogs refractive to previous chemotherapy. MOPP uses 4 drugs that give its name: M is for mechlorethamine, O is for oncovin (vincristine), P for procarbazine and prednisone. Treatment is based on 28-day cycles and 3 weeks rest periods between such cycles. Overall response to the protocol is 65%. Gastrointestinal signs like vomiting and diarrhoea are the most common side-effects with 28% of dogs having singular or reoccurring signs of gastrointestinal toxicity. Neutropoenia can happen but in much lower frequency (Rassnick *et al.* 2002).

The combination of lomustine, l-asparaginase and prednisone has been shown to have an overall response rate of 87% in dogs with previous CHOP chemotherapy. Lomustine can be given orally every 3 weeks with doses of 70 mg/m^2 for dogs over 15kg and 60mg/m^2 for dogs under 15kg. Doses are rounded down when necessary due to commercial formulations having preset concentrations. If neutrophil count is less than 500 cells/µL a week later to administration, doses are reduced by 10mg/m^2 for the rest of the treatment. L-asparaginase is given subcutaneously at doses of 400 IU/kg or 10,000 UI/m^2 on the first 2 lomustine administrations. Prednisone is given orally everyday starting at 2mg/kg, then it is tapered until doses of 1mg/kg are reached and from this point onward, prednisone is given every other day (Saba, Thamm, and Vail 2007). Side-effects include vomiting, diarrhoea, neutropoenia and possible hepatotoxicity (Saba, Thamm, and Vail 2007; Ramsey 2017).

6.5. Additional remarks regarding chemotherapy and some lymphoma entities' response to chemotherapy agents

Many chemotherapy drugs have potential nephrotoxic, hepatotoxic and myelosuppressive effects. Dogs that have compromised kidneys, liver and bone marrow are therefore at risk of developing tumour lysis syndrome and sepsis (Howard, Jones, and Pui 2011; M. Zandvliet 2016). However, chemotherapy is needed if tumour remission would reverse organ damage. For this, diuretic treatment may help avoid uric acid precipitation and accumulation of serum calcium, phosphate and potassium by increasing kidney function (M. Zandvliet 2016).

T-cell lymphomas and high-grade lymphomas have a worse response to classic chemotherapy treatment. Alternative treatments like l-asparaginase coupled with MOPP protocol can be used when dealing with T-cell lymphomas (Brodsky *et al.* 2009; M. Zandvliet 2016), however in some cases a CHOP protocol appears to have the same success rate. Case selection in these studies might be the reason for this contrasting evidence (Rebhun *et al.* 2011). The addition of cytosine arabinoside to CHOP protocols appears to improve responsiveness to chemotherapy treatment in stage V cL (L. Marconato *et al.* 2008).

Canine generalized cutaneous epitheliotropic lymphoma also responds poorly to conventional CHOP protocols (Ramsey 2017). Lomustine at doses of 70mg/m^2 every 3 weeks appears to have responsiveness rates between 70 and 83%, but remission times are reported to be about 3 months (Fontaine *et al.* 2009; Risbon *et al.* 2006; Ramsey 2017). Radiation therapy might be beneficial for focal lesions in these lymphomas and can be used in combination with other treatment methods (Fontaine *et al.* 2009).

Indolent lymphomas do not respond well to chemotherapy as chemotherapy mainly targets actively proliferating cells and as such monitoring disease progression to detect when lymphoma enters higher stages is essential to determine when chemotherapy might be appropriate (Valli *et al.* 2006; M. Zandvliet 2016; Cozzi *et al.* 2018).

Gastrointestinal lymphomas do not respond well to chemotherapy and benefit from recession procedures when obstruction occurs. The same applies to lymphomas with primary location on the nervous central system which respond poorly to chemotherapy as most drugs do not cross the blood-brain barrier. As such, using drugs like cytosine arabinoside administrated intrathecally and using radiotherapy coupled with a chemotherapy protocol is recommended (Ettinger 2003).

7. Paraneoplastic syndromes

Paraneoplastic syndromes are indirect effects resulting from tumour cells releasing biologically active substances, like hormones and cytokines, that alter bodily functions, which is a major factor in lymphoma-related morbidity (Lucas *et al.* 2007; Elliott 2014). Paraneoplastic syndromes sometimes are the first indicator of underlying neoplasia and are important prognostic factors as they can be the main cause of mortality and higher degrees of morbidity (Mellanby 2011). Treatment of the underlying neoplasia normally reverts the effects but in cases where severe clinical illness is present additional therapy focused on the paraneoplastic syndrome is needed (Mellanby 2011; Elliott 2014).

