UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

ASPECTOS EPIDEMIOLÓGICOS, PATOLÓGICOS E IMUNO-HISTOQUÍMICOS DE LINFOMA EM FELINOS NO SUL DO BRASIL

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Orientador: Saulo Petinatti Pavarini

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RESUMO

Os aspectos epidemiológicos, patológicos e imuno-histoquímicos do linfoma em felinos no Sul do Brasil foram avaliados através de um estudo retrospectivo em um período de 12 anos (2004-2016). Em 1356 necropsias de felinos, 125 diagnósticos de linfoma foram revisados com intuito de determinar idade, raça e sexo, topografia tumoral, presença do vírus da leucemia felina (FeLV) e do vírus da imunodeficiência felina (FIV) e imunofenótipo do neoplasma por imuno-histoquímica, além da categorização dos tumores de acordo com o sistema da Organização de Saúde Mundial, aplicado para uso em animais. O linfoma alimentar (33,6%) foi a mais comum, seguido do linfoma mediastinal (28%). A imuno-histoquímica do tecido neoplásico revelou 50,4% de gatos positivos para FeLV e 21.6% positivos para FIV, e 52,8% dos linfomas consistiam de células T e 47,2% de células B. A faixa etária dos gatos com linfoma de células T variou de 10 a 240 (mediana 120) meses e 6 a 204 (mediana 60) meses para gatos com linfoma de células B; 90,4% dos gatos não tinha raça definida. Entre os linfomas alimentares, o linfoma de células T associado à enteropatia do tipo 1 foi o mais frequente e, entre os linfomas mediastinais, o linfoma difuso de grandes células B foi o principal tipo de linfoma encontrado. Considerando apenas linfomas mediastinais e alimentares, houve associação estatística significativa ($p \leq 0,05$) entre infecção por FeLV e as categorias de idade. A prevalência de linfoma mediastinal entre os gatos positivos para FeLV foi de 3,21 vezes a do linfoma mediastinal entre gatos negativos para FeLV. Embora não seja estatisticamente significativo, a prevalência de linfoma de células B entre os gatos positivos para FeLV foi 1,44 vezes maior que a prevalência de linfoma de células B entre os gatos negativos para FeLV. A respeito dos padrões histológicos de infiltração pulmonar pelo linfoma, avaliamos retrospectivamente 125 casos com diagnóstico de linfoma, nos quais 16 casos apresentaram infiltração pulmonar (12,8%). Desses casos, 9 gatos eram fêmeas e 7 machos e todos não tinham raça definida. As lesões macroscópicas observadas nos pulmões consistiram em massas (25%) e nódulos (18,7%). No entanto, a maioria dos casos (56,2%) não exibiu alteração macroscópica. O padrão de infiltração neoplásico peribronquial-vascular (93,7%) foi o mais frequente, seguido do pleural (56,2%), intersticial (50%), nodular (37,5%) e alveolar (12,5%), sendo que 75% dos linfomas tiveram mais do que um padrão de infiltração. Entre os linfomas que exibiram infiltração pulmonar, 14 (87,5%) casos foram identificados como linfoma de células B e 2 (12,5%) de células T; o linfoma difuso de grandes células B foi o tipo mais frequente, representando 56% desses casos.

Palavras-chave: FeLV, imunofenótipo, linfócitos B, linfócitos T, pulmão, peribronquial-vascular.

ABSTRACT

The epidemiological, pathological and immunohistochemical aspects of lymphoma in felines in southern Brazil were evaluated through a retrospective study over a 12-year period (2004-2016). In 1,356 necropsies, 125 cats with lymphoma diagnosis were review to determine age, breed and sex, tumor topography, feline leukemia virus (FeLV) and immunodeficiency feline virus (FIV) immunohistochemistry status, immunophenotype of lymphomas, and categorization of the tumors according to World Health Organizations system applied for use in animals. Alimentary lymphoma (33.6%) was the most common, followed by mediastinal lymphoma (28%). Immunohistochemical staining of tumor tissue revealed 50.4% FeLV-positive and 21.6% FIV-positive cats, and 52.8%T-cell lymphoma and 47.2% B-cell lymphoma. The range of age of cats with T-cell varied from 10 to 240 (median of 120) months and 6 to 204 (median of 60) months for cats with B-cell lymphoma, and mixed-breed was the most frequent with 90.4% among all breeds. Alimentary lymphoma exhibited the enteropathy-associated T-cell lymphoma (type 1) as the most common and mediastinal lymphoma showed the diffuse large B-cell lymphoma as the main type of lymphoma. Considering only mediastinal and alimentary lymphomas, there was significantly statistical association ($p \leq 0.05$) between FeLV status and the categories of age. The prevalence of mediastinal lymphoma among FeLV-positive cats was 3.21 times the higher than FeLV-negative cats. Although not statistically significant, the prevalence of B-cell lineage among FeLV-positive cats was 1.44 times the prevalence of B-cell lineage among FeLV-negative cats. Regarding the histological patterns of pulmonary infiltration by lymphoma, it was retrospectively assessed 125 reports with lymphoma diagnosis, in which 16 cases showed pulmonary infiltration (12.8%). Of these, 9 cats were females and 7 were males; all of them were mixed-breed cats. Gross lesions observed in the lungs consisted of masses (25%) and nodules (18.7%); however, the majority of cases (56.2%) did not show any gross abnormality. The peribronchial-vascular infiltration pattern (93.7%) was the most frequent pattern, followed by pleural (56.2%), interstitial (50%), nodular (37.5%) and alveolar (12.5%), and 75% of the lymphomas had more than one infiltration pattern. Among the lymphomas with pulmonary involvement, there were 14 (87.5%) B-cell and 2 (12.5%) T-cell lymphomas and the diffuse large B-cell lymphoma was the most frequent type accounting for 56% of these cases.

Key-words: B-cell, FeLV, immunophenotype, lung, lymphosarcoma, peribronchial-vascular, T-cell.

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SUMÁRIO

1 INTRODUÇÃO

Os linfomas são neoplasmas hematopoiéticos que tem origem nos linfonodos ou em órgãos extranodais (VALLI; KIUPEL; BIENZLE, 2016). Na medicina humana, os linfomas são tradicionalmente divididos em linfoma do tipo Hodgkin e não-Hodgkin. O linfoma não-Hodgkin representa um amplo grupo de entidades que variam da mais indolente a mais agressiva, tendo origem a partir da proliferação clonal anormal de linfócitos T, linfócitos B, ou de ambos (ARMITAGE *et al*., 2017). Embora os linfomas do tipo Hodgkin tenham sido relatados em gatos, a grande maioria dos linfomas em animais domésticos se assemelha aos linfomas não-Hodgkin dos seres humanos, sendo referidos simplesmente como linfoma (VALLI; KIUPEL; BIENZLE, 2016).

Diversas classificações histológicas baseadas na morfologia microscópica utilizadas para classificar os linfomas em humanos, foram adotadas em medicina veterinária. Todas as classificações procuram correlacionar os tipos histológicos com o comportamento biológico tumoral, com o propósito de fornecerem informações sobre prognóstico e tratamento (BERHARD *et al*., 1997; PARODI, 2001). Dentre os principais métodos de classificação dos linfomas estão: classificação de *Rappaport*, sistema de *Kiel*, classificação de *Lukes-Collins*, *Working Formulation of Non-Hodgkin's Lymphomas for Clinical Usage* (WF) e *Revised European-American Classification of Lymphoid Neoplasms* (REAL).

A classificação de *Rappaport* incorporava o conceito do padrão de crescimento tumoral, folicular ou nodular, e sua relação com o prognóstico favorável (RAPPAPORT *et al*., 1956). Entretanto, apesar da arquitetura ser um indicativo, essa classificação não oferecia valor prognóstico, em função da maior parte dos linfomas em animais serem difusos (TESKE *et al*., 1994; PARODI, 2001). Com o aumento do conhecimento dos aspectos imunológicos dos linfomas, e um melhor entendimento da maturação e diferenciação das células linfoides, foram desenvolvidos os sistemas de classificação de *Lukes-Collins* na América do Norte (LUKES; COLLINS, 1974) e a classificação de *Kiel* na Europa (GERARD-MARCHANT *et al*., 1974). Diferente da classificação de *Kiel*, a de *Lukes-Collins* apresenta subdivisão por grau de malignidade (LUKES, 1978; TESKE; VAN HEERDE, 1995). Com o objetivo de unificar as inúmeras classificações o *National Cancer Institute* delineou um estudo multi-institucional que originou a WF, publicada em 1982. Essa classificação representa um sistema de interpretação para as classificações existentes de linfoma não-Hodgkin humano, voltada para aspectos clínicos e não morfológicos (PARODI, 2001; JACOBS; MESSICK; VALLI, 2002).

