

Hematological and biochemical aspects of cats naturally infected with feline immunodeficiency virus and feline leukemia

Aspectos hematológicos e bioquímicos de gatos naturalmente infectados pelos vírus da imunodeficiência felina e da leucemia felina

Luciana Carvalho Lacerda¹ , Aísia Nascimento da Silva² , Rebeca Dáley Santos Cruz² ,
Jéssica de Souza Freitas² , Roueda Abou Said³  & Alexandre Dias Munhoz^{3*} 

¹Veterinarian, DSc. Programa de Pós-graduação em Ciência Animal (PPGCA), Universidade Estadual de Santa Cruz (UESC), Ilhéus, BA, Brasil

²Veterinarian, MSc. PPGCA, UESC, Ilhéus, BA, Brasil

³Veterinarian, DSc. Departamento de Ciências Agrárias e Ambientais, UESC, Ilhéus, BA, Brasil

Abstract

Considering the importance and severity of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infections, information on laboratory abnormalities can determine the prognosis of infected cats. This study aimed to determine the laboratory alterations of domiciled asymptomatic cats naturally infected by FIV and/or FeLV in Northeastern Brazil. Blood samples from 200 cats were evaluated by nested-PCR and commercial immunochromatographic test for diagnosis of these infections. Complete blood count (CBC) and serum biochemistry analyses were performed to evaluate laboratory abnormalities. CBC and biochemical values of cats tested positive for FIV and/or FeLV were tabulated for the presence or absence of changes and analyzed using the chi-square test with Yates correction or Fisher's exact test for each variable, with a confidence interval of 95%. The total frequency was 6% (12/200) and 3% (6/200) for FIV and FeLV, respectively. The presence of hyperbilirubinemia (total, direct, and indirect) was the only change observed in cats positive for FIV compared to FIV-negative controls ($p < 0.05$). We believe that laboratory changes compatible with immunosuppressive conditions should be more frequent in FIV/FeLV positive cats that already present clinical signs of the disease.

Keywords: bilirubin, retroviruses, FIV, FeLV.

Resumo

Considerando a importância e a gravidade das infecções pelo vírus da imunodeficiência felina (FIV) e pelo vírus da leucemia felina (FeLV), informações sobre as alterações laboratoriais são determinantes no prognóstico dos pacientes. Objetivou-se com este estudo determinar as alterações laboratoriais de gatos assintomáticos domiciliados, naturalmente infectados pelo FIV e/ou FeLV "do nordeste do" Brasil. Amostras de sangue de 200 gatos foram avaliadas pela técnica de *nested*-PCR e pelo teste comercial imunocromatográfico para o diagnóstico dessas infecções. Hemograma completo e bioquímica sérica foram realizadas para avaliar as alterações laboratoriais. Os hemogramas e bioquímicos dos animais positivos para FIV e/ou FeLV foram tabulados quanto a presença ou ausência de alterações e analisados através do teste qui-quadrado com correção de Yates ou teste exato de Fisher para cada variável, com intervalo de confiança de 95%. A frequência total foi de 6% (12/200) e 3% (6/200) para FIV e FeLV, respectivamente. A presença de hiperbilirrubinemia (total, direta e indireta) foi a única alteração observada nos gatos positivos para FIV em relação aos animais negativos ($p < 0,05$). Acreditamos que alterações laboratoriais compatíveis com quadros de imunossupressão devam ser mais frequentes em gatos FIV/FeLV positivos que já apresentem sinais clínicos da doença.

Palavras-chave: bilirrubina, retrovírus, FIV, FeLV.

Introduction

The feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are two retroviruses that promote a progressive imbalance in the metabolism and immune system of the affected



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*Correspondence

Alexandre Dias Munhoz
Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz - UESC
Campus Soane Nazaré de Andrade, Rodovia Jorge Amado, Km 16, Salobrinho
CEP 45662-900 - Ilhéus (BA), Brasil
E-mail: munhoz@uesc.br

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animals (Hosie et al., 2009; Hartmann, 2011; Teixeira et al. 2019). Diagnosis of FIV/FeLV infections is performed by associating the clinical signs presented by the cats to complementary laboratory tests (Teixeira et al., 2007; Little et al., 2020). Diagnosis is necessary for the practice of preventive measures such as early vaccination and the initiation of necessary care in the case of positive animals (Levy et al., 2008; Westman et al., 2016).