Hypercalcaemia caused by neoplasia also referred to as hypercalcaemia of malignancy, is the most common paraneoplastic syndrome in dogs diagnosed with lymphoma (Mellanby 2011) and most characteristic of T cell lymphomas, although it can develop in B cell lymphomas as well(M. Zandvliet 2016). Tumour cells may cause osteolysis upon invasion of the bones by having an agonistic effect on osteoclasts resulting in elevated serum calcium due to bone resorption. Additionally tumour cells may secrete a hormone similar to the parathyroid hormone (PTH) called parathyroid hormone-related protein (PTHrP) which will stimulate bone resorption carried by osteoclasts (Lucas *et al.* 2007) and renal tubular absorption (Mellanby 2011). Dogs with hypercalcaemia typically present with dehydration, polyuria and compensatory polydipsia

and inability to concentrate urine due to the inhibitory effect on renal antidiuretic hormone receptors. High levels of serum calcium can cause increase in neurons' action potential thresholds leading to weakness and lethargy. Non-specific signs like nausea, anorexia, vomiting and diarrhoea can also be caused by gastrointestinal effects of hypercalcaemia (Lucas *et al.* 2007).

Therapy for hypercalcaemia for malignancy focuses on treating the underlying neoplasia and re-establishing the normal serum calcium levels. On successful chemotherapy for lymphoma, serum calcium levels should normalize, but animals that show significant clinical illness should be hospitalized and aggressive fluid therapy should be started (Mellanby 2011; Elliott 2014). Fluid therapy three times a day with a calcium-free intravenous solution and furosemide after hydration has been established is the recommended therapy protocol (Elliott 2014).

Hypoglycaemia can develop due to increased glucose usage by tumour cells but can be managed by supplementing with food containing complex carbohydrates and in more severe cases intravenous supplementation with dextrose (Elliott 2014; Idowu and Heading 2018).

Haematological paraneoplastic syndromes like anaemia, thrombocytopaenia and eosinophilia can all develop due to lymphoma. Anaemia can be a consequence of myelosuppression by tumour cells invading bone marrow or immune-mediated destruction of erythrocytes and resolution can be achieved through treatment of the lymphoma. However, a certain type of non-regenerative anaemia, anaemia of chronic disease (ACD), characterized as normocytic and normochromic can persist after chemotherapy (Mellanby 2011; Elliott 2014).

Just like anaemia, thrombocytopaenia can occur when tumour cells invade bone marrow and cause myelosuppression or immune-mediated destruction of platelets. Lower platelets count can lead to petechiation and haemorrhage. Successful treatment of the lymphoma leads to resolution, but in more severe immune-mediated thrombocytopaenia cases fresh whole blood transfusion may be needed (Mellanby 2011; Elliott 2014).

Eosinophilia occurs when tumour cells, typically in T cell lymphomas, release interleukin-5 and can be reverted with lymphoma treatment (Mellanby 2011; Elliott 2014).

Hyperglobulinaemia has been associated with lymphoma in dogs and occurs when tumour cells produce monoclonal immunoglobulins (monoclonal gammopathy). Hyperglobulinaemia can inhibit normal immunoglobulin production which put dogs at risk of infection, lead to hyperviscosity syndrome (increased blood viscosity) which can cause retinopathies, ataxia, seizures and haemorrhage due to poor platelet aggregation (Mellanby 2011; Elliott 2014). Treatment of lymphoma is necessary for reversal of hyperglobulinaemia and cyclophosphamide has been previously used to successfully treat hyperglobulinaemia (Matus *et al.* 1983).

8. Prognosis

The prognosis for dogs depends on many factors. The most significative prognostic factors are clinical substage (Table 19) and immunophenotype, that is, dogs that show clinical signs of illness (substage b) and dogs that have been diagnosed with T cell lymphomas or highly aggressive B cell lymphomas are typically associated with a poorer prognosis (Vail 2011; L. Aresu *et al.* 2015; M. Zandvliet 2016). Dogs with substage b disease have lower remission rates and smaller survival times when compared to substage a patients (Iii *et al.* 1997; Jagielski *et al.* 2002). T-cell lymphomas like T-LBL and PTCL-NOS and B cell lymphomas like immunoblastic DLBCL have shorter survival times (Valli *et al.* 2013).