Em 1994, com o objetivo de unificar aspectos morfológicos, imunofenótipicos, genéticos e clínicos, foi criada a REAL, posteriormente incorporada pela Organização Mundial de Saúde (OMS) (VALLI; KIUPEL; BIENZLE, 2016). Essa sistemática também enfatiza a distinção entre grau histológico e agressividade clínica, de maneira que a classificação histológica deve ser aplicada em tipos individuais de linfomas e não em toda a extensão de neoplasmas linfoides, como era feito anteriormente (HARRIS *et al*, 1994; HARRIS *et al*., 1999; PARODI., 2001).

A OMS utiliza como base a classificação do sistema REAL. Esta classificação foi adaptada da medicina humana e enriquecida por outras neoplasias hematopoiéticas sob a orientação de especialistas veterinários da OMS. O sistema de classificação da OMS para o linfoma dos animais domésticos é uma lista de neoplasias hematopoiéticas (Tabela 1), elaborado independentemente da origem do tumor (medula óssea ou linfonodo) e seu objetivo principal é correlacionar cada categoria de linfoma ao seu próprio comportamento e grau de malignidade (VALLI *et al*., 2000; VALLI *et al*., 2007; VONDERHAAR *et al*., 2002).

O linfoma é a neoplasia mais frequente em gatos (JACOBS *et al*., 2002; CARRERAS *et al*., 2003) e a sua alta ocorrência está associada à ação do vírus da leucemia felina (FeLV), além das suas formas espontâneas (SHELTON *et al*., 1990; MOCHIZUKI *et al*., 2011). Gatos com linfoma podem atingir a remissão tumoral quando diagnosticados de forma precoce e instituído protocolo terapêutico adequado; apesar disso, prever quais pacientes irão responder satisfatoriamente ao tratamento ainda é um desafio. Vários fatores são avaliados na tentativa de elucidar essa lacuna, como as características morfológicas e imunofenotípicas do tumor, o estadiamento clínico e as características individuais do paciente. Contudo, a grande diversidade de características clínicas, anatômicas, histológicas e de comportamentos biológicos dos linfomas em felinos aumenta essa dificuldade (CHINO *et al*., 2013). Do ponto de vista experimental, assim como os cães, os gatos representam um modelo de ocorrência espontânea para o estudo da etiologia e dos protocolos de tratamentos dos linfomas não-Hodgkin em humanos (VEZZALI *et al*., 2010).

A introdução da vacinação contra o FeLV a partir dos anos 80 resultou em uma diminuição significativa da prevalência de infecção entre a população felina e, simultaneamente, uma diminuição na proporção de casos de linfoma mediastinal nos EUA (LOUWERENS *et al*., 2005). Atualmente, em alguns países, o FeLV é menos comumente associado ao linfoma do que era há 20 anos, com taxas de prevalência

relatadas de 25,5% (Estados Unidos, 1988-1996) (VAIL *et al*., 1998) e 20,8% (Alemanha, 1996-2008) (STUTZER *et al*., 2011).

O linfoma alimentar felino representa cerca de 50% de todos os linfomas em gatos e atualmente é o local anatômico mais comum do linfoma nesta espécie (VEZZALI *et al*.,2010; VALLI *et al.*, 2000; MOORE; RODRIGUEZ-BERTOS; KASS, 2012; CHINO *et al*., 2013; SATO *et al*., 2014; SANTAGOSTINOL *et al*., 2015). O linfoma alimentar é um exemplo de linfoma que surge nos centros extranodais linfoides da mucosa, sendo exclusivo na sua localização; na verdade, em gatos, o tipo de linfoma difere entre estômago, jejuno e região ileocecal (MOORE; RODRIGUEZ-BERTOS; KASS, 2012). A maioria dos linfomas alimentares no jejuno dos gatos é de células T. No entanto, linfomas alimentares na região gástrica ou ileocecal podem ser de células T ou B (MOORE; RODRIGUEZ-BERTOS; KASS, 2012; SATO *et al*., 2014). Alguns dos linfomas alimentares são indolentes, geralmente localizados na mucosa, e outros são agressivos, geralmente transmurais. Os gatos com esta forma de linfoma transmural vivem menos de um mês pós-diagnóstico (MOORE; RODRIGUEZ-BERTOS; KASS, 2012).

Embora a imunofenotipagem ainda não seja parte de uma avaliação padrão do linfoma felino, esta ferramenta oferece informações adicionais que podem ser úteis nas decisões de tratamento e estabelecimento do prognóstico. A imunofenotipagem dos linfomas de felinos é realizada com a técnica da imuno-histoquímica que envolve o uso de anticorpos contra os antígenos de superfície nos linfócitos, deste modo é possível distinguir entre tumores de células B e células T. O imunofenótipo do linfoma felino pode ter considerável significado prognóstico (GREENLEE *et al*., 1999; DAY, 1995; RUSHLANDER *et al*., 1997; KIUPEL; TESKE; BOSTOCK, 1999; VALLI *et al*., 2000). Como a morfologia e o imunofenótipo do linfoma estão correlacionados com comportamento biológico e resposta à terapia, o conhecimento do tipo e subtipo de células será cada vez mais relevante.

Desta forma, o primeiro trabalho tem como objetivo caracterizar os aspectos epidemiológicos, patológicos e imuno-histoquímicos dos linfomas felinos no Sul do Brasil e classificar estes casos conforme o sistema de classificação das neoplasias linfoides adotadas pela OMS, aplicado para uso em animais. O objetivo do segundo trabalho é abordar os aspectos patológicos dos linfomas com envolvimento pulmonar de felinos.

Tabela 1. Classificação adaptada das neoplasias linfoides adotadas pela Organização Mundial de Saúde, aplicado para uso em animais.

Neoplasmas de células linfoides B
Neoplasmas de células B precursoras
Linfoma linfoblástico
Neoplasmas de células B maduras
Linfoma linfocítico de pequenas células
Linfoma difuso de grandes células
Anaplásico
Centroblástico
Imunoblástico
Rico em células T
Granulomatose linfomatoide
Linfoma folicular
Graus: I, II e III
Linfoma linfoplasmocítico
Linfoma de zona marginal
Nodal
Esplênico
MALT
Linfoma de células do manto
Linfoma de Burkitt
Mieloma múltiplo
Plasmocitoma extramedular
Neoplasmas de células linfoides T e células Natural Killer
Neoplasmas de células T precursoras
Linfoma linfoblástico
Neoplasmas de células T maduras
Linfoma nodal
Linfoma de zona T
Linfoma periférico não-especificado
Linfoma anaplásico de grandes células
Linfoma angioimunoblástico
Linfoma de células T do tipo enteropatia
Tipo 1: grandes células
Tipo 2: pequenas células
Linfoma extranodal
Linfoma hepatoesplênico
Linfoma hepatocitotrópico
Linfoma periférico não-especificado
Linfoma cutâneo
Linfoma cutâneo epiteliotrópico
Micose fungoide
Reticulose pagetoide
Síndrome de Sézary
Linfoma cutâneo não-epiteliotrópico
Linfoma periférico não especificado
Linfoma subcutâneo semelhante à paniculite
Linfoma anaplásico de grandes células

MALT: tecido linfoide associado a mucosa.
Fonte: VALLI; KIUPEL; BENZLE (2016, p. 217).

2 ARTIGO 1

Neste item é apresentado o artigo intitulado "**EPIDEMIOLOGY, PATHOLOGY, AND IMMUNOHISTOCHEMISTRY OF 125 FELINE LYMPHOMAS"**, que será submetido à apreciação do periódico *Veterinary and Comparative Oncology*.

EPIDEMIOLOGY, PATHOLOGY, AND IMMUNOHISTOCHEMISTRY OF FELINE 125 LYMPHOMAS

ABSTRACT

The epidemiological, pathological and immunohistochemical aspects of lymphoma in felines in southern Brazil were evaluated through a retrospective study over a period of 12 years (2004-2016). In 1356 necropsies, 125 cats with lymphoma diagnosis were review to determine age, breed, sex, tumor topography, feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) immunohistochemistry, immunophenotype of lymphomas, and categorization of the tumors according to World Health Organization system applied for use in animals. Alimentary form (33.6%) was the most common, followed by mediastinal lymphoma (28%). Immunohistochemical staining of tumor tissue revealed 50.4% FeLV-positive and 21.6% FIV-positive cats, 52.8%T-cell lymphoma and 47.2% B-cell lymphoma. The range of age of cats with T-cell varied from 10 to 240 (median of 120) months and 6 to 204 (median of 60) months for cats with B-cell lymphoma, and mixed-breed was the most frequent with 90.4% among all breeds. Alimentary tumor exhibited the enteropathy-associated T-cell lymphoma (type 1) as the most frequent and mediastinal tumor showed the diffuse large B-cell lymphoma as the major type of lymphoma. Considering only mediastinal and alimentary lymphomas, there was significantly statistical association ($p \leq 0.05$) between FeLV status and the categories of age. The prevalence of mediastinal lymphoma among FeLV-positive cats was 3.21 times higher than FeLV-negative ones. Although not statistically significant, the prevalence of B-cell lineage among FeLV-positive cats was 1.44 times the FeLV-negative cats.

