UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE VETERINÁRIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

ALTERAÇÕES CLÍNICAS E LABORATORIAIS DE GATOS NATURALMENTE INFECTADOS COM O VÍRUS DA LEUCEMIA FELINA (FeLV) E SUA CORRELAÇÃO COM A CARGA VIRAL E PROVIRAL

Naila Cristina Blatt Duda

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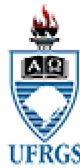

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Orientador: Félix Hilário Diaz González

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ALTERAÇÕES CLÍNICAS E LABORATORIAIS DE GATOS NATURALMENTE INFECTADOS COM O VÍRUS DA LEUCEMIA FELINA (FeLV) E SUA CORRELAÇÃO COM A CARGA VIRAL E PROVIRAL

Aprovada em 08 de março de 2018.

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RESUMO

O vírus da leucemia viral felina (FeLV) é um dos patógenos que mais causa óbito em gatos, tendo ampla distribuição mundial. Os achados mais frequentes no hemograma incluem citopenias diversas decorrentes ou não de distúrbios mieloproliferativos, uma vez que os felinos com infecção progressiva possuem maior risco para desenvolver leucemia ou linfoma. O objetivo do presente trabalho foi identificar as alterações clínicas e laboratoriais na infecção natural pelo FeLV, através da avaliação hematológica, bioquímica e de medula óssea, além de quantificar a carga viral e proviral através da PCR em tempo real (qPCR) de gatos na cidade de Porto Alegre. Foram amostrados 44 gatos domésticos, distribuídos em cinco grupos conforme a antigenemia, achados clínicos e/ou laboratoriais e cargas viral e proviral em: progressivos sintomáticos, progressivos assintomáticos, regressivos, não classificados e saudáveis (grupo controle). As variáveis foram comparadas entre os grupos e correlacionadas com a carga viral e proviral. Dos 44 gatos amostrados, 21 foram positivos no teste de ELISA (47,73%) e 23 foram negativos (52,27%). Dos negativos, 9 gatos (20,45%) apresentaram apenas baixa carga proviral na medula óssea e foram classificados como regressivos. O grupo progressivo sintomático apresentou elevada carga viral e proviral, além de alterações no eritrograma, com diferença significativa dos demais grupos. Também foram observadas variações no mielograma, sobretudo na linhagem mieloide imatura, com diversas alterações displásicas e na contagem celular dos gatos infectados. Correlações moderadas puderam ser observadas entre a carga viral no soro e na medula óssea, e entre a carga viral e proviral na medula óssea. A quantificação das cargas virais e proviral através da qPCR demonstrou ser fundamental para classificação da infecção de acordo com as categorias. Também foi possível identificar um número significativo de animais apenas com carga proviral na medula óssea, demonstrando a importância da investigação de infecção regressiva, principalmente em gatos assintomáticos. Os achados sugerem que elevadas cargas virais e proviral estão relacionadas a alterações clínicas e hematológicas e à gravidade da doença.

Palavras-chave: retrovírus felino; anormalidades laboratoriais; medula óssea; quantificação viral; neoplasia hematopoiética.

CLINICAL AND LABORATORIAL FINDINGS IN CATS NATURALLY INFECTED WITH FELINE LEUKEMIA VIRUS (FeLV) AND THEIR CORRELATIONS WITH VIRAL AND PROVIRAL LOADS

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ABSTRACT

Feline leukemia virus (FeLV) is one of the deadliest pathogens of cats, with a worldwide distribution. The most frequent finding on complete blood count (CBC) include cytopenia, resulting or not from myeloproliferative disorders, since cats with progressive infections have a higher risk of developing leukemia or lymphoma. The aim of the present study was to identify clinical and laboratorial changes in natural FeLV-infected cats through hematological, biochemical and bone marrow evaluation, as well as to quantify viral and proviral load by real-time polymerase chain reaction (qPCR) in Porto Alegre city. Forty-four domestic cats were sampled and classified into five groups according to antigenemia, clinical and/or laboratory findings and viral/proviral loads: symptomatic progressives, asymptomatic progressives, regressive, unclassified, and healthy (control group). Variables were compared between groups and correlated with viral and proviral loads. Of the 44 cats sampled, 21 were ELISA positive (47.73%) and 23 were negative (52.27%). On the negatives, 9 (20.45%) had only low bone marrow proviral load and were classified as regressive. The symptomatic progressive group presented a higher viral and proviral load, besides alterations on the erythrogram, with significant difference from the other groups. Also, it was detected some variation on the myelogram, above all on the immature myeloid lineage, with several alterations on cellular counting and dysplasia on the infected cats. Moderate correlation could be observed between serum and bone marrow viral loads and between bone marrow viral and proviral loads. The quantification of viral and proviral loads by qPCR proved to be fundamental for the classification of the infection according to the categories. It was also possible to identify a significant number of animals with only bone marrow proviral load, demonstrating the importance of investigating regressive infection, especially in asymptomatic cats. These findings suggest that higher viral and proviral loads are related to clinical and hematological disorders and disease severity.

Keywords: feline retrovirus; laboratory abnormalities; bone marrow; viral quantification; hematopoietic neoplasm.

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1 INTRODUÇÃO

O vírus da leucemia felina (FeLV) é um dos patógenos que mais causa óbito em gatos, sendo distribuído amplamente no mundo inteiro (KIM et al., 2014). No Brasil foram relatados prevalência de 47,5% em Minas Gerais (COELHO et al., 2008), 38,3% e 31% no Rio Grande do Sul (MEINERZ et al., 2010; COSTA et al., 2017), 11,52% no Rio de Janeiro (ALMEIDA et al., 2012) e 12,5% em São Paulo (HAHIWARA et al., 1997).

A infecção foi classificada conforme a antigenemia e a carga viral no sangue, em quatro categorias: progressiva, regressiva, abortiva e focal ou atípica (TORRES et al., 2005; HOFMANN-LEHMANN et al., 2008). Na infecção progressiva, se observa uma antigenemia persistente e elevada carga viral e os felinos acometidos sofrem das graves doenças relacionadas ao vírus, vindo a óbito em poucos anos (MEICHNER et al., 2012; WILLET & HOSIE, 2013). Na infecção regressiva, os gatos infectados demonstram antigenemia transitória ou não detectável, e se tornam antigenicamente negativos nos testes de rotina, mas positivos para carga proviral no sangue e tecidos hematopoiéticos (MEICHNER et al., 2012). Esses gatos podem sofrer reativação da infecção latente após imunossupressão, apresentar antigenemia novamente e desenvolver doenças relacionadas com o vírus, uma vez que ocorre a produção de partículas virais completas (HARTMANN, 2012). Inicialmente, as infecções progressivas e regressivas são acompanhadas pela persistência do DNA proviral no sangue sendo detectadas através da reação em cadeia da polimerase (PCR), mas que posteriormente estão associadas com diferentes cargas virais quando mensuradas pelo PCR quantitativo (TORRES et al., 2005; HARTMANN, 2011). Nesse sentido, estudos adicionais que quantifiquem a carga viral e proviral no sangue e em outros tecidos, como a medula óssea, devem ser realizados com objetivo de detectar cada vez mais gatos possivelmente transmissores do vírus.

A utilização de técnicas de maior sensibilidade para detecção e quantificação do vírus, como o PCR quantitativo, possibilita a identificação de animais positivos com a presença de DNA proviral e RNA viral na ausência de antigenemia (FIGUEIREDO & ARAÚJO JÚNIOR, 2011). Essa ferramenta permite classificar as infecções conforme as categorias, auxiliando na determinação do prognóstico e instituição do tratamento adequado.

