Is feline lymphoma worth managing differently?

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Abstract

T-cell lymphomas in dogs generally have a relatively worse prognosis than B-cell lymphomas with a few exceptions (e.g. T zone lymphoma). There are few reports that determine whether immunophenotype confers any prognostic value in cats. A number of other factors have been proposed by several studies however, many have only assessed specific types of lymphomas such as GI, nasal and CNS lymphomas, reporting variable survival rates.

The purpose of this study was to determine whether the immunophenotype of lymphoma as well as other variables (such as age, gender, neutering status, anatomical site, treatment or histological grade) could determine how long affected cats live with a view to determining whether any recommendations could be made regarding management of the disease. As part of this study, the proportion of cats with a laboratory diagnosis of lymphoma that are subtyped and the frequency of each anatomical location were determined. The levels of certainty of cytological diagnoses of lymphoma was also assessed.

Data for this study was obtained retrospectively from CVS group laboratory databases identifying cases diagnosed by cytology and histology between January 2014- January 2018. Following removal of exclusions, statistical analysis were performed on the remaining 1549 cases. Medical information was obtained via practice database search for the CVS-owned practices and for the non-CVS owned practice by way of questionnaire. These included lymphoma cases diagnosed by histology between February 2018 and January 2019. Data was collated in Microsoft Excel. Survival analyses were performed on a total of 140 cases that met the inclusion criteria.

The intestine was the most common anatomical location with the second most common anatomical location being the lymph nodes in the head and neck region. Diagnostic doubt was commonly expressed in cytology reports. Only a small minority of cases were immunophenotyped at initial clinicians requests (5.6%, n=87). None of the variables that were assessed proved to have any prognostic significance and in contrast to the dog, neither the B-cell nor T-cell immunophenotype influenced survival.

In summary, this study showed that immunophenotyping of feline lymphoma is seldom requested, posing a challenge both for its evaluation as a prognostic tool in large retrospective studies and potentially its future utilisation as a prognostic tool. Although the remaining variables that were assessed did not predict prognosis in this study, the relatively small numbers of treated cats in each category did not give sufficient statistical power and therefore prospective studies performed on large groups of affected cats would be required for further confirmation.

1 Introduction

Lymphoma is a malignant proliferation of lymphoid cells or tissue originating outside of the bone marrow. It represents the most commonly managed neoplastic disease in veterinary medical oncology. Feline lymphoma accounts for approximately 30% of all neoplasms and 90% of all haematopoietic tumours in cats (Dorn et al., 1967, Vail et al., 1998). In 1968, there was a reported incidence estimated at 200 per 100,000 cats (Dorn et al., 1968, Hardy, 1981). The relatively high incidence of lymphoma makes it an important disease in feline species. Feline lymphoma is a unique and complex disease and therefore our understanding of it is still emerging.

1.1 Risk factors for lymphoma

The two commonly described risk factors include infection with Feline leukemia virus (FeLV) and Feline immunodeficiency virus (FIV). Other proposed factors include chronic intestinal inflammation, inhalation of tobacco smoke and breed predisposition.

1.1.1 Feline leukaemia virus (FeLV)

FeLV is a directly oncogenic retrovirus and persistent viraemia shows a strong correlation with lymphoma, with a 60-fold or greater relative risk in antigen-positive than antigen-negative cats (Shelton et al., 1990). FeLV integrates with the *myc* oncogene leading to tumour formation (Miura et al., 1987). In the past, feline lymphoma was associated with retroviral infection (Cotter et al., 1975, Rojko et al., 1989); however, more recently, there has been a decline in FeLV-associated lymphomas (Louwerens et al., 2005, Stutzer et al., 2011, Vail et al., 1998). This decrease in prevalence is due to the widespread use of in-clinic tests for circulating FeLV antigen in addition to the introduction of effective FeLV vaccines. (O'Connor et al., 1991, Kristal et al., 2001). Despite the decrease in viral-associated disease, there has been an overall increase in the incidence of feline lymphoma. This increase has mainly been attributed to gastrointestinal lymphomas (Louwerens et al., 2005, Vail et al., 1998)

1.1.2 Feline immunodeficiency virus (FIV)

A 5-fold increased risk of lymphoma is conferred on cats infected with FIV over those that are uninfected (Shelton et al., 1990). Unlike FeLV, the incidence of FIV-associated lymphomas does not appear to have changed as, seemingly, the prevalence of FIV has not been significantly affected by the testing of household cats (Gabor et al., 1998).

1.1.3 Chronic intestinal inflammation

There is growing evidence to suggest that lymphoma can be associated with the presence of chronic inflammation. In particular, an association has been suggested between intestinal lymphoma and inflammatory bowel disease (Carreras et al., 2003, Louwerens et al., 2005) but others do not support this concept (Hart et al., 1994).

1.1.4 Environmental exposure to tobacco smoke

According to one study (Bertone et al., 2002), there was a 2.4- or 3.2-fold increased risk of pet cats developing lymphoma following exposure to tobacco smoke for 5 years or more respectively however, in another study (Smith et al., 2020), the authors found no significant difference between cats diagnosed with GI lymphoma (n=35) and those without lymphoma (n=32). Although no significant difference was found between the 2 groups in the latter study, the authors found that the percentage of cats with hair nicotine concentration (HNC) ≥0.1 ng/mg was higher for the cats with lymphoma (22.9%) than those in the control group (15.6%) therefore suggesting an association may exist.

1.1.5 Breed predisposition

Genetic factors may predispose cats to lymphoma. Studies have found that Siamese cats appear to be predisposed to developing lymphoma, most predominantly the mediastinal type with a potentially recessive mode of inheritance (Hardy, 1981, Dorn et al., 1967, Gabor et al., 1998, Louwerens et al., 2005). A heritable form of lymphoma has also been suggested to occur in a small number of Siamese-type breeds (Louwerens et al., 2005).

1.2 Diagnosis of lymphoma

The diagnosis of lymphoma usually depends on a combination of history, signalment, physical examination, clinical signs and retroviral status of the cat (Twomey and Alleman, 2005). Further tools such as cytology and histopathology and tests such as Immunohistochemistry (IHC), Polymerase chain reaction ((PCR) for antigen receptor rearrangement (PARR)) and flow cytometry can be valuable adjuncts to the diagnosis or categorisation of feline lymphoma.

1.2.1 Clinical presentation

Clinical signs of lymphoma are highly variable, non-specific and may be dependent on the affected organ or anatomic location. These signs range from malaise, weight loss, anorexia when internal organs such as the liver and spleen are involved. Other signs including lethargy, diarrhoea and vomiting may be observed when the gastrointestinal tract (GIT) is involved (Hayes, 2006). Many of these cats, however have minimal or no vomiting or diarrhea with anorexia and weight loss, therefore, it is important that when these signs are observed in a geriatric cat, gastrointestinal lymphoma should be considered as a differential diagnosis (Richter, 2003). Lymphadenopathy is also a common clinical presentation in cats with lymphoma although, it is noteworthy to mention that a number of such cats do not have lymphoma and the lymphadenopathy resolves over a 12-18 month period (Mooney et al., 1987b). An extremely rare form of lymphoma in cats; epitheliotropic cutaneous lymphoma can mimic allergic skin disease, presenting with clinical signs such as exfoliative erythroderma, patches, plaques, erosions, ulcers and lesions in the oral cavity and at mucocutaneous junctions (Fontaine et al., 2011). Since the clinical signs of lymphoma in many cases are often non-specific, further investigation into any observed masses require cytology and/or histopathology. Immunohistochemistry, PARR and flow cytometry are also useful complementary diagnostic tests to provide a more accurate diagnosis. Cytology, histopathology and immunohistochemistry are the most common methods utilised for its diagnosis.

1.2.2 Cytological features

Cytological examination of fine needle aspirate (FNA) samples or impression smears is a rapid, non-invasive and effective tool for diagnosing feline lymphoma. Cytology on samples from neoplastic lymphoid tissue most often contain a predominant population of large lymphocytes that are three to five times the size of an erythrocyte (Cowell et al., 2003). Fig 1 shows a lymph node infiltrated with a monomorphic population of large lymphoid cells.

In the majority of cases, the diagnosis of lymphoma is significantly more challenging than in other species due to the presence of certain benign hyperplastic syndromes such as idiopathic peripheral lymphadenopathy, plexiform vascularisation of lymph nodes and peripheral lymph node hyperplasia that is occasionally observed in young cats. (Lucke et al., 1987, Welsh et al., 1999, Mooney et al., 1987b)

Small cell lymphomas (in which the tumour is composed of well-differentiated small lymphocytes) can also be seen within the alimentary tract of cats. These are difficult to differentiate cytologically from a lymphoid inflammatory infiltrate or reactive hyperplasia (Briscoe et al., 2011, Mooney et al., 1987b) and therefore require biopsy for histopathology. Similarly, histopathology of the lesion may also be required if the lymphoid population is mixed but contains many large lymphocytes. In such cases, histopathologic examination is not only recommended for a definitive diagnosis but also to classify the disease subtype (Twomey and Alleman, 2005). One study showed that although overall, cytology is highly sensitive (93% sensitivity) for diagnosis of neoplastic disease in lymph nodes, a significant proportion of false negatives were T-cell lymphomas (i.e. 22/35 (63%)) and nearly 50% (20/42) of mesenteric lymph node cytology results were also falsely negative (Ku et al., 2017). The authors attributed this to reduced accessibility to affected lymphoid tissue and the higher number of small lymphocytes.

1.2.3 Histopathological features

Histopathology has an advantage over fine needle aspirate cytology in diagnosing lymphoproliferative disorders as it enables the possibility of evaluation of the architecture of the lymphoid tissue as well as the morphology of the cells (Zeppa et al., 2004). Histologic evidence of lymphoma includes the

presence of marked lymphoid cell infiltration, a monomorphic appearance of the lymphocytes, cytological immaturity of the lymphoid infiltrate, disruption, effacement and replacement of the normal structures of the involved tissue by infiltrating lymphoid cells (Moore et al., 2005). Figures 2 and 3 depict the histopathology features of a gastric wall lymphoma in a cat.

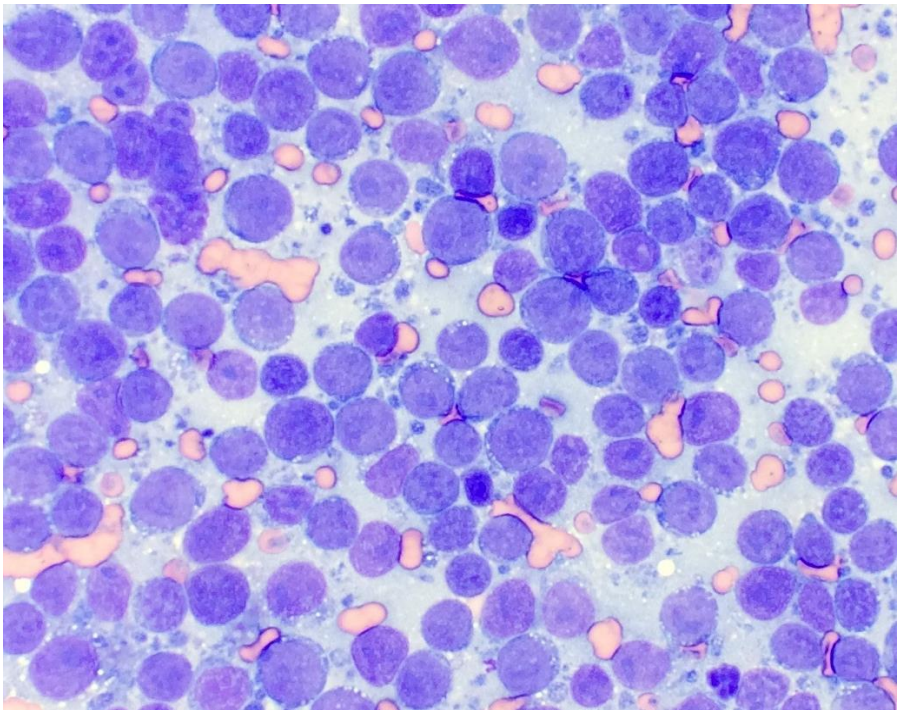

Fig 1. Intestinal lymphoma, cat; monomorphic population of large lymphoid cells with a round to oval nucleus, stippled chromatin and one or more prominent nucleoli. (Wrights-Giemsa; HP oil. (Courtesy of Niki Skeldon, Axiom Veterinary Laboratory UK.)

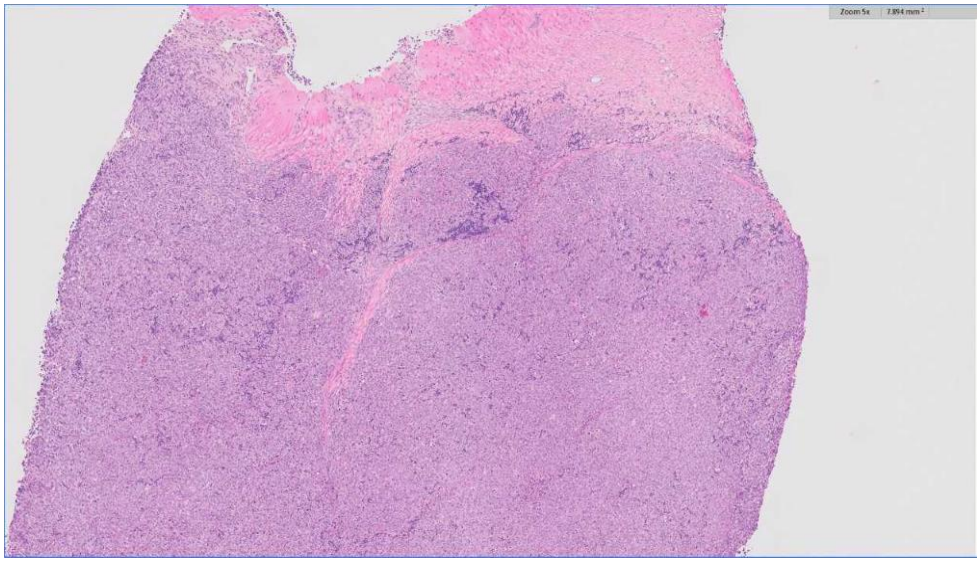

Fig 2. Gastric wall lymphoma, cat; neoplastic lymphocytes have infiltrated the muscle layers of the gastric wall, LPF (HE-stain) (Courtesy of Melanie Dobromylskyj, Finn Laboratories, Norfolk.)

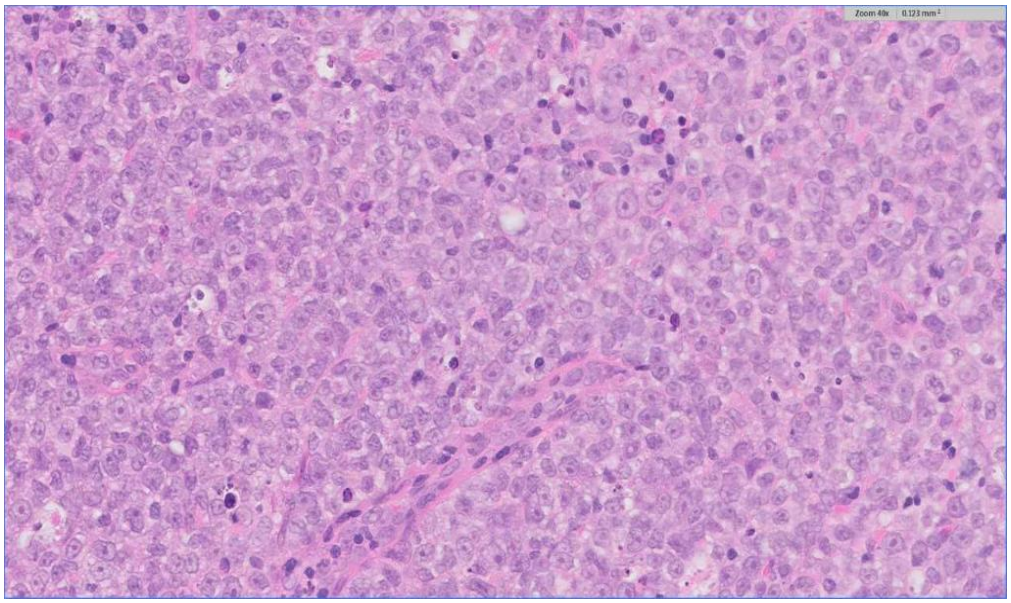

Fig 3. Gastric wall lymphoma, cat (same case as **Fig 2**); large lymphoid cells within the gastric wall. The lymphoid cells have a small amount of cytoplasm and a prominent nucleolus (H&E; HP) (Courtesy of Melanie Dobromylskyj, Finn Laboratories, Norfolk.)

1.2.4 PCR for antigenic receptor rearrangement (PARR)

PARR was first developed to detect clonally expanded lymphocyte populations in dogs (Burnett et al., 2003) and has also since been developed for use in cats (Moore et al., 2005). Rearrangement of the immunoglobulin heavy chain (IGH) variable region and (TCRG) genes through random recombination of variable (V), diversity (D) and joining (J) regions occurs in early stages of lymphoid differentiation. As a result, all tumour cells contain a unique IGH or TCRG gene rearrangement (Keller S.M et al. 2016). Since clonality is a fundamental property of neoplasia, determining the nature of a clonal population of lymphocytes within a tumour can aid in the diagnosis of B-cell (Werner et al., 2005) and T-cell lymphomas.

The diagnostic sensitivity of PARR for lymphoma was earlier reported to be approximately 65% in cats (Moore et al., 2005) however, clonal rearrangement of the TCRG V-J junction was detected in approximately 78% of cats with intestinal T-cell lymphomas (Moore et al., 2005) and more recent studies utilising four immunoglobulin primer sets (namely: IGH-VDJ, IGH-DJ, Kde and IGL) detected clonal immunoglobulin rearrangements in 87% of cats (n=38) with B-cell lymphomas (Rout et al., 2019). In this latter study, the authors also detected clonal rearrangements in 97% of T-cell leukaemias.

It should be noted that lymphoid clonality does not always correspond to neoplasia. Oligoclonal or monoclonal expansions of reactive lymphocytes with identical gene rearrangements may also be observed in chronic infections including IBD (Inflammatory Bowel Disease) leading to false-positive results. Similarly, corticosteroid administration and formalin fixation could result in fragmentation of DNA within the tissue leading to a false-negative PARR result (Moore et al., 2005).

1.2.5 Immunohistochemistry (IHC)

Immunohistochemistry (IHC) bridges 3 different scientific principles- Immunology, Histology and Chemistry. Antigens are recognized in histologic sections by specific antibodies. The antigen–antibody binding is then visualised by light or fluorescent microscopy as a coloured histochemical reaction (Meuten, 2017). Figures 4, 5 and 6 show IHC staining of lymphoid cells in a gastric wall lymphoma of a cat (same case as Figures 2 and 3). Immunophenotypic assessment is most useful in lymphocyte lineage assignment (B-cell vs. T-cell) and can also highlight distinctive lymphocyte infiltration patterns such as epithelial colonisation to aid in assessing the likelihood of lymphoma (Moore et al., 2005). Immunophenotyping of lymphoma is effective for the determination of the cell type involved and for prognostication (Twomey and Alleman, 2005).

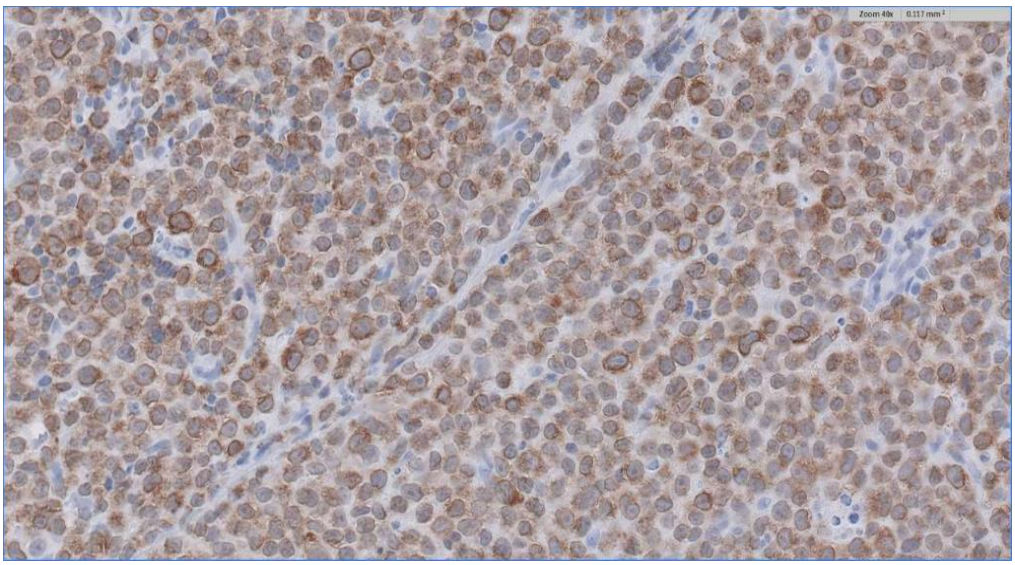

Fig 4- Gastric wall lymphoma in a cat (same case as **Fig 2 and 3**); IHC staining for CD79a (B-cell marker) showing positive cytoplasmic staining of the lymphocytes, diagnostic of B-cell lymphoma. HPF (HE-stain) (Courtesy of Melanie Dobromylskyj, Finn Laboratories, Norfolk.)

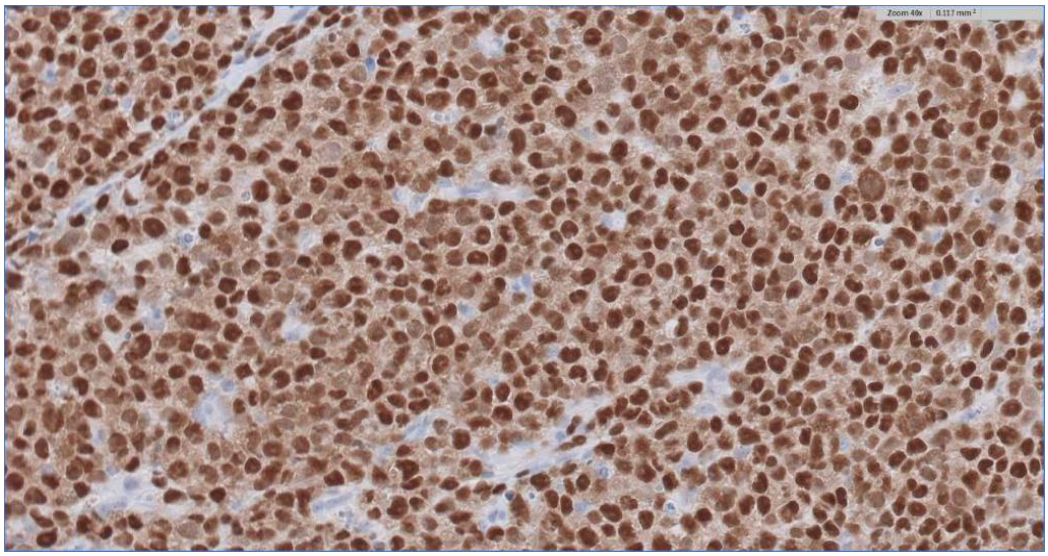

Fig 5- Gastric wall lymphoma in a cat (same case as **Fig 2 and 3**); IHC staining for PAX-5 (B-cell marker) showing positive nuclear staining of the lymphocytes, diagnostic of B-cell lymphoma. HPF (HE-stain) (Courtesy of Melanie Dobromylskyj, Finn Laboratories, Norfolk.)

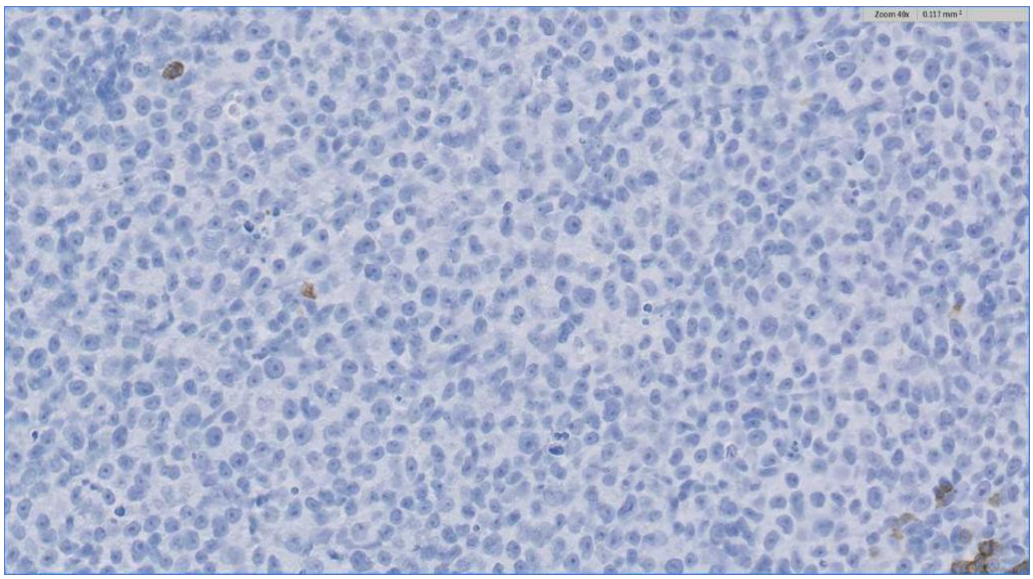

Fig 6- Gastric wall lymphoma in a cat (same case as **Fig 2 and 3**); IHC staining for CD3 lymphocytes. The lymphocytes are mostly negative for T-cells (a few scattered positively stained T-cells are seen). HPF (HE-stain) (Courtesy of Melanie Dobromylskyj, Finn Laboratories, Norfolk.)

Regarding proportions of subtypes of feline lymphoma, some authors report that the vast majority of alimentary lymphomas are of B-cell origin (Patterson-Kane et al., 2004, Gabor et al., 1999) however, more recent studies have revealed they are T-cell in origin (Sabattini et al., 2016, Wolfesberger et al., 2017, Moore et al., 2012). The latter study classified cats with GI

lymphoma by immunophenotype and by location (mucosal versus transmural) (Moore et al., 2012). Cats with mucosal T-cell lymphoma were in the majority (84/120) and a further 19 cats had transmural T-cell lymphoma. The author of a clinical review on prognostic factors of feline lymphoma (Moore, 2013) has noted that many previous studies did not separate patients according to the grade of their tumour but rather by stage and anatomic location which has therefore led to an underestimation of the contribution of immunophenotype to prognosis and has thus limited our understanding of feline lymphoma. The author has suggested that in order to improve our knowledge of lymphoma in cats, all lymphomas should be immunophenotyped as well as histologically graded.