Keywords: B-cell, FeLV, FIV, immunophenotype, T-cell.

INTRODUCTION

Lymphomas are a diverse group of neoplasms which show a great diversity of histological and biological behavior. Lymphoma represents the most common neoplasms in cats characterizing one-third of all tumors diagnosed in cats in USA.[1] Retroviral infections have been associated with development of feline lymphoma, and studies conducted before widespread vaccination of feline leukemia virus (FeLV) indicated that most cats with lymphoma were FeLV-positive and tended to be younger,[2] with mediastinal lymphoma been the most common anatomic presentation.[3] The alimentary lymphoma has been reported to be the most frequent recognized anatomical form since the implementation of vaccination programs for the control of FeLV.[4,5] Authors report that B-cell tumors predominate over T-cell tumors,[6,7,8] however the literature is contradictory, and a predominance of T-cell lymphomas also has been reported.[9]

The epidemiological characteristics of lymphomas in Brazil are likely to differ from developed countries, since the infections of FeLV and feline immunodeficiency virus (FIV) in Brazil is still frequent. To obtain a more comprehensive information of feline lymphoma in Brazilian cats, a study was conducted to characterize feline lymphoma with regard to epidemiology, location, and classification of feline lymphoma according of the World Health Organization (WHO),[10] looking for correlation between tumor location, age, and FeLV and FIV infections status.

MATERIALS AND METHODS

The study was performed on cases selected from January 2004 to December 2016, in which a diagnosis of lymphoma was originally obtained by routine necropsy examinations performed in our department. Tumors other than lymphoma (i.e. myeloproliferative malignancies) were not taken into consideration. All cats were from the metropolitan area of Porto Alegre city 30.0346° S, 51.2177° W, State of Rio Grande do Sul , Brazil. Cat's age, breed and sex were recorded.

Initially, regarding the anatomical location, cats were allotted in three groups: mediastinal, alimentary and others. Felines that exhibited large masses in the mediastinal space, with or without involvement of other organs, were classified as mediastinal (Figure 1A,B); in the alimentary group, involvement of gastrointestinal tract in the absence of mediastinal or other organs involvement (Figure 1C,D); the other

group was composed by cats with neoplasms that did not fit in the above two categories and, therefore, were organized using the anatomical classification proposed by Gabor *et al*.[4]

Systematic histopathological re-evaluation of the tumor sections stained with hematoxylin-eosin (HE) was performed with a multiobserver microscope, and the histologic characterization of each tumor was carried out through the classification of lymphoid neoplasms of the WHO, as applied for use in animals.[10] Immunophenotype was established in all cases by the use of polyclonal antibody against CD3 to demonstrate T-cell lineage, and monoclonal antibody against CD79 αcy, to demonstrate B-cell lineage on paraffin-embedded sections; the protocols were summarized in Table 1. Neoplastic tissue sections also underwent immunohistochemistry (IHQ) evaluation for FeLV and FIV; the monoclonal antibody used is also listed in Table 1. Positive and negative control slides were used. Slides were counterstained with Harris hematoxylin.

The alimentary and the mediastinal group were considered separately in the statistical analysis (Table 2). To assess the association between the type of lymphoma (mediastinal and alimentary, response variable) with the FeLV status, age, sex, and time-period (explanatory variables) a log-binomial regression was used to estimate the probability of a cat having mediastinal lymphoma. The output of the model was the Prevalence Ratio (PR). A log-binomial regression was choose due to the frequency of occurrence of mediastinal lymphoma, and using logistic regression would overestimate the PR.[11] The variable age was categorized as “kitten-young” (less than 36 months), “adult” (more or equal to 36 months and less than 96 months), and “aged” (more than 96 months). The effect of FeLV corrected by the age in the model was tested. A log-binomial regression was also used to test the association between the immunophenotype (response variable) and FeLV status (explanatory variable), considering B-cell lineage as “1” and T-cell lineage as “0” using the entire dataset (n=125). Inferential statistics were made in the software SAS using the GENMOD procedure. The link function and distribution used were *log* and binomial, respectively. Descriptive statistics were made using the software Excel.

RESULTS

The age of the affected cats varied from 6 to 240 (median of 96) months. The breed distribution showed the prevalence of Mixed-breed (113/90.4%), followed by Siamese (9/7.2%), Persian (2/1.6%) and Birman (1/0.8%). The gender distribution was

74 (59.2%) males and 51 (40.8%) females. The alimentary form (42/125-33.6%) was the most common anatomical location, followed by mediastinal (35/125-28%), mixed (13/125-10.4%), abdominal subcategory renal (13/125-10.4%), atypical (9/125-7.2%), abdominal subcategory combination (8/125-6.4%), abdominal subcategory other (3/125-2.4%), nodal subcategory multinodal (2/125-1.6%). Immunohistochemical staining of tumor tissue section revealed 63 (50.4%) FeLV-positive cats and 27 (21.6%) FIV-positive cats, of these 13 cats (10.4%) were positive for both FeLV and FIV. Overall, 66 cases (52.8%) had T-cell lymphoma and 59 (47.2%) had B-cell lymphoma. The range of age of cats with T-cell varied from 10 to 240 (median 120) months and 6 to 204 (median of 60) months for cats with B-cell lymphoma. Through microscopic observation of the histopathological specimens of lymphoma, morphological and immunohistochemistry features of lymphomas from all of the 125 cases could be graded as reported by WHO (Table 3).

Alimentary lymphoma

Alimentary lymphoma was the most common type accounting for 42 out 125 (33.6%) cases. The cats ranged in age from 12 to 240 months (median of 140). Mixed-breed cats predominated at 35 (83.3%) of 42 cats, and the remaining breeds included Siamese (11.9%), Persian (2.3%) and Birman (2.3%). There was a predominance of male cats (64.2%) and the remaining were female (35.7%). FIV/FeLV immunohistochemistry analysis was performed revealing that 16.6% (7/42) was FeLV-positive and 21.4% (9/42) was FIV-positive. One case (2.3%) was both FIV- and FeLV-positive. The immunophenotype analysis was as follows, 30 cases (71.4%) of T-cell lymphoma and 12 cases (28.5%) of B-cell lymphoma. Regarding to the category of lymphomas according to the WHO, 19 (45.2%) of 42 lymphomas was classified as enteropathy-associated T-cell lymphoma (type 1), 26.1% (11/42) as enteropathy-associated T-cell lymphoma (type 2), 23.8% (10/42) as diffuse large B-cell lymphoma (4 T-cell rich B-cell, 3 centroblastic and 3 immunoblastic) and 4.7% (2/42) as small lymphocytic B-cell lymphoma.

Mediastinal lymphoma

Mediastinal lymphoma (28%) was the second most common type encountered (35 of 125 cases). The cats age ranged from 12 to 132 months (median of 24), and all of them were Mixed-breed cats. There was a predominance of male cats, at 54.2% (19/35),

and 16 cats were female (45.7%). In the FIV/FeLV immunohistochemical analysis the results were as follows, 88.5% (31/35) was FeLV-positive and 11.4% (4/35) was FIV-positive. Three cases (8.5%) were both FIV- and FeLV-positive. The overall of immunophenotype analysis was as follows, 18 cases (51.4%) of B-cell lymphoma and 17 cases (48.5%) of T-cell lymphoma. Regarding to the category of lymphomas according to the WHO, 19 (54.2%) of 35 lymphomas was classified as diffuse large B-cell lymphoma (11 immunoblastic, 4 centroblastic and 4 T-cell rich B-cell), 34.2% (12/35) as peripheral T-cell lymphoma unspecified, 6% (2/35) as small lymphocytic B-cell lymphoma and 6% (2/35) as T-lymphoblastic lymphoma.