Além das neoplasias hematopoiéticas e distúrbios mieloproliferativos, outras alterações hematológicas já foram relacionadas com a infecção pelo vírus, como anemia, leucopenia, neutropenia, linfocitose e trombocitopenia (GLEICH & HARTMANN, 2009;

HARTMANN, 2012; WILLET & HOSIE, 2013; KIM et al., 2014). Entretanto, estudos que relacionem essas alterações com a carga viral e proviral nos tecidos hematopoiéticos e no sangue, principalmente em felinos com infecção progressiva e regressiva, ainda não foram realizados na infecção natural. Nesse sentido, essa investigação faz-se necessária para se estabelecer a ocorrência e a relevância dessas alterações no tratamento e no prognóstico de animais infectados, além de possibilitar a identificação de gatos possivelmente transmissores do vírus.

O presente estudo justifica-se pela necessidade de investigação de felinos com infecção regressiva e assintomáticos, porém com alterações hematológicas e de medula óssea. Na prática, observa-se um crescente número de gatos infectados demonstrando diversas manifestações clínicas e evoluindo ao óbito frequentemente. O controle dessa grave enfermidade enfrenta desafios tais como a vacinação e a identificação de animais portadores assintomáticos e negativos para os testes de triagem tradicionais.

2 REVISÃO BIBLIOGRÁFICA

2.1 Vírus da Leucemia Felina

O FeLV é um vírus envelopado, retrovírus RNA de cadeia simples positiva (KIM et al., 2014), classificado como um γ-retrovírus (HARTMANN, 2011), que infecta felinos domésticos e selvagens (DANIELS et al., 1999). O RNA é retrotranscrito em DNA (provírus) e é integrado ao genoma celular (FIGUEIREDO & ARAÚJO JÚNIOR, 2011). O provírus contém sequências repetidas (*Long Terminal Repeat* - LTR) nas extremidades 5' e 3', com função regulatória e de controle da expressão dos genes virais, e entre as LTRs encontram-se os genes *gag* ("group specific antigen"), *pol* (polimerase) e *env* (FIGUEIREDO & ARAÚJO JÚNIOR, 2011), necessários para replicação e formação de partículas virais (DUNHAM & GRAHAM, 2008). Estes codificam proteínas do núcleo do vírus (*gag*), enzimas responsáveis pela replicação do vírus (*pol*) e proteínas de superfície (*env*) (DUNHAM & GRAHAM, 2008). Através da comparação de variações nas sequências do *env,* foi possível se identificar quatro subgrupos do FeLV: A, B, C e T, que utilizam receptores celulares distintos para iniciar a infecção do hospedeiro (JARRET et al., 1973; SARMA & LOG, 1973; DUNHAM & GRAHAM, 2008; LEVY, 2008). O subgrupo A é o subtipo predominante, sendo responsável pela transmissão exógena entre os gatos (JARRETT & RUSSEL, 1978; JARRETT et al., 1978; DUNHAM & GRAHAM, 2008). Os subgrupos B, C e T se originam a partir do FeLV-A através de mutações, inserções e/ou recombinações com sequências endógenas do FeLV (enFeLV), durante a replicação do vírus no animal infectado (LEVY, 2008). Esses subgrupos estão associados com tumores (DONAHUE, et al. 1991; HARTMANN, 2012), anemia aplásica (MACKEY et al., 1975) e imunodeficiência, respectivamente (DONAHUE, et al. 1991; HARTMANN, 2012). Outros subgrupos foram descritos recentemente, tais como o D (ANAI et al., 2012) e o E, sendo esse último relatado como intermediário entre o A e B, decorrente de mutações no gene *env*, possivelmente ocasionadas por diversas etapas de pressão seletiva dos gatos, como por exemplo, a vacinação (MIYAKE et al., 2016).

As formas de contágio incluem contato direto, principalmente pela via oronasal, através da saliva e secreções nasais, que contêm elevada carga viral, durante o processo de lambedura mútua ou compartilhamento de vasilhas de comida e água (KIM et al., 2014). A contaminação também pode ocorrer através do sangue contaminado, motivo pelo qual todos os gatos doadores de sangue devem ser testados (NESINA et al., 2015;

PENNISI et al., 2015; WARDROP et al., 2016). Os fatores de risco para infecção incluem idade jovem, alta densidade populacional, falta de higiene ambiental (LUTZ et al., 2009), comportamento agressivo e co-infecção pelo FIV (GLEICH et al, 2009).

O período de progressão da doença é altamente variável, entretanto, cerca de 50% e 83% dos gatos infectados vão a óbito em dois e três anos após o diagnóstico (HARTMANN, 2012; WILLET & HOSIE, 2013; KIM et al., 2014). Esse período pode ser encurtado dependendo da gravidade das consequências da infecção na medula óssea e no sistema imune do gato. Ainda não existem estudos que estabeleçam exatamente essa sobrevida, bem como a relação com a gravidade dos sinais hematológicos e o subgrupo viral.

A infecção pelo vírus foi classificada em quatro categorias, de acordo com antigenemia e carga viral no sangue periférico: progressiva, regressiva, abortiva e infecção focal ou atípica (TORRES et al., 2005; HOFMANN-LEHMANN et al.; 2008). Na infecção progressiva se observa uma antigenemia persistente e elevada carga viral, em que esses indivíduos padecem das graves consequências relacionadas ao vírus, vindo a óbito em cerca de 2,4 anos (LEVY et al., 2006; MEICHNER et al., 2012; WILLET & HOSIE, 2013). Nesses casos, os gatos positivos podem ser detectados através de testes rápidos, amplamente utilizados na prática clínica veterinária e avaliados quanto a sensibilidade e especificidade. Na infecção regressiva, os gatos infectados mostram uma antigenemia transitória ou não detectável, e se tornam antigenicamente negativos nos testes de rotina, mas positivos para cargas provirais identificadas através da PCR do sangue e tecidos, uma vez que ocorre a integração do DNA proviral do vírus no genoma do hospedeiro (CATTORI et al., 2006). Esses gatos podem sofrer reativação da infecção latente após imunossupressão, apresentar antigenemia e desenvolver doenças relacionadas com o vírus, uma vez que ocorre a produção de partículas virais completas (HARTMANN, 2011). Gatos antigenicamente negativos e que apresentam citopenias periféricas inexplicáveis são frequentemente suspeitos de infecção latente pelo FeLV (SHELTON & LINENBERGER, 1995).

Inicialmente as infecções progressivas e regressivas são acompanhadas pela persistência do DNA proviral no sangue sendo detectadas pela técnica da PCR, mas que posteriormente estão associadas com diferentes cargas virais quando mensuradas por PCR quantitativo (TORRES et al., 2005; HARTMANN, 2012). Na infecção abortiva, não se detecta antígenos ou provírus nos gatos infectados, mas eles se tornam protegidos frente a uma nova infecção e na infecção focal ou atípica, a replicação do vírus é

confinada em certos tecidos, como baço, linfonodos, glândula mamária e intestino delgado (HARTMANN, 2012; MEICHNER et al., 2012). Nesses gatos, a replicação pode levar à produção intermitente ou de baixo grau de antígenos, e, portanto, podem ter resultados fracamente positivos ou discordantes nos testes (HARTMANN, 2012).