The presence of severe clinical illness due to paraneoplastic syndromes can be a poorer prognostic factor (Mellanby 2011). Poorer response to initial chemotherapy has been associated with poorer prognosis as well (Jagielski *et al.* 2002).

Other factors strongly associated with a poorer prognosis are previous corticosteroid treatment (Laura Marconato *et al.* 2011), extranodal lymhphoma locations like the central nervous system, gastrointestinal tract and skin (M. Zandvliet 2016), and T-cell lymphoma associated hypercalcaemia (Rosenberg, Matus, and Patnaik 1991).

However, each dog will have different disease progression and response to treatments, so, monitoring signs of disease progression, response to treatment and adapting treatment methods to better reach remission is essential in good lymphoma management and ensuring that dogs have a good quality of life (Ettinger 2003; Vail 2011; M. Zandvliet 2016).

9. Conclusion

Non-Hodgkin canine lymphoma has unknown aetiology and is the primary haematopoietic neoplasia diagnosed in companion animals like the dog. Due to the many clinical presentations, immunophenotypes with different behaviours and each dog's different response to treatment it is a case-by-case disease.

Chemotherapy is the treatment of choice for most canine lymphoma cases as it has been proven to be the best method in controlling the disease for the longest periods of time possible and is very well tolerated by dogs. Chemotherapy protocols and alternative treatment methods need to be tailored to the specific presentation and immunophenotype of the lymphoma while also being in accordance with the time availability and financial situation of each owner because treatment is essential in ensuring the patients have the best quality of life possible.

Part III - Clinical case: Henry

1. Introduction

Henry was a neutered, 9-year-old Border Collie and weighed around 20 kilograms. Henry was diagnosed with malignant lymphoma and subjected to CHOP chemotherapy. He achieved complete remission, but lymphoma reappeared later. In the end he was euthanized.

2. Anamnesis

Henry had previous history of an axillary mass, which at the time was diagnosed as lipomas after fine needle aspiration samples were analysed. On the 3rd of December 2018 the mass situated on his left axilla was disc shaped, measured 6cm by 3cm, had a slight muscle attachment but was still moveable. There were no signs of inflammation, pain and discomfort and was described as having a soft and fatty texture. On the 13th of June 2019 the mass had retained the same shape and size and the only notable finding during physical examination was a slightly tense abdomen. On the 23rd of December 2019 the axillary mass was still the same size.

However, on the 14th of April 2020 the owner contacted the practice and pointed out that Henry had developed two new masses on the neck and was eating less than normal and promptly booked a consult for the following day, the 15th.

On the 15th of April 2020, upon physical examination, the retromandibular, inguinal and left popliteal lymph nodes were visibly enlarged and an FNA of each was performed.

3. Diagnosis

Fine needle aspiration was performed on the affected lymph nodes and samples were sent for cytology. Results were obtained on the same day (the 15th of April 2020) and the predominant cell type (over 80% of the cell population) was identified as large immature neoplastic lymphocytes with a large population of lymphoglandular bodies.

With all the evidence available, Henry was diagnosed with malignant lymphoma. No further classification was pursued by the owner who decided on following a chemotherapy protocol as soon as possible.

4. Pre-chemotherapy clinical chemistry and haematological profile

Before starting chemotherapy, on the 23rd of April 2020 a comprehensive profile was established by measuring a variety of parameters including a complete blood count, ion content, blood gases and liver/kidney function enzymes.

Multiple parameters had values that did not fall in the normal range set for dogs as seen in table 21. Besides these parameters presence of polychromasia was pointed out by the equipment.

Table 21. Henry's pre-chemotherapy comprehensive profile. Reference ranges adapted from (Fielder 2015).