1.2.6 Flow cytometry

Flow cytometry can be used to determine the presence of surface markers on the neoplastic lymphocytes in cell suspensions and thus determine the cell types involved (Chabanne et al., 2000). It can therefore be used to differentiate lymphoid cells from other cell lines such as granulocytic and monocytic cells (Ramos-Vara, 2005). Flow cytometry assists in the assessment of phenotypic patterns and in confirming a diagnosis of lymphoma (Guzera et al., 2016)

1.3 Classification of lymphoma

Classification of lymphoma is usually based on anatomical location, histological grade as well as immunophenotype (Barrs and Beatty, 2012), the latter being an important component in determining the histological grade of the tumour.

1.3.1 Classification based on anatomical location

There have been inconsistent attempts to classify feline lymphoma by anatomical location and these usually require categorising them as multicentric, mixed, miscellaneous and unclassified extranodal (Gabor et al., 1998). Several authors have classified feline lymphoma into mediastinal, multicentric, alimentary and extranodal (Gabor et al., 1998, Louwerens et al., 2005, Vail et al., 1998). The most common extranodal anatomic site however, is the gastrointestinal tract (Teske et

al., 2002, Taylor et al., 2009), followed by mediastinal and nodal lymphoma (Sato et al., 2014). Feline lymphoma is frequently located in the gastrointestinal tract or mediastinum, and compared with dogs, fewer cats develop multicentric lymphoma (Carreras et al., 2003).

1.3.2 Classification based on immunophenotype

Immunophenotyping of lymphoma aids in the determination of the cell type as well as in prognostication (Twomey and Alleman, 2005). Cluster of differentiation (CD) antigens on the surface of cells that have been considered clinically important and can be detected by immunophenotyping include CD3 (T cell) and CD79 (B cell) (Fan, 2003).

1.3.3 Classification based on histopathological features

The histological classification systems that are most frequently utilised for feline lymphomas include the Revised European-American classification of lymphoid neoplasms/ World Health organisation (REAL/WHO classification) and The National Cancer Institute Working Formulation (NCIWF) scheme. Both schemes are complementary since neither consider both histological grade and immunophenotype (Barrs and Beatty, 2012).

1.3.3a The Revised European-American classification of lymphoid neoplasms/ World Health organisation (REAL/WHO) scheme

The Revised European-American classification of lymphoid neoplasms (REAL/WHO) scheme divides lymphoma into B- and T- lymphocyte origin on the basis of immunophenotyping, cell structure, genetic features and clinical features (Harris et al., 2000). Box 1 summarises the REAL/WHO classification of lymphoma.

Using the REAL/WHO system, diffuse large B-cell lymphoma (DLBCL) is one of the most common types of lymphoma in cats (Valli et al., 2000, Sato et al., 2014, Chino et al., 2013). Cats also have large cell lymphomas, T-cell-rich B-cell lymphomas (TCRLBCL), that are "Hodgkin's-like (Sato et al., 2014, Chino et al., 2013). Mucosal T-cell lymphoma of small to intermediate cell type (WHO EATCL type II) is the dominant

lymphoma in the gastrointestinal tract of cats (Moore et al., 2012).

It has been over a decade and a half ago since Valli et al. (2002) emphasized the need for lymphoma subtyping using the WHO classification and stated that 'a simple diagnosis of lymphoma is not sufficient for veterinary oncologists to provide optimal tumour management or to assess data prospectively'. Nevertheless, reports that actually utilise the WHO classification to subtype feline lymphomas are rare (Vezzali et al., 2010, Moore et al., 2012, Wolfesberger et al., 2018). There is a possibility that the immunophenotype has prognostic significance in cats, although, there is currently not enough evidence to support this.

Box 1- Summary of the Revised European–American Lymphoma (REAL) classification of lymphoid neoplasms adopted by the World Health Organisation as applied for use in animals. The most common feline lymphomas are enteric, large B-cell (includes T-cell-rich large B-cell lymphoma (TCRLBCL)), nasal, mediastinal, and Burkitt's in some studies. Valli et al 2017. Adapted from 'Tumors in domestic animals' edited by Meuten D.J. * Enteropathy associated T-cell lymphoma (EATCL).

B-cell neoplasms	**T-cell and putative NK-cell neoplasms**
Precursor B-cell neoplasms	*Precursor T-cell neoplasm*
Lymphoblastic leukaemia/lymphoma	Lymphoblastic lymphoma (LBL)/leukaemia
Mature (peripheral) B-cell neoplasms	*Mature (peripheral) T-cell and NK-cell neoplasms*
Chronic lymphocytic leukaemia/small lymphocytic lymphoma	Chronic lymphocytic leukaemia (CLL)/small cell lymphoma (SLL)
Prolymphocytic leukaemia Lymphoplasmacytic lymphoma	Prolymphocytic leukaemia
Plasmablastic lymphoma	Large granular lymphocytic (LGL) leukaemia/lymphoma
Mantle cell lymphoma (MCL)	T-zone lymphoma (TZL), nodal
Follicular lymphoma	*Intestinal T-cell lymphoma (enteropathy associated)
Diffuse large B-cell lymphoma (DLBCL)	Hepatosplenic γδ T-cell lymphoma
Subtypes: T-cell-rich large B-cell (TCRLBCL); primary mediastinal (thymic)	Mycosis fungoides/Sézary syndrome
Angiocentric B-cell lymphoma (lymphomatoid granulomatous)	Intravascular lymphoma (angiocentric)
Marginal zone lymphoma (MZL)	Subcutaneous panniculitis–like T-cell lymphoma Angioimmunoblastic T-cell lymphoma
Nodal, splenic, extranodal marginal zone lymphoma of mucosa- associated lymphoid tissue type (MALT)	Aggressive natural killer (NK)-cell leukaemia/lymphoma
Burkitt's lymphoma/Burkitt's cell leukemia	Adult T-cell lymphoma/leukaemia
Provisional entity: high-grade B-cell lymphoma Burkitt's-like	Anaplastic large cell lymphoma; cutaneous and systemic
Plasma cell myeloma	Peripheral T-cell lymphoma not otherwise specified (PTCL-NOS)
Plasmacytoma	

1.3.3b The National Cancer Institute Working Formulation (NCI WF) scheme

The NCI WF scheme classifies lymphoma according to its natural state of progression into 3 histological grades; high (rapidly progressive), intermediate and low (indolent) on the basis of the frequency of mitosis (Non-Hodgkins's Lymphoma Pathologic Classification Project, 1982). This scheme has been successfully used to classify lymphoma in cats (Valli et al., 2000) as well as in dogs (Teske et al., 1994) and cattle (Vernau et al., 1992). Seventy-five percent of feline alimentary lymphomas are histologically classified as low-grade small cell lymphomas; they are generally T-cell in origin and most commonly arise in the small intestine (Sabattini et al., 2016).

1.4 Distinguishing between Inflammatory Bowel Disease (IBD) and Intestinal T-cell lymphoma

Immunophenotyping has become an important diagnostic tool to differentiate between inflammatory bowel disease and intestinal lymphoma when there are indefinite histological changes (Waly et al., 2005) and presents a diagnostic challenge to both veterinary clinicians and pathologists (Willard et al., 2002). IBD and intestinal T-cell lymphomas in cats are both characterised by marked intestinal infiltration of small lymphocytes (Carreras et al., 2003, Moore et al., 2005). Expansion of T-cell population occurs in diffuse mucosal-associated lymphoid tissue (MALT) in feline inflammatory bowel disease and feline intestinal lymphoma (Vail et al., 1998). It has even been suggested that inflammatory bowel disease (IBD) can progress to lymphoma (Louwerens et al., 2005). The inability to distinguish between these two diseases presents a diagnostic dilemma for the clinician, rendering it difficult to assign the best therapeutic options for such cats. Differentiation between these two conditions can be reliably carried out by identifying clonality or by immunohistochemistry (Carreras et al., 2003, Moore et al., 2005). A diagnostic algorithm to assist in distinguishing between feline intestinal lymphoma and inflammatory bowel disease in cats is presented in Figure 7.

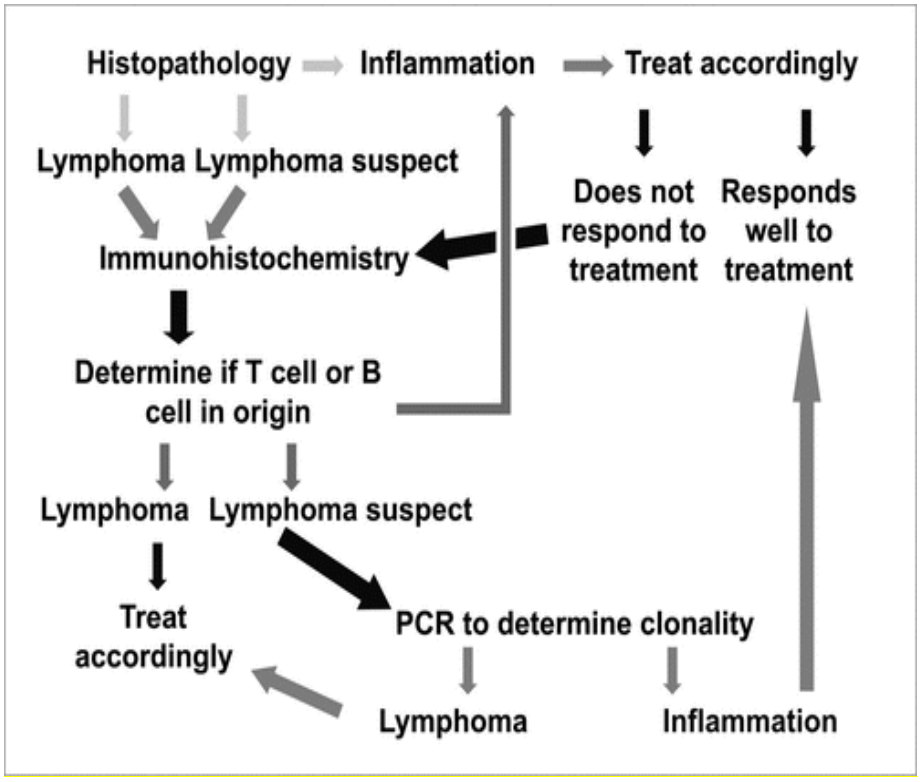

Figure 7- Diagnostic algorithm to differentiate feline intestinal lymphoma from inflammatory bowel disease in small intestinal surgical biopsy specimens, (Kiupel et al., 2011).

1.5 Treatment of lymphoma

Treatment for lymphoma in most cases requires the use of chemotherapeutic agents. Other options may include radiation therapy, or where possible, in cases of localised neoplasia, surgical removal of the tumour can be performed.

1.5.1 Chemotherapy

Chemotherapy is the most effective treatment for lymphoma in cats (Kristal et al., 2001). Clinical remission and return to a good quality of life can be achieved when appropriate treatment is instituted for many of these cats; however, predicting which cats will respond to therapy is often a challenge for the clinician.

Several different chemotherapeutic options for lymphoma have been described with variable outcomes (Teske et al., 2002, Kristal et al., 2001, Vail et al., 1998). The COP protocol (cyclophosphamide, vincristine and prednisolone) however, is the standard combination treatment for feline lymphoma (Vail

et al., 1998, Cotter, 1983). In the latter study, complete response was observed in 74% of cats (n=38) treated with COP and the duration of complete response ranged from 2-42 months. In another study, a similar proportion (72.7% (n=110)) treated using the COP protocol achieved complete response (Taylor et al., 2009).

The Wisconsin-Madison (WM) protocol, a combination chemotherapy that includes vincristine, l-asparaginase, cyclophosphamide, prednisolone, doxorubicin and methotrexate achieved complete response in 64% of affected cats (n=16) with median survival times of 112 days (Taylor et al., 2009). In this study also, the median survival times in cats treated using the COP protocol and prednisolone were 171 days and 60 days respectively. Out of the 110 cats treated, only 10 cats were treated with corticosteroids alone.

The use of L-asparaginase as a single agent therapy for lymphoma achieved complete remission in only 15% of cases (n=13) (LeBlanc et al., 2007). Chemotherapy using a single agent such as doxorubicin has also been explored, however this has been found to be poorly effective in induction and maintenance of remission in cats with lymphoma with complete response seen in 26% in one study (Kristal et al., 2001) and 32% in another (Peaston and Maddison, 1999).

The use of metronomic therapy, a treatment regime in which low doses of chemotherapeutic agents are administered on a continuous or frequent, regular schedule (e.g. daily or weekly), usually over a long time have been shown to achieve relatively good survival rates. One such study carried out on 28 cats with GI small-cell lymphoma achieved a median survival rate of 786 days using chlorambucil and glucocorticoids (Stein et al., 2010). In another study, 95% of the cats (n=41) with histologically confirmed low-grade lymphoma were shown to have responded to treatment with prednisone and chlorambucil with survival rates of 428 days for cats achieving a partial response and 897 days for cats achieving a complete response (Kiselow et al., 2008). Table 1 summarises the response to treatment and survival times in these studies.

Authors	No of cats	Treatment protocol	No achieving complete response (CR)	MST (days)
Kristal et al, 2001	19	Doxorubicin only	26% (n=5)	92
Teske et al, 2002	61	COP	75.4% (n=46)	251
Stein et al, 2010	28*	Glucocorticoid and chlorambucil	96% (n=27)	786
Kiselow et al, 2008	41**	Glucocorticoid and chlorambucil	56% (n=23)	704
LeBlanc et al, 2007	13	L-asparaginase	15% (n=2)	NR
Taylor et al, 2009	66	COP	72.7% (n=48)	171
	25	WM	64% (n=16)	112
	10	Prednisolone	30% (n=3)	60

Table 1- Response to treatment and survival according to medical treatment in some studies. MST (Median survival time),*study involved cats with GI small-cell lymphoma only, ** study involved cats with low grade lymphoma only, NR- not reported, COP (cyclophosphamide, vincristine and prednisolone), WM- Wisconsin-Madison protocol.

When taking into account the response to treatment, the COP protocol achieves the best complete response proportions (75.4% and 72.7%) and the least percentage complete response proportions were observed following treatment with L-asparaginase and Doxorubicin (15% and 26% respectively). Interestingly, the longest median survival times (MST) were observed following the use of a combination of chlorambucil and prednisolone, although all the patients in this group had GI small-cell lymphoma.

1.5.2 Radiation therapy

Lymphoid neoplasms are not only responsive to chemotherapy, they are also sensitive to radiation therapy suggesting that localised forms such as nasal lymphoma will respond well to treatment using radiation therapy (Evans and Hendrick, 1989, Haney et al., 2009). Nineteen cats that had received radiotherapy alone achieved a complete response rate of 93% with a median survival time of 1431 days (range 12-1971 days); much higher than that observed following any of the chemotherapeutic treatments (Haney et al., 2009).

1.6 Survival times and prognosis of lymphoma

Survival times following treatment of feline lymphoma are suggested to be dependent on several factors including anatomic location, clinical stage, subtype, FeLV status, chemotherapy, corticosteroid treatment prior to chemotherapy and potentially, mitotic index. Although feline lymphoma is routinely diagnosed cytologically, histopathology is not always performed (Moore et al., 2012, Valli et al., 2000, Sato et al., 2014) and as such, prognostic information for affected patients is sparse.

1.6.1 Survival time based on anatomical location

Anatomical location has been reported to predict survival in cats with lymphoma and several studies have reported its significance (Teske et al., 2002, Sato et al., 2014, Cotter, 1983).

A study by Taylor et al. (2009) involving cats with nasal lymphoma found that cats achieving complete remission following treatment had the longest survival (749 days) while cats with central nervous system (CNS) lymphoma had the shortest (70 days). Although, it is worth noting that the cats selected for this study were all from referral centres and so their anatomic presentations are likely biased towards forms of lymphoma that first opinion practitioners have either had difficulty diagnosing or treating. Also, the number of cats with the CNS and laryngeal forms of lymphoma (n=7 and n=8 respectively) in this study were too small for adequate survival analysis.

1.6.2 Survival time based on clinical stage

For most cancers in cats, determining the prognosis is important for developing the optimum treatment procedure; however, clinical stage is difficult to ascertain in feline lymphomas because of the various anatomic forms of this disease (Moore, 2013). Moreover, studies that relate the clinical stage of lymphoma to survival are rare. Clinical stage was shown in a study by (Mooney et al., 1989) to be significantly related to both the response to treatment and to survival rates. In this study, cats with stage I lymphoma had higher (93%) response rates compared with 40-60% response

rates observed in cats with stage IV/V. One study in which 28 cats with renal lymphoma were staged showed that cats with stage-II lymphomas that were FeLV negative had the best response to treatment (Mooney et al., 1987a). In this study the staging system utilised was based on the number of organs or the location of the tumour. Table 2 below shows the staging system used in the study.

Clinical stage	Staging criteria
Stage I	Single tumour (extranodal) or single anatomic area (nodal)
Stage II	Single tumour (extranodal) with regional lymph node involvement. Two or more nodal areas on the same side of the diaphragm, Two (extranodal) tumours with or without regional lymph node involvement on the same side of the diaphragm. A primary, resectable, gastrointestinal tract tumour usually in the ileocaecocolic area with or without involvement of associated mesenteric lymph nodes only
Stage III	Two tumours (extranodal) on opposite sides of the diaphragm. Two or more nodal areas cranial and caudal to the diaphragm. All extensive primary unresectable intra-abdominal disease. All paraspinal or epidural tumours, regardless of other tumor site(s)
Stage IV	Stages 1 to 3 with liver and/or spleen involvement
Stage V	Stages 1 to 4 with initial involvement of CNS and/or bone marrow

Table 2 Staging system used for lymphoma at the Donaldson-Atwood Cancer Clinic (Mooney et al., 1987a).

1.6.3 Survival time based on the immunophenotype

Shorter survival times and an increased likelihood of relapse have been observed in dogs with T-cell lymphomas than those with B-cell lymphomas (Teske et al., 1994, Borska et al., 2009) with the exception of certain T-cell lymphomas such as T-zone lymphomas with reported median survival rates similar to that observed in dogs with B-cell lymphomas (Martini et al., 2016). In cats however, there is insufficient data on immunophenotyping of B- versus T-cell lymphomas as a predictor of survival (Patterson-Kane et al., 2004).

The use of the REAL/WHO classification provides more detailed information on the disease than labelling the tumours as low-, intermediate- or high-grade lymphoma (Wolfesberger et al., 2017). A study of 30 cats showed that cats with Intestinal T-cell lymphoma (ITCL) and T-cell rich B-cell lymphoma

(TCRBCL) lived significantly longer than those with diffuse large B cell lymphoma (DLBCL) or peripheral T-cell lymphoma (PTCL) (Wolfesberger et al., 2017). In this study, the prognostic value and survival rates in a group of cats based on their WHO classification was investigated. Their aim was to determine if there were significant differences between survival rates based on mitotic rate, subtype and pre-treatment with corticosteroids, however, the small number of cats (n=30) employed in this study likely increased the probability of misrepresentation of survival rates in these cats. In general, there is little information regarding the differences in the rate of dissemination and progression between different histological types and grades of lymphoma in cats (Patterson-Kane et al., 2004).

1.6.4 Survival time based on the FeLV status

FeLV status has been shown to be significantly related to survival; FeLV-test positive cats have shorter survival times than FeLV negative cats (Mooney et al., 1989, Vail et al., 1998). However, the clinical importance of FeLV status in veterinary oncology is now relatively low due to the marked decrease in prevalence (Moore, 2013, Vail et al., 1998).

1.6.5 Survival time based on the histological grade

One study (Fondacaro et al., 1999), showed that histological grade was strongly related to clinical outcome. Cats with low-grade lymphoma treated with oral prednisolone and chlorambucil had significantly higher remission rates (69% versus 18%) and survival times (17 versus 2.7 months) than did cats with high-grade lymphoma treated with multiagent chemotherapy.

1.6.6 Survival time based on chemotherapy

Several studies have been carried out to determine survival and prognostic factors following chemotherapy for feline extranodal lymphoma. In one study by Haney et al. (2009), only 3 out of the 97 cats were immunophenotyped and in another (Taylor et al., 2009), none of the 110 cats were immunophenotyped or histologically graded and so survival rates based on phenotype and tumour grading were not assessed. Immunophenotyping and histological grading of feline lymphomas would help determine any potential relationships between subtype or grade and survival rates.

1.6.7 Survival time based on response to therapy

One of the most important prognostic factors noted in several feline lymphoma studies, is response to therapy (Milner et al., 2005, Vail et al., 1998, Teske et al., 2002, Vail et al., 2010, Collette et al., 2016).

Unfortunately, this cannot be assessed prior to treatment. Cats that have complete response (CR) to therapy had significantly longer progression-free interval (PFI) and survival time (MST) than those with partial or no response (Collette et al., 2016).

1.6.8 Effect of pre-chemotherapy corticosteroid treatment on survival time

There is conflicting evidence on survival rates in cats treated with corticosteroids prior to chemotherapy. Taylor et al. (2009) showed that pre-treatment with corticosteroids significantly reduced survival times while studies by Wolfesberger et al. (2017) and Fabrizio et al. (2014) found that treating cats with glucocorticoids prior to chemotherapy did not worsen their prognosis. It is noteworthy that a very low number of cats pre-treated with corticosteroids in the latter 2 studies (n=12 and n=14, respectively) were employed in contrast to the former, whose study involved a larger number of cats (n=37). The difference in sample size could be a possible cause of the discordant results. Nevertheless, studies involving large numbers of pre-corticosteroid treatments in cats are necessary to confirm whether or not pre-chemotherapy treatment has an effect on survival rates. Furthermore, survival times were not fully defined in these studies.

Taylor et al. (2009) and Wolfesberger et al. (2017) calculated survival times from the date of lymphoma diagnosis to the date of death but did not clearly state whether or not deaths were due to euthanasia. Similarly, in addition to not mentioning euthanasia, Fabrizio et al. (2014) calculated survival time from the date of diagnosis to the date of death from any cause. This could lead to misleading conclusions as deaths due to non-lymphoma related causes could potentially be included in their survival analysis. Furthermore, although this study involved a larger number of cats (n=50), survival times were only reported in cats with mediastinal lymphoma.

Other forms of lymphoma likely present with longer or shorter survival rates.

1.6.9 Survival time based on mitotic index

Another potential predictor of survival is the mitotic index. The mitotic index indirectly measures the degree of cell proliferation based on the quantification of mitotic figures in a histopathological microscopic field. It has been shown to be a strong predictor of the outcome of human and canine patients with cancers such as breast carcinoma and mast cell tumours (Biesterfeld and Reitmaier, 2001, Romansik et al., 2007). However, little is known about whether mitotic index can predict survival in feline cancers and specifically feline lymphoma. One study showed that mitotic rate had no effect on survival rates in cats with lymphoma (Wolfesberger et al., 2017).

Unfortunately, the prognostic factors in cats are not as straightforward as in dogs. In dogs, immunophenotype (B-cell vs T-cell) still remains an important prognostic factor: However, in cats, different immunophenotypes of lymphoma tend to be associated with different anatomical locations but these do not necessarily predict the outcome therefore the value is not currently recognised.

The best prognostic system for cats appears to include a combination of staging the extent of disease along with certain anatomical categories that include gastrointestinal or renal lymphoma (Moore, 2013)

2 Aims and objectives of research project

The main aim of this study was

- To determine the survival efficacy of treatments employed in a group of cats with a laboratory diagnosis of lymphoma with a view to determining whether or not such cats should be managed any differently based on our knowledge of different variables they present with. These variables include:
    - Age, gender and neutering status
    - Anatomical site
    - Treatment status and type of treatment
    - Immunophenotype (B-cell versus T-cell)
    - Histological grade

Further aims of this study were:

- To determine the frequency of use of the common laboratory tests for lymphoma based on laboratory submissions.
- To determine the frequency of the use of specific terms to describe the probability of feline lymphoma diagnosis on cytology reports.
- To determine the frequency of lymphoma at each anatomical site based on numbers received at our laboratories in a specified period.

2.1 Research questions:

- What is the frequency of use of cytology, histopathology and immunohistochemistry in cats with a laboratory diagnosis of lymphoma?
- What is the frequency of each anatomical form of lymphoma?
- Are there significant differences between survival times in affected cats based on the variables listed above?
- Based on these findings, should affected cats be managed any differently?

3 Materials and methods

3.1 Ethical approval

Ethical approval was received for this project from the Research Ethics Committee at the School of Veterinary Medicine and Science, University of Nottingham.

3.2 Data collection at Consolidated Veterinary Services (CVS)

The data for this study was obtained from laboratories under the CVS division. CVS Group plc is the largest integrated veterinary services provider in the UK encompassing four main business areas; veterinary practices, veterinary diagnostic laboratories, pet crematoria and e-commerce division. FINN pathologists and Axiom clinical pathology laboratory are two diagnostic laboratories operated by CVS laboratories division. The recruitment of case material for this study was through interrogation of the FINN and Axiom laboratory databases (MiLab®). Once cases were identified by this method, the medical history and outcome of those cases that were from CVS-owned veterinary clinics could be reviewed within CVS practice management software (Robovet®)

3.3 Framework of project

The flow diagram (Fig 8) below summarises the framework of the retrospective case series for survival in feline lymphoma:

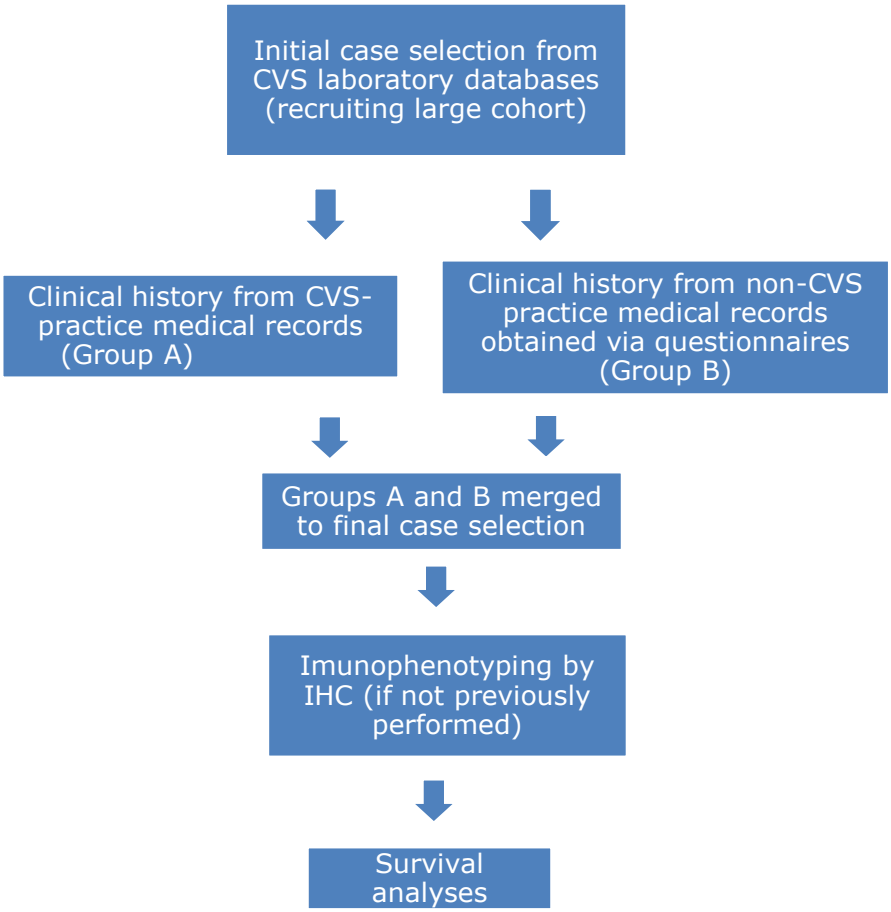

Fig 8- Flow diagram summarising the research project.