2.1.1 Etiopatogenia

Felinos com infecção progressiva possuem 62 vezes mais risco para desenvolver linfoma ou leucemia, pelo efeito direto do vírus na oncogênese (HARTMANN, 2012; MEICHNER, 2012). Existem diversos mecanismos patofisiológicos através dos quais a infecção pelo vírus pode levar a formação de tumores. A oncogênese pode ser ocasionada indiretamente pelos efeitos imunossupressores do vírus ou, ainda, diretamente, através da ativação de proto-oncogenes ou supressão de genes anti-tumorais nos locais de integração do DNA proviral (mutagênese insercional), levando à transformação neoplásica da célula (HARTMANN, 2012; MEICHNER, 2012). Algumas células mantêm sua função intacta, enquanto outras podem acumular algumas proteínas virais (p15E e *gp* 70) no citoplasma e interferir na função. Outro efeito adverso verificado em alguns tipos de FeLV é a falência em integrar com o DNA proviral no genoma felino que leva ao acúmulo de DNA viral no citoplasma que pode desregular a função celular e causar toxicidade (SPARKES & PAPASOULIOTIS, 2012).

O vírus se replica em células que apresentam elevada taxa de multiplicação e possui um tropismo pelas células precursoras hematopoiéticas da medula óssea, além das células das criptas intestinais (DUNHAM & GRAHAM, 2008), e os linfócitos nos folículos linfoides. A infecção na medula óssea leva a uma grande quantidade de vírus reproduzido, sendo muito direcionado para o sistema imune do hospedeiro e, com isso, provocando uma persistente viremia. Na medula óssea, as células da linhagem mielomonocítica desenvolvem uma carga viral, assim como nas células maduras, enquanto nas células precursoras dos eritrócitos essa carga viral permanece nas células não diferenciadas. A viremia está particularmente persistente na medula óssea, embora o vírus possa ser encontrado livre no plasma.

2.1.2 Alterações clínicas e laboratoriais na infecção pelo FeLV

A evolução da infecção e o curso clínico são determinados pela combinação de fatores virais e do hospedeiro (HARTMANN, 2011). Já foram relatadas diversas doenças relacionadas ao FeLV, como neoplasias hematopoiéticas, distúrbios mieloproliferativos, alterações hematológicas, doenças neurológicas, imunomediadas, falhas reprodutivas em fêmeas e numerosas infecções secundárias ocasionadas pelos efeitos supressivos do vírus na medula óssea e no sistema imune (DUNHAM & GRAHAM, 2008; HARTMANN, 2011), como os hemoplasmas (BERGMANN et al., 2017). Essas alterações estão intimamente relacionadas com as propriedades do vírus, onde os subgrupos acabam por determinar diferenças no quadro clínico, sendo que as neoplasias têm sido associadas primariamente com o subgrupo B, enquanto que as anemias não regenerativas estão relacionadas com o subgrupo C (HARTMANN, 2011). Por se tratar de um vírus altamente mutagênico, não existem estudos correlacionando, na infecção natural, todos os subgrupos com as alterações hematológicas, bem como com a gravidade dos sinais hematológicos.

As alterações hematológicas associadas com o FeLV incluem anemia, neutropenia persistente, transitória ou cíclica, anormalidades plaquetárias e pancitopenias (HARTMANN, 2011). Essas alterações podem ser observadas tanto em animais com infecção progressiva devido à replicação viral ativa, quanto em animais regressivos, uma vez que ocorre supressão da medula óssea em ambos as infecções (HARTMANN, 2011).

Diversas alterações na medula óssea podem ser relacionadas com a infecção pelo FeLV, como aplasia, hipoplasia ou hiperplasia das diferentes linhagens hematopoiéticas (megacariocítica, mieloide e eritroide), além de alterações displásicas (diseritropoiese, disgranulopoiese e dismegacariocitopoiese), neoplasias linfoides (HARVEY, 2012) e mielofibrose (HARTMANN, 2011). Essas alterações são comumente observadas nos distúrbios mieloproliferativos, como a síndrome mielodisplásica (SMD) e as leucemias mieloides, que resultam frequentemente em citopenias periféricas (HARVEY, 2012).

2.1.3 Diagnóstico

Diversos meios de diagnóstico para o FeLV estão descritos na literatura como o teste da hemaglutinação passiva, teste de fixação do complemento, ensaio imunofluorescente, ensaio imunoenzimático (ELISA), teste salivar e teste de diagnóstico rápido (KIM et al., 2014). A maioria dos testes utilizados detectam antígenos virais no sangue total, plasma ou soro (BOENZLI, et al., 2014). O alvo antigênico para esses testes

é a proteína p27 do capsídeo viral, que é a proteína mais abundante no plasma de gatos virêmicos. A maior parte dos testes são baseados na imunocromatografia rápida e ELISA, apresentando alta especificidade (cerca de 100%). Entretanto, a sensibilidade desses testes é menor, uma vez que uma parcela dos gatos infectados pelo FeLV (infecções regressivas) será antigenicamente negativa, não sendo detectada por esses métodos. O diagnóstico desses pacientes deve ser feito através de técnicas mais sensíveis, como a PCR e a PCR em tempo real que detectam e, no caso da qPCR, quantificam o DNA proviral do FeLV e o RNA viral, uma vez que o DNA proviral permanece integrado no genoma das células da medula óssea e tecidos linfoides (WILLET & HOSIE, 2013).

A PCR em tempo real pode detectar níveis muito baixos do RNA do vírus no plasma ou secreções, como a saliva, que possivelmente não seriam detectados pelos métodos convencionais antigênicos. Esse teste pode ser importante para confirmação diagnóstica e quantificação da carga viral, quando os testes de detecção de antígenos p27 forem discordantes, fracamente positivos ou limítrofes, podendo sugerir infecção latente na medula óssea (WILLET & HOSIE, 2013).

A utilização de técnicas de maior sensibilidade para detecção e quantificação do vírus, como o PCR quantitativo, possibilita a identificação de animais positivos com a presença de DNA proviral e RNA na ausência de antigenemia (FIGUEIREDO & ARAÚJO JÚNIOR, 2011). Essa ferramenta permite classificar as infecções conforme as categorias, auxiliando na determinação do prognóstico e instituição do tratamento adequado.

3 ARTIGO CIENTÍFICO

Os materiais e métodos, bem como resultados e discussão estão descritos no artigo científico.

Title page – original research

Laboratory and clinical findings and their association with viral and proviral loads in cats naturally infected with feline leukemia virus

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23

24 **Highlights**

25 - Myelodisplastic disorders are associated with higher bone marrow proviral loads
26 - Viral and proviral loads in serum or bone marrow classify the infection stage
27 - Molecular methods to detect feline leukemia virus for endemic areas
28 - Severity of hematological disease depends of leukemia viral and proviral loads

29

30

31 **Abstract**

32 This study was conducted to correlate clinical, laboratory, and bone marrow (BM)
33 changes in cats naturally infected with feline leukemia virus and their association with
34 viral loads in blood and BM and proviral loads in BM. Cats were classified into five
35 groups based on antigenemia, clinical and/or laboratory findings and viral/proviral loads,
36 according to a prospective study: symptomatic progressive (GI); asymptomatic
37 progressive (GII); regressive (GIII); unclassified (GIV); or healthy (GV). |Correlations
38 between these five groups and viral/proviral loads were evaluated. High viral and proviral
39 loads were detected in GI and GII and viral loads were significantly associated with
40 laboratory signs. Proviral loads detected in BM were significantly lower in GIII and GIV.
41 GI cats were more likely to develop hematopoietic disorders than those from the other
42 groups. The findings suggest that higher viral blood and proviral BM loads are related to
43 hematological and clinical disorders and to disease severity.