Others

Here is grouped the remaining categories, which were less frequent. The mixed category of lymphoma, (which involves two or more anatomical forms) accounted for 13 of 125 (10.4%) cases. The age of the cats varied from 9 to 156 (median of 60) months and the breed distribution showed the frequency of Mixed-breed (92%) and Siamese (8%). There was a predominance of female cats (61.5%). In the FIV/FeLV immunohistochemistry analysis the results were as follows, 53.8% (7/13) was FeLV-positive and 30.7% (4/13) was FIV-positive. Two cases (15.3%) were both FIV- and FeLV-positive. The immunophenotype analysis was as follows, 10 cases (76.9%) of B-cell lymphoma and 3 cases (23%) of T-cell lymphoma. Regarding to the category of lymphomas according to the WHO, 8 (61.5%) of 13 lymphomas were classified as diffuse large B-cell lymphoma (6 centroblastic, 1 T-cell rich B-cell and 1 anaplastic), 2 cases (15.3%) as small lymphocytic B-cell lymphoma and one (7.6%) of each of the following: hepatosplenic T-cell lymphoma, peripheral T-cell lymphoma unspecified and T-lymphoblastic lymphoma.

The abdominal subcategory renal lymphoma (which involves either or both kidneys) accounted for 13 of 125 (10.4%) cases. The age of the cats varied from 12 to 216 (median of 108) months and the breed distribution showed the frequency of Mixed-breed (84.6%), followed by Persian (7.6%) and Siamese (7.6%). There was a predominance of male cats (69.2%). In the FIV/FeLV immunohistochemical analysis the results were as follows, 61.5% (8/13) was FeLV-positive and 23% (3/13) was FIV-positive. Two cases (15.3%) were both FIV- and FeLV-positive. The immunophenotype analysis was as follows, 9 cases (69.2%) of T-cell lymphoma and 4 cases (30.7%) of B-cell lymphoma. Regarding to the category of lymphomas according to the WHO, 9

(69.2%) of 13 lymphomas were classified as peripheral T-cell lymphoma unspecified and 30.7% (4/13) as diffuse large B-cell lymphoma (3 T-cell rich B-cell and 1 centroblastic).

The atypical category of lymphoma (related to non-lymphoid such as the central nervous system) accounted for 9 of 125 (7.2%) cases. The age of the cats varied from 6 to 180 (median of 48) months and all of them were Mixed-breed cats. There were 5 male cats (55.5%) and 4 cats were female (44.4%). In the FIV/FeLV immunohistochemical analysis the results were as follows, 66.6% (6/9) was FeLV-positive and 33.3% (3/9) was FIV-positive. Three cases (33.3%) were both FIV- and FeLV-positive. The immunophenotype analysis was as follows, 7 cases (77.7%) of B-cell lymphoma and 2 cases (22.2%) of T-cell lymphoma. Regarding to the category of lymphomas according to the WHO, 6 (66.6%) of 9 lymphomas were classified as diffuse large B-cell lymphoma (3 immunoblastic, 2 centroblastic and 1 T-cell rich B-cell), 22.2% (2/9) as peripheral T-cell lymphoma unspecified and 11.1% (1/9) Burkitt-like lymphoma.

The abdominal subcategory combination lymphoma (which involves organs of the gastrointestinal tract, liver, spleen and/or kidney) accounted for 8 of 125 (6.4%) cases. The age of the cats varied from 12 to 180 (median of 96) months and the breed distribution showed the frequency of Mixed-breed (75%), followed by Siamese (25%).There were 5 male cats (62.5%) and 3 cats were female (37.5%). In the FIV/FeLV immunohistochemical analysis the results were as follows, 37.5% (3/8) was FeLV-positive and 37.5% (3/8) was FIV-positive. One case (12.5%) was both FeLV- and FIV-positive. The immunophenotype analysis was as follows, 6 cases (75%) of B-cell lymphoma and 2 cases (25%) of T-cell lymphoma. Regarding to the category of lymphomas according to the WHO, 5 (62.5%) of 8 lymphomas were classified as diffuse large B-cell lymphoma (2 T-cell rich B-cell, 1 anaplastic, 1 centroblastic and 1 immunoblastic), 25% (2/8) as peripheral T-cell lymphoma unspecified and 13% (1/8) as follicular B-cell lymphoma.

The abdominal subcategory others lymphoma (which involves organs such as liver or spleen in the absence of gastrointestinal or renal involvement) accounted for 3 of 125 (2.4%) cases, as follows: a 24-month-old male cat, FIV-negative and FeLV-positive, which had a hepatosplenic T-cell lymphoma; a 48-month-old male cat, FIV- and FeLV-positive, which had a hepatosplenic T-cell lymphoma; and a 120-month-old

male cat, FIV- and FeLV-negative, which had a diffuse large B-cell lymphoma (anaplastic).

The nodal subcategory multinodal lymphoma (which involves many or all peripheral lymph nodes) accounted for 2 of 125 (1.6%) cases; a 60-month-old female, FIV-negative and FeLV-positive, which had a diffuse large B-cell lymphoma (immunoblastic) and a 144-month-old male, FIV- and FeLV-negative, which had a peripheral T-cell lymphoma unspecified.

Inferential statistics

Considering only mediastinal and alimentary lymphomas (n=77), there were significantly statistical association ($p \leq 0.05$) between FeLV status and the categories of age in the unavailable model. The PR of mediastinal lymphoma among FeLV-positive cats correct by age was 3.21 (CI, 95%: 1.08 – 9.54), which means that the prevalence of mediastinal lymphoma in FeLV-positive was 3.21 times the prevalence of FeLV-negative cats. Results of the model can be seen in Table 4. It could be also observed that the prevalence of mediastinal lymphoma increases inversely with the age, considering the "aged" as a reference. No association ($p>0.05$) were found between type of lymphoma with the variable sex and the time the diagnosis was made. Although not statistically significant ($p=0.06$), the prevalence of B-cell lineage among FeLV-positive cats was 1.44 times (CI, 95%: 0.98-2.13) the prevalence of B-Cell lineage among FeLV-negative cats.

DISCUSSION

Lymphoma is considered the most common neoplasm in felines, it may present a wide variety of clinical-pathological presentations and this variation is associated to different factors. The epidemiological and pathological aspects observed, with more than 50% of FeLV-positive felines and the high number of mediastinal lymphomas, are similar to those observed in earlier studies before 1990.[2,3]

Prophylaxis of FeLV and FIV in cats is based on identification and isolation of infected animals and vaccination for FeLV of those at risk. The adoption of these measures resulted in a considerable reduction in cases of FeLV infection in some countries.[4,5] The prevalence of FeLV infections can vary, for example, in Southeast Brazil, the observed prevalence of 11.52% is high when compared to the prevalence

among cats in Southern Australia and Japan, at 2% and 2.9%, respectively.[12,13,14] FeLV and FIV have been associated with lymphoma, especially before the widespread vaccination for FeLV.[5] Previous studies have reported that lymphoma occurs most frequently in young FeLV-positive cats,[2,15,16] in contrast to most recent reports.[5,17,18,19,20]

In our study it was remarkable the great frequency of mediastinal lymphoma represented by large masses occupying the mediastinal space. There was a higher prevalence of mediastinal lymphoma in young FeLV-positive than FeLV-negative cats. The mediastinal lymphoma in FeLV-positive cats was a frequent form in previous studies that reported almost 50% of cases of mediastinal lymphoma in USA and UK and around 70% in Japan.[3,21] Recent studies have suggested that the incidence of feline lymphoma has decreased, and the relative frequency of different anatomical forms has changed, demonstrated by the increased prevalence of older cats with extranodal types of lymphoma instead of younger cats with mediastinal lymphoma.[22,23,24] In the USA and Australia, for example, recent studies have reported a frequency of mediastinal lymphoma lower than 15% and approximately 25%, respectively.[5,16,25]

Recent studies also have demonstrated that the frequency of older FeLV-negative cats with mediastinal lymphoma has increased and these changes, in addition with the incidence and locations of feline lymphoma, may be due, in part, to the use of testing and vaccination for FeLV.[5,19] The median age of cats with mediastinal lymphoma in the present study was 24 months, which is comparable to previous reports for cats with mediastinal lymphoma.[27] Our study indicates that in southern Brazil, FeLV-positive cats up to 3 years-old with lymphoma, probably have the mediastinal form. The Mixed-breed of cats is over-represented in the current study, meaning that firm conclusions about breed predisposition could not be made.