3.3.1 Initial case selection from CVS laboratory databases

Clinical details of selected cats were collected retrospectively from MiLab® using the search terms- lab report/diagnosis and keywords including "feline'' ,"lymphoma'', "consistent with lymphoma" , "compatible with lymphoma" or "probable lymphoma'' in samples submitted for histopathological investigation from January 2014 to January 2017 and for cytological investigation from January 2015 to January 2018. Data was collated in Microsoft Excel.

Lymphoma cases recruited to the study included those that were confirmed by one or more of the following methods:

- **Cytology** – Presence of a monomorphic population of lymphoid cells or a predominance of medium- or large-sized lymphoid cells.
- **Histopathology** – Presence of a lymphocytic infiltration of malignant lymphocytes with significant variation in nuclear size and/or shape into the affected area (for example, the epithelium or lamina propria in intestinal lymphoma).
- **PARR** (PCR for antigenic receptor rearrangement) - Demonstration of a clonal B-cell or T-cell population.
- **IHC** (Immunohistochemistry) – Predominance of B-or T-cell positive staining cells or occasionally, a mixed population of B-cell and T-cell staining cells.

3.3.2 Describing levels of certainty of feline lymphoma on cytological reports

Cytological reports were assessed on 688 laboratory submissions from 2015 to 2018. Cases were categorised based on the different terms used to describe the cytologist's confidence in lymphoma diagnosis. These modifiers were grouped under 2 broad categories; "certainty 1" in which the diagnosis was made with a low level of certainty and "certainty 2", with a high level of certainty. A subcategory of certainty 1 ("certainty 1A") was also created which consisted of cases in which the diagnosis could either have been lymphoma or other inflammatory or neoplastic conditions.

3.3.3 Medical information retrieval

All cats with a diagnosis of "lymphoma", "probable lymphoma", "compatible with lymphoma", "consistent with lymphoma", "likely lymphoma", "presumptive lymphoma", "suggestive of" and "typical of lymphoma" were included. Once these cases had been identified, medical information relating to the affected cats from CVS-owned Vet practices were obtained through searching of the practice software database (Robovet®) and for the non-CVS owned practices, via survey questionnaires.

3.3.4 Final case selection

The following criteria were used for inclusion and exclusion of cases:

3.3.4a Inclusion criteria:

- Cats that had been diagnosed with lymphoma by either one or more of cytology, histopathology, PARR and/or IHC.
- For the purposes of survival data analyses
    - Cats that had received treatment for lymphoma.
    - Cats that had not undergone treatment for lymphoma but have survived for 28 days or more following a diagnosis.

3.3.4b Exclusion criteria:

- Cats in which lymphoma was not the diagnosis following cytology, histopathology and/or IHC
- For the purposes of survival analyses
    - Cats that did not receive any treatment for lymphoma and survived for less than 28 days following a diagnosis.

A minimum survival of 28 days was selected with the intention of including cases that were given a genuine opportunity to live with their diagnosis but to exclude cases in which the diagnosis itself may have directly led to euthanasia.

3.3.5 Immunophenotyping by immunohistochemistry (IHC)

If a request for IHC had not been made on the initial submission and tissue biopsy sample was received, IHC was then carried out on stored paraffin-embedded tissue sections held at FINN histopathology laboratory. After the tissue blocks had been retrieved from storage, freshly cut sections were made and these samples were sent to Glasgow University Veterinary School where they were stained with appropriate lymphoid cell markers (CD3, PAX-5 and CD79a) using the following protocol:

- At room temperature, tissue sections were rinsed in buffer (Tris buffer) pH 7.5 + Tween for 5 minutes.
- Antigen retrieval was performed using the HIER (Heat-Induced Epitope Retrieval) using Menarini Access

Retrieval Unit which utilizes a sodium citrate buffer at pH6 for 1 minute and 40 seconds at 125^0C full pressure.

- Tissue sections were loaded unto the Dako Autostainer followed by a rinse in buffer.
- Treatment was performed with Dako Real TM Peroxidase blocking solution for 5 minutes and then followed by a 5-minute rinse in buffer.
- Primary antibody (CD3, CD79a or PAX 5) was then added followed by addition of Dako universal diluent for 30 minutes and then rinsed for 10 minutes in buffer.
- Secondary antibody (Goat anti-rabbit IgG conjugated with HRP (Horse- Radish Peroxidase)) was then added followed by a 10-minute rinse in buffer.
- Then treatment with Dako K5007 DAB (3,3'-Diaminobenzidine) chromogen was performed followed by a 3-minute rinse in water.
- Counterstaining was then performed with Gills Haematoxylin for 27 seconds followed by a rinse in water (Blue in Scotts Tap water substitute (STWS)).
- Tissue was dehydrated, cleared and mounted in synthetic resin.
- The negative control was treated exactly the same as the tissue sections with the exception that the diluent was left on the sections with no primary or second antibodies added.

3.4 Definition of categories for data analyses

3.4.1 Retrospective analysis in the large cohort group

Data for this group was obtained following the initial database search on MiLab® from 2014 to 2017. The following data for the affected cats were obtained;

1. Laboratory identification number
2. Age, gender and breed
3. Anatomical site of lymphoma
4. Cytology and histopathology reports
5. IHC and PARR result
6. WHO subtype (where assigned)

7. FeLV status

3.4.2 Retrospective analysis in the smaller cohort group involving CVS practices (Group A)

This study group partially constituted a combination of a subset of cases in the large cohort from 2014 to 2017 as well as those obtained from a further MiLab® database search for cases obtained from January 2018 to March 2019. Medical information was then obtained via a Robovet® practice management, clinical records database search. The following data were obtained;

1. Laboratory identification number

2. Age, gender and breed

3. Anatomical site of lymphoma

4. IHC result

5. Histological grade

6. Treatment administered

7. Survival time (in days)

3.4.3 Retrospective analysis in smaller cohort group involving non-CVS practices (Group B)

This study group was a subset of cases in the large cohort group obtained following the initial database searching on MiLab® from 2014 to 2017. Medical information for this group was requested via questionnaires (see Appendix C1). These were focused at determining the following:

1. Whether lymphoma was the working diagnosis

2. The method of lymphoma diagnosis

3. The FeLV status of the patient

4. Information regarding any lymphoma-related treatment or surgical intervention if performed

5. Whether the patient was alive, deceased or lost to follow-up

6. The cause of death or euthanasia if the patient was deceased

7. Whether death or euthanasia was lymphoma-related if deceased

8. Date of death/ euthanasia (if deceased) or date last seen at the practice (if alive or lost to follow-up)

Using all the available medical information obtained via Robovet® clinical database searching (Group A) and questionnaires (Group B), the results were collated in Excel and data was categorised on the basis of age (0-3y, 4-6y etc), gender and neutering status, breed, organs affected (e.g stomach, intestinal, lymph nodes of the head and neck etc), and were information was available, histological grade (high grade vs low grade), treatment (e.g chemotherapy, cortosteroids, non- treated cats surviving >28 days) and IHC result (B-cell, T-cell, B-cell and T-cell).

The term "gastrointestinal" was used to categorise lymphoma affecting the stomach and intestine only.

Demographics were compared between the large cohort and subgroup A and B to check for any bias in case selection in subgroups.

Survival times were determined from the date of a laboratory confirmed diagnosis of lymphoma to the date of death or euthanasia. Cats were censored if they were alive at the end of the study or if they were lost to follow up by the 31st of March 2020.

3.5 Statistical analyses

- The Graph Pad Prism® statistical software was utilised for the following:
    - Kaplan-Meier survival analysis
    - "Log rank" test to compare survival data for each of the groups of cats
    - Statistical significance (set at $P<0.05$)

4 Results

4.1 Case selection from CVS laboratory databases

The initial case selection identified 1,702 cats of which 1,549 cats met the inclusion criteria. A summary of the number of inclusions and exclusions for the 2 diagnostic methods utilised is shown in table 3 below:

Diagnostic method	No of cases initially selected	No of exclusions	No of inclusions
Cytology	688	134	554
Histology	1014	19	995
Total	**1702**	**153**	**1549**

Table 3: Number of cases selected, inclusions and exclusions.

Data was obtained following laboratory searching over one 3-year period for cytology cases (January 2014 to January 2017) and a separate 3-year period for histopathology cases (January 2015 to January 2018). Following removal of exclusions, more diagnoses were made by histology; 995/1549 (64%); than by cytology (554/1549 (36%)). Figure 9 shows the flow chart for case selection.

Initial exclusions were made for the following reasons:

1 Cases where cytological or histological diagnoses were inconclusive or where the terms "possible lymphoma", "concern for lymphoma", "cannot rule out lymphoma", "lymphoma or other" or "primary differential is lymphoma" were used in the reports.
2 Cases where reactive hyperplasia could not be excluded
3 Cases in which the diagnosis was not confirmed as lymphoma at cytology

A total of 117 cases were excluded at this stage

Final exclusions were made for the following reasons:

1. Cases in which following histology and IHC testing, lymphoma could not be confirmed

A further 17 cases were excluded at this stage

Inclusions were made using these criteria:

1. Cases where cytological diagnoses were stated as "Lymphoma", "suspect lymphoma", "suspicious for lymphoma", "suspicion for lymphoma", "compatible with lymphoma", "consistent with lymphoma", "likely lymphoma", "presumptive lymphoma", "probable lymphoma", "suggestive of lymphoma", "typical of lymphoma"
2. Cases in which the diagnosis was confirmed as lymphoma at cytology, histology or IHC testing.

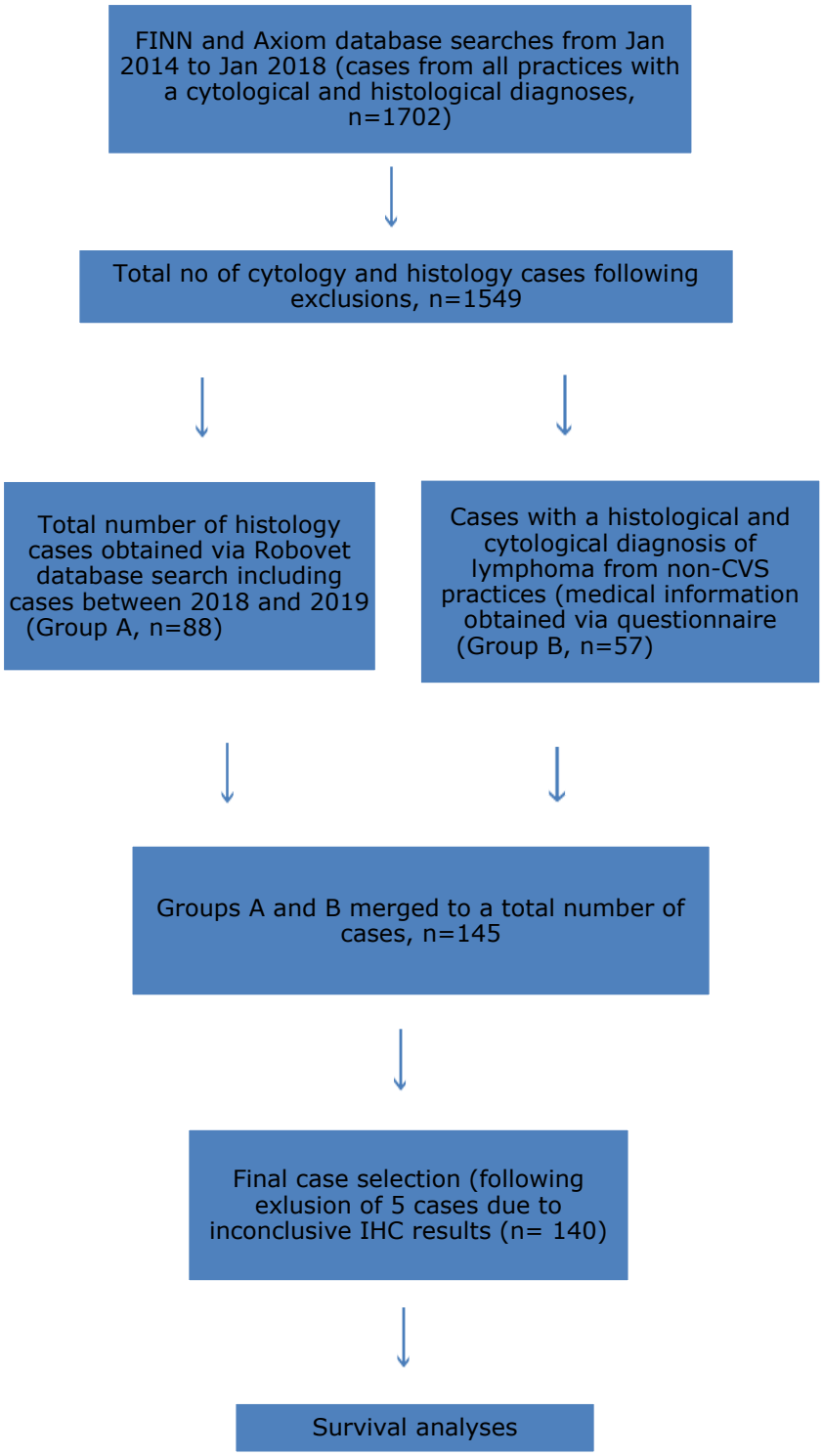

Fig 9: Flow chart for case selection.

4.1.1 Signalment

Patient characteristics of the large cohort are summarised in Table 4.

The majority of cats were between the ages of 8-11 years. Regarding breed distribution, the highest proportion was domestic shorthair (74%, n = 1147), followed distantly by domestic longhair (6.8%, n=105).
With respect to gender, neutered males constituted the highest proportion (43%, n=666), closely followed by neutered females at 33.8% (n=524), 22.9% (n=19) and 35.1% (n=20).

Variable	Category	Number (%)
Age (years)	0-3	77 (5.0)
	4-7	279 (18.0)
	8-11	560 (36.2)
	12-15	460 (29.7)
	>15	95 (6.1)
	Unknown	78 (5.0)
Breed	Domestic shorthair	1147 (74)
	Domestic longhair	105 (6.8)
	Siamese	37 (2.4)
	Maine Coon	34 (2.2)
	British shorthair	31 (2.0)
	Burmese	31 (2.0)
	Bengal	22 (1.4)
	Unknown	21 (1.4)
	Domestic mediumhair	13 (0.8)
	Oriental shorthair	11 (0.7)
	Persian	11 (0.7)
	Birman	8 (0.5)
	Norwegian Forest	7 (0.5)
	British Blue	6 (0.4%)
	Burmilla	3 (0.2)
	Russian Blue	2 (0.1)
	Burmese cross	1 (0.06)
	Other breeds (including cross breeds)	59 (3.8)
Gender	Male	169 (10.9)
	Male neutered	666 (43)
	Female	124 (8.0)
	Female neutered	524 (33.8)
	Unknown	66 (4.3)

Table 4: Patient characteristics and frequencies of categories of cats with lymphoma, n= 1549.

4.1.2 Anatomical locations

The distribution of the affected cats based on anatomical location of disease in the large cohort is shown in the bar chart below (Fig. 10) and these proportions are tabulated (Table 5).

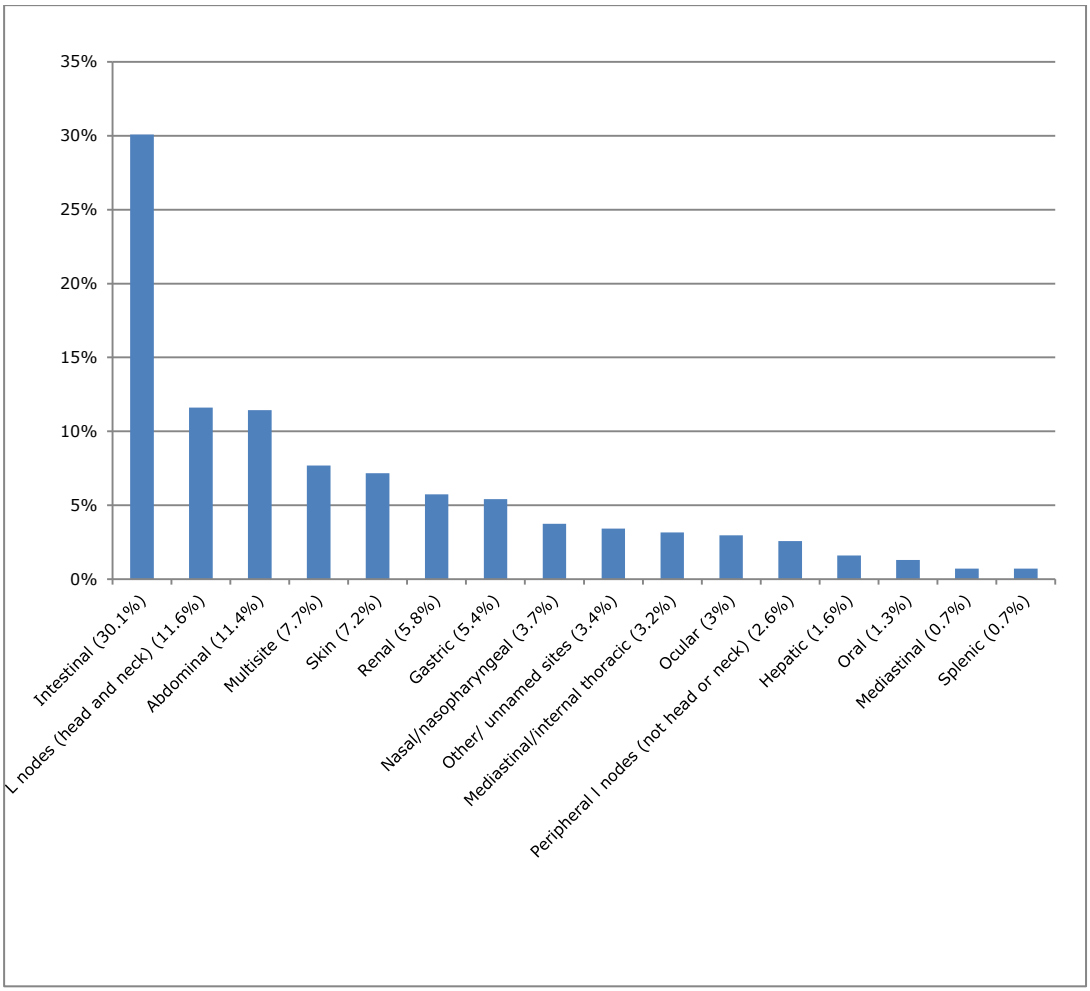

Fig 10: Classification based on anatomical location, n= 1549.

Anatomical location category definitions are shown in Appendix A.

The most common anatomical location of lymphoma was the intestine at 30.1% (n=466) and in combination with the gastric form, gastrointestinal lymphomas were most prevalent at 35.5% (n=550). The second most prevalent site was the lymph nodes in the head and neck region at 11.6% (n=180).

Anatomical location	Large cohort
Intestinal	466 (30.1%)
Lymph nodes (head and neck region)	180 (11.6%)
Abdominal	177 (11.4%)
Multisite	119 (7.7%)
Skin	111 (7.2%)
Renal	89 (5.8%)
Gastric	84 (5.4%)
Nasal/nasopharyngeal	58 (3.7%)
Hepatic	25 (1.6%)
Mediastinal/ internal thoracic	49 (3.2%)
Ocular	46 (3%)
Peripheral lymph nodes (not in head or neck region)	40 (2.6%)
Oral	20 (1.3%)
Mediastinal	11 (0.7%)
Splenic	11 (0.7%)
Other/ unnamed sites	53 (3.4%)
Total	**1549 (100%)**

Table 5: Proportions of different anatomical locations

4.1.3 Describing levels of certainty of feline lymphoma on cytological reports

Cytological reports were assessed on 688 laboratory submissions from 2015 to 2018. Ten cases were excluded as lymphoma was not diagnosed. Of the remaining cases (n=678), 22% (n=149) had an unmodified diagnosis of "Lymphoma". In the remaining 529 cases, a total of 15 different descriptive terms were used to describe the cytologist's confidence in lymphoma diagnosis. There were 231 cases in "certainty 1" category, 292 cases in "certainty 2" category and 6 uncertainty cases ("certainty 1A"). There were far more cases in "certainty 2" and the "unmodified lymphoma" categories combined (65%, n= 441) than in "certainty 1" and "certainty 1-A" categories combined (35%, n=237). Table 6 shows the descriptive terms used in the reports and the frequencies of their use.

Descriptive term	Number of cases	Percentage
Certainty 1	**231**	**34.1%**
cannot rule out	3	0.4%
concern for	54	8.0%
possible	43	6.3%
primary differential is	1	0.1%
suspect	106	15.6%
suspicion	2	0.3%
suspicious for	22	3.2%
Certainty 1-A	**6**	**0.9%**
lymphoma or other	6	0.9%
Certainty 2	**292**	**43.1%**
compatible with	16	2.4%
consistent with	100	14.7%
likely	37	5.5%
presumptive	1	0.1%
probable	110	16.2%
suggestive of	27	4.0%
typical of	1	0.1%
Unmodified lymphoma	**149**	**22.0%**
Unmodified Lymphoma	149	22.0%
Grand Total	**678**	**100.0%**

Table 6: Descriptive terms and modifiers used in feline lymphoma cytology reports (n=678). Unmodified lymphoma included cases in which lymphoma diagnoses were made without the use of any modifiers or descriptive terms.

For the purpose of this study a total of 107 cases with the following diagnoses were excluded: "possible lymphoma", "concern for lymphoma", "cannot rule out lymphoma", "lymphoma or other" or "primary differential is". A further 17 cases were later excluded as the histological diagnoses were either suggestive of reactive hyperplasia or inconclusive for lymphoma therefore making a total of 554 cytology cases that were included in this study.

4.1.4 Subtyping results following initial clinician requests in the large cohort

Of the 1,549 cats diagnosed with lymphoma by histopathology and cytology, between 2014 and 2018, clinician requests for subtyping were made in 6.7% of cases (n= 104), see Figure 11. Immunohistochemistry was performed in the majority of cases (5.6%, n= 87) and PARR testing was performed in only 1.1% of cases (n = 17). The following results were obtained:

- 58 B-cell lymphomas of which 5 were further classified (according to WHO) as T-cell rich B-cell lymphoma (TCRBCL)
- 41 T-cell lymphomas of which 4 were further classified (according to WHO) as Enteropathy-associated T-cell lymphoma (EATCL)
- 5 were inconclusive (i.e. IHC staining pattern were neither suggestive of T-cell or B-cell lymphoma)

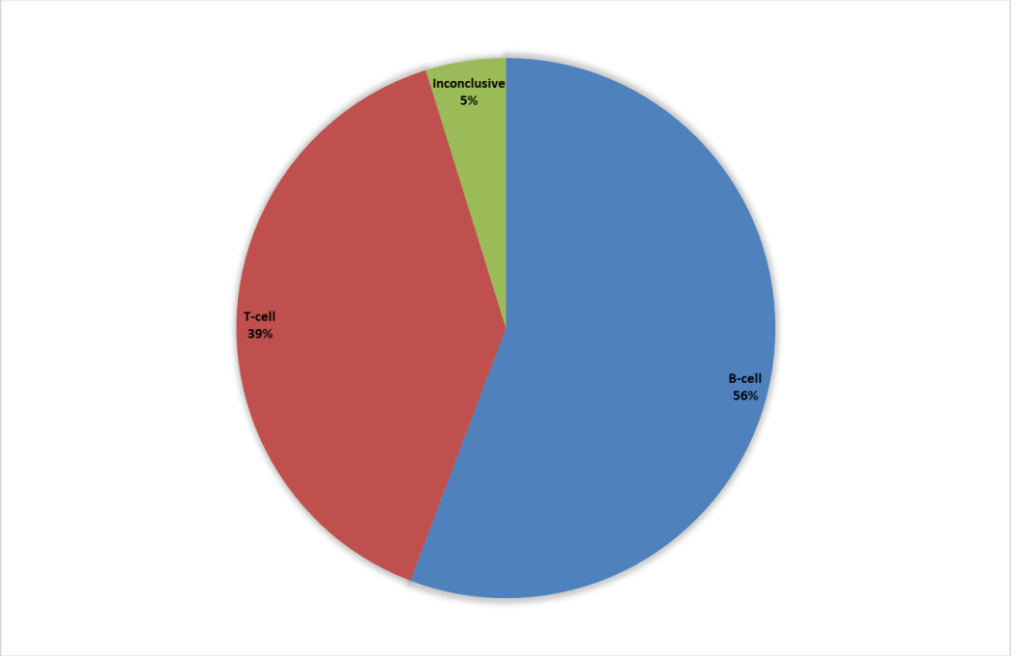

Fig 11: Immunohenotyping results of initially requested cases (n=104).

4.1.5 FeLV status

Very little information was available regarding the FeLV status of the cats. According to data held in the laboratory, only 2 of these cats in the large cohort were reported as FeLV positive; one of which had a cranial intrathoracic mass and the other, pleural effusion and ascites.

4.2 Case selection from CVS-owned practices (Group A)

4.2.1 Subtyping cases for survival study

Following searching of the CVS practice clinical database (Robovet®) to determine which cats diagnosed by histopathology had either received lymphoma-related treatment or not received treatment but survived for >28 days, a total of 88 cases were identified comprising 56 cases from the large cohort (obtained from 2014-2017) and an additional 32 cases from a further database search from January 2018 to March 2019). See Figure 9. Eighteen of the cases had been immunophenotyped by IHC on initial clinician requests. For the remaining 70 cases that had not already been phenotyped, stored tissue sections obtained from the affected cats were processed for IHC and the results were determined by the author under the supervision of a qualified anatomic pathologist who is a Fellow of the Royal College of Pathologists (FRCPath), anatomic pathology. The number of immunophenotyped cases is shown in Table 7:

Category	Number
Initially immunophenotyped	18
Retrospectively immunophenotyped	70
Excluded as not lymphoma	1
Excluded due to inconclusive IHC results	4
Total no of immunophenotyped lymphoma cases	83

Table 7: Number of immunophenotyped survival study cases from 2014 to 2019 (Group A).

Five of the 88 cases were excluded following IHC; 3 cases due to lack of expression of a distinct immunophenotype (i.e. neither B-cell nor T-cell origin), one case because cell population on histopathology and IHC stain pattern was not suggestive of lymphoma and the fifth case due to very poor uptake of immunohistochemical stain therefore precluding definitive determination of the immunological origin.

4.2.2 Patient characteristics, treatment, outcome and prognostic factors

Two hundred and fifty one submissions were reviewed in the Robovet database between 2014 and 2019 of which 83 cases met the inclusion criteria. The patient characteristics, immunophenotype, treatment and survival times are summarised in Table 8 (see Appendix B).