44

45 **Keywords:** feline retrovirus; laboratory abnormalities; hematopoietic disorders; bone
46 marrow; virus quantification

47

48 **Abbreviations:**

49 ALL, acute lymphocytic leukemia; ALP, alkaline phosphatase; AML, acute myeloid
50 leukemia; AML-M6, erythroleukemia; BM, bone marrow; CBC, complete blood count;
51 ELISA, enzyme-linked immunosorbent assay; FeLV, feline leukemia virus; FIV, feline
52 immunodeficiency virus; Hb, hemoglobin; Ht, hematocrit; IgG, immunoglobulin G; IgM,
53 immunoglobulin M; IMHA, immune-mediated hemolytic anemia; LTR, long terminal
54 repeat; MCHC, mean corpuscular hemoglobin concentration; MDS, myelodysplastic
55 syndrome; MDS-Er, myelodysplastic syndrome with erythroid predominance; MCV,
56 mean corpuscular volume; M:E, myeloid to erythroid ratio; PCR, polymerase chain
57 reaction; qPCR, quantitative real-time PCR; RBC, red blood cell; WBC, white blood cell.
58

59 **1 Introduction**

60

61 Feline leukemia virus (FeLV) infections occur worldwide and account for the
62 majority of deaths among cats [1]. In Brazil, the prevalence of FeLV infections in cats is
63 high, ranging in some studies between 11.5% and 38.5% when ELISA and indirect
64 immunofluorescence are used [2-4] and approximately 47.5% in cases in which blood
65 proviral DNA was detected [5].

66 The stages of FeLV infection have been classified according to antigenemia and
67 to blood viral/proviral loads as abortive, focal or atypical, progressive, or regressive [1,6].
68 In progressive infection, antigenemia and high viral and proviral loads are associated with
69 clinical disease, leading to death within a few years. Immunocompetent cats produce
70 abortive infection since they produce high levels of neutralizing antibodies and do not
71 have antigenemia or viral/proviral loads in their blood. Focal or atypical infection is
72 characterized by localized viral replication with low or intermittent antigen production.
73 In regressive infection, cats present a transient viremia (within weeks or months) which
74 leads a negative result in antigen and viral load tests; however, proviral loads can be
75 identified in blood and hematopoietic tissues. Regressive cats may reactivate infection
76 following immunosuppressive events that culminate in antigenemia and virus-associated
77 diseases. Initially, progressive and regressive infections are characterized by the
78 persistence of proviral DNA in the blood, detected through PCR, but they are later
79 associated with different viral loads when qPCR is performed [7,8].

80 In the bone marrow (BM), FeLV induces several alterations in peripheral blood,
81 such as anemia, neutropenia, leukopenia, platelet disorders, thrombocytopenia,
82 lymphocytosis, pancytopenia, hematopoietic neoplasms, and myeloproliferative
83 disorders [1,9]. Cats with progressive or regressive infections are more commonly

84 affected by BM disorders, since virus-induced BM suppression and integration of proviral
85 DNA into hematopoietic cells may occur [8]. Cats with latent FeLV infection and
86 negative antigen blood test results are strongly suspected of viral infection when
87 unexplained cytopenia is detected in blood examinations [10]. Progressive and regressive
88 infections have not been completely understood and characterized. In endemic areas,
89 studies relating hematopoietic disorders, clinical manifestations associated with
90 molecular tests must be conducted to elucidate the role of viral/proviral loads in blood
91 and BM in naturally infected cats.

92 The aim of the present study was to correlate regressive and progressive FeLV
93 infections with clinical and laboratory findings, including hematological, biochemical,
94 and BM evaluations. In addition, an attempt was made to associate viral and proviral
95 loads with the clinical stages of infection.

96

97

98 **2 Materials and methods**

99

100 *2.1 Animals and inclusion criteria*

101

102 A total of 51 male and female cats older than six months admitted to the Feline
103 Medicine Outpatient Clinic of the Veterinary Teaching Hospital affiliated with the
104 Federal University of Rio Grande do Sul were included in this prospective study. Thirty-
105 three of these cats were clinically healthy; 18 showed FeLV-related disease as defined by
106 clinical and laboratory findings. The definition criteria were: hematological alterations in
107 peripheral blood (anemia, leukopenia, lymphopenia, thrombocytopenia, and
108 leukocytosis); no prior therapy with immunosuppressive drugs, erythropoiesis- or

109 granulopoiesis-stimulating agents, or antimicrobials; and sufficient BM specimen for
110 cytological analysis. The study protocol was approved by the institutional Committee on
111 Animal Care and Experimental Usage (process no. 28928). All cat owners agreed to
112 participate in the study.
113
114
115 *2.2 Blood sample collection and laboratory parameters*
116
117 All selected cats were subjected to clinical and laboratory evaluations, when most
118 of the blood samples were collected. A complete blood count (CBC) was performed using
119 an automated hematology analyzer (ProCyte DX Hematology Analyzer, Idexx
120 Laboratories, USA). White blood cell (WBC) differential counts were performed on all
121 samples. The CBC included red blood cell (RBC) count, hemoglobin (Hb), hematocrit
122 (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration
123 (MCHC), WBC total and differential counts, platelet count, and manual reticulocyte
124 count [11]. The complete differential count consisted of all nucleated cells, including
125 immature mononuclear cells. Cytochemical stains such as myeloperoxidase and Sudan
126 Black B were used to differentiate immature mononuclear cells in blood smears.
127 Serum blood chemistry included albumin, alanine aminotransferase, creatinine,
128 urea, alkaline phosphatase (ALP), glucose, and total proteins, obtained by the
129 colorimetric and enzymatic method performed on an automated spectrophotometer (CM
130 200, Wiener Lab Group, Argentina). Globulin levels were quantified.
131 FeLV antigenemia was determined in all serum samples by a rapid immunoassay
132 for feline immunodeficiency virus (FIV) and FeLV (Snap FIV/FeLV Combo Test, Idexx
133 Laboratories, USA), which simultaneously detects FeLV antigen p27 and FIV antibodies.

134 After the analyses, the remaining sera were stored at -70 °C for later RNA extraction and
135 qPCR.
136
137
138 *2.3 Bone marrow cytology*
139
140 BM aspiration was performed in all cats under general anesthesia and analgesia
141 following the previously described methodology [12]. The BM aspirate was immediately
142 placed on a Petri dish for the selection of spicules and squash preparation. The residual
143 BM specimen was stored in microtubes and kept at -70 °C for a maximum of 24 months
144 for RNA and DNA extraction. All BM smears were stained with Wright-Giemsa and the
145 microscopic analysis followed previously described guidelines [12]. Cytological
146 examination consisted of the evaluation of general cell population and bone spicules
147 under a low-power objective (10x), followed by a differential count from 500 to 1,000
148 nucleated cells and morphological analysis under high-power objectives (100x in
149 immersion oil). The overall degree of cellularity, content of particles (iron stores,
150 megakaryocytes, stromal cells, hematopoietic cells, and other cells), myeloid to erythroid
151 ratio (M:E ratio), sequence and completeness of maturation order, followed by
152 morphological evaluation of hematopoietic lineages, were determined for all BM
153 specimens. Counts of all nucleated cells and non-erythroid cells and of other cell types
154 (lymphocytes, plasma cells, mononuclear phagocytes, mast cells, and neoplastic cells)
155 were obtained. BM cytological findings were interpreted with a recent CBC obtained up
156 to 24 hours prior to sampling. Complementary cytochemical staining, with either
157 myeloperoxidase or Sudan Black, was performed, whenever necessary, to identify the
158 origin of undifferentiated nucleated cells [12].