Since the introduction of FeLV vaccination, the most common type of lymphoma in cats is enteric T-cell lymphoma, which makes up approximately 50% of all feline lymphomas.[22,23,24,26,27] The findings of this study reflect the publish literature, where alimentary lymphoma predominates.[4,7,8,22,23] Our findings, specifically in the alimentary lymphoma, the T-cell phenotype consistently predominates (71.4%; 30 out of 42), which is linked to previous reports,[26] but in contrast to other studies that report mainly B-cell lymphoma.[6,7,8,22] Although in our study the mean age of cats with B- or T-cell alimentary lymphoma did not differ significantly (130 versus 143 months), most of them were old FeLV-negative cats agreeing with studies that report alimentary lymphoma occurring predominantly in older FeLV-negative cats.[9,16,18,28] The alimentary

lymphoma was predominantly of enteropathy-associated T-cell lymphoma (EATL) (type 1, 45.2 % and type 2, 26.1%), representing 71.4% of all alimentary lymphomas. The most common subtype reported by Vezzalli *et al.*[26] was the enteric T-cell lymphoma (i.e., EATL) at 33% followed by Peripheral T-cell lymphoma at 23%. Transmural EATL in cats have been reported to be most commonly type 1,[10] as occurred in our study. The predominance of T-cell lymphoma in the alimentary form is supported by the reports that identifying the origin of this cells as the diffuse mucosal-associated lymphoid tissue of the intestine.[8,30]

Most of the cats that had renal lymphoma were FeLV-positive male cats with a median age of 108 months. Cats with renal lymphoma are usually older and FeLV-negative, although in one Australian study 54% (15/28) cats with renal lymphoma were FIV-positive.[31] Here a slight majority of renal lymphomas showed T-cell origin, however some authors report that renal lymphoma appears to be mainly B-cell origin, but this was not examined for or correlated to prognosis.[7,16]

Overall there was a predominance of DLBCL with 39 (31.2%) of 125 cases. DLBCL have been associated with FeLV but there also are DLBCL that is not virally induced.[23,24,32] By means of WHO, DLBCL is one of the most common type of lymphoma in cats.[22,23,24,26] There are subtypes of DLBCL, represented for mild morphologic variants that occurs as heterogeneous populations within the same neoplasm, but distinguishing these subtypes has not been associated with differences in clinical behavior or therapeutic response behavior.[10]

The results of this study suggests the higher frequency of intestinal and mediastinal anatomical forms of lymphoma in the southern Brazil.

Conflict of Interest Statement

The authors do not have any potential conflicts of interest to declare.

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Table 1. Antibodies used in immunohistochemistry procedures.

Antibody	Antigen retrieval	Dilution	Detection method	Chromogen
Mouse antifeline leukemia virus gp 70 [a]	40 min/100ºC, Tris EDTA buffer pH 9.0	1:500	MACH 4	AEC
Mouse antifeline immunodeficiency virus p24gag [a]	40 min/100ºC, Citrate buffer pH 6.0	1:200	MACH 4	AEC
Mouse antihumam CD79αcy [b]	20 min/100ºC, Tris EDTA buffer pH 9.0	1:100	MACH 4	DAB
Polyclonal rabbit antihuman CD3 [b]	15 min/RT Protease XIV	Ready-to-use	MACH 4	AEC

[a]: Bio-rad®; [b]: Dako®; MACH 4 = Universal HRP-Polymer (Biocare®); AEC = 3-amino-9-etilcarbazol (Dako); DAB = 3,3'Diaminobenzene (Dako®), RT: room temperature.

Table 2. Anatomical classification of lymphoma in 125 necropsied cats.

Types	Total	%
Alimentary	42	33.6%
Mediastinal	35	28.0%
Other	48	38.4%
Total	**125**	**100%**

Table 3. Feline lymphomas classified according to the WHO classification.

Number of cats	Abbreviation	WHO classification type
54	DLBCL	Diffuse large B-cell lymphoma
27	PTCL	Peripheral T-cell lymphoma unspecified
19	EATL-1	Enteropathy-associated T-cell lymphoma (type 1)
11	EATL-2	Enteropathy-associated T-cell lymphoma (type 2)
6	SLBCL	Small lymphocytic B-cell lymphoma
3	HTCL	Hepatosplenic T-cell lymphoma
3	TLL	T-lymphoblastic lymphoma
1	FBCL	Follicular B-cell lymphoma
1	Burkitt-like	Burkitt-like lymphoma

Table 4. Results of the multivariable model to test the association between the type of lymphoma (alimentary and mediastinal) and FeLV status corrected by age of 77 cats analyzed over the period 2004-2016.

Variable	PR*	CI (95%)		*P*-value
Categories of age				
Aged	1,00	*	*	*
Adult	3,70	1,22	11,25	0,0211
Kitten-young	4,06	1,35	12,19	0,0125
FeLV	3,21	1,08	9,24	0,0365

*Prevalence ratio

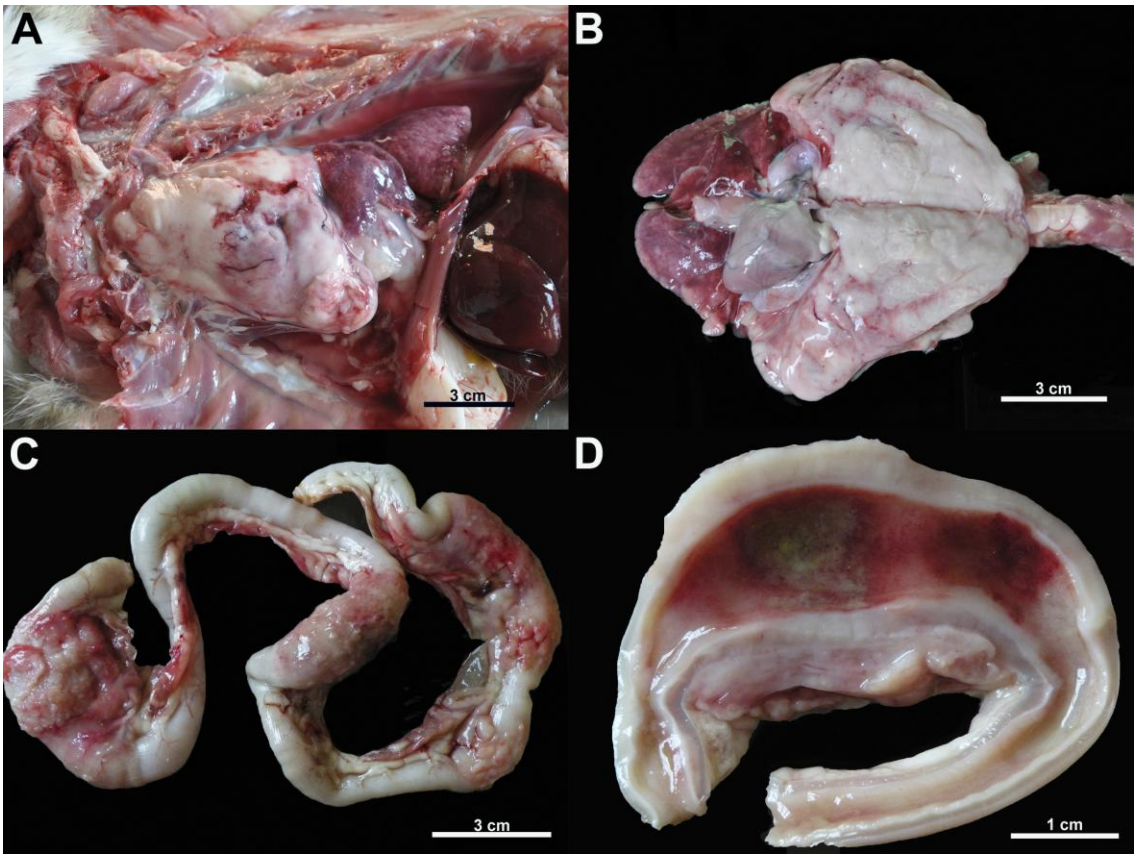

Figure 1. **Gross features of feline lymphoma**. **A.** Mediastinal lymphoma in a cat. A large irregular whitish neoplastic mass is observed in the mediastinum, obliterating part of the thoracic cavity. It is also possible to visualize the accumulation of moderate amount of fluid in the cavity and areas of atelectasis in some pulmonary lobes. **B.** Mediastinal lymphoma in a feline. Cut surface of neoplastic mediastinal mass. It is observed that tumor proliferation is cranial to the heart and the surface is homogeneously white. **C.** Alimentary lymphoma in a cat. Multifocal areas of thickening of the small intestine (jejunum). The mesenteric nodal involvement is also noted on the left of the image. The segments containing the neoplastic infiltration are white, slightly irregular and with areas of hemorrhage. **D.** Alimentary lymphoma in a cat. Small intestine (jejunum). A marked diffuse and whitish thickening of the intestinal wall is observed. There is also area of mucosal ulceration and moderate edema.