Parameters	Reference range	Henry's results
Urea	2.9-10 mmol/L	5
Creatinine	44-150 µmol/L	98
ALT (Alanine Transaminase)	10-109 U/L	135
ALP (Alkaline Phosphatase)	1-114 U/L	294
TP (Total Protein)	54-75 g/L	68
Albumin	23-31 g/L	26
Globulin	27-44 g/L	42
Glucose	4.2-6.6 mmol/L	3.5
Bile Acid	<15 µmol/L	24
Sodium	142-152 mmol/L	141
Potassium	3.9-4.1 mmol/L	4.6
Chloride	110-124 mmol/L	112
Calcium	2.3-2.9 mmol/L	2.1
Phosphorus	0.9-1.7 mmol/L	1.3
cPL (Canine Pancreatic Lipase)	<200 µg/L	296
TT4 (Total Thyroxine)	6.14-45.5 nmol/L (Hegstad-Davies *et al.* 2015)	16
Cholesterol	3.5-7.2 mmol/L	5.6
CK (Creatine Kinase)	52-368 U/L	147
Total Bilirubin	0-5.1 µmol/L	5
Hb (Haemoglobin)	11.9-18.9 *10 g/L	10.5
PCV (Packed Cell Volume)	0.35-0.57 L/L	0.28
RBC (Red Blood Cells)	4.95-7.87 $*10^{12}$/L	4.38
MCV (Mean Corpuscular Volume)	66-77 fL	64.8
MCHC (Mean Corpuscular Haemoglobin Concentration)	32-36.3 *10 g/L	37
WBC (White Blood Cells)	5-14.1 $*10^9$/L	6.4
Neutrophils (Segmented)	2.9-12 $*10^9$/L	4.9
Lymphocytes	0.4-2.9 $*10^9$/L	1.2
Monocytes	0.1-1.4 $*10^9$/L	0.3
Reticulocytes	0-1%	3.7
Platelets	211-621 $*10^9$/L	187
pH	7.35-7.45 (Waddell 2012)	7.38
pCO2 (Venous)	40-50 mmHg (Waddell 2012)	36
pO2 (Venous)	30-42 mmHg (Waddell 2012)	31
HCO3 (Venous)	20-24 mEq/L	21.4

	(Waddell 2012)	
BE (Base Excess)	-4 - +4 mmol/L (Waddell 2012)	-4

5. Chemotherapy

Henry's body surface area (BSA) was determined to be 0.744m^2 at 20kg (BSA (m^2) = 0.101 × (weight in kg$^{(2/3)}$)) when the protocol started but was adjusted as his weight varied. The protocol chosen was a CHOP based chemotherapy protocol similar to the University of Wisconsin – Madison Lymphoma CHOP protocol. His protocol is represented in table 22 with the protocol week and the day of administration, the drugs given and any remarks between weeks. The first day of chemotherapy was the 25th of April 2020 and last day 6th of October 2020.

Henry had weekly haematological tests, typically a day prior to the day of chemotherapy administration, in order to monitor neutrophil population.

Table 21. Henry's induction chemotherapy protocol.

Week/Day	Drugs/Dosage/Route	Remarks
1 – 25/04	Vincristine 0.52ml IV Prednisolone 2mg/kg SID	-
2 – 2/05	Cyclophosphamide 172.5mg PO Prednisolone 1.5mg/kg SID	Rash on vincristine IV site Lymph nodes reduced in size Thrombocytopaenia resolved
3 – 12/05	Vincristine 0.52ml IV Prednisolone 1mg/kg PO SID	2kg weight gain Lymphopaenia
4 – 19/05	Doxorubicin 22.3mg (11ml) IV (over 20 minutes) Prednisolone 0.5mg PO SID Maropitant 1mg/kg SC Chlorphenamine 1 vial (1ml) IM	Lymphopaenia
5	-	No treatment
6 – 2/06	Vincristine 0.52ml IV	Really sick after doxorubicin administration Delayed due to *E.coli* infection
7 – 9/06	Cyclophosphamide 150mg PO	Leucopenia Weight gain
8 – 23/06	Vincristine 0.52ml IV	Delayed due to low neutrophil count
9 – 30/06	Doxorubicin 23.55mg (11.78ml) IV *only* 7ml given (over 20 minutes) Maropitant 1mg/kg SC Chlorphenamine 1ml IM	Partial doxorubicin dose due to previous side effects
10	-	No treatment
11 – 14/06	Vincristine 0.45ml IV	Weight gain Leucopenia
12 – 21/07	Cyclophosphamide 200mg PO	Leucopenia Neutrophil count near borderline
13- 4/08	Vincristine 0.45ml IV	Delayed due to neutrophil count too low

14– 12/08	Doxorubicin 23,78mg (11.89 ml) IV (over 20 minutes) *only* 8ml given Maropitant 1mg/kg SC Chlorphenamine 1ml IM	Partial doxorubicin dose due to previous side effects
15	-	No treatment
16- 8/09	Vincristine 0.36ml IV	Delayed due to low neutrophil count New mass appeared (non-neoplastic) Vincristine dose reduced due to neutrophilia
17 – 15/09	Cyclophosphamide 200mg PO	Responding well to reduced doses
18 – 29/09	Vincristine 0.4ml IV	Delayed due to low neutrophil count Mid-range vincristine dose as no leucopaenia developed following a 0,36ml dose
19 – 6/10	Doxorubicin 11.89ml IV *only* 8ml given Maropitant 1mg/kg SC Chlorphenamine 1ml IM	Leucopaenia Partial doxorubicin dose due to previous side effects