159

160

161 *2.4 Molecular analysis*

162

163 Total RNA from serum and BM samples was extracted using TRIzol (Invitrogen),
164 following the manufacturer's instructions. In addition, genomic DNA was extracted from
165 BM samples using PureLink DNA Mini Kit (Invitrogen). DNA quantification was
166 performed with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific). The
167 extracted RNA and DNA were immediately stored at -80 ºC and -20 °C, respectively.
168 First, to qPCR and RT-qPCR, a standard curve was constructed by cloning a FeLV
169 amplicon into a pCR2.1 vector from TOPO TA Cloning vector (Invitrogen), according to
170 the manufacturer's instructions. The amplicon was obtained by conventional PCR using
171 the following primers: FeLV_U3-F (2): 5'-GCTCCCCAGTTGACCAGAGT-3 '[13] and
172 FeLV_U3-exo_r: 5'-TTTATAGCAGAAA GCGCGCG -3'[14]. The target region is a 93-
173 base pair (bp) fragment in the U3 region of FeLV's LTR [13]. Competent *E. coli* (NEB
174 10-beta) were used for transformation and plasmid DNA was extracted according to
175 standard methods [15]. In order to confirm the FeLV clone insertion, the clones were
176 sequenced and quantified using Qubit Fluomer (Thermo Fisher Scientific). Tenfold serial
177 dilutions (10^8 - 10^0) were used for plotting the standard curves of amplification.
178 Viral and proviral genome loads were evaluated by qPCR. For viral load
179 evaluation, RNA extracted from serum and BM samples was subjected to cDNA
180 synthesis. Reactions were performed with 10 µL of RNA, 200 µM of dNTPs, 0.2 µM of
181 random primers, 1 U of Invitrogen SuperScript reverse transcriptase, and water q.s.p. 20
182 µL. Cyclings were carried out in an Epperdorf Mastercycler Personal following the

183 manufacturer's instructions.

184 The viral genome loads were determined by qPCR as follows: fifteen microliters
185 containing 3 μL of standard curve or 2 μL of cDNA, 1X Fast EvaGreen qPCR Master
186 Mix (Biotium, USA), 0.2 μM of each primer (FeLV_U3-F (2) and _FeLV_U3-exo_r),
187 1X ROX, and water up to the final volume. The reactions were performed in a
188 StepOnePlus Real-Time PCR system (Applied Biosystems, Life Technologies) under the
189 following conditions: initial denaturation at 95 ºC for 2 min, followed by 45 cycles of
190 amplification (5 s at 95 ºC and 30 s at 60 ºC) and melting curve analysis range extending
191 from 60 °C to 95 °C. All reactions were performed in triplicate and the results were
192 reported as means (copies/mL). Fluorescent measurements were carried out during the
193 elongation stage and a threshold cycle (Ct) value was calculated by comparison with the
194 standard curve.

195 The proviral load of the DNA extracted from BM samples (100 ng) was evaluated
196 by qPCR under the same conditions described above.

197

198

199 *2.5 Groups and statistical analysis*

200

201 Based on antigenemia, serum and BM viral loads and BM proviral loads, and
202 presence or absence of clinical signs, the cats were distributed into five groups: group I
203 (GI) corresponded to cats with symptomatic progressive infection (antigenemia, high
204 viral and proviral loads, and clinical signs); GII refers to samples from cats with
205 asymptomatic progressive infection (antigenemia, high viral and proviral loads, and
206 absence of clinical signs); GIII refers to cats with regressive infection (absence of
207 antigenemia and viral load, presence of proviral load, with or without clinical signs); GIV

208 corresponded to cats with unclassified infection (absence or presence of antigenemia, low
209 viral and proviral loads, with or without clinical signs); and GV, which included samples
210 from cats with no signs of infection (absence of antigenemia and of viral and proviral
211 loads).
212 To statistics analysis, Kolmogorov-Smirnov test was used to determine the
213 normality of data. Asymmetrically distributed variables were described as median,
214 minimum and maximum, and the Kruskal-Wallis test was utilized for between-group
215 comparison. Those variables with a symmetric distribution were described as mean and
216 standard deviation and the comparison between groups was made by ANOVA. Tukey's
217 post-hoc test was used whenever significant differences were found. A logarithmic
218 transformation was performed for asymmetric variables before the post-hoc test.
219 Spearman's correlation coefficient was used for quantitative variables. SPSS v20.0 was
220 used for the analysis and the level of significance was set at 5%.
221
222
223 **3 Results**
224
225 *3.1 Cats and clinical signs*
226
227 Forty-four out of 51 cats were selected for this study. Seven of them were
228 excluded due to lack of sufficient material for complete BM cytology (n=2), death before
229 BM sampling (n=2), hemolytic anemia unrelated to FeLV infection (n=2), and positivity
230 for FIV (n=1). Thirty-one cats were female (70.5%) and 13 were male (29.6%). Mean age
231 was 2.5 years (6 months to 11 years).
232 Considering clinical signs, cats with symptomatic progressive infection (GI)

233 presented with nonspecific clinical signs, such as apathy, dyspnea, coughing, hyporexia,
234 anorexia, loss of appetite, progressive weight loss, or oral lesions. Clinical signs such as
235 apathy, hyporexia, and progressive weight loss were found in one cat with regressive
236 infection (GIII) and in one cat from GIV (unclassified infection).

237

238

239 *3.2 Blood and bone marrow parameters*

240

241 Symptomatic cats from GI presented nonregenerative anemia, among other
242 individual alterations in peripheral blood in CBC and WBC count (Table 1). Individual
243 biochemical changes varied slightly, with groups presenting differences only in creatinine
244 and glucose levels (Table 2).

245 BM cytology interpretation, based on previous studies [12] and classifications
246 [16,17] (Table 3), revealed that cats from GI, GIII, and GIV presented myelodysplastic
247 syndrome with erythroid predominance (MDS-Er; n=3), immune-mediated hemolytic
248 anemia (IMHA; n=3), acute lymphocytic leukemia or leukemic lymphoma (ALL; n=2),
249 erythroleukemia (AML-M6; n=1), and hypereosinophilic syndrome (n=1). A difference
250 was observed between GII and GV in the percentage of immature myeloid cells (P =
251 0.027).

252 Antigenemia was detected in 21/44 cats (47.7%) and one cat presented a weakly
253 positive result. According to molecular analysis, FeLV RNA was detected in the serum
254 of 22/44 cats (50%) and in the BM of 24/44 (54.5%) cats, whereas FeLV DNA was found
255 in the BM of 34/44 (77.3%) cats. The viral and proviral loads differed between the groups
256 (Table 4).

257

258

259 *3.3 Molecular analysis*

260

261 Serum viral loads were higher in GI cats (P = 0.009). Differences were also
262 observed for BM viral loads between GI and GIV and between GII and GIV (P = 0.007).
263 BM proviral loads were higher in GI and GII than in GIII and GIV (P < 0.001).
264 GI and GII cats presented antigenemia associated with high serum and BM viral
265 loads and high BM proviral loads. Four cats from IV were negative for antigenemia with
266 a low viral in serum or BM and with a low BM proviral load. One cat from GIV was
267 weakly positive in the rapid immunoassay test, with low serum and BM viral loads
268 (105.92 and 40.8 copies/mL, respectively) and low BM proviral load (79.15 copies/mL).
269 Considering all studied cats, a moderate correlation was observed between viral
270 loads in serum and BM (R= 0.574; P = 0.006) and between viral and proviral loads in BM
271 (R= 0.568; P = 0.004). Blood parameters showed a moderately positive correlation
272 between serum and BM viral loads with the WBC count (R= 0.625; P = 0.002 and R=
273 0.420; P = 0.041, respectively). In biochemical parameters, a moderately negative
274 correlation was found between BM viral and proviral loads and glucose (R=-0.442; P =
275 0.030 and R=-0.539; P = 0.001, respectively) and between BM proviral load and ALP
276 (R=-0.423; P = 0.013) and albumin (R=-0.466; P = 0.006). Correlation was found
277 between BM proviral load and globulins (R= 0.342; P = 0.047). BM parameters showed
278 a moderately negative correlation between serum viral load and plasma cells (R=-0.468;
279 P = 0.028) and a moderately positive correlation between proviral load and percentage of
280 immature myeloid cells (R=0.402; P = 0.018).