3 ARTIGO 2

Nesse item é apresentado o artigo intitulado "**PATHOLOGIC CHARACTERIZATION OF LYMPHOMA WITH PULMONARY INVOLVEMENT IN CATS"**, que será submetido à apreciação do periódico *Journal of Comparative Pathology*.

PATHOLOGIC CHARACTERIZATION OF LYMPHOMA WITH PULMONARY INVOLVEMENT IN CATS

ABSTRACT

Studies that have attempted to evaluate histological patterns of lung infiltration by lymphoma in cats are lacking. We retrospectively evaluated the histological patterns of pulmonary involvement during a 12-year period. In 1,356 necropsies, 125 reports with lymphoma diagnosis were review histologically, and 16 showed lung infiltration (12.8%). Nine cats were females, 7 cats were males, and all of them were Mixed-breed cats. Gross lesions observed in the lungs consisted of masses (25%) and nodules (18.7%); however, the majority of cases (56.2%) did not show any gross abnormality. The peribronchial-vascular infiltration pattern (93.7%) was the most frequent pattern, followed by pleural (56.2%), interstitial (50%), nodular (37.5%) and alveolar (12.5%), and 75% of the lymphomas had more than one pulmonary infiltration pattern. Among the cases, there were 14 (87.5%) B-cell and 2 (12.5%) T-cell lymphomas. Six cats (37.5%) were FeLV-positive and 3 cats (18.7%) were FIV-positive. Diffuse large B-cell lymphoma was the most frequent type accounting for 56% of all cases.

Key words: cat; lung; lymphosarcoma; peribronchial-vascular

INTRODUCTION

Lymphoma is a proliferative disease arising from lymphoid tissues involving any organ or tissue and accounts for 50 to 90% of all hematopoietic tumors in the cat. Since hematopoietic tumors (lymphoid and myeloid) account for approximately one-third of all feline tumors, it is estimated lymphoid neoplasia accounts for an incidence of 200 per 100,000 cats at risk (Vail, 2013). Involvement of lymphoma cells in organs other than lymph node is well described, including the alimentary tract, kidney, central nervous system, trachea, skin, nasal cavity, eye, retrobulbar space and skin (Bree *et al*., 2017). Lymphoma can involve the lungs as a primary disease or secondary to multicentric or extranodal lymphoma (Vail, 2007).

In humans, primary pulmonary lymphoma is rare, representing <1% of all lymphoma, and approximately 3% to 4% of extranodal lymphoma (Cadranel *et al*., 2002). Primary pulmonary lymphoma is defined as a clonal lymphoid proliferation affecting one or both lungs (parenchyma and/or bronchi) in a patient with no detectable extrapulmonary involvement at diagnosis or during the subsequent 3 months (William *et al*., 2013). Primary lymphoma lesions are centered on the airways, often contain air bronchograms, and occasionally result in bronchial dilation. Nodules, masses, and areas of consolidation are most frequently reported patterns (King *et al*., 2000; Lee *et al*., 1997; Wislez *et al*., 1999). Histologically, malignant lymphocytes have a preferential peribronchoquial-vascular distribution (King *et al*., 2000; Lee *et al*., 1997; Wislez *et al*., 1999).

In humans, the prevalence of secondary pulmonary involvement is 4–12% at initial presentation and 30–40% during the course of the disease (Lee *et al*., 1997). Pulmonary infiltration in humans due to secondary pulmonary lymphoma is varied. Nodular, bronchovascular, alveolar, and a miliary interstitial pattern have all been reported. Additionally, thoracic lymphadenopathy and pleural effusion are common in secondary pulmonary lymphoma (Lewis *et al*., 1991; Mentzer *et al*., 1993; Lee *et al*., 1997). Pulmonary involvement in feline lymphoma is rare and there are no data regarding the prevalence of abnormal pulmonary infiltrates in cats with lymphoma (Gabor *et al*., 1999; Gabor *et al*., 1998; Louwerens *et al*., Taylor *et al*., 2009). To our knowledge, reports specifically describing the histological pattern of pulmonary lymphoma in the cat limited to a few individual reports (Darbes *et al*., 1998; Krecic and Black, 2000). The purpose of this report is to describe the pulmonary histological patterns of secondary pulmonary lymphoma in 16 cats.

MATERIALS AND METHODS

All cases of lymphoma with pulmonary involvement during the period of January 2004 to December 2016 were included in this study. All cats were from the metropolitan area of Porto Alegre city, State of Rio Grande do Sul, Brazil. All postmortem data were recorded. The distribution of lesions at the time of necropsy was used to categorise cases anatomically, therefore cats were grouped in three groups: mediastinal, alimentary and others. Cats that exhibited large masses in the mediastinal space, with or without involvement of other organs, were classified as mediastinal; in the alimentary group, involvement of gastrointestinal tract in the absence of mediastinal or other organs involvement; in the other group was grouped the cats that did not fit in the above two categories and, therefore, they were organized using the anatomical classification proposed by Gabor *et al*. (1998). Systematic histopathological evaluation of the lung sections, stained with hematoxylin-eosin (HE), was performed, and the pulmonary lymphoma cases were classified morphologically according to the distribution patterns using the classification proposed by Costa *et al*. (2004): peribronchial-perivascular; when the lymphoma cells were present in the peripheral parts of perivascular and peribronchial space; interstitial, when lymphoma cells infiltrated predominantly along the alveolar septa; nodular, unique or multiple, coalescent; pleural, when lymphoma cells were present in the pleura; and alveolar, when lymphoma cells filled the alveolar spaces. These patterns were related to the macroscopic findings of the lung parenchyma of each case: nodule was considered a small round or oval-shaped growth in the lung, smaller than 3 centimeters in diameter; mass or mass-like if the growth is larger than that; and peribronchial thickening. The histologic characterization of each tumor was performed through the classification of lymphoid neoplasm adopted by the World Health Organization (WHO), as applied for use in animals (Valli *et al*., 2016). Immunophenotype was established in all cases by the use of polyclonal antibody against CD3, as pan-T marker, and monoclonal antibody against CD79 αcy, as pan-B marker, on paraffin-embedded sections; the protocols are summarized in Table 1. Neoplastic tissue sections also underwent immunohistochemistry (IHQ) evaluation for FeLV (feline leukemia virus) and FIV (feline immunodeficiency virus); the monoclonal antibody used is listed in Table 1. Positive and negative control slides were used. Slides were counterstained with Harris hematoxylin.

RESULTS

Out of 125 cases, 16 (12.8%) of them showed secondary extranodal pulmonary involvement. There was no case of primary extranodal pulmonary involvement of lymphoma. The anatomical distribution of lymphomas was as follows: mixed lymphoma (8/50%) was the most common form, followed by mediastinal (4/25%), alimentary (2/12.5%) and atypical form (2/12.5%). Macroscopic pulmonary abnormalities were absent in nine cases, ((56.2%), and the gross findings found were masses (4/25%) (Fig. 2.A), nodules (3/18.7%) and peribronchial thickening (1/6.2%) (Fig. 2.B); specifically one case showed two gross findings: nodules and peribronchial thickening. Of lymphomas, four (25%) of 16 showed one infiltration pattern and 12 (75%) of 16 showed two or more infiltration patterns as follows: one case showed five infiltration patterns, four cases showed four infiltrations patterns, two cases showed three infiltration patterns, five cases showed two infiltration patterns, therefore the average number of infiltrations per case was 2.5%. The peribronchial-perivascular (15/93.7%) (Fig. 1.A,B) was the most frequent pattern, followed by pleural (9/56.2%) (Fig. 1.E), interstitial (8/50%) (Fig. 1.C), nodular (6/37.5%) (Fig. 1.D), and alveolar (2/12.5%) (Fig. 1.F). There were 14 B-cell and 2 T-cell lymphomas. Of the 16 cats, six cats (37.5%) were FeLV-positive, 10 cats (62.5%) were FeLV-negative, three cats (18.7%) were FIV-positive and 13 cats (81.2%) were FIV-negative. Two cats were both FeLV- and FIV-positive; one was a 60-month-old female and the other a 24-month-old female. Both had a diffuse large B-cell lymphoma (DLBCL) and mixed anatomical form. Table 2 shows cat's median age, sex ratio, FIV and FeLV ratio, and the infiltration patterns of B-cell and T-cell lymphomas. Peribronchial-perivascular involvement was the most frequent infiltration pattern in both B- and T-cell lymphoma, and alveolar the least frequent infiltration pattern in B-cell lymphoma, while one case of T-cell lymphoma showed both interstitial and pleural infiltration pattern. Diffuse large B-cell lymphoma (DLBCL) was the most frequent type. Among the T-cell lymphomas, we find 2 cases of extranodal T-cell lymphoma (ETCL). Table 3 shows the age and sex of the cats and infiltration patterns of the B- and T-cell subtypes.