At the end of the study, most treated cats had been euthanized (73/83; 88%). One cat was still alive and 9 were lost to follow up. Based on the clinical history available, the cause of euthanasia was due to progression of lymphoma in the deceased cats.

4.3 Case selection from non-CVS practices (Group B)

4.3.1 Patient characteristics, treatment, outcome and prognostic factors

Medical information of cats with lymphoma was requested from practices outside the CVS Group by questionnaires (see appendix C1). Of the 1298 questionnaires sent out electronically, 57 completed questionnaires were returned. The patient characteristics, immunophenotype, treatment and survival times of these cats are summarised in Table 9 (see Appendix C2):

Based on the medical information provided in the questionnaires, majority of the cats (55/57; 96%) had died. One cat was lost to follow up. The other was still alive. Lymphoma was the cause of death in all the deceased except for 2 cats, in which the cause of death was not confirmed.

Only 5 cases in this group where immunophenotyped at initial clinicians request.

4.4 Summary of anatomical forms and phenotype of all immunophenotyped cases

The outcome measures were compared between cases that IHC was applied on initially versus retrospectively as shown in Table 10. No differences were found allowing both groups of data to be combined for further analysis.

No of cases	B-cell	T-cell	Inconclusive	B-cell and T-cell
II (n=104)	58 (55.8%)	41 (39.4%)	5 (4.8%)	0 (0%)
RI (n=69)	41 (59.4%)	22 (31.9%)	4 (5.8%)	2 (2.9%)
Total (n=173)	**99 (57.2%)**	**63 (36.4%)**	**9 (5.2%)**	**2 (1.2%)**

Table 10: Summary of immunophenotyping results by IHC (n=173). II- initially immunophenotyped, RI- retrospectively immunophenotyped.

The relationship between anatomical location and tumour cell phenotypes is shown in Table 11. The majority of lymphomas were of intestinal origin (42.1%, n=69), followed distantly by those originating in the head and neck region (11.6%, n=19). Overall, there was a slightly higher percentage of B-cell lymphomas than T-cell lymphomas in the intestinal form (53.6%, n= 37) versus (44.9%, n= 31) and the majority of lymphomas in the head and neck region were of B-cell origin (89.4%, n=17). When combining the gastric and intestinal forms (gastrointestinal lymphomas), a total of 81 cases were found, of which a higher percentage were assigned B-cell than T-cell i.e. (58%, n=37) versus (40.7%, n=33).

Anatomical location	B-cell	T-cell	B-cell + T-cell	Total
Intestinal	37	31	1	69 (42.1%)
Lymph nodes (head and neck region)	17	2	0	19 (11.6%)
Cutaneous	5	7	0	12 (7.3%)
Gastric	10	2	0	12 (7.3%)
Nasal/nasopharyngeal	7	0	0	7 (4.3%)
Multisite	3	4	0	7 (4.3%)
Peripheral l nodes (not in head or neck region)	3	3	0	6 (3.7%)
Ocular	4	2	0	6 (3.7%)
Site not confirmed	1	2	0	3 (1.8%)
Renal	1	1	0	2 (1.2%)
Abdominal	0	2	0	2 (1.2%)
Gastrointestinal	1	1	0	2 (1.2%)
Abdominal lymph node	1	1	0	2 (1.2%)
Intestine and liver	0	1	0	1 (0.6%)
Intestinal and omentum	0	1	0	1 (0.6%)
Laryngeal/pharyngeal	1	0	0	1 (0.6%)
Spleen	1	0	0	1 (0.6%)
Lymph node (site not specified)	1	0	0	1 (0.6%)
Oral	0	1	0	1 (0.6%)
Submandibular and prescapular lymph nodes	1	0	0	1 (0.6%)
Tarsal joint and tendon	1	0	0	1 (0.6%)
Hepatic	0	1	0	1 (0.6%)
Laryngeal	1	0	0	1 (0.6%)
Tonsilar	0	1	0	1 (0.6%)
Middle ear	1	0	0	1 (0.6%)
Oral	1	0	0	1 (0.6%)
Rectal	1	0	0	1 (0.6%)
Oculonasal	0	0	1	1 (0.6%)
Total	**99 (60.4%)**	**63 (38.4%)**	**2 (1.2%)**	**164**

Table 11: Summary of anatomical forms and phenotype of all immunophenotyped cases. (9 cases with an inconclusive IHC result were excluded from this analysis.)

4.4.1 Anatomical locations and immunophenotype for patients with well-defined survival data

The relationship between anatomical location and tumour cell phenotypes for Groups A (n=83) and B (n=5) are shown in Table 12. Fifty-two cases (59.1%) were determined as B-cell

origin, 34 cases (38.6%) as T-cell origin and 2 cases (2.3%) demonstrated co-expression (i.e. T-cell and B-cell origin). In the intestinal form, there was a slighter higher number of T-cell than B-cell phenotypes (n=21 and n=17 respectively) however, when combining the gastric and intestinal forms (i.e. gastrointestinal lymphoma), the numbers were similar (n=22 versus n=23).

	Immunophenotype			
Anatomical location	**B-cell**	**T-cell**	**B-cell and T-cell**	**Total**
Intestinal	17	21	1	39
Lymph nodes (head and neck region)	10	2		12
Gastric	6	1		7
Nasal/ nasopharyngeal	5			5
Cutaneous	3	1		4
Ocular	3	2		5
Multisite	2	4		6
Anal	1			1
Oral	1			1
Peripheral l nodes (not in head or neck region)	1	1		2
Renal	1			1
Abdominal	1	1		2
Laryngeal	1			1
Face		1		1
Eye and nose			1	1
Total	**52 (59.1%)**	**34 (38.6%)**	**2 (2.3%)**	**88**

Table 12: Anatomical forms and immunophenotype of 88 cats with lymphoma in which survival times were well defined.

Regarding other anatomical forms, the majority of cases diagnosed in the head and neck area were of B-cell origin (10/12 cases). Similarly, 6/7 cases of gastric lymphomas were of B-cell origin. All 5 nasal/nasopharyngeal lymphomas were of B-cell origin.

4.5 Anatomical locations and histological grade

With respect to histological grading for cats in Groups A and B, 43% (60/140) were histologically graded and their proportions are depicted in Table 13. The majority of cases (85%, n=51) were designated high grade with only few a cases designated intermediate- or low- grade.

Anatomical location	Histological grade			
	High	Intermediate	Low	Total
Intestinal	21	1	2	24
Lymph nodes (head or neck region)	7	2		9
Multisite	4	1		5
Gastric	4		1	5
Peripheral lymph nodes (not head or neck region)	3			3
Cutaneous	3			3
Nasal/nasopharyngeal	3			3
Abdominal	2	1		3
Anal	1			1
Oral	1			1
Ocular	1		1	2
Liver and mesenteric lymph node	1			1
Total	**51 (85%)**	**5 (8.3%)**	**4 (6.7%)**	**60 (100%)**

Table 13: Anatomical location and histological grade of cats with lymphoma (n=60). The remaining 80 cats were not histologically graded.

Nearly all gastrointestinal lymphomas assessed (25/29; 86%) were classified as high grade. Similarly, 7/9; 78% of those located in the head or neck region were also high grade.

4.6 Survival analyses (Groups A and B)

4.6.1 Survival time and anatomical location

Of the 140 cases in Groups A and B, for which there was survival data, 110 cats had received treatment. The median

survival times following treatment are shown in Table 14 for the most common anatomical locations:

Anatomical location	Treated cats		Non-treated cats (surviving >28 days)	
	Number	MST in days (range)	Number	MST in days (range)
Intestinal	45	62 (3-1325)	7	58 (29-379)
Lymph nodes (head or neck region)	16	90 (8-668)	3	NC
Gastrointestinal	54	71 (3-1325)	8	72 (29-379)

Table 14: Survival time of treated cats with lymphoma for the more common anatomical locations. MST = median survival time. The intestinal group is a subgroup of the gastrointestinal group. NC- not calculated as too few numbers in this category. Non-treated cats surviving less than 28 days were not included in this analysis.

The MSTs for the treated cats were in the range of 2-3 months for the 3 location groups. The intestinal and gastrointestinal groups were categorised separately for the purpose of comparison with studies that determined MSTs in these groups.

4.6.2 Survival time and treatment

The proportions and median survival times (MST) according to treatment type are shown in Table 15. Corticosteroids were the most common treatment employed (42.7%, n= 47), closely followed by chemotherapy (37.3%, n=41). The least utilised treatments were homeopathy, and a combination of chemotherapy and radiotherapy (each at 0.9%, n= 1). The highest MST was achieved following treatment with a combination of chemotherapy and surgery (177 days, range 16-360 days, n=5) and lowest with surgery only (37 days, range 0-941days, n=13).

Treatment	Number (%)	MST in days (range)
Corticosteroid only	47 (42.7%)	52 (0-1117)
Chemotherapy	41 (37.3%)	71 (3-1325)
Surgery only	13 (11.8%)	37 (0-941)
Chemotherapy and surgery	5 (4.5%)	177 (16-360)
Surgery and corticosteroid	2 (1.8%)	NC
Chemotherapy and radiotherapy	1 (0.9%)	NC
Homeopathy	1 (0.9%)	NC
Total	**110 (100%)**	

Table 15: Survival data of treated cats with lymphoma based on the type of treatment, n=110. MST = median survival time. NC- Not calculated as too few cases for range determination.

With regards to the specific protocols used, MSTs are shown in comparison to corticosteroids and surgery in Table 16:

Type of treatment	Number of cases	MST in days (range)
Chlorambucil and corticosteroid	15	86 (10-901)
COP	18	79 (3-1325)
Corticosteroid only	47	52 (0-1117)
Surgery only	13	37 (0-941)

Table 16: Survival data of treated cats with lymphoma based on specific treatment, n=93. MST = median survival time. Data for the remaining 17 treated cats not included as too few cases in their categories.

4.6.3 Survival time and histological grade

The proportions and MSTs according to histological grade are shown in table 17:

Histological grade	Number of cases	MST in days (range)
High grade	45	48 (6-1325)
Intermediate grade	5	43 (34-452)
Low grade	4	486 (35-1460)

Table 17: Survival data based on histological classification (n=54). MST = median survival time. Six non-treated cats surviving less than 28 days were not included in this analysis.

Similar MSTs were observed between the cats with high grade lymphoma and those with intermediate grade lymphoma. The

four cats with low grade lymphoma had an overall MST that was longer than the other grades.

4.6.4 Survival time and immunophenotyped

The proportions and MSTs according to immunophenotype are shown in Table 18 below:

Immunophenotype	Number of cases	MST in days (range)
B-cell	52	84 (0-1460)
T-cell	33	52 (6-799)
B-cell and T-cell	2	NC

Table 18: Survival data based on immunophenotype (n=87). One non-treated cats surviving less than 28 days was not included. NC – Not calculated as too few in category.

4.7 Kaplan-Meier survival analysis

Survival comparisons by Kaplan-Meier analyses for the following groups are represented in Figures 12-18:

1. Age (≥11 years or ≤10years)
2. Gender, gender and neutering status (female vs female neutered vs male vs male-neutered)
3. Treatment status (treated vs non-treated cats surviving >28 days)
4. Type of treatment (corticosteroid alone vs chemotherapy alone vs surgery alone vs chemotherapy and surgery vs non-treated cats surviving >28 days)
5. Immunophenotype (B-cell vs T-cell)
6. Type of treatment in the gastrointestinal category (Treated vs non-treated group)
7. Histological grade (high grade vs intermediate grade vs low grade).

   A value of $P< 0.05$ was considered significant.

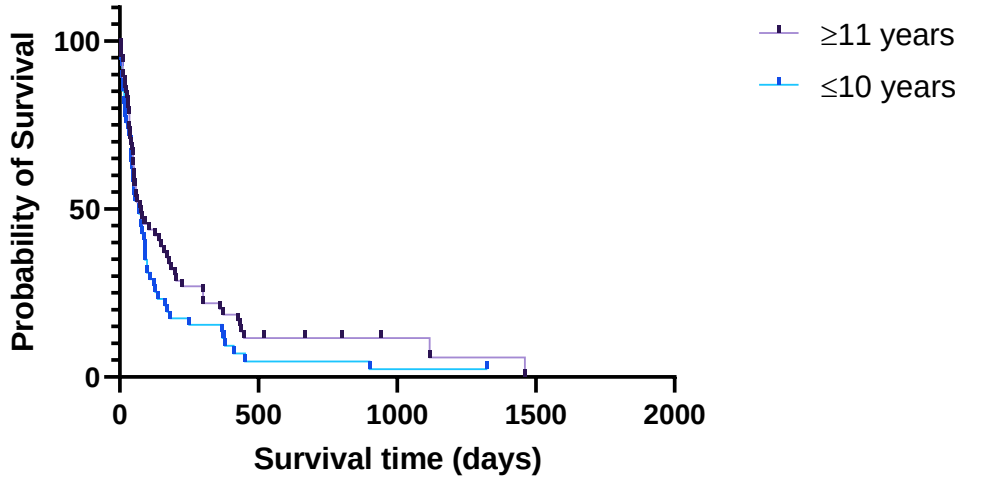

Fig 12: Kaplan-Meier curve survival proportions based on age (n= 120). Analysis excluded a total of 19 cases (12 cats surviving <28 days, 5 cats with unknown ages and 2 treated cats with survival time of 0 days). No significant difference was found between survival times in cats ≥11 years (Median Survival Time; (MST) = 75 days, n=57) and cats ≤10 years (MST = 71 days, n=50): (P value= 0.23).

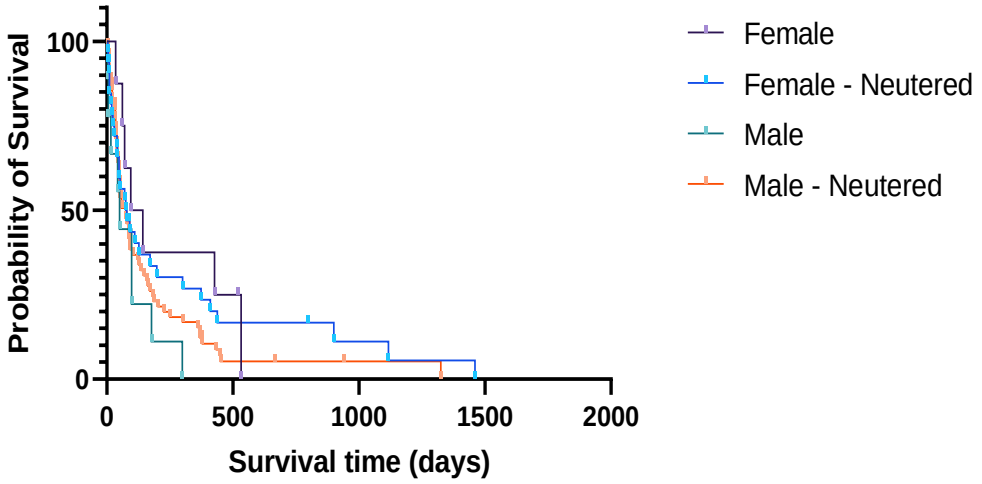

Fig 13: Kaplan-Meier curve survival proportions based on gender and/or neuter status (n= 120). A total of 20 cases were excluded (13 cats surviving < 28 days, 5 as ages were unknown and 2 as survival time was 0 days). No significant differences were observed between the different groups (P value= 0.15).

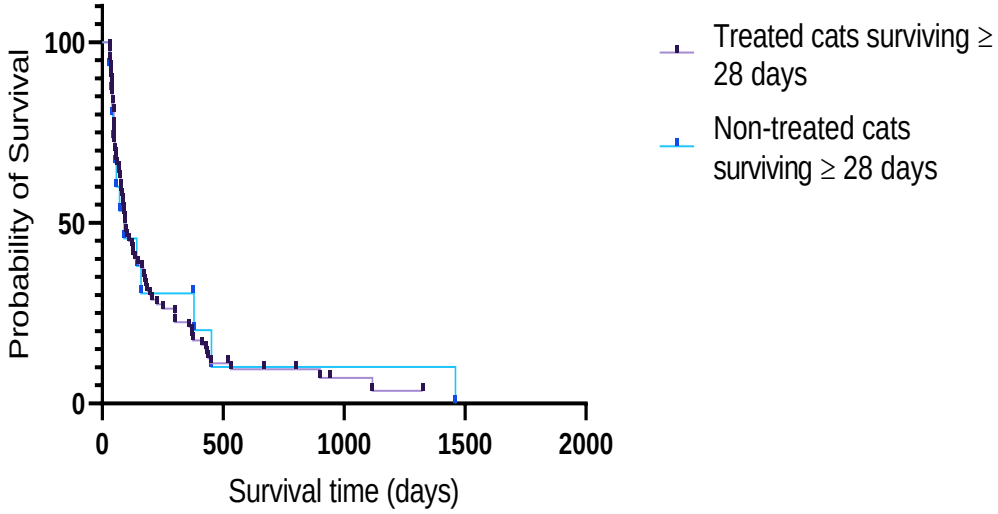

Fig 14: Kaplan-Meier survival curves stratified according to treatment status (n= 98). No significant difference was found between survival times in the treated cats (MST) = 95 days) and non-treated cats (MST = 91 days) that survived for >28 days: (P value =0.752).

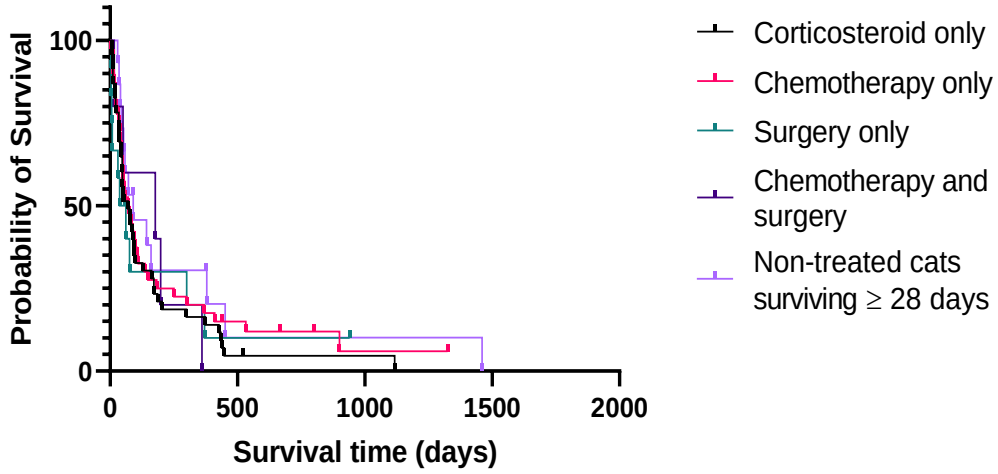

Fig 15: Kaplan-Meier curve depicting survival proportions based on treatment (n= 119). Fifteen non-treated cats that survived for >28 days were included for comparison. Survival times were not dependent on treatment status or type of treatment (P value= 0.73).

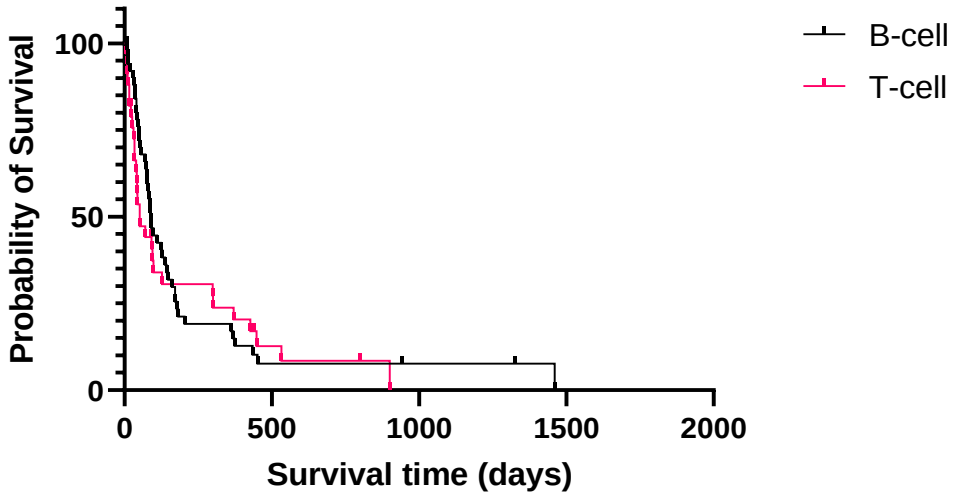

Fig 16: Kaplan-Meier curve showing survival proportions based on immunophenotype (n= 85). Three immunophenotyped cases were excluded (2 cases with dual expression (both B-cell and T-cell) due to very low numbers. One case because cat was euthanized prior to diagnosis). No significant difference was found between the T-cell subtype (MST = 52 days, n=34) and B-cell subtype of cats (MST = 89 days, n=51): P value =0.606.

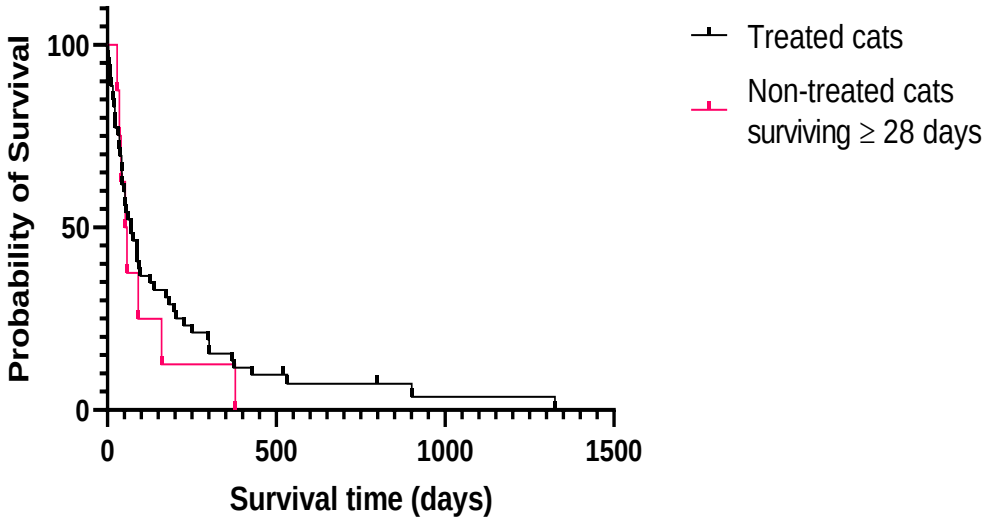

Fig 17: Kaplan-Meier curve showing survival proportions of cats with gastrointestinal lymphoma (n= 61). No significant difference was found between the treated cats (MST = 71 days, n=53) and the non-treated cats surviving >28 days (MST = 55.5 days, n=8): P value = 0.881.

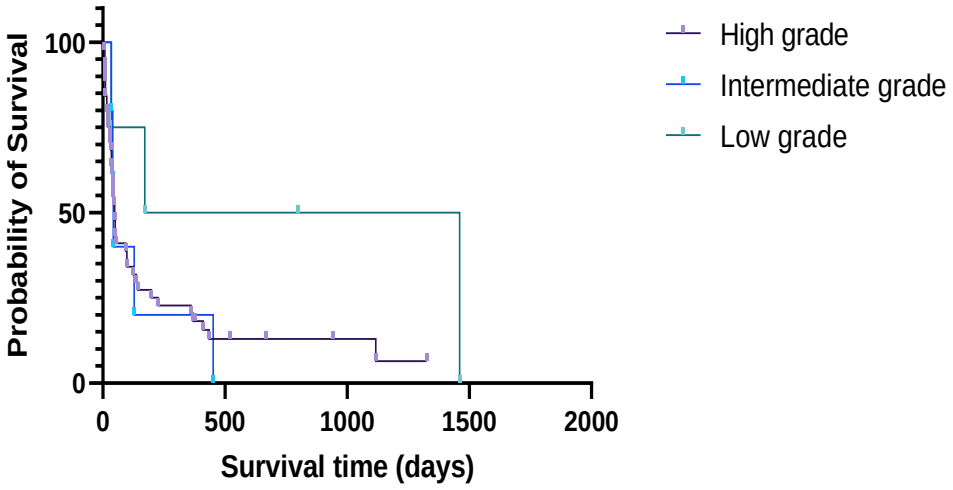

Fig 18: Kaplan-Meier curve showing survival times based on histological grade (n=54). Six un-treated cats surviving <28 days were excluded from this analysis. No significant differences were found between the 3 curves; P= 0.144.

5 Discussion

In this study, lymphoma was more commonly diagnosed by histology than by cytology, and in cytology reports diagnostic doubt was commonly expressed. The most frequent anatomical form of lymphoma in cats was intestinal and both B- and T-cell immonophenotypes were present in this location. The second most common anatomical location was in the lymph nodes in the head and neck region and the B-cell phenotype was more common. The T-cell immunophenotype was less common in other locations.
Regarding treatment, affected cats are commonly not treated or are only treated using corticosteroids alone. Whether treatment was administered or which specific treatment was administered did not confer any significant survival benefit and in contrast to the dog, in this study, immunophenotype did not influence survival either.

5.1 Frequency of use of cytology for feline lymphoma diagnosis

In conjunction with immunohistochemistry, histopathology is now considered the current gold-standard test for the majority of feline lymphoma cases (Paulin et al., 2018). However, fine needle aspirate cytology confers several advantages over histopathology in that it provides a quick turnaround time, is inexpensive, relatively convenient and causes less discomfort to the patient thus allowing biopsy of multiple sites and serial sampling especially when results are inconclusive (Valli, 2008, Caniatti et al., 1996, Nikousefat et al., 2016). Cytology is often used on its own to achieve a diagnosis of lymphoma in dogs and cats (Sharkey et al., 2007, Twomey and Alleman, 2005). In this study, the initial database screening revealed that 554 cytological diagnoses of lymphoma were made in a 3-year period in comparison 995 histological diagnoses in another 3- year period confirming histopathology as the preferred method of diagnosis however, it suggests that in a significant number of instances, cytology may be the only diagnostic test employed by clinicians for cats with lymphoma. Possible reasons could include the relatively cheaper cost, ease of sampling and quicker turnaround times associated with cytology in comparison to histopathology.

Also, a significant finding in the survey of cases obtained via questionnaire (subset B) showed that 14/57 cats (24.6%) were diagnosed by cytology alone of which the majority (9/14;

64%) had undergone treatment (5 cases using chemotherapy, 3 cases using corticosteroids and 1 case by homeopathy) based on this diagnosis. This could further suggest that in many cases, a cytological diagnosis of lymphoma is considered sufficient for clinicians to make a clinical judgement regarding medical treatment of such cats and highlights its relevance in current clinical practice. In contrast to canine lymphoma however, it would appear that there are fewer feline lymphomas diagnosed by cytology alone. A recent report showed that 64% of canine lymphoma diagnoses were based on cytology alone (Pittaway et al., 2019). Conversely, this difference could be due to the contrasting prevalence of intestinal lymphoma in cats versus nodal lymphoma in dogs and perhaps an increased likelihood that if laparotomy investigation is performed in a cat, biopsy material would more likely be submitted for histological examination than the aspiration accessible peripheral nodes.