281

282

283 **4 Discussion**

284

285 Most laboratory and clinical findings on FeLV natural infection are correlated
286 with higher viral and proviral loads in serum and BM. Our study was the first to correlate
287 hematological disorders and BM cytology with FeLV loads. Regressive and progressive
288 infections are a frequent consequence after natural infection of cats with FeLV. We
289 attributed all abnormal hematological findings in peripheral blood to a FeLV natural
290 infection, particularly to a progressive stage. Nonregenerative anemia and
291 thrombocytopenia, as well as other hematopoietic disorders, are mainly caused by the
292 suppressive effect of the virus on the BM, resulting from infected hematopoietic stem
293 cells and BM stromal cells [8].

294 First, we suggest that all findings associated with erythroid lineage observed in
295 FeLV-positive cats are multifactorial and related to IMHA, inflammation, and
296 hematopoietic neoplasms [1]. Even though the Coombs test was not performed, four cats
297 from GI, GIII, and GIV, presented hematological manifestations suggestive of IMHA
298 [18]. Nonregenerative to poorly regenerative macrocytic anemia, icteric plasma,
299 dyserythropoiesis, erythrophagocytosis, mild plasmacytosis, and iron stores present in
300 BM cytology could be associated with IMHA. The actual mechanism that triggers IMHA
301 in FeLV-positive cats has not been completely elucidated. Nonspecific increase of
302 immunoglobulins, loss of T-cell activity, and antigen antibody complexes can lead to
303 overactive or dysregulated immune response in FeLV infection. These mechanisms
304 leading to immune-mediated disorders include IMHA [8].

305 Considering all selected cats, lymphopenia concomitant with lymphoid
306 hypoplasia was not associated with FeLV loads. Most cats from GIV, which presented
307 low viral and proviral loads and variable antigenemia, presented with lymphopenia and

308 lymphoid hypoplasia, suggesting immune system involvement, regardless of the viral
309 load in blood and BM. In FeLV infection, the virus could affect lymphoid organs such as
310 thymus, lymph nodes, and BM, leading to atrophy and depletion of important zones that
311 are responsible for lymphocyte production, specifically of T-cells [19]. Our study
312 demonstrates that the number of viral/proviral particles in the BM does not have any
313 influence on this mechanism, since all groups demonstrate lymphoid hypoplasia. In
314 clinical practice, lymphopenia associated with neutropenia plays an important role,
315 increasing the predisposition to secondary infection, which could deteriorate the clinical
316 status and lead to death quickly.

317 Our study suggests hematopoietic disorders in one or more BM cell lineages
318 associated with peripheral cytopenia are frequent in FeLV naturally infected cats. Since
319 that the FeLV replies in hematopoietic and BM accessory cells, the proposed mechanism
320 is related to viral insertional mutagenesis, which triggers the neoplastic transformation of
321 these cells [1,20]. Consequently, there are myeloproliferative disorders characterized by
322 hematopoietic tissue replacement with bone marrow suppression, resulting in peripheral
323 cytopenia and dysplastic changes in distinct cell lineages [1]. Myelodysplastic syndromes
324 (MDS), as a pre-stage of acute myeloid leukemia (AML), are related to a mutagenesis
325 that promotes myeloid dysfunction followed by suppression of myeloid differentiation
326 and proliferation [21]. Our study demonstrates that positive cats with BM disorders
327 presented with dysplastic changes in blood and BM. Macrocytic erythrocytes without
328 reticulocytosis observed in four cats were associated with viral infection. This alteration,
329 observed in peripheral blood, is associated with incomplete cell division in the BM and
330 is considered an important finding related to ineffective erythropoiesis in cats with MDS
331 [22].

332 Correlations between BM proviral loads and globulin and albumin levels, possibly

333 associated with increased IgG and IgM, have been found in a previous study [23].
334 Additionally, the moderate positive correlation between proviral loads and percentage of
335 immature myeloid cells in BM may be explained by the presence of effective myeloid
336 hyperplasia, which results in peripheral neutrophilia. The moderate positive correlations
337 found between serum and BM viral loads with WBC count may also be explained by
338 peripheral neutrophilia combined with lymphocytosis, verified in leukemic conditions.
339 The moderate positive correlation for viral loads in serum and BM and between viral
340 loads and proviral loads in BM suggests some relationship between viremia and the
341 presence of the virus in BM, in addition to active integration of the DNA hematopoietic
342 cells.

343 So far, few studies have assessed the relationship between BM proviral loads,
344 related to FeLV infection and hematological outcome. One study on FeLV naturally
345 infected cats did not identify the presence of regressive infection [24]. Two studies
346 conducted in England and Switzerland reported that 9.3% and 10%, respectively, were
347 positive only for DNA proviral load in blood [25,26], but there was no attempt to detect
348 proviral particles in the BM. This finding highlights the importance of using qPCR to
349 detect animals with regressive infection [7] in selected populations, especially in endemic
350 areas, since apparently healthy cats that are negative for antigenemia can be infected
351 through blood transfusion [27,28]. Additionally, cats may present reactivation of latent
352 infection after immunosuppression and antigenemia, and then develop virus-related
353 diseases [1].

354 No discordant results were found between serum antigen detection and qPCR. All
355 positive cats in the immunoassay presented viral and proviral loads in qPCR (n=21).
356 Likewise, all cats without concomitant viral and proviral loads were negative in the
357 immunoassay (n=10). A weak reaction to qualitative ELISA could be observed in one cat

358 that demonstrated low viral load in blood (105.92 copies/mL), but the viral load was
359 higher than that of another cat (71.04 copies/mL) with undetectable antigenemia in the
360 test. Absence of antigenemia associated with the proviral load detected by conventional
361 PCR has been already described in naturally infected cats [26]. A previous study has
362 suggested that the cats were either in the acute phase of the infection or did not have the
363 ability to induce antigenemia in persistent infection. In agreement with other studies
364 [7,25,26], a significant share of cats with proviral load was found through qPCR and
365 undetectable antigenemia (13/44), in addition to some cats presenting viral loads in the
366 serum and/or BM with undetectable antigenemia (4/44). Considering these studies and
367 the data presented, molecular methods for FeLV detection in blood and BM must be
368 performed in endemic areas to detect carrier cats and to control infection in the cat
369 population since clinical signs are nonspecific.

370 Actually, the classification of infection stages based on blood and BM proviral
371 loads has not been used in any study. Due to the absence of threshold values of viral and
372 proviral loads in the literature for groups classification in the present study, five cats were
373 allocated to a distinct group, as they could be classified through previous studies as
374 regressive, focal, or atypical. Cats with low viral and proviral loads must be re-evaluated
375 to verify whether the disease progressed or the infection was eliminated, because cats can
376 fight off viremia within 2 to 16 weeks [29]. Therefore, qPCR is important for assessing
377 the course of infection in naturally infected cats and can indicate whether there is a need
378 for additional tests or whether routine tests should be repeated in negative or suspected
379 cats, especially among blood donors, breeders, or cats living with other cats, particularly
380 kittens.