DISCUSSION

Pulmonary involvement in cats with lymphoma is unusual (Geyer *et al*., 2010) and generally represents less than 3% of the neoplasms in felines (Gabor *et al*., 1998; Taylor *et al*., 2009). Primary pulmonary lymphoma has not been described in the cat. In the present study, all cases of lymphoma that showed pulmonary involvement had secondary origin, probably occurring due to direct extension, via hematogenous or lymphatic dissemination from distant sites or from foci of lymphoid tissue within the lung parenchyma itself (Gabor *et al*., 1998; Lee *et al*., 1997).

Although there was frequently more than one type of infiltration pattern, lung infiltration is predominantly peribronchial-perivascular, similar to what occurs in human cases (Costa *et al*., 2004). Histologically, primary pulmonary lymphoma in humans has a peribronchoquial-vascular distribution while the histological appearance of secondary pulmonary lymphoma is more variable (Geyer *et al*., 2010). The histological pattern of pulmonary lymphoma in cats is still not well characterized.

A lymphoid aggregate located within peribronchial lymph nodes or inside the bronchial mucosa is the so-called bronchus associated lymphoid tissue (BALT) (Sminia *et al*., 1989). Even though some authors reported that BALT is absent in domestic cats (Pabst and Gehrke, 1990), the presence of BALT in normal lungs is still debatable and it is actually being considered that the lung has the capacity to form BALT under certain stimulatory conditions. Al-Tikriti *et al*. (2012) have reported the presence of both nodular and diffuse lymphoid tissues in health domestic cats; the nodular form is found in interstitial tissue, adventitia of pulmonary vessels, bronchi and bronchiole walls reaching the terminal bronchioles of the lung. Therefore these findings suggest that the predominantly peribronchial-vascular infiltration pattern in the present study suggest hematogenous or lymphatic dissemination to the peribronchial tissues in the lung after stimulation.

Grossly, the lesions are relatively well-defined nodules of variable size, grey, white or yellowish. Nodules and masses are the major findings of lung infiltration by secondary lymphoma. Well-demarcated nodules and/or masses within the pulmonary parenchyma are the most commonly described radiographic feature of feline pulmonary lymphoma (Gabor *et al*., 1998; Geyer *et al*., 2010; Krecic and Black, 2010). It was observed that although the peribronchial-perivascular pattern of infiltration was the most common, gross findings was identified only in one case.

Lymphoma is the most common malignancy of domestic cats and FeLV is the most widely recognized cause. In Brazil, some FeLV epidemiologic studies have been carried out indicating that FeLV infections remains common in this country (Almeida *et al*., 2012). Related studies are still scarce, although a survey of data from several regions in Brazil yielded prevalence ranging from 4 to 21% for FIV and from 10 to 32% for FeLV (Teixeira *et al*., 2012). Our study cannot draw any firm conclusion concerning the relationship between the presence of FIV and FeLV and the development of certain pulmonary patterns in lymphoma due the low frequency of FIV- and FeLV-positive cats.

The majority of the studies dealing specifically with lung infiltration by different B- and T-cell lymphomas are case reports (Darbes *et al*., 1998; Krecic *et al*., 2000; Brown *et al*. 2011). In the present study the proportion of B-cell lymphoma exceeded that of T-cell tumors, as observed by others (Vezzali *et al*., 2010; Pohlman *et al*., 2009). In canine, as in human medicine, the vast majority of lymphoid neoplasm (80–85%) is of B-cell origin. The inherent genomic instability of B-cell germinal centers might explain why they are more susceptible to lymphomas than mature T-cells, which have fixed and stable T-cell receptor genes (Vezzali *et al*., 2010). The predominance of DLBCL is similar to reported by Moore (2006) and Pohlman *et al*. (2009).

The mixed anatomical category, in which there was involvement of two or more anatomical forms, was the most common type of lymphoma, accounting for 50% of cases. Gabor *et al*. (1998) reported the mixed category as the second most common type. Likewise, it was not possible to determine which tissues or organ systems were primarily involved in the mixed types.

All cases of lymphoma in our study were of secondary origin, so these cases reflect cases of lymphoma dissemination, and the results have to be interpreted in this context, such as the multiple patterns of lung infiltration in all cases. There was a predominance of peribronchial-perivascular patterns and the vast majority was classified as DLBCL by means of the WHO. Accurate localization and staging of the disease are essential for deciding the treatment strategy.

Declaration of Conflicting Interests

The authors do not have any potential conflicts of interest to declare.

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Table 1. Antibodies used in immunohistochemistry procedures.

Antibody	Antigen retrieval	Dilution	Detection method	Chromongen
Mouse antifeline leukemia virus gp 70 [a]	40 min/100ºC, Tris EDTA buffer pH 9.0	1:500	MACH 4	AEC
Mouse antifeline immunodeficiency	40 min/100ºC, Citrate buffer pH 6.0	1:200	MACH 4	AEC

virus p24gag [a]				
Mouse antihumam CD79αcy [b]	20 min/100ºC, Tris EDTA buffer pH 9.0	1:100	MACH 4	DAB
Polyclonal rabbit antihuman CD3 [b]	15 min/RT Protease XIV	Ready-to-use	MACH 4	AEC

[a]: Bio-rad®; [b]: Dako®; MACH 4 = Universal HRP-Polymer (Biocare®); AEC = 3-amino-9-etilcarbazol (Dako); DAB = 3,3'Diaminobenzene (Dako®), RT: room temperature.

Table 2. Cases of B-cell and T-cell lymphoma that showed lung infiltration (N=16).

Cell type	No. (%) of cases	Median age (month)	Sex ratio (M:F)	FIV ratio (P:N)	FeLV ratio (P:N)	Infiltration pattern				
						Peribronchial-vascular	Nodular	Alveolar	Interstitial	Pleural
B-cell	14 (87.5)	78	7:7	2:12	5:9	13 (92.8)	6 (42.8)	2 (14.2)	7 (50)	8 (57.1)
T-cell	2 (12.5)	57	0:2	1:1	1:1	2 (100)	0	0	1 (50)	1 (50)

Data are given as number (percentage); FIV: feline immunodeficiency virus; FeLV: feline leukemia virus; M: male; F: female; P: positive; N: negative.

Table 3. Histologic subtypes of B-cell lymphomas (n= 14) and T-cell lymphoma (n= 2).

Histologic subtype	No. (%) of cases	Median age (month)	Sex ratio (M:F)	FIV ratio (P:N)	FeLV ratio (P:N)	Infiltration pattern				
						Peribronchial-vascular	Nodular	Alveolar	Interstitial	Pleural
DLBCL	12 (85.7)	78	5:7	2:10	5:7	11 (91.6)	5 (41.6)	2 (16.6)	6 (50)	8 (66.6)
SLBCL	2 (14.2)	108	2:0	0:2	0:2	2 (100)	1 (50)	0 (0)	1 (50)	0 (0)
PTCL	1 (50)	18	0:1	0:1	1:0	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
HTCL	1 (50)	96	0:1	1:0	0:1	1 (100)	0 (0)	0 (0)	0 (0)	0 (100)

Data are given as number (percentage); DLBL: diffuse large B-cell lymphoma; SLBCL: small lymphocytic B-cell lymphoma; PTCL: peripheral T-cell lymphoma unspecified; HTCL: hepatosplenic T-cell lymphoma; F: female; Male: male; P: positive; N: negative.