The inability to directly compare the proportion of cases performed by cytology and histopathology within the same 3-year period due to data availability is one limiting factor of this study. Retrospective data were obtained over separate 3-year periods with some time overlap and therefore it is possible data obtained within the same 3-year period could possibly have higher or lower proportions.

5.2 Levels of certainty in cytological diagnosis of feline lymphoma

The level of certainty of a cytological or histological diagnosis can be affected by the quality of sample, nature of the lesion, level of experience of the pathologist, and availability of patient medical information (Christopher and Hotz, 2004, Pitman and Black-Schaffer, 2017). In cytological reports, the probability or level of certainty of a diagnosis is often conveyed using descriptive terms such as “probable,” “suggestive,” and “compatible with” (Christopher and Hotz, 2004, Christopher and Ku, 2018, Idowu et al., 2013), although the use of each modifier may be due to preference of the pathologist thus resulting in significant variability in their pathology reports. Due to this wide variation and overlap in interpretation of these terms by pathologists and clinicians, there is potential for miscommunication which has been shown to affect clinical decision-making, including the decision to

euthanize (Christopher et al., 2010, Vivero et al., 2014). A study by (Christopher and Ku, 2018) reported that cytologic interpretations of neoplasia in 367 dogs and cats were 3.3 times more likely to be modified than non-neoplastic lesions. Lymphoma was twice as likely to have a modified descriptive term than metastatic neoplasia and (25/39; 64%) had an "unmodified'' feline lymphoma diagnosis. Another study on 26 cats with lymphoma in the lymph nodes (Amores-Fuster et al., 2015) showed that 50% of cases were confidently diagnosed. The remaining were assigned a "probable" or "possible" lymphoma.

In this present study, the confidence in cytological diagnosis appeared to be less than previously reported- majority of cases having a "modified" diagnosis (78%, n=529) with a total of 15 different modifiers used.

In order to avoid miscommunication with clinicians due to variability in use of descriptive terms in cytology reports, (Christopher and Ku, 2018) have further suggested that the following categories may be appropriate: 96–100% probability (high risk, evidence of malignancy), 60–83% probability (suspicious for malignancy), and 12–49% probability (low risk, probably benign). This categorisation could therefore be adapted to feline lymphoma cases describing them as one of three categories; "High risk of lymphoma", "suspicious for lymphoma" and "low risk for lymphoma". These terms could potentially also be used to categorise other neoplastic lesions. Cases where this would be beneficial would include intestinal lesions where it is particularly difficult to distinguish between small cell lymphoma and inflammatory bowel disease.

5.3 Frequency of the use of immunohistochemistry for subtyping feline lymphomas according to the WHO classification

The usefulness of IHC in preventing a misdiagnosis of lymphoma cannot be overemphasized. One study on cats with nasal lymphomas showed that 3% of cases (4/125) could not be verified as lymphoma as they were immunonegative for all the IHC markers used (Nagata et al., 2014). Similarly, of the 69 cases retrospectively immunophenotyped in this study, one case could not be verified as lymphoma following IHC testing. This therefore reflects the need for immunophenotyping to be performed in combination with histopathology in feline

lymphoma cases in order to prevent misdiagnoses of lymphoma.

Regarding the frequency of subtyping requests, of the 1549 cats diagnosed with lymphoma, clinician requests for subtyping were made in only 6.7% of cases (n= 104) of which IHC testing predominated at 5.6% (n= 87). Of the phenotyped cases, the WHO classification was determined in only 10% (n=9; 5 of which were further classified as T-cell rich large B-cell lymphoma (TCRLBCL) and 4 as Enteropathy-associated T-cell lymphoma (EATCL). Therefore, in proportion to the total number of lymphoma cases, only 0.6% (9/1549) were categorised according to the WHO classification. This is one limitation to this study and further supports previous stating that reports which utilise the WHO classification to subtype feline lymphomas are rare (Vezzali et al., 2010, Moore et al., 2012, Wolfesberger et al., 2018). One could argue that further classifying these tumours may just be an academic exercise as this information would not necessarily alter the current treatment options however, the authors of a study (Wolfesberger et al., 2017) on 30 cats with lymphoma showed that the WHO classification has prognostic importance and thus may be necessary to optimise treatments in future.

5.4 Frequency of B-cell versus T-cell immunophenotypes

Immunophenotyping of lymphoma is useful in determining the cell type involved and in providing prognostic information (Twomey and Alleman, 2005). In one study (Valli et al., 2000) classifying 602 cases of lymphoma, 67% was derived from B cells, 27% from T cells, and 6% from null cells. Similarly, following the retrospective assessment of cell phenotypes on 88 cases in this study (table 11), 59.1% (n = 52) were derived from B cells, 38.6% (n=34) from T cells and 2.4% (n=2) co-expressed B-cell and T-cell. Contrary to Twomey and Alleman (2005), at least under the treatment approaches employed on cases in this study, immunophenotype did not appear to be prognostic in terms of survival.

It is possible that the 3 excluded cases where the cells did not express a distinct phenotype (i.e. neither B-cell nor T-cell origin), were “null-cell” lymphomas. However, this could not

be confirmed as further assessment was not carried out by other diagnostic methods such as PARR or flow cytometry.

Regarding the different anatomical locations, of the 81 gastrointestinal lymphoma cases that we found, a higher percentage were assigned B-cell than T-cell i.e. (58%, n=37) versus (40.7%, n=33). This agrees with findings in one study (Patterson-Kane et al., 2004) in which the majority of GI lymphomas (65%, n= 15) were of B-cell origin but disagrees with another (Moore et al., 2012) in which the majority of GI lymphomas (83%, n=124) were of T-cell origin. One possible explanation for this discrepancy that has been suggested by the authors in the latter study is the possibility of underestimating the number of T-cell lymphoma cases from lymphoplasmacytic IBD due to the similarity in histological presentation. A study of 50 cats with gastrointestinal lymphoma (Pohlman et al., 2009) showed that T-cell lymphomas may be more prevalent in the small intestine of cats than previously thought.

All 5 nasal/-nasopharyngeal lymphomas were of B-cell origin, a similar finding to that of 2 studies (Haney et al., 2009, Wolfesberger et al., 2017) (n=3 and n=4 respectively) and in a third study, all 10 nasal/ orbital lymphomas were of the B-cell subtype (Chino et al., 2013).

5.5 Frequency of histological grading

The majority of histologically graded cases were designated high-grade (51/60; 85%), far fewer were intermediate-grade (5/60; 8.3%) and low-grade (4/60; 6.7%). These findings did not correlate with that of another large study (n=602) that reported 54% (n=323) as high-grade, 35% (n=210) as intermediate-grade and 11% (n=69) as low-grade (Valli et al., 2000). Although the individual proportions that were histologically graded in this study did not correlate with that of the larger study, a combined total of 92% were graded either intermediate- or high- grade which is similar to that of several studies reporting approximately 90% of lymphomas in cats are classified histologically as intermediate- or high- grade (Holmberg et al., 1976, Valli et al., 1981, Valli et al., 2000).

The relatively low numbers of cats with low- and intermediate-grade lymphomas in comparison to those with high grade lymphomas resulted in lack of statistical power for survival analysis in this study. Similar proportions of numbers in each category would be needed to determine whether or not there is an association between histological grade and survival.

5.6 Signalment

The domestic shorthair (DSH) was the most common breed in the large cohort study (74%, n=1147) in keeping with the high prevalence of this breed in the UK and the findings of other studies (Louwerens et al., 2005, Vail et al., 1998, Taylor et al., 2009, Mooney et al., 1989, Waite et al., 2013).

The median age of the cats in the large cohort (n=1471) was 10 years which is identical to that of two studies (Taylor et al., 2009, Mahony et al., 1995). The mean age in this study was also 10 years and is comparable to one study (Wolfesberger et al., 2017)). The age range of the cats in this study (8 months to 22 years) was also similar to that reported in some studies (Valli et al., 2000, Vail et al., 1998).

Neutered males constituted the highest proportion (43% n=666) in the large cohort which is comparable to that of a large study on 602 cats (Valli et al., 2000)

5.7 Anatomical locations

In this study, lymphoma was confined to the gastrointestinal tract in the highest proportion of cases (35.5%, (n=550)). This agrees with 2 studies (Vail et al., 1998, Waite et al., 2013). With this knowledge, one could imagine that cases in this study in which the requesting clinician reported the tumour site as “abdominal mass” or those in which the anatomical location was not mentioned could indeed be originating from the stomach or intestine therefore implying a higher prevalence of GI lymphoma. Given that the proportion designated as “abdominal mass” constituted 11.4% of the large cohort, the “true” proportion of GI lymphoma cases could indeed be greater than 40% along with the originally reported GI lymphoma cases.

Another significant finding in this study was that the second most common site of diagnosis of feline lymphoma was the lymph nodes in the head and neck region based on the observations in the large cohort group (11.6%). Such an observation was not found in previously reported literature.

The relatively fewer numbers of lymphomas affecting other anatomical sites in comparison to those originating in the intestine and the head/neck region is one limiting factor to this study. Future studies assessing larger numbers of lymphoma cases with other anatomical forms as well as prospective case controlled studies should be performed to determine the prognostic value of anatomical location.

The lack of recorded medical information regarding full clinical staging limited the assessment of its relationship to survival times. There is a possibility that although lymphoma was diagnosed in an organ, other organs or lymph nodes could potentially have been affected. Without full clinical staging, this would be impossible to ascertain.

5.8 Treatments

In this study, of the 110 cats that received treatment, the largest proportion were treated using corticosteroids only (42.7%, n= 47), closely followed by those treated using chemotherapy (37.3%, n=41). Interestingly, both achieved a similar MST; 52 (0-1117) for corticosteroid-treated cats versus 71 (3-1325) for chemotherapy-treated cats.

The proportion of feline lymphoma cases treated with corticosteroids alone has rarely been reported in literature. In one study (Taylor et al., 2009), fewer cats (10/110) were treated with corticosteroids alone and achieved an MST of 60 days comparable to that observed in this study (i.e. 52 days). The authors of that study also reported that 70% of the cats responded to treatment with only 30% achieving complete response. Unfortunately, medical information regarding response to therapy was not available for the cats in this current study.

Regarding the specific treatment, 18 cats that were treated using the COP protocol in this study achieved an MST of 79 days. Other studies (Teske et al., 2002, Taylor et al., 2009)

have reported higher MSTs (251 days (n=61) and 171 days (n=66) respectively) for this treatment category. One possible explanation for the poor correlation could be that these studies involved referral practices in contrast to the current study that primarily involved primary care practices.

In this study, 15 cats that were treated using a combination of glucocorticoid and chlorambucil had an MST of 86 days. In contrast, much higher MSTs were recorded in 2 previous studies (Stein et al., 2010) and (Kiselow et al., 2008) reporting 786 days and 704 days respectively, although it is worth mentioning that the first study involved only cats with GI small cell lymphoma and the latter study only involved cats with low grade lymphoma.

It was somewhat unexpected in this study that specific chemotherapy protocols did not demonstrate any survival benefit over corticosteroid alone. It may have been possible to demonstrate a statistically significant difference of MST between 52 and 71 days, if a greater number of treated cases were recruited. However, even if there is truly an advantage that there was insufficient statistical power to detect, it would appear based on this study that chemotherapy may only offer extended survival of only a few weeks compared to corticosteroids alone.

Poor survival was observed across all patient groups. There reasons are not entirely clear but this might include factors such as the owners decision to euthanize due to poor quality of life, poor response to treatment, age or cost of treating lymphoma resulting in such cats being euthanized prior to deterioration in their condition. Unfortunately, these factors could not be assessed in this study as data regarding the clinical stage or condition of the patient at the time of euthanasia was not available. Prospective studies may help to determine the reasons for this.

5.9 Survival time assessment

In order to satisfy the main aim of this retrospective study which was to identify variables that might assist veterinary clinicians determine the prognosis of lymphoma in cats, a number of variables were assessed. These included; age, gender and neutering status, treatment status, types of treatment, immunophenotype and histological grade. None of these variables correlated with survival times. This agrees with

reports stating that none of age, gender, neutering status confer any survival benefits in cats with lymphoma (Mooney et al., 1989)

5.9.1 Relationship between anatomical location and survival time

The anatomical site of lymphoma is a well-known prognostic factor according to several studies (Teske et al., 2002, Collette et al., 2016, Sato et al., 2014, Cotter, 1983). However, the authors of one study of 103 cats (Mooney et al., 1989) reported that it did not significantly affect survival time consistent with this study's finding that the MSTs in treated cats with the intestinal form and gastrointestinal forms were similar (62 days, n=45 and 71 days, n=54). Unfortunately, there were too few cases to determine survival times in cats with lymphomas in the head and neck region. The MSTs in cats with GI lymphoma varied in one study (Moore et al., 2012) depending on the infiltrative pattern observed histologically; mucosal T-cell lymphoma group had an MST of 29 months (n =54) in contrast to the transmural T-cell lymphoma group with an MST of 1.5 months (n=13).

5.9.2 Relationship between histological grade and survival time

The effect of histological grade on survival has been speculated and in many previous studies, affected cats were not separated according to histological grade (Moore, 2013). One study reported a median survival time of 704 days in 41 cats with low grade lymphoma (Kiselow et al., 2008). Only 4 cases of this present study were designated as low grade with an overall MST of 486 days. The intermediate- and high- grade lymphomas had similar MSTs which were much lower (43 days versus 42 days). The longer MSTs in the 4 cats with low grade lymphomas could suggest higher survival rates for this grade however, this low sample number (possibly coupled with the fact that not all were histologically graded) did not give us the statistical power to confirm. Prospective studies involving higher numbers of cases particularly in the intermediate- and low- grade categories are required to validate these findings.

5.9.3 Relationship between immunophenotype and survival time

Since the prognostic value of immunophenotyping feline lymphomas is not yet appreciated, it is not necessarily performed for prognostic purposes. In many cases, it is done either to confirm the diagnosis of lymphoma especially where cytology or histopathology are inconclusive (Dr Owen Davies, personal communication) or merely as an academic exercise. Although there is no convincing evidence that immunophenotype is of prognostic relevance in cats with lymphoma, too few cats are subtyped or classified to WHO/REAL to definitively prove this theory.

Notwithstanding, the lack of statistical difference in survival times between the B- and T-cell subgroups of cats in this study correlates with previous reports stating that immunophenotype does not confer any survival benefits to cats with lymphoma (Wolfesberger et al., 2017, Patterson-Kane et al., 2004).

It is possible that more cases of feline lymphoma that are immunophenotyped as well as histologically graded, the better understanding clinicians would have regarding their prognostic use.

There were several limitations to performing survival analyses. These included:

- The retrospective and multi-institutional nature of this study- Analysis and comparisons between survival times amongst a variety of practices have the potential for variations in treatment protocols (particularly for chemotherapeutic agents). However, these results reflect the broad experience of veterinary practices.
- The difficulty in adequately measuring survival in veterinary species. The majority of the cats were euthanized and did not die a natural death and so it is probable they may have lived longer had they not been euthanized.

6 Conclusion

Although lymphoma is the most commonly diagnosed neoplasm in cats, its varied presentations has limited any significant advancements in predicting the biological behavior of the disease in each case. This study has helped to shed some more light on the complexity of the disease and the challenges this poses in the diagnosis, classification and prognostication from the viewpoint of a pathologist.

Regarding the frequency of diagnostic modalities for feline lymphoma, histopathology was found to be more commonly utilised than cytology. It also appears that cytology is currently considered important and possibly as important as histopathology (with or without immunohistochemistry) in reaching a clinical diagnosis.

Regarding the frequency of use of specific terms to describe the probability of lymphoma in cytology reports, a significant number of cases were not diagnosed with a high level of certainty and many descriptive terms were used in such cases. This may have potentially added an extra complication to the confidence clinicians placed in such diagnoses and their decision to treat or euthanize the patients. Guidelines on reporting of such cases by cytologists may therefore need to be re-evaluated and implemented in order to minimise the number of descriptive terms that can result in miscommunication between cytologists and clinicians.

With respect to the frequency of anatomical locations, analysis of data in a large group of cats revealed a predominance of gastrointestinal lymphomas, with lymphomas in the head and neck region being the second most common anatomical form.

With regards to prognosis of lymphoma in cats which was the main aim of this study, none of the variables assessed in this study (i.e. age, gender, neutering status, treatment status, types of treatment, immunophenotype or histological grade) demonstrated a significant prognostic relevance. Therefore, they have not been proven to be useful in deciding whether or not to treat or for determining the type of treatment employed (where treatment is considered) and in answering the original question “Is feline lymphoma worth managing differently?” The choice of whether or not to treat or which treatment to administer should therefore depend on a combined decision between the owner and the clinician which may take into

account the clinical stage and the quality of life of the patient; factors which were not assessed in this study.

In summary, based on the inability to demonstrate any prognostic association with many variables in this study, one could suggest that there are currently no obvious simplistic recommendations for change that would improve the way feline lymphoma is being managed in clinical practice. However, in order to advance this area of study and thereby improve the knowledge of the disease, prospective studies on cats with lymphoma are recommended. At the time of diagnosis, these lymphomas would need to be immunophenotyped with full histological grade and WHO classification performed by the same group of histopathologists, utilising the same template for measuring and recording the observed histological features (e.g. mitotic index, cell size). Information on full clinical staging should be recorded at the time of diagnosis. The cats in such studies would ideally need to have been treated within the same practice or group of practices using the same treatment protocols. Where treatment is administered, response to therapy would also need to be measured during the course of treatment. At the time of euthanasia, the reason for euthanasia would be determined prior to performing survival analyses. Such studies would likely be more beneficial in determining whether there are any possible prognostic features that predict survival in such cats not evident in this study. Currently, it would appear that response to therapy may be the most important prognostic factor for cats with lymphoma.

7 Clinical training

7.1 Introduction

This combined residency in clinical pathology is a 3-year programme in which candidates are trained both, at a University and at a diagnostic laboratory. It was designed to provide the foundational knowledge, technical skills and experience necessary to competently practice clinical pathology in an academic environment as well as in a commercial setting. This includes developing knowledge of basic pathologic processes and skills needed to interpret laboratory data, as well as to make clinicopathologic correlations.

This residency is a CVS-sponsored programme which meets the eligibility requirements for the Royal College of Pathologists (RCPath) examination in that it provides a largely work-based training alongside acquiring skills to conduct research. Upon successful completion of two examinations, I would acquire a credential of Fellow of the Royal College of Pathologists (FRCPath) - an internationally recognised veterinary pathology qualification.

This MVM programme includes a research project which I have been working on this for the past 3 years, investigating the diagnosis, treatment and survival in feline lymphoma supported initially by Rachel Dean (original secondary supervisor) and the Centre for Evidence-based Medicine (CEVM) group.

In order to achieve this, I spent the majority of my first year at Nottingham University; the remaining 2 years were spent at Axiom Veterinary Laboratory, Devon (one of the 2 CVS-owned laboratories).

At the University of Nottingham, under the supervision of Dr Peter Graham, I assisted in undergraduate group teaching and demonstrating sessions which involved analysing and interpreting clinical pathology case reports as well as cytology and haematology slide training sessions. I also assisted in providing a clinical pathology service, 2 days in a week at the laboratory in Pride Veterinary Centre, Derbyshire. This entailed

reading and reporting cytology slides and performing basic laboratory management under Peter's supervision.

As part of my training at Nottingham during the course of my first year, cytology and haematology slides that had previously been read and reported to submitting practices were sent from Axiom Veterinary Laboratory, Devon to give me an opportunity to practice reading and interpreting such slides.

Towards the end of my first year as well as the early part of my second year, I embarked on an externship programme (for a 1-month period across 2 years) at the RVC (Royal Veterinary College) under the supervision of Dr Balazs Szladovits who contributed significantly to my training. During this one month period, my role as an extern at the RVC involved working with a team that included qualified clinical pathologists, clinical pathology residents and technicians to offer veterinary diagnostic laboratory services. This involved reading and interpreting cytology and haematology slides, participating in journal club meetings, attending cytology-, anatomic pathology- and neurology rounds in which clinical cases that involve a wide variety of animal species were discussed. I was also given the opportunity to present cases during these rounds.

At the beginning of October 2018, I embarked on the laboratory-based aspect of my residency at Axiom where I started reading 3 cytology cases per day. Over the next few months, my caseload was gradually increased to a minimum of 10 cases comprising a mixture of cytology and haematology cases. From the end of July 2020, I started reading and interpreting 25 cases per day. Complicated cases are secondarily reviewed by a board-certified clinical pathologist.

Since the beginning of April 2020, there has been a significant change in my working pattern due to the COVID-19 pandemic and every member of the clinical pathology team at Axiom (including myself) are expected to work from home, where possible in order to comply with the government's "social distancing'' guidelines. This presented some challenges in the latter part of my residency training as the majority of it was done remotely with limited face-to-face contact with my senior

colleagues. Despite these challenges, I managed to complete my residency training at the end of October 2020.

I sat the FRCPath (part I) examination in September 2020 and the second part (part II) in spring of 2021 and was successful in both of them at first attempt.

This chapter demonstrates my clinical and professional developments and achievements during the clinical training scholarship.

7.2 Objectives of training

This training is expected to cover the core areas of veterinary clinical pathology as stated by the RCPath namely:

1. Clinical biochemistry and other testing (including biochemistry, endocrinology, ELISA tests, blood gas and acid-base evaluation, and protein electrophoresis)
2. Haematology / Coagulation
3. Urinalysis
4. Miscellaneous topics (including the light microscope, miscellaneous equipment, Pharmaceutical/Toxicological Pathology and other types of testing)
5. Cytology/Histology
6. Laboratory Quality and Management

In addition to these core areas, an understanding of medical microbiology, virology and histology is necessary as these areas are important in the diagnosis and differentiation of infectious and non-infectious conditions. The training should emphasise the common domestic species (dogs, cats, horses, and farm animals [cattle, sheep, pigs, goats]). Furthermore, knowledge of common birds kept as pets, as well as ferrets, rabbits, hamsters, gerbils and other small exotic pets and zoo animal and wildlife laboratory medicine is also expected, but these species will not receive the emphasis that the common domestic species will receive in the examination.

The main objectives of an RCPath resident are to:

- Recognise and accurately describe normal and abnormal cytological features of various body systems, organs, tissues and cell types
- Read and interpret normal and abnormal haematology samples
- Interpret clinical biochemistry laboratory results
- Identify or suggest likely causes of abnormal cytological and haematological features observed
- Provide a concise cytology, haematology and/or a biochemistry report that provides relevant information that may assist the veterinary clinician in diagnosing a disease or condition, or rule out the presence of a disease or condition
- Perform basic laboratory management procedures
- Develop skills in scientific methods, study design and analysis and scientific writing.

In order to meet these objectives and to achieve those of my sponsor, my work was largely composed of clinical pathology duties but also of conducting a Masters research project.

7.3 Clinical work

7.3.1 General training

In my first year, I primarily underwent training at the SVMS with shorter periods (approximately 4 weeks) spent at Axiom Vet lab in Devon and 1 week spent at the RVC (Royal Veterinary college) as an extern.

Training during my second year took place mainly at Axiom Vet lab with 3 weeks spent at the Royal Veterinary College (RVC) as an extern.

The entirety of my third year took place at Axiom Vet Lab. During this period, all meetings with my main supervisor where held on a weekly- or fortnightly- basis on Microsoft Teams.

7.3.1.1 Training at the School of Veterinary Medicine and Sciences (SVMS) in Nottingham

My training at the SVMS and at Axiom involved learning the following:

- The mechanisms involved in disease processes (i.e. the pathologic basis of veterinary diseases)
- How to read and interpret cytology and haematology slides and to write corresponding reports
- How to interpret and write clinical chemistry case reports based on laboratory findings and to correlate such findings with the reported or expected clinical presentations.
- Basic laboratory management procedures applicable to analyser performance and trouble-shooting such analysers were necessary.
- Teaching skills both by observing and participating in teaching or instructing sessions of Vet students at the University

During the first year of my training at the University of Nottingham, a total of 170 haematology and cytology slides that had been previously reported were chosen by senior members of the clinical pathology team at Axiom. These cases were chosen based on the different cytological abnormalities associated with different anatomical locations/ organs and a variety of haematological abnormalities. These cases were sent to me in Nottingham for reading and interpretation (as practise). My written reports were then reviewed under the guidance of my supervisor on a weekly basis.

7.3.1.2 Pathology rounds and teaching at Nottingham

General pathology rounds

I joined the anatomic pathology residents every 3-4 weeks for a short quiz based on a chosen chapter of 'general pathology' textbooks (Robbins and Cotran, Zachary) that is included on the RCPath reading list.

Student anatomic pathology rounds

Every 2 weeks, at the end of pathology rotations, we attended undergraduate student presentations along with residents in pathology and lecturers. At these rounds, cases where presented by the students and these cases were discussed in detail.

Student clinical pathology rounds

I participated in student practical demonstration sessions in cytology, haematology and urinalysis. On several occasions I was an instructor in those teaching sessions.

Pride laboratory visits

Once or twice a week, along with my fellow resident we visited the in-clinic veterinary laboratory at Pride where we were involved in

- Reading an interpreting a small number of cytology and haematology cases under my supervisors guidance
- Assisting the lab manager with performing QA, QC and trouble-shooting analysers.

7.3.1.3 Training during externship programme at the RVC

My 4-week externship programme at the RVC's diagnostic lab entailed the following activities:

- Reading and interpreting haematology and cytology slides.
- Attending and presenting cytology cases at clinical pathology rounds.
- Participating in medicine, neurology and histopathology rounds
- Attending seminars and journal club meetings.

7.3.2 Case log at RVC and Axiom

During my clinical time I recorded a total of 65 cases (including 35 cytology and 30 haematology cases) at the RVC

(see Appendices 1 and 2) and a total of 2,465 cases at Axiom (including 463 haematology cases, 1945 cytology cases and 57 protein electrophoresis cases (see Appendices 3 to 5)

7.4 Conferences, seminars, CPD and externships

Date of course/ event	Course/event	Location	Activity
31st October to 3rd November 2020	Joint ACVP and ASVCP congress	Online	Delegate
12th August 2020	X-WOW careers in pathology	Online webinar	Presenter*
November 9th to 13th 2019	Joint ACVP and ASVCP congress	Marriott Rivercenter Hotel, San Antonio, Texas	Delegate
17th November to 20th March 2019	Quality Management for the Veterinary Clinical Pathology Laboratory Part I	Veterinary Information Network (VIN)- Online	Course attendee
5th April 2019	BSAVA congress	ICC Birmingham	Presenter **
28th January to 1st February 2019	Clinical pathology externship	Royal Veterinary College, Hawkshead Lane, Hertfordshire	Extern
25th January 2019	Highcroft Veterinary Referrals CPD day	Highcroft Veterinary Referrals, Bristol	Delegate
3rd to 7th December 2018	Clinical pathology externship	RVC , Hawkshead Lane Hertfordshire	Extern
10th to 14th September 2018	Clinical pathology externship	RVC , Hawkshead Lane Hertfordshire	Extern
19th April 2018	Postgraduate seminar	Sutton Bonington campus, University of Nottingham	Presenter***
23rd and 24th June 2018	Clinical pathology practice examinations	Idexx laboratory, Wetherby	Candidate
4th October to 22nd November 2017	Statistics and Experimental Design for Bioscientists D24C02	Sutton Bonington campus, University of Nottingham	Course attendee
Monthly	ASVCP and ESVCP online rounds in clinical pathology	Online webinar	Course attendee

* X-WOW careers in Veterinary Pathology- “Why I chose a career in veterinary clinical pathology. ** BSAVA congress (project abstract presentation) – “Is feline lymphoma worth treating differently? *** Postgraduate seminar (poster for project) – “Is feline lymphoma worth treating differently?”