6. Chemotherapy Follow-up

After protocol completion, during a few weeks Henry had low levels of white blood cells that with constant monitoring were determined to be improving at each check-up. For example, on the 19th of October 2020, henry's white blood cells count was $3.6*10^9$/L (normal range is 5-14.1 $*10^9$/L), but on the next examination on the 2nd of November 2020, white blood cell count had normalized ($5*10^9$/L).

The multiple areas of alopecia especially his tail, had started growing back after the first week post chemotherapy. Additionally, the owner reported that Henry was behaving normally at home.

A complete blood count on the 7th of January 2021 revealed reduced white blood cell count at $3.7*10^9$/L (5-14.1$*10^9$/L) and slight neutropaenia at $2.8*10^9$/L (2.9-12$*10^9$/L). However, atypical white cell morphology was not detected, and lymph nodes were not visibly enlarged.

On the 22nd of January Henry had a consult because for the past three days he had lost his appetite and was slightly lethargic. No enlarged lymph nodes were observed during physical examination. Blood samples were taken for a comprehensive haematological profile. He was given maropitant for three days to help improve his appetite.

The comprehensive profile was done on the 23rd of January by an external clinical pathology laboratory (AXIOM). According to AXIOM's standards Henry had leukopaenia and lymphopaenia, but no longer had neutropaenia. His albumin levels were slightly decreased, his ALT, glutamate dehydrogenase (GLDH), bile acids, amylase and DGGR (1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester) lipase were all elevated. Results can be seen in Table 23 bellow. These values can be seen in table 23

Table 22. Abnormal parameters in Henry's comprehensive profile on the 23rd of January 2021.

Parameter	Normal Range provided by AXIOM	Result
WBC	5-14.1$*10^9$/L	5.3
Lymphocytes	0.4-2.9$*10^9$/L	0.4
Albumin	26-40 g/L	25.9

ALT	13-78 U/L	108
GLDH	2-6 U/L	9.2
Bile Acids	0-15 µmol	15.5
Amylase	100-948 U/L	1271
DGGR lipase	0-90 U/L	95

Due to these altered values, Henry was prescribed a low-fat diet for a suspected pancreatitis and an abdominal ultrasound was planned given his previous history with lymphoma. His appetite had continuously improved while on medication (maropitant).

On the 28th he was submitted to the abdominal ultrasound. The ultrasound revealed a right pancreatic limb increased in size, distended common bile duct at 7.2mm maximal diameter measured (normal diameter is less than 4mm). His spleen appeared to be larger than normal but had normal echogenicity. Liver parenchyma was normal and no lymph nodes were found to be enlarged. Canine pancreatic lipase immunoreactivity (cPLI) test result was 145.1 µg/L (0-200 µg/L). No signs of abdominal pain or discomforted were observed.

On the 3rd of February 2021, Henry once again had a consult because of loss of appetite while on medication (omeprazole and maropitant). On the physical examination popliteal and retromandibular lymph nodes were visibly enlarged again. Blood samples was taken for haematology, which revealed lymphocytes were on the lower end of the normal range at $0.8*10^9$/L (0.4-$2.9*10^9$/L) and low MCV at 63.2fL (66-77fL), and FNA of the popliteal lymph node was sent for cytology. The cytology results once again, had evidence of lymphoma, that is, a great percentage (>70%) of lymphocyte population was described as immature and neoplastic and a large presence of lymphoglandular bodies.

To determine the next step, the practice contacted an oncology specialist who suggested that attempting to re-induce remission with a CHOP protocol was advantageous. The owner was informed that the next remission would be shorter. The owner agreed to proceed with chemotherapy.

7. Re-induction chemotherapy

The same CHOP protocol was followed and is represented in table 24 bellow.

Table 23.Henry's re-induction chemotherapy protocol.