381 The quantitative results of viral loads found in blood were similar to those reported
382 by previous studies for FIV-positive cats [30, 31]. Clinically sick animals older than 5

383 years with hematopoietic disorders presented plasma viral loads significantly higher than
384 those observed in healthy cats with no clinical signs [30], while viral loads increased as
385 the disease progressed [31]. In one study that monitored viral and proviral loads in the
386 tissues of cats experimentally infected with FeLV, the highest values were observed in
387 cats with viremia, followed by healthy cats, and the lowest proviral loads were observed
388 in regressive cats [32]. But no studies on the viral and proviral loads of cats naturally
389 infected with FeLV have assessed the progress of the disease or of its symptoms. It has
390 been recently reported that antigenemia is correlated with DNA loads in blood [33], but
391 the relationship with clinical and hematological symptoms is still unknown.

392 A limitation of this study was the low adherence of cat owners to BM cytology,
393 because of the risk of anesthesia among cats with poor clinical/laboratory parameters.
394 Another limitation was the failure to assess the viral and proviral loads of GIV cats
395 considered unclassified based on the molecular parameters. These factors should be
396 considered in further studies to obtain information about carrier status or development of
397 disease and about the syndromes associated with FeLV infection.

398

399

400 5 Conclusions

401

402

403 The detection of proviral loads in BM using qPCR is important for the detection
404 of animals that persistently test positive or have regressive infection, especially
405 asymptomatic cats in endemic areas. Additionally, quantifying viral and proviral loads is
406 essential for classifying the infection and implementing proper clinical management of
407 patients and follow-up of focal or regressive infections considering their potential clinical

408 manifestations and potential reactivation of infection and subsequent spread of the disease
409 to other cats.
410
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413
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546

547 **Tables**

548

549 **Table 1. Median (range) and mean ± standard deviation of hematological values**
550 **from FeLV-infected cats according to group classification based in clinical signs,**
551 **antigenemia and viral/proviral loads.**

552

553 **Table 2. Median (range) and mean ± standard deviation of biochemical values**
554 **from FeLV-infected cats according to group classification based in clinical signs,**
555 **antigenemia and viral/proviral loads.**
556
557 **Table 3. Median (range) and mean ± standard deviation of bone marrow cytology**
558 **from FeLV-infected cats according to group classification based in clinical signs,**
559 **antigenemia and viral/proviral loads.**

560

561 **Table 4. Median (range) of proviral (DNA loads), viral loads in serum (RNA – SR**
562 **loads) and bone marrow (RNA – BM loads) per mL of blood or bone marrow and**
563 **presence of antigenemia and clinical signs of the five groups of cats.**

564

565

Table 1. Median (range) and mean ± standard deviation of hematological values from FeLV-infected cats according to group classification based in clinical signs, antigenemia and viral/proviral loads.

Parameter	Reference*	GI (symptomatic progressive, n=10)	GII (asymptomatic progressive, n=10)	GIII (regressive, n=9)	GIV (unclassified; n=5)	GV (control, n=10)	*P* value
RBCs (10^6/µL)	6-10	3.8 ±2.5[a]	9.1 ± 1.2[b]	8.2 ± 2.3[b]	6.5 ± 3.34[a,b]	8 ± 1.39[b]	< 0.001
Hb (g/dL)	8-15	6.1 ± 3.4[a]	12.7 ± 1.6[b]	11.4 ± 2.7[b]	9.6 ± 4.7[a,b]	10.6 ± 1.5[b]	< 0.001
Ht (%)	24-45	19.6 ± 10.3[a]	39.6 ± 4.0[b]	35.8 ± 7.8[b]	30.2 ± 14.5[a,b]	33.8 ± 4.6[b]	< 0.001
MCV (fL)	39-55	54.7 (43.1 -78.6)[a]	43.4 (41.1 -48.9)[b,c]	44 (40-60.9)[b,c]	46.1 (44.3-65.4)[a,b]	42.6 (37-48.8)[c]	0.002
MCHC (%)	31-35	30.4 ± 1.8	31.9 ± 1.4	31.6 ± 1.1	31.7 ± 1.1	31.4 ± 0.8	0.106
TPPs (g/L)	60-80	70.6 ± 8.1	76.8 ± 10.4	74.7 ± 5.3	70.4 ± 16.3	68.2 ± 4.8	0.221
Aggregate reticulocytes (%)	0-0.4	0.35 (0 - 7.2)	0.2 (0.1-0.2)	0.2 (0.1-0.4)	0.3 (0.1-1.7)	0.2 (0.1-0.5)	0.169
Aggregate reticulocytes (10^3/µL)	≤ 60	25.2 (0-1100)	14.9 (8.5-23.0)	16.7 (6.7-29.2)	15.8 (7.3-35.9)	16.4 (8.1-28.2)	0.383
nRBCs	0 - 3	0.5 (0-24)[a]	0 (0-0)[b]	0 (0-1)[a,b]	0 (0-61)[a,b]	0 (0-0)[b]	0.015
Platelet count (10^3/µL)	200-800	195.2 ± 151.6	245.2 ± 97.2	277.2 ± 100.1	317.7 ± 181.1	317.9 ± 97.1	0.249
WBCs (/µL)	5000-19500	18300 (7200-437000)[a]	10700 (7500-17600)[b]	13900 (4300-17500)[a,b]	9900 (7500-10800)[b]	11000 (7500-18800)[a,b]	0.019
Band neutrophil (/µL)	0-300	0 (0-4370)	0 (0-225)	0 (0-119)	0 (0-101)	0 (0-188)	0.614
Segmented Neutrophils (/µL)	2500-12500	9898.2 ± 5232.8	7705 ± 2140.4	7841 ± 3711.3	6680.6 ± 1068.1	7999.2 ± 3869.7	0.529
Eosinophils (/µL)	100-1500	286 (0-36080)	534 (75-1898)	595 (0-5053)	525 (0-891)	673 (110-3388)	0.903
Lymphocytes (/µL)	1500-7000	3011 (762-423890)	2061 (760-5808)	3094 (1333-8225)	1386 (1020-3996)	1985 (1218-7062)	0.471
Monocytes (/µL)	0-850	494 ± 402.6	271.8 ± 160.6	298.7 ± 174.6	247.4 ± 104.2	265.3 ± 210.2	0.198

RBCs - red blood cells; Hb - hemoglobin; Ht - hematocrit; MCV - mean cell volume; MCHC - mean cell hemoglobin concentration; TPPs - total plasma proteins; nRBCs - nucleated red blood cells; WBCs - white blood cells. Different letters indicate significantly difference between groups, using to Kruskal-Wallis or ANOVA to parametric values. *Reference values from laboratory.

Table 2. Median (range) and mean ± standard deviation of biochemical values from FeLV-infected cats according to group classification based in clinical signs, antigenemia and viral/proviral loads.