7.5 Sample cases

7.5.1 Sample receipt, preparation, distribution and testing/ diagnosis

Samples that have been submitted to our laboratory for diagnostic purposes are first received by our "post room" staff members who unpack, process and distribute the samples to the appropriate departments (e.g. cytology, haematology, chemistry, microbiology etc.). These samples are further processes and tested within the departments.

As a member of a team of clinical pathologists, approximately 75% of my workload involves cytology, approximately 20% involves haematology and the remainder involves a combination of interpretation and validation of biochemistry, endocrinology and electrophoresis cases.

For the purpose of demonstrating aspects of my clinical training during the course of my residency, I have chosen the following 3 cases which I was involved in interpreting and reporting:

CASE REPORT 1

PATIENT DETAILS

An 8-year-old neutered female Greyhound was presented for a booster dose of vaccination. The dog was well in itself. On clinical examination, all but one of the peripheral lymph nodes were enlarged.

A total of 5 fine needle aspirates (one from each site) were taken from the right mandibular lymph node, left mandibular lymph node, left prescapular lymph node, right prescapular lymph node and right popliteal lymph node. A peripheral blood sample was concurrently obtained and these were submitted to the laboratory along with blood smears made at the practice.

CYTOLOGICAL FINDINGS

Cytological findings on all lymph node aspirates were similar. Over 90% of the cell population consisted of a monomorphic population of small lymphocytes (measuring between 1-1.5 times the diameter of an RBC). The lymphocytes had a condensed round to oval nucleus, with no visible nucleoli and a small amount of pale basophilic cytoplasm (see Fig 19). Occasional morphologically unremarkable intermediate-sized and large lymphocytes, mature plasma cells and non-degenerate neutrophils were also observed. Mast cells were rarely seen and no infectious agents were noted.

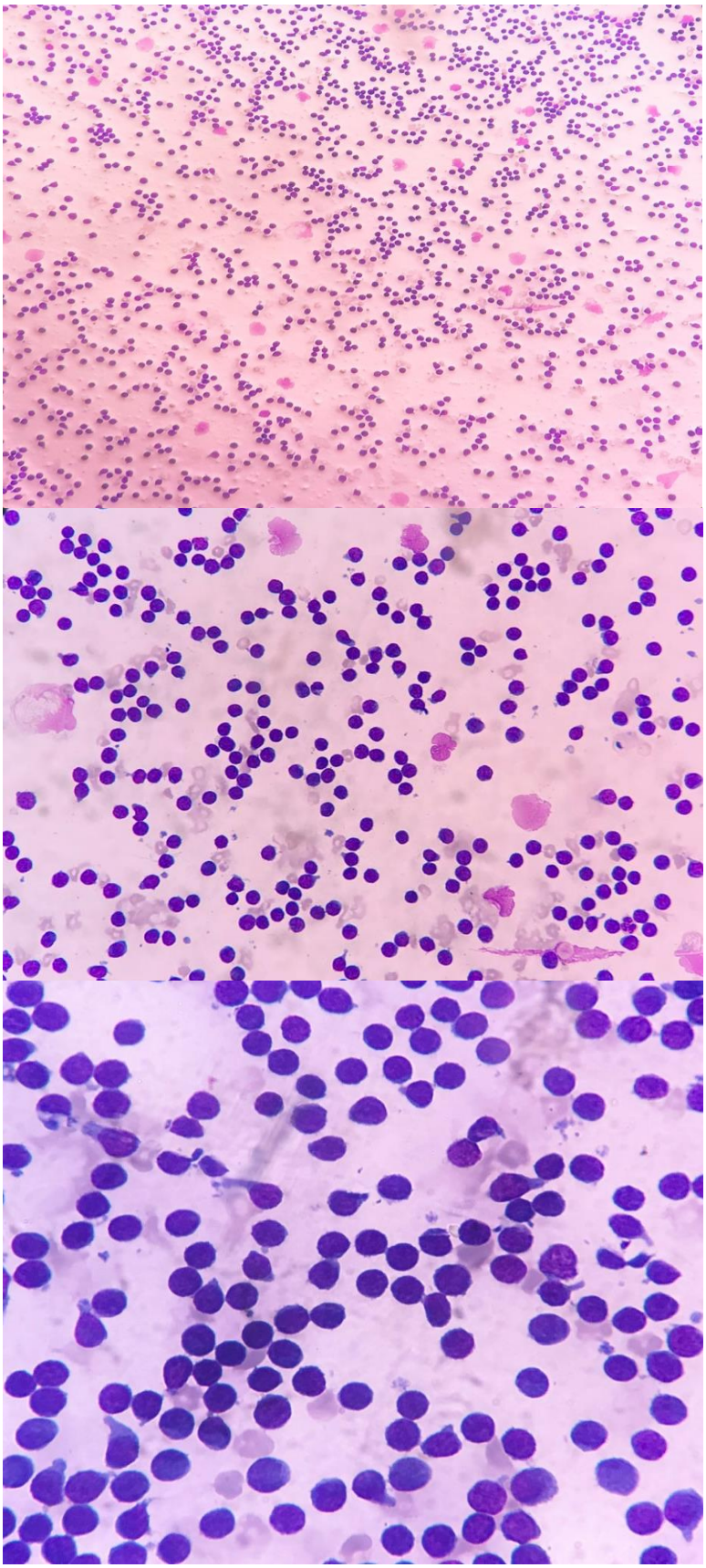

Figure 19- Prescapular lymph node aspirate from a Greyhound with small cell lymphoma. Note the monomorphic population of dense basophilic, round nucleus measuring with similar size to adjacent erythrocytes. (Wright-Giemsa stain. Original magnification at 200x, 500x and 1000x respectively.)

CYTOLOGICAL INTERPRETATION

Cytological findings were interpreted as strong suspicion for lymphoma (small-cell type)

HAEMATOLOGICAL FINDINGS

The EDTA blood sample for this patient was analysed using the Sysmex XT-2000i. There was a mild leucocytosis (16.8 x 10^9/L, RI 3.0-10.0) and a marked lymphocytosis (12.6 x 10^9/L, RI-0.6-2.5). All other haematological parameters were unremarkable. Upon blood smear examination, the density of leukocytes was mildly increased with a predominance of small to intermediate-sized lymphocytes. The lymphocytes had a round to oval or angular nucleus, dense chromatin and had small amounts of pale basophilic cytoplasm. Small clumps of platelets were frequently observed in the smear but the platelet count was normal (220 x 10^9/L, RI 90-235). No morphological abnormalities were noted in the remaining leukocytes or platelets.

HEMATOLOGIC INTERPRETATION

Haematological findings were reported as moderate lymphocytosis with predominance of small to intermediate-sized lymphocytes: strong concern for lymphoproliferative disease.

COMMENTS ON CYTOLOGICAL AND HAEMATOLOGICAL FINDINGS

Given the presence of a monomorphic population of small lymphocytes with minimal evidence of antigenic stimulation to suggest reactive lymphoid tissues in the lymph node aspirates, a strong concern for small cell lymphoma was raised with reactivity (antigenic stimulation) considered less likely. Tissue biopsy for histopathology (including immunohistochemistry) were recommended on the prescapular or the popliteal lymph nodes. These lymph nodes were a preferable choice for biopsy than the mandibular lymph nodes as they are less prone to reactivity. Taking into account both the cytological and haematological findings, there was a strong concern that this dog was in the leukemic phase of lymphoma (given the peripheral lymphocytosis and the presence of a similar lymphocyte population in circulation). Other tests that were

recommended included the PCR for antigen receptor rearrangement (PARR) to confirm the presence of a clonal population and flow cytometry for phenotyping. Consultation with a veterinary oncologist was also advised.

The submitting clinician rung for advice on the recommended tests and following this conversation, the clinician proceeded to submit biopsy samples from the popliteal and prescapular lymph nodes for histopathology:

HISTOLOGY FINDINGS

Histological examination of the popliteal and prescapular lymph nodes revealed only a small number of structures characteristic of follicles but an expansion of the paracortical tissue. In the paracortical cells, a uniform population of lymphocytes were seen having nucleoli that are small or indistinct and occasionally multiple. There was also thickened fibrous trabeculae within the nodes. Some mitotic figures were present.

HISTOLOGICAL DIAGNOSIS

A histological diagnosis of low grade lymphoma was reported.

HISTOLOGISTS COMMENT

Given the presence of a fairly uniform monomorphic population of cells within the paracortical tissue the findings were more suggestive of neoplasia rather than hyperplasia and the lesions were thought to have been longstanding due to the thickened fibrous trabeculae present within the nodes. The lesions were considered low grade and slowly progressing and the full prognosis for this type of lesion was uncertain. Due to the presence of some mitotic figures (indicating actively dividing cells), the histologist also reported that some response to chemotherapy and steroids may be achieved but the long term prognosis was exceedingly guarded.

SUMMARY OF FINDINGS

Taking into account the presence of a moderate peripheral lymphocytosis, the cytology and histological findings indication a low grade, small cell lymphoma, this patient was likely to have Stage V lymphoma.

CASE REPORT 2

PATIENT DETAILS

A 10-month-old neutered female Siberian cat with a history of lethargy and anorexia post spay via laparotomy. Ultrasonography revealed the presence of free fluid in the abdomen. Samples that were submitted included fluid obtained from the abdomen, serum and whole blood in EDTA.

HAEMATOLOGY

The EDTA blood sample was analysed using the Sysmex XT-2000i, to reveal a mild anaemia (21%, RI-27-50) and lymphopenia (0.4 x 10^9/L, RI-.5-7) On smear examination there was moderate rolueaux formation in the erythrocytes (reflecting elevated globulins due to inflammation) and there was no evidence of regeneration (i.e no polychromasia or reticulocytes were seen).

CHEMISTRY

The serum sample appeared icteric prior to testing. Analysis was performed using the Beckman Coulter AU480 analyser to reveal a mild hypoalbuminaemia (19.4g/l, 26-42), mild hyperglobilinaemia (57.9 g/l, RI-15-57) with an albumin to globulin ratio (A:G) of approximately 0.3, mildly decreased urea (5.2mmol/l, RI-6.1-12.5) and a moderately elevated total bilirubin (38.6mmol/l, RI-0-10).

VALIDATORS COMMENT

Non-regenerative anaemia could reflect chronic disease or could be pre-regenerative if there has been peracute haemorrhage/haemolysis where the bone marrow has had insufficient time to respond. Differentials for lymphopenia would include corticosteroids/stress, acute inflammation or infection, loss i.e. chylothorax, lymphangiectasia, decreased production i.e., immunosuppressive therapy or destruction of lymphoid tissue.
Hypoalbuminaemia could be associated with increased intestinal losses, urinary losses, inflammation, third spacing into body cavity effusions which was noted on your history or decreased hepatic production. Hyperglobulinaemia can be caused by chronic inflammation/antigenic stimulation, certain infectious diseases (e.g. feline infectious peritonitis (FIP),

feline immunodeficiency virus (FIV) infections, glomerulonephropathy and neoplasia (e.g. lymphoma, multiple myeloma).
The elevation in biliburin, could be pre-hepatic (a mild anaemia is noted), hepatic or post-hepatic.
Differentials for a low urea include severe starvation, diuresis, portosystemic shunts and hepatic disease.

FELINE CORONA VIRUS (FCoV) IMMUNOFLOURESCENCE ASSAY (IFA)

The FCoV IFA test was performed on the abdominal fluid and the titre was determined as positive at 1:640.

FLUID ANALYSIS

The abdominal fluid was also analysed on the chemistry analyser to reveal a total protein of 66.7g/l, albumin of 17.7 g/l, globulin of 49 g/l and an A:G ratio of 0.4.

CYTOLOGICAL EXAMINATION

Examination of direct and concentrated smears revealed low to high numbers of nucleated cells, occasional lysed cells and occasional to low numbers of erythrocytes on an eosinophilic granular background. There were high numbers of non-degenerate and poorly preserved neutrophils, moderate numbers of large mononuclear cells, frequently activated and occasional small lymphocytes. No micro-organisms were found.

CYTOLOGICAL INTERPRETATION
The cytological findings were consistent with an exudate and mixed, predominantly neutrophilic inflammation.

CYTOLOGISTS COMMENT
Cytology was consistent with an exudate and mixed, predominantly neutrophilic inflammation. Although no micro-organisms were identified differentials included a bacterial infection, FIP or a fungal infection. Culture was also recommended.

SERUM PROTEIN ELECTROPHORESIS (SPE)

SPE was performed on the patient's serum sample. The agarose gel tracing revealed a mild peak in the alpha-2 region

and a broad-based, moderate peak in the globulin region (see figure 20). This, together with hypoalbuminemia, was compatible with inflammation/infection. FIP was suspected.

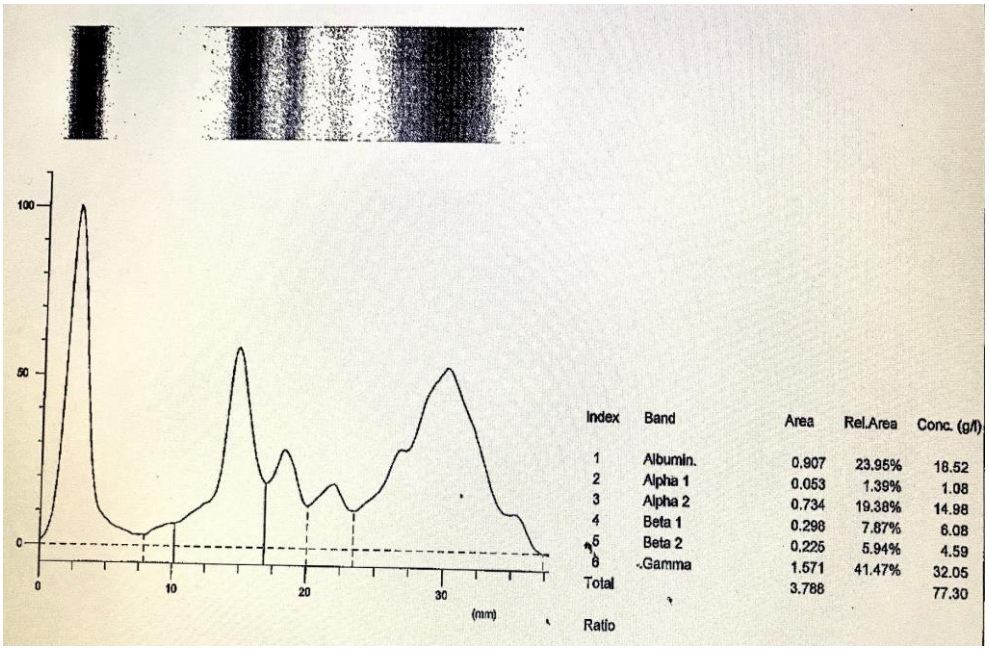

Fig 20- Electrophoretogram of a cat diagnosed with FIP infection.The trace shows a mild acute phase response and polyclonal gammopathy. The first (narrow) peak corresponds to albumin, the second prominent peak represents alpha 2 globulin which is mildly elevated. The peak at the far right represents the gamma globulin fraction which is moderately elevated and has a broad base.

SUMMARY OF FINDINGS

The presence of a combination of serum A:G ratio of 0.3, fluid A:G ratio of 0.4, positive FCoV antibody positive titre (1:640), hypoalbuminaemia and hyperglobulinaemia in a young cat with a clinical history of an abdominal effusion was most compatible with FIP.

CASE REPORT 3

PATIENT DETAILS:

A 3-year-old ferret with a firm 1 cm mass at the end of the tail.

CYTOLOGICAL FINDINGS

These aspirates were of low nucleated cellularity and adequate cell preservation. Low numbers of large foamy, ovoid to polygonal cells with finely granular nuclei and basophilic cytoplasm were seen on a densely eosinophilic, proteinaceous background. These cells are mostly individualised and rarely observed in small, loosely-cohesive clusters (see figure 21). The cells have variable N:C ratio, a round to oval nucleus measuring between 2-8 times the diameter of an RBC with stippled chromatin, 1-2 variably prominent nucleoli and a variable amounts of pale basophilic cytoplasm (interpreted as physaliferous cells). There was marked anisocytosis and anisokaryosis. Binucleation and multinucleation (up to 7 nuclei) with variably-sized nuclei were frequently observed. Nuclear moulding was also commonly observed. Occasional non-degenerate neutrophils were also noted. No infectious agents were seen.

CYTOLOGIC INTERPRETATION

Mesenchymal neoplasm: Consistent with chordoma

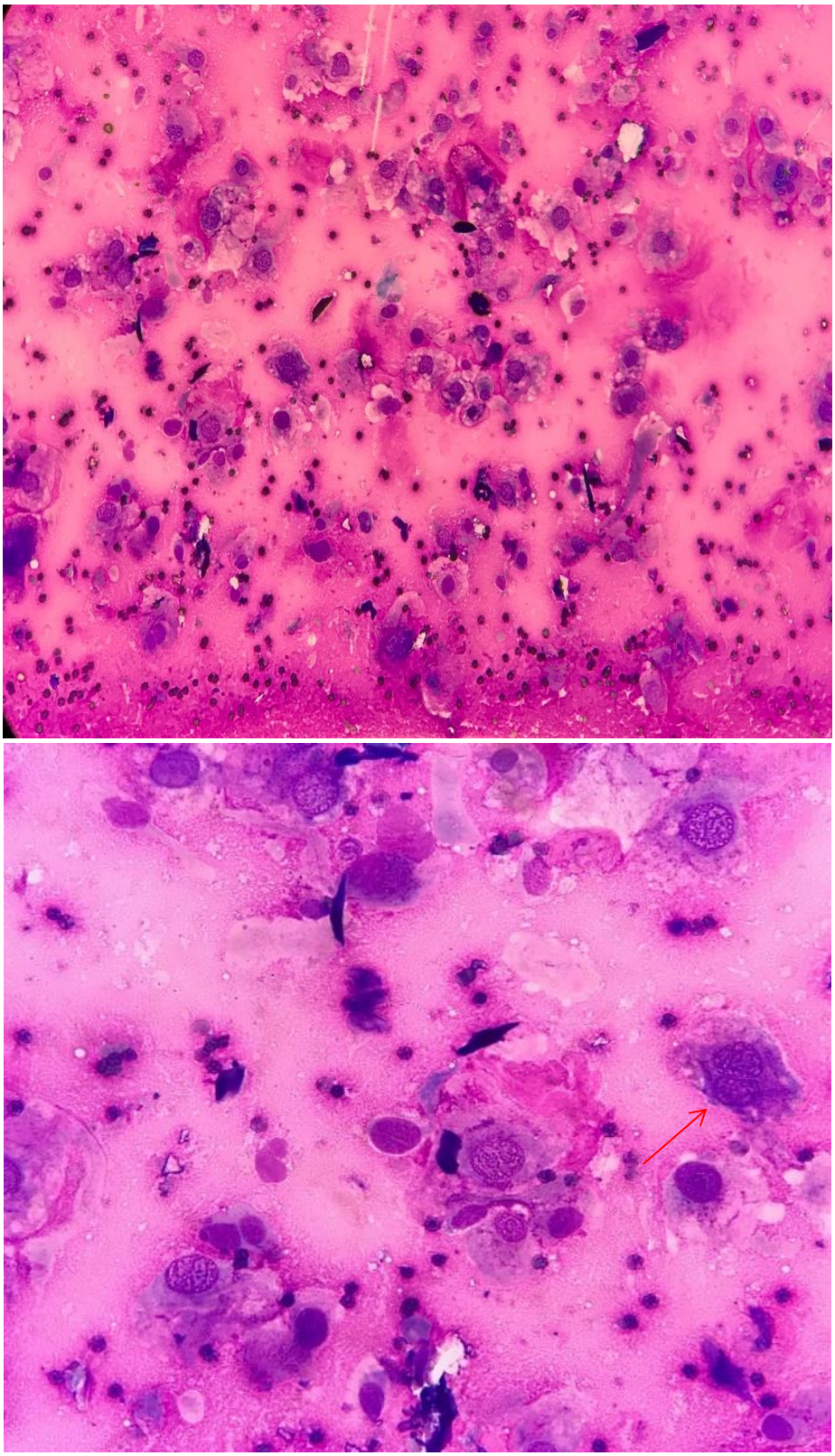

Fig 21- **Fine needle aspirate from a tail mass of a ferret with chordoma**. Physaliferous cells are recognised as large and foamy, ovoid to polygonal cells with finely granular nuclei and basophilic cytoplasm. The background is densely eosinophilic and proteinaceous. A binucleated form is visible (red arrow). (Wright-Giemsa stain. Original magnification at 500x and 1000x.)

CYTOLOGISTS COMMENT:

The presence of a mesenchymal cell population on a tail mass was most consistent with a chordoma. Chordomas are the most common musculoskeletal tumour in ferrets. They arise from remnant of the notochord of embryonic mesoderm and are commonly located at the tip of the tail but may also be seen in the cervical and thoracic vertebrae. These tumours are characterised by the presence of hyaline cartilage-like matrix between the lobules of "physaliferous cells." These tumours are generally considered to be locally aggressive with little metastatic potential, although local and distant metastasis has been reported. Surgical removal with histopathology was recommended.

Word count (excluding references- 26,568)

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Appendix A Definition of anatomical location categories

Anatomical location	Description
Gastrointestinal	Mass in gastrointestinal tract with or without abdominal lymph node involvement
Mediastinal/ internal thoracic	Presence of effusion with suspected/ confirmed mass within the mediastinal/ internal thoracic cavity
Mediastinal	Confirmed mediastinal mass
Abdominal	Mass located within the abdomen but organ, lymph node or intestinal origin not specified
Multisite	Mass present in three or more organs/ locations

Table A- Definition of anatomical location categories

Appendix B Patient characteristics (Group A)

Age (years/ months)	Sex/ neuter status	Breed	Anatomic location	Grade	IHC result	Treatment	Survival (days)
13y	**FN**	**DSH**	**Eye**	**Low**	**B-cell**	**None**	**1460**
10y	MN	DSH	Intestine	High	B-cell	COP	1325
14y	MN	DSH	Skin	High	B-cell	Hind limb amputation	*941
11y	FN	DSH	Intestine	Low	T-cell	Chlorambucil and prednisolone	**799
Unknown	F	Bengal	Intestine	NP	T-cell	Chlorambucil and prednisolone	533
9y	**MN**	**Siamese**	**Neck**	**Intermediate**	**B-cell**	**None**	**452**
12y	MN	DSH	Skin	NP	T-cell	Prednisolone	448
11y	FN	DSH	Jejunum, duodenum and liver	NP	T-cell	Chlorambucil and Prednisolone	*438
12y	MN	DMH	Skin	High	B-cell	Prednisolone	435
12y	F	DSH	Intestine and mesenteric lymph node	NP	T-cell	Prednisolone	427
9y	**MN**	**DSH**	**Intestine, mesenteric lymph node and liver**	**NP**	**B-cell**	**None**	***376**
13y	FN	DSH	Intestine and mesenteric lymph node	NP	B-cell	Prednisolone	374
7y	MN	DSH	Neck	NP	T-cell	Surgical removal	370
7y	MN	DSH	Intestine	High	B-cell	Chlorambucil and prednisolone	369
17y	MN	DLH	Nasopharyngeal	High	B-cell	Chlorambucil and prednisolone + debaulking	360
14y	MN	DSH	Intestine	NP	T-cell	Chlorambucil and prednisolone	301
11y	M	BSH	Intestine	NP	T-cell	Prednisolone	299

14y	MN	DSH	Stomach	NP	B-cell	Prednisolone	204
6y	MN	DSH	Stomach and mesenteric lymph node	NP	B-cell	COP	183
12y	M	DSH	Eye	NP	B-cell	Enucleation, Chlorambucil and prednisolone	177
9y	MN	DSH	Neck	Low	B-cell	Prednisolone	172
12y	FN	Maine Coon	Stomach	NP	B-cell	Prednisolone	172
13y	**MN**	**DSH**	**Intestine**	**NP**	**B-cell**	**None**	**160**
11y	MN	DSH	Nose	NP	B-cell	COP , Lomustine	148
13y	**F**	**DLH**	**Neck**	**High**	**B-cell**	**None**	**143**
6y	MN	BSH	Intestine	High	B-cell	Lomustine and prednisolone	137
10y	MN	DSH	Neck	Interm ediate	B-cell	Prednisolone and surgical removal	128
13y	FN	DSH	Mesenteric lymph node	NP	T-cell	Prednisolone	127
10y	MN	DSH	Nasopharyng eal	High	B-cell	COP and radiotherapy	124
8y	FN	DSH	Neck	NP	B-cell	COP	110
10y	M	Burmilla	Mouth	High	B-cell	Prednisolone	98
8y	M	DSH	Intestine	High	T-cell	COP	98
Unknown	F	DSH	Face	NP	T-cell	Prednisolone	95
8y	MN	DSH	Intestine and mesenteric lymph node	NP	T-cell	Chlorambucil and Prednisolone	92
9y	**FN**	**DSH**	**Intestine**	**NP**	**B-cell**	**None**	**91**
13y	**FN**	**DSH**	**Pancreas, mesenteric lymph node and spleen**	**NP**	**T-cell**	**None**	***89**
9y	MN	DSH	Rectal	NP	B-cell	Prednisolone	89
8y	MN	DSH	Intestine	NP	B-cell	Prednisolone	87
13y	MN	DSH	Mesenteric lymph node	NP	B-cell	Chlorambucil and prednisolone	86
7y	MN	DSH	Kidney	NP	B-cell	COP	82
14y	FN	DSH	Skin	NP	B-cell	prednisolone	79
9y	MN	DSH	Stomach	NP	B-cell	Prednisolone	76
11y	**Unkno**	**DSH**	**Neck**	**NP**	**B-cell**	**None**	**72**

	wn						
9y	FN	DSH	Mesenteric lymph node	NP	B-cell	Chlorambucil and prednisolone	71
8y	F	DSH	Intestine and mesenteric lymph node	NP	T-cell	Prednisolone	70
14y	MN	DSH	Eye	NP	B-cell	Enucleation	*57
15y	MN	DSH	Nose	NP	B-cell	None	*56
10y	Unkno wn	Siamese	Intestine	NP	B-cell	Prednisolone	56
12y	**FN**	**DSH**	**Stomach and lymph node**	**High**	**B-cell**	**None**	**53**
16y	MN	DSH	Intestine and mesenteric lymph node	NP	T-cell	Chlorambucil and prednisolone	52
10y	MN	DSH	Mesenteric lymph node	NP	T-cell	Prednisolone	52
11y	M	DSH	Liver, stomach, gastric and mesenteric lymph nodes	High	B-cell	COP	51
5y	MN	DSH	Nose	NP	B-cell	Prednisolone	49
Unknown	Unkno wn	DSH	Neck	High	B-cell	Prednisolone	47
16y	**MN**	**DSH**	**Skin**	**NP**	**T-cell**	**None**	**44**
Unknown	MN	DSH	Intestine and mesenteric lymph node	High	T-cell	Prednisolone	43
8y	FN	DSH	Intestine	High	B-cell	COP	42
15y	MN	DSH	Mesenteric lymph node	NP	T-cell	prednisolone	42
7y	**MN**	**DSH**	**Intestine**	**High**	**B-cell**	**None**	**40**
9y	FN	OSH	Unknown lymph node in abdomen	Interm ediate	B-cell	COP	40
9y	MN	Burmese	Neck	NP	T-cell	COP	39
14y	MN	DSH	Eye and nose	NP	B-cell and T-cell	Enucleation	37
13y	MN	Burmese	Stomach and lymph node	High	B-cell	prednisolone	36
17y	MN	DSH	Intestine and mesenteric lymph node	High	B-cell	Prednisolone	35
15y	MN	DSH	Liver, jejunum,	High	T-cell	Prednisolone	34

			lymph node and pancreas				
6y	MN	DSH	Popliteal lymph node	Interm ediate	T-cell	Prednisolone	34
13y	MN	DLH	Eye	NP	T-cell	Enucleation	31
10y	MN	DSH	Intestine and mesenteric lymph node	High	B-cell	Chlorambucil and prednisolone	30
11y	**FN**	**DSH**	**Intestine and mesenteric lymph node**	**High**	**B-cell**	**None**	**29**
5y	MN	DSH	Intestine	High	T-cell	Prednisolone	22
9y	MN	DSH	Mesenteric lymph node	NP	T-cell	Prednisolone	*20
9y	MN	DSH	Neck	NP	B-cell	Prednisolone	18
6y	MN	NF	Intestine and omentum	High	T-cell	COP	16
14y	FN	DSH	Unknown lymph node in abdomen	High	T-cell	Prednisolone	11
15y	MN	DSH	Neck	High	B-cell	Prednisolone	11
13y	MN	DSH	Intestine	High	B-cell	Prednisolone	10
10y	MN	DSH	Intestine	High	T-cell	Prednisolone	9
8m	FN	DSH	Popliteal lymph node	NP	B-cell	Vincristine and prednisolone	9
2y	FN	DSH	Eye	High	T-cell	Enucleation	*8
9y	MN	DSH	Liver, mesenteric lymph node and ileum	High	T-cell	COP	8
7y	MN	Russian Blue	Neck	High	B-cell	Dexamethas one	*8
12y	FN	DSH	Intestine	NP	T-cell	Prednisolone	6
14y	M	DSH	Mesenteric lymph node	NP	B-cell and T-cell	COP	3

Table 8: Patient characteristics, immunophenotype, anatomical location, histological grade, treatment and survival times of 83 cats diagnosed with lymphoma by histology (2014 – 2019). (Non-treated cats surviving >28 days (**in bold)**, COP- vincristine, cyclophosphamide and prednisolone, NP- Histological grading not performed. *Lost to follow up, ** Alive).