Week/Day	Drugs/Dosage/Route	Remarks
1 – 10/02	Vincristine 0.518ml IV Prednisolone 2mg/kg SID	-
2 – 17/02	Cyclophosphamide 200mg PO Prednisolone 1.5mg/kg SID	Leucopaenia Lost weight
3 – 24/02	Vincristine 0.518ml IV Prednisolone 1mg/kg PO SID	Leucopaenia Lymph nodes appear to be normal Slight muscle weakness
4 – 4/03	Doxorubicin 20mg (10ml) IV (over 20 minutes) *only* 8ml given Prednisolone 0.5mg PO SID Maropitant 1mg/kg SC	Lost more weight Periods of appetite loss Partial doxorubicin dose due to previous side effects
5	-	No treatment
6 – 17/03	Vincristine 0.53ml IV	Leucopaenia Gained weight
7 – 24/03	Cyclophosphamide 200mg PO	Gained weight
8 – 31/03	Vincristine 0.53ml IV	Leucopaenia
9 – 8/04	Doxorubicin 20mg (10ml) IV *only* 8ml given (over 20 minutes)	Urinary tract infection Partial doxorubicin dose

	Maropitant 1mg/kg SC	due to previous side effects
10	-	No treatment

On the 3rd of April 2021, Henry was brough to the practice due to loss of appetite and blood in his urine. The owner brought a urine sample which was sent for analysis. Urinalysis confirmed the presence of haematuria and proteinuria (4+ or >16mmol/L and 3+ or >20g/L respectively) which indicated possible urinary tract infection confirmed by microscopic analysis of the sample that had revealed the presence of white blood cells, increased population of epithelial cells, red blood cells and was positive for cocci. He was treated for a urinary tract infection with a combination of amoxicillin and clavulanic acid.

On the 19th of April, Henry was admitted for hospitalization for dyspnoea, lethargy, no appetite and pyrexia. Lymph nodes were found to be enlarged regarding previous times. A comprehensive blood profile was done. Pancreatic lipase was very high measuring at 1697.7 µg/L, there was evidence of anaemia (Red blood cells count was $3.8*10^{12}$/L, normal range 4.95-$7.87*10^{12}$/L) and leucopaenia ($3.6*10^{9}$/L, normal range 5-$14.1*10^{9}$/L) with lymphocyte and neutrophil count being on the lowest margin of the normal range, $0.4*10^{9}$/L (0.4-$2.9*10^{9}$/L) and $3*10^{9}$/L (2.9-$12*10^{9}$/L) respectively.

On the next few days (20th – 22nd), fever continued even with anti-inflammatory medication, his appetite had not fully returned, and he was having trouble moving and lymph nodes were still enlarged. Repetitive tachycardia had developed.

On the 26th of April 2021, Henry was visibly distressed and at physical examination lymph nodes were still enlarged, arrythmias were present, a large mass on the cranial abdomen could be felt and his mucosa was pale.

His owner had chosen to continue treatment while Henry showed signs of improvement, however, due to his worsening condition he was euthanised and passed away peacefully near his owner.

8. Discussion

A variety of clinical factors in this case indicated a possible lymphoma diagnosis. Henry was 9 years old at the time which falls into the median age range for cL development (Vail 2011) and was a Border Collie which has some degree of overrepresentation of cL and is considered an at-risk breed (Edwards *et al.* 2003; Cheng *et al.* 2019).

Painless lymphadenopathy with no other specific signs of disease is the characteristic signalment of multicentric lymphoma (Ettinger 2003). Additionally, Henry had lost his normal appetite which is a non-specific clinical sign associated with multicentric lymphoma in a smaller percentage of cases (Vail 2011).

No further classification was pursued by Henry's owner, but with the current knowledge of cL and evidence provided by the fine needle aspiration, Henry probably developed multicentric lymphoma as he only had lymphadenopathy (Vail 2011) and the multicentric presentation is by far the most common presentation in dogs (Vail 2011; M. Zandvliet 2016).

The large amount of lymphoglandular bodies found in the samples is characteristic of lymphoid malignancies, especially lymphoma, and are most common in B-cell lymphomas (Bavle 2014). The multicentric, high-grade B-cell lymphoma appears to be one of the most common lymphomas in the Border Collie (Cheng *et al.* 2019).

Many of the altered parameters on Henry's first comprehensive biochemical profile are possible of being attributed to lymphoma. Changes in ALT and ALP can be indicative of loss of hepatic function. A reduction in ALT and ALP levels when no previous acute hepatic injury has been reported can indicate loss of functioning hepatocytes, while increase in ALT levels can

indicate necrosis of hepatocytes or changes in cell permeability. However, only increases of over two-fold in ALT and ALP levels are indicative of acute cellular damage. Henry had a very small increase in ALT and ALP levels which can happen when lymphoma invades the liver and causes a small degree of cell destruction, but also happens in chronic hepatopathies (Villiers and Ristić 2016), reactive hepatopathy and dehydration (M. Zandvliet 2016).