Parameter	Reference*	GI (symptomatic progressive, n=10)	GII (asymptomatic progressive, n=10)	GIII (regressive, n=9)	GIV (unclassified; n=5)	GV (control, n=10)	*P* value
Albumin (g/L)	21-33	28.7 ± 3.3	29 ± 1.7	31 ± 2.1	29.4 ± 7.64	31.3 ± 1.9	0.313
ALT (U/L)	< 83	96 (5-2372)	58 (3-78)	26 (0-77)	18 (1-1752)	66 (27-101)	0.333
Creatinine (mg/dL)	0.8-1.8	0.9 ± 0.3[a]	1.0 ± 0.3[a,b]	0.9 ± 0.25[a]	1.4 ± 0.6[b]	0.9 ± 0.1[a]	0.014
ALP (U/L)	< 93	34 (0-1262)	35 (0-187)	48.2 (24-244)	42.2 (0-43.6)	57.9 (27.3 -141)	0.229
Glucose (mg/dL)	73-134	93.8 ± 42.1[a,b]	86.4 ± 12.7[a]	123.7 ± 27.3[a,b]	140.6 ± 20.9[b]	103.4 ± 42.5[a,b]	0.019
Urea (mg/dL)	32-54	52.3 ± 28.9	57.9 ± 11.3	52.3 ± 10.1	52.3 ± 10.1	56.3 ± 11.8	0.943
TP (g/L)	54-78	71.9 ± 5	77.1 ± 9.1	72.9 ± 5.0	71.4 ± 15.1	74.6 ± 8	0.605
Globulins (g/L)	27-50	43.2 ± 5	48.1 ± 9.9	41.9 ± 5.8	42 ± 9.6	43.3 ± 8.7	0.404

ALT = alanine aminotransferase; ALP = alkaline phosphatase; TP = total protein. Different letters indicate significantly difference between groups, using to Kruskal-Wallis or ANOVA to parametric values. *Reference values from laboratory.

Table 3. Median (range) and mean ± standard deviation of bone marrow cytology from FeLV-infected cats according to group classification based in clinical signs, antigenemia and viral/proviral loads.

Parameter	Reference[*]	GI (symptomatic progressive, n=10)	GII (asymptomatic progressive, n=10)	GIII (regressive, n=9)	GIV (unclassified; n=5)	GV (control, n=10)	*P* value
Cellularity (%)	25-75	87.5 (50-95)	75 (0-75)	75 (25-95)	50 (0-85)	50 (50-80)	0.052
Fat (%)	25-75	12.5 (5-50)	25 (0-50)	25 (5-75)	50 (0-50)	50 (20-50)	0.090
Megakaryocytes (field/10x)	> 5	2.2 ± 2	2.8 ± 1.6	2.6 ± 2.5	1.9 ± 1.4	2.9 ± 1.8	0.844
M:E ratio	1.21-2.16	0.91 ± 0.8	1.1 ± 0.5	0.9 ± 0.6	0.7 ± 0.4	0.8 ± 0.5	0.642
Immature erythroid cells (%)	0-2.4	3.9 (0-14)	2.7 (0-5.7)	2 (0.7-4.7)	2 (0-11.7)	1.8 (0.3-7.6)	0.274
Mature erythroid cells (%)	11.2-39.8	35.3 ± 21.5	34.7 ± 14.2	47.4 ± 16.9	35 ± 20.6	51.7 ± 14.4	0.117
Immature myeloid cells (%)	0-3.4	3.2 ± 2[a,b]	3.8 ± 2.9[a]	1.7 ± 0.9[a,b]	2 ± 1.2[a,b]	1.4 ± 0.4[b]	0.027
Mature myeloid cells (%)	24.6-59.8	28.4 ± 19.0	40.9 ± 15.8	37.4 ± 16.5	29.6 ± 17.5	34.6 ± 12.3	0.459
Lymphocytes (%)	11.6-21.6	8.5 (2.5-90)	4.2 (0-10.4)	8 (3.1-21.2)	7.3 (0-10)	5.4 (1.7-22.6)	0.312
Plasma cells (%)	0.2-1.8	0.4 (0-5.4)	1.1 (0-4.9)	0.2 (0-3.5)	1.6 (0-2.7)	1 (0.2-2)	0.239
Macrophages (%)	0-0.2	0.1 (0-0.5)	0.3 (0-0.8)	0.2 (0-0.4)	0.6 (0-0.8)	0.2 (0-1)	0.226
Monocytes (%)	0.2-1.6	0.9 ± 0.6	0.9 ± 0.6	0.9 ± 0.5	1.1 ± 0.9	0.6 ± 0.3	0.413
Eosinophils (%)	0.8-3.2	1.7 (0.2-42.2)	2.2 (0 -5.8)	2.7 (0-7.8)	2.2 (0-3.5)	2.4 (1.7-5.5)	0.786

M:E - myeloid:erythroid ratio. Different letters indicate significantly difference between groups, using to Kruskal-Wallis or ANOVA to parametric values. [*]R.E. Raskin, J.B. Messick, Bone marrow cytologic and histologic biopsies: indications, technique and evaluation, Vet. Clin. North Am. Small Anim. Pract. 42 (1) (2012) 23-42. https://doi.org/10.1016/j.cvsm.2011.10.001.

Table 4. Median (range) of proviral (DNA loads), viral loads in serum (RNA – SR loads) and bone marrow (RNA – BM loads) per mL of blood or bone marrow and presence of antigenemia and clinical signs of the five groups of cats.

Group	n	DNA loads	RNA-SR loads	RNA-BM loads	Antigenemia
I	10	$6.52x10^6$ ($2.06x10^5$ – $3.69x10^7$)[a]	2.42 $x10^6$ ($1.62x10^4$ - $6.68x10^9$)[a]	$6.26x10^6$ ($1.62x10^4$ – $3.82x10^8$)[a]	Present
II	10	$1.08x10^7$ ($1.04x10^6$ – $5.95x10^7$)[a]	$3.26x10^5$ ($1.17x10^4$ – $7.4x10^6$)[b]	$4.38x10^6$ ($2.33x10^6$ – $7.72x10^6$)[a]	Present
III	9	67.7 (33.05-568.65)[b]	Absent	Absent	Absent
IV	5	96.55 (36.85-218.95)[b]	88.48 (71.04-105.92)[b]	48.24 (32.68-128.92)[b]	Absent/Present
V	10	Absent	Absent	Absent	Absent
P value		< 0.001	0.009	0.007	

Different letters in columns indicate significantly using Kruskal-Wallis. Groups: I – symptomatic progressive; II – asymptomatic progressive; III – regressive; IV – unclassified; V – control cats.

5 CONSIDERAÇÕES FINAIS

Esse estudo permitiu verificar diversas alterações hematológicas e de medula óssea de felinos com infecção natural pelo FeLV. A quantificação da carga viral e proviral foi fundamental para a classificação da infecção conforme as categorias em progressivos e regressivos, sendo observado um número elevado de animais com positividade apenas na medula óssea. A identificação desses animais é extremamente importante, uma vez que podem transmitir a infecção para felinos saudáveis através de transfusão sanguínea e sofrer reativação da infecção após imunossupressão, desenvolvendo diversas doenças relacionadas com o vírus. Na literatura, não foi encontrado nenhum estudo que avaliasse a correlação da carga viral e proviral com as alterações hemato-bioquímicas e de medula óssea de felinos com infecção natural pelo FeLV, fazendo deste trabalho um estudo pioneiro.

A correlação entre a carga viral no soro e a carga viral na medula óssea, e entre a carga viral na medula óssea e carga proviral, sugerem que há relação entre a viremia e a presença do vírus na medula óssea, além de integração ativa no DNA das células hematopoiéticas. Os gatos com infecção progressiva e com elevadas cargas viral e proviral demonstraram diversas alterações hematológicas e sinais clínicos, sugerindo que a gravidade da doença possa estar associada a várias partículas virais no sangue e na medula óssea.

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