Appendix C1 Sample questionnaire (group B)

Unique Identification Number:
1. Is lymphoma your working diagnosis for this patient? (Yes/No) (Please note if no is selected, then no further questions will be asked and survey will end)
2. Laboratory identification number (provided in the invitation letter)
3. How was lymphoma diagnosed? Histopathology/ cytology/ other (tick any/ all that apply)
3.a. If you selected Other, please specify:
4. Please select the organ(s) or site(s) of diagnosis
5. Is the cat FeLV positive?
6. Is the cat alive, deceased or lost to follow up?
6.a. If deceased, please state date of death or euthanasia (DD/MM/YYYY)
6.b. Was death or euthanasia lymphoma-related? (Yes/ No/ unsure)
6.b.i. If unsure, please explain why you are unsure of the cause of death
6.c. If lost to follow up, please state date last seen at practice (DD/MM/YYYY)
7. Did the cat receive any treatment or surgery for lymphoma? (Yes/No)
7.a. Please select treatment or surgical procedure (if applicable)
7.a.i. Please specify treatment (if applicable)
7.a.ii. Please state chemotherapeutic drug or protocol (if applicable)

Appendix C2 Patient characteristics (Group B)

Age (yrs)	Sex/ neuter status	Breed	Site	Dx method	Mitosis	Grade	IHC	Treatment	Survival time (days)
16	FN	DSH	Liver and mesenteric lymph node	Histo	8	High	NP	Corticosteroids	1117
10	FN	DSH	Intestine	Histo	5-10	None	T-cell	Chlorambucil and prednisolone	901
14	MN	DSH	Submandibular lymph node	Histo	9	High	NP	COP	**668
12	F	DSH	Intestine	Histo	Up to 6	High	NP	Corticosteroids	*520
17	FN	DSH	Submandibular lymph nodes and spleen	Cyto	None	None	NA	Corticosteroids	438
7	FN	DSH	Neck area	Histo	4-5	High	NP	COP	411
10	**MN**	**DSH**	**Intestine and lymph node**	**Histo**	**0.8**	**None**	**NP**	**None**	**379**
16	FN	DSH	Intestine	Histo	Up to 10	None	NP	Surgical excision	301
10	MN	Unknown	Stomach	Histo	3-5	None	NP	COP	251
12	MN	DSH	Intestine	Histo	Numerous	High	NP	Surgical excision and corticosteroids	226
11	FN	British Blue	Intestine	Histo	Up to 1.1	High	NP	Surgical excision and COP	198
13	MN	DSH	Neck area	Histo	Up to 10	None	NP	Corticosteroids	186
10	MN	DLH	Nasal/nasopharyngeal	Histo	None	None	NP	Corticosteroids	163

14	MN	OSH	Submandibular and neck area	Cyto	None	None	NA	COP	104
U	MN	Siamese	Anus	Histo	8	High	NP	Corticosteroids	94
10	MN	DSH	Lymph node in neck	Histo	Moderate	None	NP	Surgical excision	76
14	FN	DSH	Laryngeal region	Histo	4	None	B-cell	COP	75
13	F	DSH	Intestine and abdominal lymph node	Histo	5-10	None	NP	Surgical excision	62
14	MN	DSH	Submandibular lymph node	Cyto	None	None	NA	Chlorambucil and prednisolone	58
13	**MN**	**DSH**	**Mesentery**	**Histo**	**4**	**None**	**NP**	**None**	**58**
13	MN	DSH	Submandibular lymph node	Histo	8.4	High	NP	Cyclophosphamide and prednisolone	51
6	MN	DSH	Intestine	Histo	Up to 11	None	NP	Surgical excision, cyclophosphamide and prednisolone	50
12	FN	OSH	Nasopharyngeal	Histo	5 or more	High	NP	Corticosteroids	48
10	FN	DLH	Kidney	Cyto	None	None	NA	Homeopathy	48
13	MN	DSH	Skin and skeletal muscle	Histo	10	High	NP	Corticosteroids	48
11	MN	Birman	Pharyngeal	Histo	Up to 3	None	NP	Corticosteroids	47
9	M	DSH	Mesenteric lymph node	Histo	3-5	Intermediate	NP	COP	43
14	**F**	**DSH**	**Stomach**	**Histo**	**3-4**	**High**	**NP**	**None**	**35**

14	MN	DSH	Intestine and lymph node	Histo	1	Low	NP	Chloramb ucil and prednisol one	35
11	**MN**	**DSH**	**Intestine**	**Histo**	**5**	**High**	**T-cell**	**None**	**26**
9	FN	Burmese X	Intestine	Cyto	None	None	NA	Chemoth erapy (type not mentione d)	24
13	MN	DSH	Stomach and small intestine	Cyto	None	None	NA	Corticost eroids	22
13	MN	DSH	Abdominal mass	Cyto	None	None	NA	Chemoth erapy (type not mentione d)	21
10	FN	DLH	Mesenteric lymph node	Histo	10	High	NP	CHOP	19
14	FN	BSH	Abdominal mass	Cyto	None	None	NA	Corticost eroids only	17
6	M	DLH	Intestine and mesenteric l node	Histo	Up to 5	High	T-cell	Surgical excision and COP	16
13	**F**	**DSH**	**Stomach**	**Histo**	**2-3**	**None**	**NP**	**None**	**13**
13	**FN**	**DSH**	**Kidney**	**Histo**	**2-3**	**None**	**NP**	**None**	**13**
9	**MN**	**DSH**	**Abdominal mass**	**Cyto**	**None**	**None**	**NA**	**None**	**12**
14	FN	DLH	Intestine, mesentery and abdominal lymph node	Histo	2.4	None	NP	Chloramb ucil and corticost eroids	11
11	**U**	**DSH**	**Mesenteric adipose tissue**	**Histo**	**Up to 7 or more**	**High**	**NP**	**None**	**11**
6	MN	DSH	Abdominal mass	Cyto	None	None	NA	Chloramb ucil and corticost	10

								eroids	
16	**MN**	**DSH**	**Nose, soft palate, retropharyngeal, prescapular, submandibular region**	**Cyto**	**None**	**None**	**NA**	**None**	**10**
11	**FN**	**DSH**	**Stomach, omentum and mesenteric lymph node**	**Histo**	**Up to 3**	**High**	**NP**	**None**	**6**
7	M	Unknown	Intestine	Histo	Up to 11	High	NP	Surgical excision	6
10	**MN**	**DLH**	**Skin**	**Histo**	**2-4**	**None**	**NP**	**None**	**6**
4	**FN**	**DSH**	**Stomach**	**Histo**	**5-12**	**High**	**NP**	**None**	**5**
13	FN	DSH	Stomach and lymph node	Histo	6-8	None	NP	Surgical excision	4
10	MN	DSH	Intestine	Histo	None	None	NP	Surgical excision	3
14	**U**	**DSH**	**Abdominal mass**	**Cyto**	**None**	**None**	**NA**	**None**	**2**
9	**MN**	**DSH**	**Submandibular lymph node**	**Histo**	**Frequent**	**High**	**NP**	**None**	**1**
12	FN	DSH	Intestine and abdominal lymph node	Histo	2.5	None	NP	Corticosteroids	0
13	FN	DSH	Anus	Histo	2-4	None	B-cell	Surgical excision	0
14	**FN**	**DSH**	**Stomach and lymph node**	**Histo**	**3-4**	**None**	**NP**	**None**	**0**
6	**MN**	**DSH**	**Abdominal**	**Histo**	**5.2**	**High**	**NP**	**None**	**0**

			mass						
9	**MN**	**DSH**	**Liver, spleen and lymph node**	**Cyto**	**None**	**None**	**NA**	**None**	**0**
10	**MN**	**DSH**	**Skin**	**Histo**	**Infrequent**	**None**	**NP**	**None**	**0**

Table 9: Patient characteristics, immunophenotype, anatomical location, histological grade, treatment and survival times of 57 cats diagnosed with lymphoma by cytology and histology. (U-unknown, Non-treated cats surviving >28 days- in bold, COP- vincristine, cyclophosphamide and prednisolone. NP- Not performed. CHOP- vincristine, cyclophosphamide, hydroxyrubicin and prednisolone, Mitosis- per 40x HPF, Dx- Diagnostic method, NA- not applicable for IHC testing as cytology only performed, *Lost to follow up, **Alive)

Appendix 1 Haematology case load at the RVC

Case number	Species	Breed	Age	Sex
P330836	Tortoise	Hermann's	5 y	F
P330795	Camelid	Alpaca	5/6 y	F
P330844	Canine	Cross Breed	1y 10m	FN
P330849	Feline	Burmese	8y 8 m	FN
P330893	Canine	Bichon Frise	8y 9m	MN
P330929	Canine	Whippet	9y 2m	FN
P330959	Canine	Boston Terrier	8 y	MN
P330998	Canine	Jack Russel Terrier	4y	MN
P331076	Canine	Cavalier King Charles Spaniel	7 y	M
P335476	Canine	Schnauzer	8y 1m	M
P335173	Canine	Unknown	9y 3m	MN
P335440	Avian	Rosy Flamingo	48y	F
P337924	Canine	Pug	14y 5m	FN
P337932	Amphibian	Mountain chicken Frog	8y 6m	F
P337954	Reptile	Frilled Lizard	4y 2m	M
P338006	Canine	French Bulldog	5m	M
P338022	Canine	Crossbreed	11m	FN
P338026	Rodent	Guinea Pig	6y 3m	F
P338087	Feline	Domestic Shorthair	4y 2m	F
P340404	Feline	Scottish wild Cat	9m	F
P340416	Feline	Domestic Shorthair	8y 11m	MN
P340439	Canine	Unknown	9y 11m	MN
P340509	Avian	African Grey Parrot	7y	F
P340537	Canine	Mini Schanuzer	6y 5m	FN
P340544	Feline	Ragdoll	5y 3m	FN
P340595	Feline	Russian Blue	9y 9m	FN
P340612	Canine	Shih Tzu	5m	F
P340687	Feline	Maine Coon	7m	Unknown
P335476	Canine	Schnauzer	8y 1m	M
P335279	Feline	Domestic Shorthair	7y 10m	FN

Appendix 2 Cytology case load at the RVC

Case number	Species	Breed	Age	Sex	Organ/location
P330839	Canine	Staffordshire bull terrier	8 y 10 m	MN	Skin
P330911	Canine	Chihuahua	13y 4m	FN	Liver
P330981	Canine	Border Terrier	10y 2m	MN	Cerebrospinal fluid
P330982	Canine	Crossbreed	6y	FN	Joint fluid
P331031	Canine	Crossbreed	11y 2m	MN	Liver
P331005	Canine	Crossbreed	11 yrs	MN	Lung
P330839	Canine	Staffordshire Bull Terrier	8y 10m	MN	Cutaneous
P330911	Canine	Chihuahua	13y 4m	FN	Liver
P335201	Canine	Cross Breed	7y 3m	MN	Cutaneous
P335214	Feline	Domestic longhair	10y	MN	Rectum
P330959	Canine	Boston Terrier	8y	MN	Peritoneal fluid
P335256	Canine	Unknown	12y 4m	MN	Salivary gland
P335409	Equine	Thoroughbred	19y	F	Peritoneal fluid
P335435	Canine	Border Terrier	11y 11m	FN	Cerebrospinal fluid
P335494	Canine	Border Collie	4y 11m	Unknown	Cerebrospinal fluid
P337925	Canine	Pug	11y 4m	M	Urine
P337987	Canine	Pug	3y 6m	MN	Cerebrospinal fluid
P338032	Feline	Domestic Shorthair	5y 7m	MN	Salivary gland
P338075	Canine	Cavalier King Charles Spaniel	10y 7m	FN	Liver and spleen
P338087	Feline	Domestic Shorthair	4y 2m	F	Peritoneal fluid
P338167	Canine	Bulldog	1y 1m	MN	Joint fluid
P338213	Canine	Boxer	8y 11m	F	Heart base
P340401	Canine	French Bulldog	30w	M	Endotracheal wash fluid
P340414	Feline	Domestic Longhair	13y 7m	MN	Spleen
P340447	Canine	Cocker Spaniel	11y 4m	MN	Lymph node
P340487	Canine	Standard Poodle	6y 11m	MN	Lymph node
P340551	Feline	Maine Coon	3y 10m	FN	Lymph node
P340583	Feline	Domestic Shorthair	16y 8m	FN	Mammary tissue
P340622	Equine	Arab	20y	Unknown	Cutaneous
P340641	Feline	Maine Coon	3y 11m	F	Kidney
P340666	Canine	Lurcher	11y	MN	Pleural fluid
P335390	Canine	German Shepherd	11y 4m	FN	Cutaneous
P340507	Feline	Domestic Shorthair	2y 8m	FN	Bile
P338129	Equine	Unknown	19y	F	Peritoneal fluid
P335341	Canine	West Highland White Terrier	12y 5m	FN	Joint fluids and liver

Appendix 3 Haematology case load at Axiom

Case number	Species	Breed	Age	Sex
19051626659	Canine	Mini Schnauzer	11y 12m	MN
19051626660	Canine	Bichon Frise	11y 8m	MN
19052127954	Canine	Sealyham Terrier	12y 9m	M
19053030796	Canine	Labrador Retriever	13y	F
19053131129	Canine	Golden Retriever	8y 8m	FN
19060131430	Canine	Border Collie	11y 1m	FN
19060532493	Canine	Crossbreed	1y 1m	MN
19060532656	Canine	Cocker Spaniel	6y	M
19060632819	Canine	Labrador Retriever	9y 6m	FN
19060632615	Canine	Yorkshire Terrier	13y 9m	F
19061134068	Canine	Cocker Spaniel	2y 10m	MN
19061134377	Canine	Staffordshire Bull Terrier	13y 5m	FN
19061234704	Canine	Dachshund	12y 8m	FN
19061234530	Canine	Labradoodle	12y 5m	FN
19062137590	Canine	English Springer Spaniel	5y 8m	MN
19062237941	Canine	Boxer	7y 10m	MN
19062037303	Canine	German Shepherd	6y 2m	MN
19062037450	Canine	Labrador Retriever	9y 11m	Unknown
19062038186	Canine	Cocker Spaniel	12y 1m	MN
19062238156	Canine	Crossbreed	7y	MN
19062238155	Canine	Cavalier King Charles Spaniel	9y 7m	F
19062238125	Canine	Cavalier King Charles Spaniel	13y	FN
19062538353	Canine	Springer Spaniel	6y 3m	MN
19062638769	Canine	Crossbreed	10y 6m	F
19062638880	Canine	Boxer	1y 1m	F
19062739270	Canine	Crossbreed	12y	FN
19070240553	Canine	Lurcher	3y	MN
19071243540	Canine	Shih Tzu	12y 8m	MN
19071143577	Canine	Unknown	13y	MN
19071644477	Canine	Springer Spaniel	4y 11m	M
19071745150	Canine	Whippet	10y 1m	FN
19071644537	Canine	Crossbreed	Unknown	M
19071745329	Canine	Standard Schanuzer	8m	FN
19071745035	Canine	Crossbreed	6y 9m	MN
19071745027	Canine	Crossbreed	8y 5m	MN
19071745025	Canine	Sealyham Terrier	11y 10m	M
19071644822	Canine	Golden Retriever	11y	MN
19071645034	Canine	Labrador Retriever	10y 7m	MN
19071745216	Canine	Cavalier King Charles Spaniel	13y 1m	FN
19071845364	Canine	Vucciriscu	2y	FN
19072346608	Canine	Staffordshire Bull Terrier	Unknown	MN
19072447093	Canine	Staffordshire Bull Terrier	11y 1m	M
19072447098	Canine	Bichon-Frise	7y	FN

19072447053	Canine	Spaniel x Dax	8y 1m	F
19072447058	Canine	Labrador Retriever	5y 10m	FN
19080650638	Canine	Collie	3y 4m	M
19080751118	Canine	Crossbreed	11y 4m	Unknown
19080751117	Canine	German Shepherd	11y	FN
19080751113	Canine	Crossbreed	8y 5m	MN
19080851555	Canine	Bichon-Frise	5y 10m	FN
19080851506	Canine	Cocker Spaniel	Unknown	M
19080851559	Canine	Boxer	9y	F
19080851557	Canine	English Springer Spaniel	9y 11m	MN
19080851588	Canine	Cocker Spaniel	14y	F
19080952010	Canine	Cocker Spaniel	7y 11 m	F
19080851939	Canine	Crossbreed	6y 4m	FN
19080975290	Canine	German Shepherd	2y	F
19080952213	Canine	Labrador Retriever	9y 11m	MN
19080951623	Canine	Cocker Spaniel	5y 11 m	FN
19080952122	Canine	Jack Russel Terrier	12y 9m	FN
19080952164	Canine	Springer Spaniel	11y	M
19080952227	Canine	Alaskan Malamute	11y	FN
19081382868	Canine	Shih-Tzu	9y 9m	MN
19081052515	Canine	Golden Retriever	11y 6m	FN
19081352968	Canine	Greyhound	7y	FN
19081453249	Canine	Domestic Shorthair	18y	F
19081453527	Canine	Border Collie	8 months	F
19081443413	Canine	Yorkshire Terrier	7y 7m	F
19081553718	Canine	Crossbreed	7y 6m	Unknown
19081053735	Canine	Miniature Schnauzer	6y	FN
19081654368	Canine	Pointer	10y 6m	MN
19082356226	Canine	Cocker spaniel	8y 8m	MN
19082356225	Canine	Standard Schnauzer	8y 7m	FN
19082356405	Canine	Labrador Retriever	11y 9m	FN
19082756861	Canine	Labrador Retriever	10y	FN
19082456578	Canine	Jack Russel Terrier	6y 10m	MN
19082356325	Canine	Golden Retriever	1y 4m	M
19082857004	Canine	Crossbreed	6m	F
19082856979	Canine	English Springer Spaniel	9y	FN
19082856904	Canine	Whippet	6y 2m	FN
19082857278	Canine	Labrador Retriever	11y 9m	FN
19082857060	Canine	Pointer	10y	F
19082857009	Canine	Cockapoo	6y	MN
19082857281	Canine	Crossbreed	1y 7m	Unknown
19082957461	Canine	West Highland White Terrier	13y	MN
19082957458	Canine	Staffordshire Bull Terrier	8y 2m	FN
19082957455	Canine	Cocker Spaniel	Unknown	MN
19083158635	Canine	Border Collie	12y	FN
19090459244	Canine	Cocker Spaniel	10y 4m	MN
19090359043	Canine	Labrador cross	Unknown	F

19090459251	Canine	Crossbreed	4y	F
19090459355	Canine	Crossbreed	1y 2m	MN
19090459252	Canine	Golden Retriever	8y 11m	FN
19090559750	Canine	Springer Spaniel	6y 6m	MN
19090559641	Canine	German Shepherd	7y 9m	FN
19090559677	Canine	Labrador Retriever	9y 4m	M
19090559889	Canine	Lurcher	1y 2m	F
19090660178	Canine	Crossbreed	1y 3m	M
19090660405	Canine	Labrador Retriever	6y 6m	MN
19090660187	Canine	Lurcher	1y 10 m	MN
19090760592	Canine	Labradoodle	9y 10m	FN
19090760509	Canine	Springer Spaniel	5y 1m	FN
19091061276	Canine	Golden Retriever	6y	Unknown
19091261816	Canine	Crossbreed	3y 4m	Unknown
19091261816	Canine	Cocker Spaniel	6y 6m	MN
19091362310	Canine	Cavalier King Charles Spaniel	7y 2m	FN
19091763007	Canine	Dachshund	8y 1m	FN
19091763002	Canine	Whippet	8y 5m	FN
19091863530	Canine	Labrador Retriever	9y 2m	MN
19091863508	Canine	Labrador Retriever	8y 6m	MN
19091863529	Canine	Chihuahua	7y 8m	MN
19091963965	Canine	Shar Pei	1y 2m	F
19092465222	Canine	Portuguese Water Dog	11y 5m	FN
19092465133	Canine	English Springer Spaniel	9y 1m	FN
19092465567	Canine	Labrador Retriever	3y 10m	FN
19092465371	Canine	Lurcher	1y 2m	F
19092565713	Canine	Briard	7y	MN
19092565895	Canine	Crossbreed	9y 9m	FN
19092565665	Canine	Mini Schnauzer	Unknown	Unknown
19092666172	Canine	Yarmouth Toller	6y 4m	M
19092666089	Canine	Crossbreed	7y 8m	M
19100167283	Canine	Labrador Retriever	10y 1m	F
19100368206	Canine	Greyound	6y 1m	FN
19100368101	Canine	Jack Russell Terrier	11y 3m	F
19100969960	Canine	Staffordshire bull Terrier	8y 1m	M
19100969869	Canine	Springer Spaniel	5y 2m	FN
19100910087	Canine	Crossbreed	7y	FN
19101010381	Canine	Lurcher	10m	F
19101010420	Canine	Crossbreed	1y 4m	M
19101010382	Canine	Male neutered	6y 10m	MN
19100568924	Canine	Crossbreed	11y 3m	FN
19101411532	Canine	Whippet	7y 5m	F
19101511677	Canine	Australian Shepherd	9y 5m	M
19101511720	Canine	German Shepherd	3y	M
19101511654	Canine	Whippet	5y	MN
19101612232	Canine	Beagle	10y 6m	MN
19101512042	Canine	Bichon Frise	10y 6m	FN

19101511778	Canine	Crossbreed	10y	MN
19101712618	Canine	German Shepherd	13y 1m	MN
19101712624	Canine	German Mastiff	7y 11m	M
19101712619	Canine	Labrador Retriever	5y 5m	M
19101712543	Canine	Samoyed	8y 9m	F
19101712585	Canine	Standard Smooth-Haired	2y 3m	MN
19101712670	Canine	Crossbreed	1y 7m	Unknown
19101712753	Canine	Cocker Spaniel	4y 1m	FN
19101913526	Canine	Crossbreed	7y	FN
19101913654	Canine	Labrado Retriever	11y 6m	MN
19102213811	Canine	Whippet	5y	MN
19101913490	Canine	Shetland Sheepdog	11y	MN
19102213838	Canine	Standard Scnauzer	8y 9m	FN
19102213774	Canine	Crossbreed	4y 5m	MN
19102314358	Canine	Labrador Retriever	1y 5m	F
19102314344	Canine	Pointer	10y 2m	MN
19102314326	Canine	English Cocker Spaniel	1y 2m	M
19102414758	Canine	Cocker Spaniel	6d	MN
19102314451	Canine	Schnoodle	1y	MN
19102615565	Canine	Crossbreed	5y 2m	MN
19102615738	Canine	Crossbreed	7y 1m	MN
19102915892	Canine	Cocker Spaniel	8y 5m	F
19102915852	Canine	Cocker Spaniel	10y 5m	MN
19102916215	Canine	Labrador Retriever	6y 6m	MN
19102915949	Canine	Crossbreed	11y 1m	MN
19103016429	Canine	English Springer Spaniel	7y 2m	FN
19103016326	Canine	Springer Spaniel	5y 2m	FN
19103016414	Canine	Labrador Retriever	4y 9m	MN
19103116839	Canine	Bichon Frise	9y 2m	MN
19103116851	Canine	Pug	8y 10m	M
19103116841	Canine	Dachshund	8m	M
19110217785	Canine	Crossbreed	7y 2m	MN
19110518025	Canine	Miniature Poodle	12y 4m	MN
19111622154	Canine	Cavapoo	3y	FN
19111622182	Canine	Labrador Retriever	10y 7m	M
19111521602	Canine	Cockapoo X	4y	MN
19112123476	Canine	Miniature Schnauzer	12y 1m	MN
19112123471	Canine	Crossbreed	3y	FN
19112624765	Canine	Crossbreed	3y	FN
19112624698	Canine	Crossbreed	5y 11m	FN
19112725340	Canine	Miniature Poodle	11y	M
19112725338	Canine	Crossbreed	3y	FN
19112725519	Canine	Labrador Retriever	10y 8m	MN
19112825755	Canine	Jack Russell Terrier	10y 8m	MN
19112825774	Canine	Spaniel	5y	MN
19112825990	Canine	Crossbreed	7y 2m	MN
19112825736	Canine	Pug	4y	M