Hypoglycaemia is a non-specific signal, but has been documented in a previous lymphoma case (Zhao *et al.* 1993). In Henry's case his loss of appetite might have caused the hypoglycaemia or even an increase in glucose usage by neoplastic cells (Idowu and Heading 2018). It is worthy of note that the normal range of glucose can vary depending on the source of information, while (Fielder 2015) defines the normal range as 4.2-6.6 mmol/L, other sources like (Idowu and Heading 2018) for example, use a range of 3.3-6.2 mmol/L which would classify Henry as normoglycaemic.

Henry's bile acid levels were increased which can be a consequence of hepatocyte damage due to lymphoma infiltrating the liver, however, bile acid levels are not a common indicator of neoplasia, but of hepatic dysfunction and cholestasis (Villiers and Ristić 2016). Levels of 1-381 mmol/L have been reported in dogs with neoplasia but the median is around 11.7 mmol/L (Pena-Ramos *et al.* 2021)

Elevated pancreatic lipase can be indicative of pancreatitis especially if levels are above 400 µg/L where the specificity of the tests is 100% and sensitivity is 70%. However, his test has low sensitivity (43%) when it comes to detecting mild pancreatitis (Trivedi *et al.* 2011) which could have been Henry's case due to his lipase levels not being over the 400 µg/L threshold and him not having clear clinical signs of pancreatitis except for his loss of appetite.

Looking at Henry's abnormal blood parameters it's possible to see that he was anaemic which can be a common finding in canine lymphoma cases. The haematocrit and PCV indicated moderate anaemia and his RBC and reticulocyte count point towards a regenerative anaemia (Villiers and Ristić 2016; Parachini-Winter, Carioto, and Gara-Boivin 2019). The presence of polychromasia is also indicative of regenerative anaemia (Hodges and Christopher 2011). Henry's lower MCV and normal mean corpuscular haemoglobin concentration (MCHC) indicate microcytic normochromic cells, although the degree by which MCV is lower is minimal. Even though Henry's parameters indicate a regenerative anaemia, a non-regenerative, normocytic, normochromic anaemia with elevated reticulocytes, known as anaemia of chronic disease, has been described in dogs with lymphoma (M. D. Lucroy *et al.* 1998; Miller *et al.* 2009).

Henry's platelet levels revealed thrombocytopaenia, which is a relatively common finding in canine lymphoma (Grindem *et al.* 1994) and is a sign of bone marrow infiltration by neoplastic cells (Ettinger 2003).

Additional classification of Henry's lymphoma wasn't pursued by the owner, but with the cytological evidence and clinical signs observed, multicentric lymphoma was the most likely diagnosis. Although no further classification was pursued by the owner, current literature and guidelines support the decision of starting chemotherapy with just cytological information of lymphoma. However, radiography and echography could have been performed to make sure there were no abdominal or thoracic evidence compatible with enlarged lymph nodes, masses or enlarged organs like the liver and spleen (Ettinger 2003; M. Zandvliet 2016).

A multi-agent CHOP based protocol was chosen which is the most efficient first-line protocol for multicentric lymphoma with the highest remission rates and longest survival times (Ettinger 2003). Before starting chemotherapy, neutrophils levels must be above 1500/µL (Vail 2011), which was Henry's case, and he was clear to proceed.

After the first dose of vincristine Henry developed a rash near the site where the catheter was placed, which can be a consequence of vincristine administration (Ramsey 2017), however, it was determined that the rash was provoked by the fur clipping. On week 4, after the

first doxorubicin administration, Henry felt really sick with episodes of vomiting and doxorubicin was then given at 70% of the dose for the rest of the protocol. Maropitant and chlorphenamine were given before doxorubicin administration to minimize chances of allergic reaction to doxorubicin and its gastrointestinal effects, doxorubicin was always administered in conjunction with free flowing 0,9% NaCl solution over 20 minutes and heart rate monitoring was done in order to detect any arrythmias due to the cardiotoxic effect of doxorubicin (Ramsey 2017).