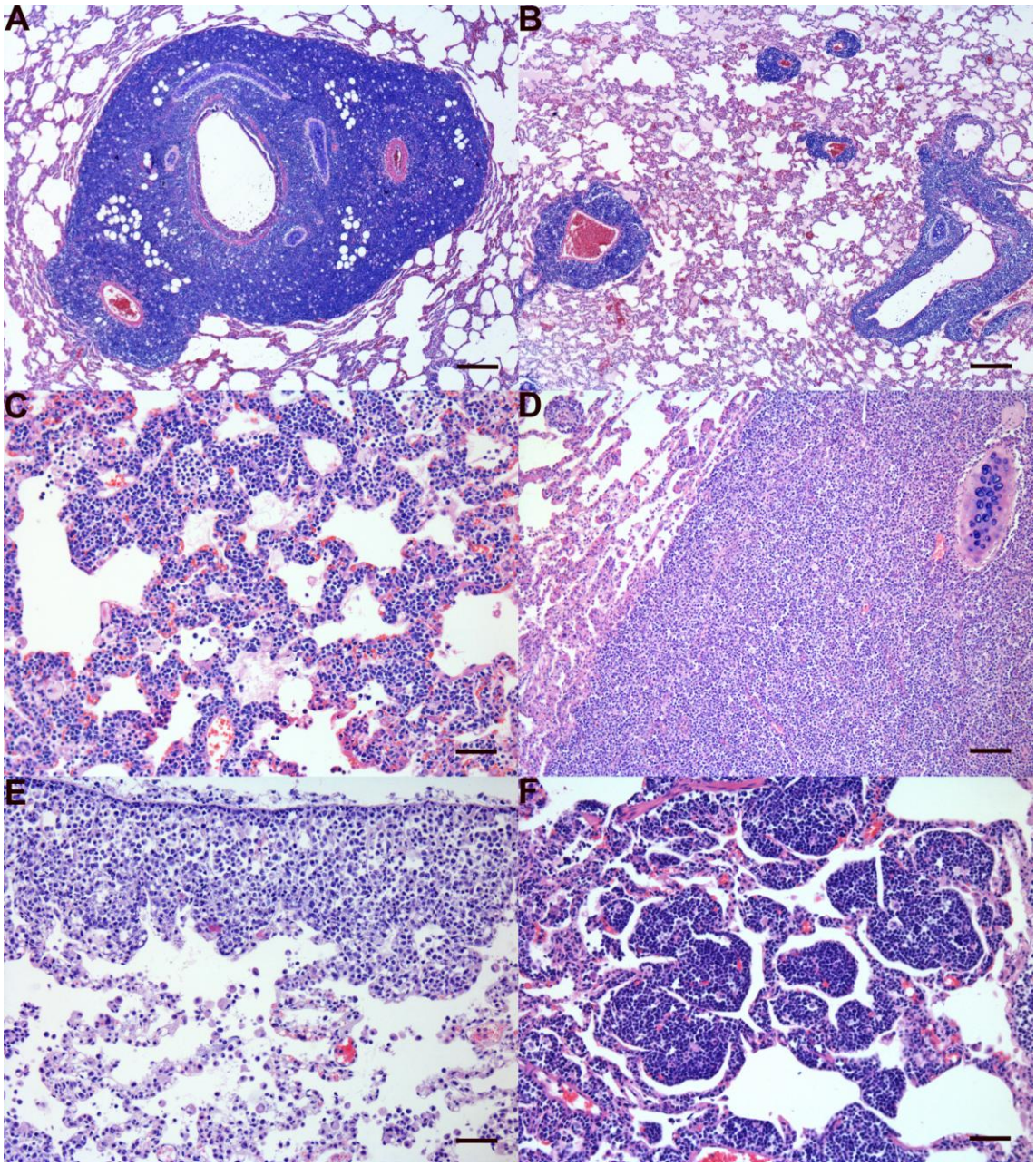

Fig. 1. Feline lymphoma with pulmonary involvement. A. Peribronchial-vascular pattern of lung infiltration, marked infiltrate of neoplastic cells surrounding bronchi and vascular structures. Bar. 330µm, HE. **B.** Peribronquial-vascular pattern of lung infiltration at low power. Bar. 330µm, HE. **C.** Interstitial pattern of lung infiltration, lymphoma cells infiltrate along the alveolar septum that became thickened. Bar. 110µm, HE. **D.** Nodular pattern of lung infiltration. Tumor infiltration consists of a solid mass, forming a nodule. Bar. 230µm, HE. **E.** Pleural infiltration pattern, lymphoma cells are present beneath the pleural, which is visibly thickened. Bar. 110µm, HE. **F.** Alveolar pattern of lung infiltration, lymphoma cells fill the alveolar spaces, conferring a pneumonic aspect to the tissue. Bar. 110µm, HE.

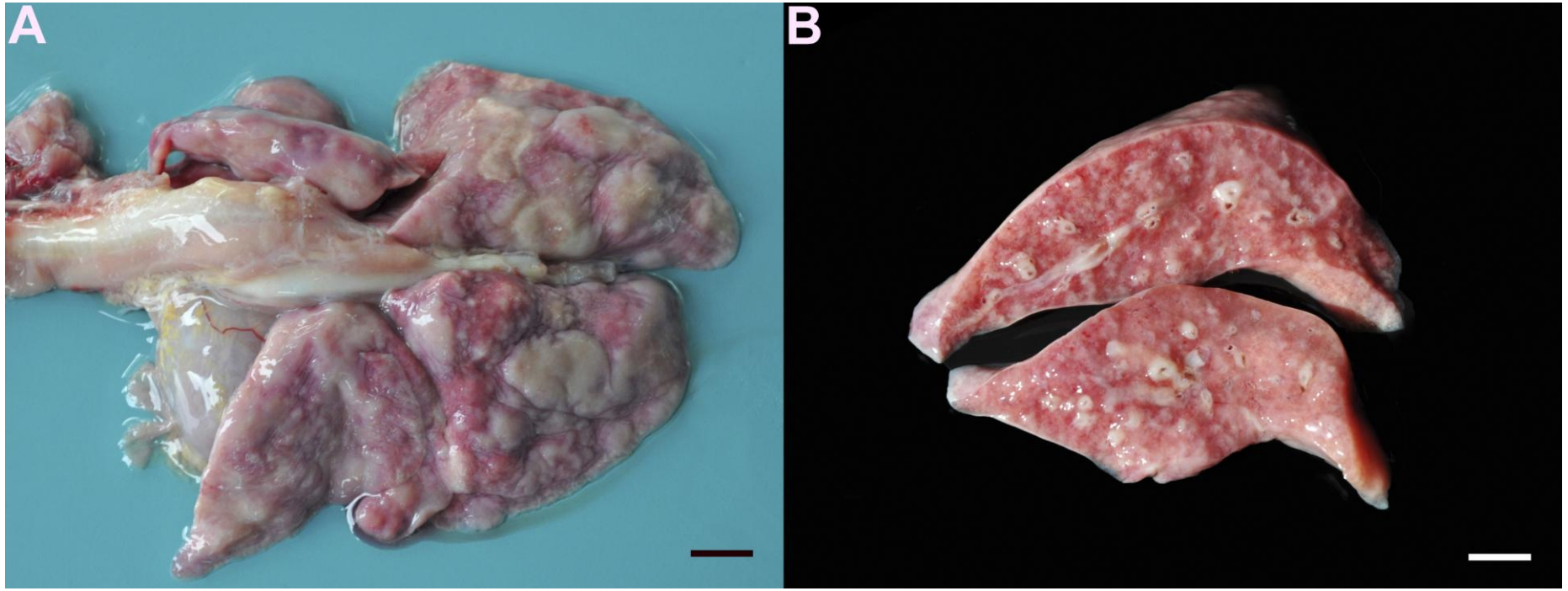

Fig. 2: Feline lymphoma with pulmonary involvement. A. Multiple variably-sized masses of lung infiltration by secondary lymphoma are seen in all lung fields. **B.** Circumscribed, multiple variably-sized nodules by secondary infiltration in lung of lymphoma, also evident with predominance of peribronchial thickening is seen.

4 CONCLUSÃO

O linfoma alimentar foi a forma topográfica mais frequente, com mediana de 140 meses de idade, seguido da forma mediastinal (28%), com mediana de 24 meses de idade. A maioria dos gatos com linfoma alimentar era negativa para FeLV (83%), no entanto no linfoma mediastinal, a grande maioria dos gatos foi identificado como positivo para FeLV (88.5%). O tipo de linfoma mais frequente na forma alimentar foi o linfoma de células T do tipo enteropatia e na forma mediastinal a categoria mais frequente foi o linfoma difuso de grandes células B. Considerando apenas linfomas mediastinais e alimentares, houve associação estatística significativa ($p \leq 0,05$) entre infecção pelo FeLV e as categorias de idade. A prevalência de linfoma mediastinal entre os gatos positivos para FeLV é de 3,21 vezes a prevalência de linfoma mediastinal entre gatos negativos para FeLV. Embora não seja estatisticamente significativo, a prevalência de linfoma de células B entre os gatos positivos para FeLV foi 1,44 vezes a prevalência de linfoma de células B entre os gatos negativos para FeLV.

O envolvimento pulmonar foi observado em 12,8% dos felinos com linfoma, sendo o padrão peribronquial-vascular o mais frequente, seguindo do pleural, intersticial, nodular e alveolar. Entre os linfomas com envolvimento pulmonar, a categoria do linfoma difuso de grandes células B foi a mais frequente totalizando 56% dos casos.

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