19113026533	Canine	Cocker Spaniel	10y 6m	MN
19113026733	Canine	Cocker Spaniel	3y 4m	Unknown
19113026534	Canine	Crossbreed	11y 2m	FN
19121029385	Canine	Labrador Retriever	10y	MN
19121029279	Canine	Dachshund Jack Russell Terrier	3y 1m	M
19121230069	Canine	Sheepdog	11y 6m	FN
19121230120	Canine	Pug	5y 4m	FN
19121230234	Canine	Border Collie	9y	FN
19121230123	Canine	Crossbreed	6y 9m	MN
19121230061	Canine	Bernese Mountain Dog	8y 4m	FN
19121731379	Canine	Jack Russell Terrier	5y 10m	MN
19121731378	Canine	French Bulldog	4m	M
19121731442	Canine	Weimaraner	10y 11m	FN
19121932300	Canine	Crossbreed	5y 10m	FN
19121932326	Canine	German Shepherd	2y 3m	M
19122433455	Canine	Crossbreed	9y 7m	FN
19122433474	Canine	Border Collie	8y 4m	FN
19122834071	Canine	Crossbreed	7y 6m	FN
19122833990	Canine	Pug	5y 4m	FN
19123134465	Canine	Lab cross Whippet	3y	M
19123134354	Canine	Border Collie	10y 4m	MN
20010736025	Canine	Labradoodle	12y 2m	FN
20010736040	Canine	Bernese Mountain Dog	6y 2m	FN
20010836660	Canine	Labrador Retriever	7y 5m	MN
20010836666	Canine	Golden Retriever	9y 2m	FN
20010836760	Canine	Crossbreed	8y	FN
20010736435	Canine	Boxer X	11y 6m	MN
20011138140	Canine	Greyhound	7y 2m	MN
20011138461	Canine	Cocker Spaniel	6y 2m	FN
20011138204	Canine	Crossbreed	2y 8m	MN
20011138259	Canine	Crossbreed	7y 11m	FN
20011438712	Canine	West Highand White Terrier	12y 8m	FN
20011539485	Canine	Griffon X	7y	FN
20012442547	Canine	Border Collie	9y	FN
20012843926	Canine	Springer	10y 6m	MN
20012843761	Canine	Standard Poodle	11y 8m	MN
20012843417	Canine	Bichon Frise	14y 2m	MN
20012843414	Canine	French Bulldog	1y 3m	F
20012843343	Canine	Crossbreed	6y 7m	MN
20012944030	Canine	Tibetan Terrier	9y 1m	MN
20012944016	Canine	Mini Schnauzer	11y 3m	FN
20012944018	Canine	Crossbreed	13y 6m	MN
20012943999	Canine	French Bull dog	5y 5m	FN
20012843416	Canine	Bichon Frise	7y 3m	FN
20013044473	Canine	Jack Russell Terrier	8y 9m	MN
20013044471	Canine	Labrador Retriever	7y 5m	MN
20020145607	Canine	Cavalier King Charles Spaniel	6y 10m	MN

20020747386	Canine	Golden Retriever	10y 11m	FN
20020546350	Canine	Crossbreed	10y 10m	MN
20020747611	Canine	Labrador Retriever	7y 11m	M
20021048184	Canine	German Pointer	10y 11m	FN
20021148284	Canine	English Springer Spaniel	6y	FN
20021449733	Canine	English Springer Spaniel	6y	FN
20021470871	Canine	Yorkshire Terrier	9y	F
20021850689	Canine	Lurcher	6y	FN
20021850604	Canine	West Higland White Terrier	13y 10m	MN
20022051908	Canine	Lurcher Cross	7y 2m	M
20031459390	Canine	Whippet	2y	FN
20031860443	Canine	Cocker Spaniel	12y	MN
20031960951	Canine	Collie X	2y	M
20031960840	Canine	Labradoodle	11y 10m	FN
20032161624	Canine	Labrador Retriever	2y	F
20032461985	Canine	ShihTzu	2y 6m	MN
20032462100	Canine	Boxer	9y	FN
20032662485	Canine	Cavalier King Charles Spaniel	8y	F
20032662505	Canine	English Boxer	3y 5m	Unknown
20032862803	Canine	German Shepherd	2y 6m	MN
20032862847	Canine	Crossbreed	9y 6m	FN
20033162927	Canine	Pug X	6y 10m	F
20033162959	Canine	Cockapoo	3y	F
20053014470	Canine	Chihuahua	15y 1m	MN
20060214902	Canine	Bulldog	3y	M
20060214866	Canine	Weimaraner	5y 7m	F
20060315354	Canine	Cavalier King Charles Spaniel	10y 1m	MN
20060315395	Canine	Crossbreed	4y 4m	F
20060315347	Canine	Pomeranian	6y 4m	F
20060315335	Canine	Beagle	9y 3m	MN
20060315382	Canine	Labrador Retriever	6m	F
20060415799	Canine	Collie x Huntaway	8y	M
20060415732	Canine	Lurcher	5y	MN
20060516167	Canine	Labrador Retriever	6y 9m	FN
20060516207	Canine	German Longhaired Pointer	10y 2m	M
20060516205	Canine	Standard English Bull Terrier	3y 1m	M
20060516206	Canine	Standard English Bull Terrier	13y 11m	MN
20060917110	Canine	Labrador Retriever	6y 9m	FN
20060917056	Canine	Miniature Schnauzer	1y 8m	FN
20060917051	Canine	Pug	5y 5m	M
20061218557	Canine	Lurcher	1y 11m	F
20061619186	Canine	Dachshund	3m	M
20061720099	Canine	Dachshund	11y 8m	MN
20061519130	Canine	Collie	10y 3m	M
20061719998	Canine	Collie X	9y 5m	M
20061719959	Canine	Rottweiler	5y 9m	FN
20061820211	Canine	German Shepherd	8y 2m	MN

20061920775	Canine	Whippet	12y	MN
20061920637	Canine	Labrador Retriever	7y 11m	FN
20061920638	Canine	Labrador Retriever	6m	M
20061920679	Canine	English Springer Spaniel	9y 7m	FN
20061920689	Canine	Labrador Retriever	12y 3m	MN
20061975209	Canine	Terrier X	10y	FN
20061975167	Canine	Labrador Retriever	9y	FN
20061975178	Canine	Crossbreed	5y 10m	MN
20061975183	Canine	Welsh Collie	10m	F
20061975195	Canine	Border Collie	7y	FN
20061975200	Canine	Ibizan hound	11y 7m	MN
20061975201	Canine	Miniature Schnauzer	8y	FN
20061975216	Canine	Boxer	8y 4m	FN
20061975233	Canine	Hungarian Vizsla	8y 9m	F
20061975213	Canine	German Shepherd	8y	FN
20061975214	Canine	Labrador Retriever	7y 7m	MN
20061975226	Canine	Crossbreed	9y 8m	FN
20061975228	Canine	Bulldog	2y 1m	M
20062021029	Canine	Staffordshire Bull Terrier	6y 11m	M
20062021208	Canine	Greyhound	8y 8m	FN
20062321451	Canine	Labrador Retriever	8y 2m	FN
20062321532	Canine	Staffordshire Bull Terrier	6y 11m	MN
20061920863	Canine	Labrador Retriever	8y 10m	M
20062422064	Canine	Pug	5y 5m	M
20062321484	Canine	West Highland White Terrier	8y 8m	F
20062321481	Canine	Tibetan Terrier	9y 9m	M
20062422013	Canine	Cavachon	11m	F
20062422054	Canine	Long haired German Pointer	10y 2m	M
20062422055	Canine	Lurcher	8y 8m	FN
20062422058	Canine	Border Terrier	10y 9m	FN
20062623171	Canine	Siberian Husky	12y 5m	MN
19060331843	Equine	Donkey	11y 1m	M
19080650663	Equine	Cob	13y	Unknown
19111622081	Equine	Horse/ Cob X	19y	unkown
19051726947	Feline	Domestic cat	10y 1m	MN
19052228387	Feline	Ragdoll	15y 11m	FN
19052930352	Feline	Domestic Shorthair	13y 3m	MN
19052930031	Feline	Domestic Shorthair	12y 9m	MN
19061335020	Feline	Domestic cat	10y 9m	M
19062037298	Feline	Domestic Shorthair	15y 7m	FN
19062038185	Feline	Domestic Shorthair	5y 3m	Unknown
19062238153	Feline	Domestic Shorthair	4y 10m	MN
19062238119	Feline	British Shorthair	7y 10m	FN
19070240516	Feline	Domestic Shorthair	15y 10m	FN
19070240296	Feline	Domestic Shorthair	1y	FN
19062940016	Feline	Ragdoll	11y 3m	MN
19071945836	Feline	Domestic Shorthair	14y 5m	FN

19080751084	Feline	Domestic Shorthair	Unknown	FN
19080751014	Feline	Oriental Shorthair	7y 2m	FN
19080851691	Feline	Domestic Longhair	3m	F
19080951937	Feline	Domestic Shorthair	Unknown	FN
19080751048	Feline	Domestic Shorthair	18y	F
19080951992	Feline	Bengal	14y 2m	MN
19080751425	Feline	Domestic Shorthair	11y	FN
19081052617	Feline	Devon Rex	11y 2m	MN
19081352867	Feline	Domestic Shorthair	11y 10m	FN
19081576205	Feline	Domestic Shorthair	11y	MN
19081553942	Feline	Domestic Longhair	3m	F
19081052445	Feline	Domestic Shorthair	12y 2m	MN
19082456621	Feline	Tonkinese	12y 1m	FN
19082857007	Feline	Siberian	1y	FN
19082857005	Feline	Domestic Shorthair	11y 4m	FN
19082957659	Feline	Domestic Longhair	3m	F
19090459241	Feline	Domestic Shorthair	12y 1m	FN
19090559751	Feline	Domestic cat	6y 4m	MN
19090559723	Feline	Domestic Longhair	15y	MN
19090559773	Feline	Domestic Longhair	13y 3m	MN
19090559990	Feline	Bristish Shorthair	4y 7m	MN
19090660250	Feline	Oriental Shorthair	9y 2m	FN
19090660273	Feline	Domestic Shorthair	Unknown	Unknown
19090760537	Feline	Domestic cat	2y 4m	F
19090760805	Feline	Bengal	12y 8m	M
19091061111	Feline	Domestic Shorthair	15y	FN
19091262015	Feline	Norwegian Forest	13y 8m	MN
19091362226	Feline	Domestic Shorthair	18y	Unknown
19091763042	Feline	Domestic Shorthair	4m	M
19091863511	Feline	Domestic Shorthair	11y 1m	MN
19091863514	Feline	Domestic Shorthair	1y	MN
19091863681	Feline	Unknown	3y 3m	FN
19091963954	Feline	Domestic Shorthair	12y 3m	MN
19100167163	Feline	Domestic cat	5months	M
19100569143	Feline	Domestic Longhair	5m	F
19100969956	Feline	Domestic shorthair	15y 1m	MN
19100569006	Feline	Bengal	16y 1m	
19101010507	Feline	Domestic Shorthair	10y 5m	FN
19101010377	Feline	Domestic Shorthair	7y 5m	M
19100969920	Feline	Domestic Shorthair	10m	FN
19100910165	Feline	Domestic Shorthair	15y 6m	FN
19100910149	Feline	Domestic Longhair	5y 1m	
19101211178	Feline	Domestic Shorthair	5y 4m	MN
19101010539	Feline	Domestic Shorthair	12y	MN
19101511686	Feline	Domestic Shorthair	15y 8m	MN
19101211203	Feline	Crossbreed	13y 8m	MN
19101511651	Feline	Domestic Shorthair	14y	FN

19101511633	Feline	Unknown	12y 1m	F
19101712616	Feline	British Blue	15y	FN
19101712620	Feline	Domestic Shorthair	11y 6m	MN
19101913383	Feline	Bengal	9y 9m	MN
19102213857	Feline	Domestic Shorthair	8y 1m	F
19102214023	Feline	Domestic Shorthair	12y 2m	MN
19102213988	Feline	Domestic Shorthair	14y	M
19102314322	Feline	Domestic Longhair	5y 3m	F
19102314576	Feline	Domestic Shorthair	13y 1m	FN
19102615476	Feline	Domestic Shorthair	11y 8m	FN
19102615606	Feline	British Shorthair	3m	F
19102615531	Feline	Domestic Longhair	14y 1m	FN
19102615758	Feline	Domestic Shorthair	11y 9m	MN
19102414667	Feline	Domestic Shorthair	2y 8m	MN
19103016400	Feline	Domestic Shorthair	2y	MN
19103016418	Feline	domestic Longhair	3y 3m	MN
19110518011	Feline	Ragdoll	4m	MN
19110517986	Feline	Domestic Shorthair	2y	MN
19110518023	Feline	Norwegian Forest	6y 5m	FN
19110518146	Feline	Domestic Shorthair	9y 1m	F
19111622027	Feline	Domestic Shorthair	4y 5m	MN
19111622014	Feline	Domestic Shorthair	3y 6m	FN
19111922420	Feline	Domestic cat	7y	F
19111922537	Feline	Domestic Shorthair	5y	FN
19112023128	Feline	Domestic Shorthair	10y 8m	Unknown
19112023093	Feline	Bengal	1y	F
19112624857	Feline	Siamese	18y	MN
19112624747	Feline	Domestic Shorthair	Unknown	MN
19121230183	Feline	Domestic Shorthair	12y	MN
19121230125	Feline	Domestic Shorthair	11y 7m	MN
19121129941	Feline	Domestic Shorthair	18y	FN
19121731357	Feline	Pekingnese	6y	M
19121731563	Feline	Domestic Longhair	11y 1m	MN
20010836664	Feline	Domestic Shorthair	8y 10m	MN
20011138144	Feline	Siamese	3y 6m	Unknown
20011138328	Feline	Domestic longhair	3y	F
20011740267	Feline	Domestic shorthair	3y 2m	M
20011740384	Feline	Domestic shorthair	12y 7m	MN
20012442554	Feline	Domestic Shorthair	4y 3m	MN
20012543188	Feline	Unknown	15y 11m	F
20012843426	Feline	Persian	5y 5m	MN
20012843428	Feline	Domestic cat	7y 2m	MN
20012843466	Feline	Domestic shorthair	12y 6m	MN
20013144924	Feline	Crossbreed	3y 6m	FN
20021248837	Feline	Domestic medium hair	7y	F
20021349252	Feline	Domestic Shorthair	7y	MN
20021449747	Feline	Domestic shorthair	12y 2m	FN

20021449706	Feline	Domestic shorthair	3y	FN
20021850718	Feline	Domestic Shorthair	7y 8m	MN
20021449936	Feline	Domestic Shorthair	Unknown	M
20021951137	Feline	Domestic Shorthair	14y 8m	M
20031759902	Feline	Domestic shorthair	8y 9m	MN
20031459519	Feline	Persian	6y	FN
20031960836	Feline	Domestic shorthair	9y 5m	FN
20032461938	Feline	Siberian	5 months	M
20032562298	Feline	Norwegian forest	5y	FN
20032461885	Feline	Ragamuffin	6y 11m	MN
20032662494	Feline	Persian	12y	MN
20032662578	Feline	Domestic shorthair	7y	MN
20032662477	Feline	British Blue	9y 9m	MN
20032862798	Feline	Domestic shorthair	9y 10m	FN
20032862871	Feline	Domestic shorthair	15y 1m	FN
20033162976	Feline	Sphynx	4y 2m	FN
20033163006	Feline	Domestic longhair	5y 2m	FN
20052713140	Feline	Domestic shorthair	17y	MN
20060214951	Feline	Domestic shorthair	14y	MN
20060214946	Feline	Domestic shorthair	13y 9m	F
20060315368	Feline	Ragdoll	6m	F
20060415757	Feline	Ragdoll	1y 10m	M
20060415729	Feline	Domestic shorthair	13y 1m	FN
20060516121	Feline	Domestic shorthair	12y 10m	MN
20060516209	Feline	Maine Coon	11y	F
20060917234	Feline	Domestic shorthair	17y 7m	FN
20060917214	Feline	Crossbreed	7y 10m	MN
20060917058	Feline	Domestic shorthair	14y 2m	FN
20060917049	Feline	Feline Burmese	1y 3m	FN
20060917054	Feline	Bengal	9y 5m	MN
20061218581	Feline	Domestic Shorthair	7y 4m	FN
20061619278	Feline	Siamese X	11y	MN
20061619200	Feline	Domestic shorthair	19y 6m	M
20061619184	Feline	Maine Coon	5y	MN
20061720050	Feline	Domestic shorthair	1y	MN
20061920644	Feline	Ragdoll	1y 10m	MN
20061920615	Feline	Domestic shorthair	3y 11m	FN
20062321531	Feline	Domestic shorthair	9y 3m	MN
20062422248	Feline	Domestic shorthair	6y 7m	MN
20062422249	Feline	Domestic shorthair	6y 7m	MN
20062623171	Feline	Domestic shorthair	10y 11m	MN
20062522498	Feline	Domestic shorthair	8y 7m	MN
20062622556	Feline	Border Collie	10y 10m	MN
20062522442	Feline	Exotic shorthair	1y 3m	MN

Appendix 4a Cytology case load at Axiom (feline and canine)

Anatomical/ sample location	Canine	Feline	Total
Skin	1324	111	1435
Lymph node	77	18	95
Anal mass	38	2	40
Urine	32	13	45
Lymph nodes	24	4	28
Mammary gland	23	3	26
Joint fluid	16	2	18
Anal gland	15		15
Liver	13	8	21
Spleen	11	1	12
Salivary gland	9	3	12
Prostate	7		7
Mammary mass	6	1	7
Bronchoalveolar lavage	5	4	9
Abdominal fluid	5	5	10
Possible lymph node	4	4	8
Perianal	4		4
Vaginal smear	4		4
Ear	4		4
Perianal gland	4		4
Digit	3		3
Possible joint fluid	3	2	5
Eye	3	1	4
Abdominal mass	3	7	10
Thyroid region	3	1	4
Bone	3		3
Pericardial fluid	3		3
Thoracic mass	2		2
Cerebrospinal fluid	2		2
Tongue	2	4	6
Lip	2		2
Skin and lymph nodes	2		2
Liver and Spleen	2	2	4
Thyroid	2	1	3
Lung	2		2
Tracheal wash	2	2	4
Vulva	2		2
Cystic dental fluid	1		1
Round cell tumour	1		1
Possible mammary gland	1	1	2
Mouth	1	2	3
Conjuctiva	1		1
Muscle	1		1
Lymph node and bone marrow	1		1
Nasal fluid	1	1	2
Rectal area	1		1
Neck region	1	1	2
Sciatic nerve	1		1
Nipple	1		1
Lymph node and anus	1		1
Oral	1		1
Tail and prepuce	1		1
Oral lesion	1		1
Lymph node probable	1		1
Paw region	1		1
Possible salivary gland or lymph node	1		1

Pawpad	1		1
Rectum	1		1
Penis	1		1
Eyelid	1		1
Perianal	1		1
Sinus	1		1
Perianal mass	1		1
Skin and lymph node	1		1
Tracheal mucus	1		1
Skin/ possible lymph node	1		1
Joint fluids	1		1
Tail	1		1
Pinna	1		1
Tail mass	1		1
Possible anal sac	1		1
Lymph node and intestinal mass	1		1
Possible cutaneous	1		1
Bronchial brush	1		1
Possible joint	1		1
Endotracheal tube	1		1
Abdominal lymph node	1	1	2
Perivulva	1		1
Mammary skin		1	1
Pleural fluid		2	2
Bile		1	1
Abdominal mass and kidney		1	1
Stomach		1	1
Kidney		3	3
Intestine		3	3
Respiratory wash sample		1	1
Umbilical mass		1	1
Ear canal		1	1
Spleen and lymph node		1	1
Ileocaecocolic mass, lymph node and liver		1	1
Bone marrow		1	1
Intestinal		1	1
Liver and panceas		1	1
Nasal flush		1	1
Thoracic fluid		2	2
Nasal mass		1	1
Skin (lip)		1	1
Intestinal mass		1	1
Intestinal lymph node		1	1
Mediastinal mass		1	1
Cystic fluid		1	1
Possible renal		1	1
Nasal region		2	2
Oral cavity		1	1
Total	**1707**	**238**	**1945**

Appendix 4b Cytology case load at Axiom (all other species)

Anatomical/ sample location	Equine	Ferret	Guinea Pig	Hedgehog	Rabbit	Rat	Rodent	Fox	Total
Skin	2		1	1	4	1	6		15
Mammary gland							1		1
Bronchoalveolar lavage	2								2
Eye	1								1
Thyroid region								1	1
Cerebrospinal fluid	1								1
Tracheal wash	1								1
Tail		1							1
Possible mammary mass							1		1
Endometrium	2								2
Tendon sheath	1								1
Nasal bone	1								1
Respiratory wash sample	1								1
Peritoneal fluid	1								1
Peritoneal fluid	1								1
Perineal mass							1		1
Total	**14**	**1**	**1**	**1**	**4**	**1**	**9**	**1**	**32**

Appendix 5 Protein electrophoresis cases at Axiom

Case number	Species	Breed	Age	Sex	Interpretation
191004 68614	Feline	Bengal	8y 7m	MN	Polyclonal gammopathy
191004 68728	Feline	Domestic Shorthair	11m	MN	Polyclonal gammopathy
191005 69269	Feline	Domestic Shorthair	15y 5m	FN	Monoclonal gammopathy
191015 11913	Feline	Domestic Shorthair	8y 2m	M	Polyclonal gammopathy
191025 15290	Canine	Whippet	10y 11m	FN	Polyclonal gammopathy
191025 15414	Canine	Brittany, American Spaniel	6y	FN	Polyclonal gammopathy
191026 15643	Canine	Lurcher	10y 6m	FN	Polyclonal gammopathy
191031 16823	Canine	Border Terrier	11y 10m	M	Polyclonal gammopathy
191102 17575	Canine	Brittany Spaniel	3y	MN	Polyclonal gammopathy
191105 18050	Canine	Wire-haired Pointer	3y 3m	FN	Polyclonal gammopathy
191105 18336	Feline	British Shorthair	4y 8m	MN	Polyclonal gammopathy
191105 18417	Canine	Crossbreed	7y 8m	MN	Polyclonal gammopathy
191115 21665	Canine	Crossbreed	3y 9m	M	Polyclonal gammopathy
191116 21994	Canine	Spaniel	4y 4m	M	No comments provided
191116 22053	Reptile	Snake	24y	F	Polyclonal gammopathy
191116 22335	Canine	Rhodesian Ridgeback	10y 7m	FN	Unremarkable
191116 22687	Canine	Labrador Retriever	10y 7m	M	Gammapathy, possible monoclonal
191122 23873	Canine	Greyhound X	6y 8m	FN	Gammopathy (Gamma globulins raised)
191122 24043	Canine	Pointer	9y 6m	FN	Polyclonal gammopathy
191122 24221	Canine	Pug	7y 6m	MN	Hypoproteinemia
191123 24552	Feline	Domestic Shorthair	9y	MN	Monoclonal gammopathy
191126 24677	Canine	Yorkshire Terrier	13y 10m	FN	Urine EP and unremarkable Protein EP
191126 25238	Canine	Mini Wire-haired Dachshund	6y	Unknown	Unremarkable
191231 34367	Bird	Penguin	13y 6m	F	Polyclonal gammopathy
191231 34747	Feline	Crossbreed	17y 2m	FN	Polyclonal gammopathy
200103 34963	Canine	Bernese Mountain Dog	4y	FN	Gammopathy (Gamma globulins raised)
200103 35374	Canine	Fox Terrier	7y 6m	M	Polyclonal gammopathy
200103 35374	Canine	Fox Terrier	7y 6m	M	Polyclonal and monoclonal gammopathy
200103 35377	Canine	Golden Retriever	6y 10m	Unknown	Polyclonal gammopathy and marked hypoalbuminaemia

200104 35448	Feline	Domestic Shorthair	13y 10m	MN	Polyclonal gammopathy
200107 36452	Feline	Domestic Shorthair	16y	Unknown	Mild hypoalbuminaemia
200108 36615	Equine	Thoroughbred	5y	Unknown	Polyclonal gammopathy
200108 36756	Canine	Spaniel x Dax	9y	Unknown	No significant abnormalities
200110 37972	Canine	Crossbreed	7y 3m	F	Polyclonal gammopathy- Leishmania PCR positive
200110 38016	Reptile	Loggerhead Turtle	Unknown	F	Not interpreted
200113 38548	Canine	Standard Doberman	10y 6m	MN	Polyclonal gammopathy and UPE
200114 38578	Feline	Domestic longhair	8y 6m	MN	Polyclonal gammopathy/ possible FIP
200114 38817	Canine	Crossbreed	8y 8m	MN	Unremarkable
200114 39102	Canine	German shepherd	12y 5m	MN	Marginally elevated beta globulins
200114 39109	Canine	English Shepherd	10y	FN	Marginally elevated Beta globulins
200114 39125	Canine	Shih-Tzu	4y 6m	Unknown	Monoclonal gammopathy
200115 39486	Feline	Maine Coon	2y 7m	FN	Polyclonal gammopathy/ probable FIP
200117 40533	Feline	Domestic shorthair	3y	FN	Unremarkable
200118 40635	Canine	Collie	3y	FN	Marginally elevated beta globulins
200118 40887	Canine	English Setter	6y 7m	MN	Marginally elevated beta globulins
200121 41202	Canine	West Highland White Terrier	11y	M	Monoclonal gammopathy
200125 43298	Canine	Crossbreed	3y 8m	MN	Polyclonal gammopathy
200204 46003	Equine	Dale	28y 2m	MN	Acute inflammation and Polyclonal gammopathy
200207 47248	Feline	Border Terrier	12y 7m	M	Polyclonal gammopathy
200207 47401	Canine	Crossbreed	5y 2m	M	Marginally elevated beta globulins
200207 47473	Canine	Crossbreed	5y 1m	FN	Marginally elevated beta globulins
200208 48124	Turtle	Turtle	Unknown	F	Not interpreted
200211 48709	Canine	Spaniel	5y 7m	MN	Marginally elevated beta globulins
200325 62353	Feline	Siberian	6m	FN	Acute phase response and polyclonal gammopathy- Probable FIP
200325 62390	Canine	Bernese mountain Dog	8y	MN	Acute phase response
200325 92887	Feline	Domestic shorthair	12y	FN	Possible Bence Jones proteinuria
200326 62552	Feline	Domestic shorthair	12y	FN	Unremarkable