During every chemotherapy session lymph nodes were palpated to determine any change in size of the lymph nodes, but more concrete measuring could have been done. After week 2 they reduced in size and continued until the end of the protocol. During the protocol Henry had an *E.coli* lower urinary tract infection which delayed chemotherapy for week 6, but made a recovery when treated with amoxicillin. Neutropaenia delayed some chemotherapy sessions due to neutrophil count being lower than 1500/µL and leucopaenia was a common occurrence in many weeks. Leucopaenia could have been caused by the bone marrow depression effects of chemotherapy agents like vincristine (Northrup *et al.* 2002; Ramsey 2017) and it resolved when vincristine doses were reduced. Another side effect of chemotherapy was alopecia and coat thinning which can be a consequence of doxorubicin and cyclophosphamide administration in dogs (Falk *et al.* 2017; Ramsey 2017).

Henry completed 19 weeks of chemotherapy and on the 6th of October 2020 was considered in complete remission as there was complete reduction of measurable disease (Ettinger 2003), as lymph nodes were no longer visibly enlarged and appeared to be of normal size at palpation.

A few weeks after chemotherapy was completed Henry had lower levels of white and red blood cells which is a characteristic side effects of chemotherapy agents that can affect the bone marrow (Northrup *et al.* 2002; Ramsey 2017).

After protocol ending, Henry was submitted to regular blood testing on a monthly basis. Results obtained on the 23rd of January 2021 showed an increased amylase and DGGR lipase characteristic of pancreatitis (Trivedi *et al.* 2011; Hope *et al.* 2021) and decreased albumin coupled with increased ALT is indicative of liver disease which could be due to neoplastic cell infiltration (Villiers and Ristić 2016). An enlarged pancreas found in the abdominal ultrasound supports the pancreatitis diagnosis (French *et al.* 2019).

On the 3rd of February 2021 physical examination revealed enlarged lymph nodes and cytological evidence of FNA samples collected from the lymph nodes revealed neoplastic cells supporting a diagnosis of lymphoma relapse (Ettinger 2003; M. Zandvliet, Rutteman, and Teske 2013). Following the advice of an oncology specialist and current literature (Ettinger 2003; Vail 2011; M. Zandvliet, Rutteman, and Teske 2013), a re-induction chemotherapy protocol using the same CHOP protocol was tried.

Henry responded well to the new protocol and lymph nodes reduced in size. Typical chemotherapy side-effects were noticed, but at this time, muscle weakness was noticed after the week 2, which was suspected to be from prednisolone as it can induce some degree of myopathy (Faludi, Mills, and Chayes 1964).

On the 19th of April 2021, Henry was hospitalized with sepsis-like symptoms characteristic of systemic inflammatory response syndrome (SIRS) possibly as a result of the cytotoxic effect and immune suppression chemotherapy provokes (Ettinger 2003; Campbell 2011). His symptoms were pyrexia, lethargy, tachycardia and hypoglycaemia. His pancreatic lipase was also very elevated which indicated an underlying pancreatitis flare-up. His lymph nodes were also enlarged which means he was in a state of progressive disease and lymphoma had acquired drug resistance (Ettinger 2003; Maurice Zandvliet and Teske 2015).

On the 26th of April 2021, his condition had not improved and on physical examination a cranial abdominal mass was found which meant that probably multiple organs had been

affected by the lymphoma and had probably developed stage IV/V substage b multicentric lymphoma (Ettinger 2003; M. Zandvliet, Rutteman, and Teske 2013).

The high clinical stage, progressive disease and the presence of systemic clinical signs indicate a poor prognosis (Jagielski *et al.* 2002) and with his quality of life deteriorating the owner agreed that euthanasia was the best option.

Henry's overall survival time since starting therapy was 12 months and remission time was around 4 months. Dogs treated with a CHOP protocol typically have a median survival time of 12 months like Henry, but Henry's remission time fall short the overall median remission time of 8 months (Ettinger 2003; Vail 2011; M. Zandvliet 2016).

Conclusion

The internship at Priory Veterinary Surgeons allowed the author to experience a multitude of cases and the workflow of a veterinary practice. The professional environment and high standard of care enabled the author's growth as a person and a future practitioner.

Writing this report was important for the consolidation of knowledge across all areas of veterinary medicine acquired throughout the years at university and the months spent as an intern at Priory.

It was also important in expanding the knowledge of canine lymphoma which is one of the most common neoplasias found in dogs, and even though the concrete aetiology is currently unknown, if left untreated the average survival time of dogs is extremely slow and morbidity is very high.

In conclusion, both the internship and report were crucial in further growing as a future veterinary medicine professional.

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