Clinical laboratory changes presented by positive FIV/FeLV animals depend on the infection stage (Gleich & Hartmann, 2009). Animals can present with fever, anorexia, lymphadenopathy, leukemias, and changes in hematological aspects such as anemia, neutropenia, and lymphopenia, which indicates immunosuppression (Collado et al., 2012; Novo et al., 2016; Costa et al., 2017; Cristo et al., 2019). The biochemical findings described are diverse and nonspecific, with reports of increased concentrations of glucose, total protein, urea, and creatinine (Hofmann-Lehmann et al., 1997; Hosie et al., 2009; Poli et al., 2012).

Considering the severity of these infections, information about laboratory changes in positive FIV and FeLV animals can be useful in determining the prognosis of infected felines. This study aimed to determine the laboratory alterations of domiciled cats naturally infected by FIV and/or FeLV in the microregion of Ilhéus-Itabuna, Bahia, Brazil.

Materials and methods

Location of the study and sampling design

Between February 2012 and April 2013, an epidemiological study of analytical cross-sectional design was conducted in the cities of Ilhéus (14°47'S; 39°02'W) and Itabuna (14°47'S; 39°16'W), which are within the microregion of Ilhéus-Itabuna, Bahia, Brazil. Through non-probability sampling, 200 cat owners were selected from the records of veterinary clinics in the region. The inclusion criteria were as follows: 1) Asymptomatic cats, i.e., clinical examination did not show any abnormalities suggestive of systemic disease such as vomiting, diarrhea, weight loss, nasal secretion, or neoplasia (Collado et al., 2012); and 2) cats aged 6 months or over. The study was conducted in accordance with the principles of bioethics and animal welfare, under protocol number 011/12 (CEUA/UJESC).

Serological tests for FIV and FeLV

Three milliliters of blood was collected by venous, cephalic, or jugular puncture from each cat, where 1 mL was reserved for performing the complete blood count (CBC) and extracting DNA, and 2 mL for analysing the biochemical and serological profiles. The samples were tested for retroviruses using the commercial immunochromatography test: FIV ac/FeLV Ag Test Kit (Alere®), following the manufacturer's recommendations.

Extraction of genomic DNA and polymerase chain reaction (PCR) for FIV and FeLV

Genomic DNA was extracted from total blood using a commercial kit (QIAamp DNA Blood Minikit; Qiagen™), following the manufacturer's recommendations. All samples were subjected to nested PCR for detection of FIV and FeLV. The samples that presented negative results were subjected to GAPDH gene amplification in order to check the integrity of DNA and the absence of potential inhibitors. Table 1 presents the primers used for each PCR (FIV, FeLV, and *GAPDH*).

For amplification of the proviral DNA of FIV, nested PCR with two reactions were set up, each with a final volume of 25 µL composed of 10X buffer, 2.0 mM MgCl₂, 0.2 mM of each dNTP, 1 µM of each primer, and 1.25 U Taq DNA polymerase. In the first reaction, 5 µL DNA of sample DNA was added and then 2 µL of the amplification product from this reaction was used in the second reaction. Sterile ultrapure water was used to complete the final reaction volume. The two reactions followed the same amplification protocol: initial denaturation at 95 °C for 5 min, followed by 30 cycles at 95 °C for 45 s, 58 °C for 45 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min. The composition of the reactions and amplification protocols was adapted from a previously described report (Hohdatsu et al., 1998).

To assess the proviral DNA of FeLV, nested PCR was performed. Both reactions presented final volumes of 25 µL, containing 10X buffer; 2.0 mM MgCl₂; 0.2 mM of each dNTP; 0.4 µM of each

Table 1. Primers used in nested-PCR for FIV and FeLV, and *GAPDH*. Oligonucleotide sequence, gene and amplified product size.

	Sequence 5'-3'	Region	Product	References
FIV				
A2	AAT ATG ACT GTA TCT ACT GC	<i>gag</i>		Hohdatsu et al. (1998)
S2	TTT TCT TCT AGA GTA CTT TCT GG			
NS	TAT TCA AAC AGT AAA TGG AG	<i>gag</i>	329pb	Hohdatsu et al. (1998)
NA	CTG CTT GTT GTT CTT GAG TT			
FeLV				
U3-F	ACA GCA GAA GTT TCA AGG CC	U3/ <i>gag</i>		Miyazawa & Jarrett (1997)
G-R	GAC CAG TGA TCA AGG GTG AG			
U3-F(2)	GCT CCC CAG TTG ACC AGA GT	U3/ <i>gag</i>	601pb	Miyazawa & Jarrett (1997)
G-R(2)	GCT TCG GTA CCA AAC CGA AA			
GAPDH				
GAPDH F	CCT TCA TTG ACC TCA ACT ACAT		400pb	Birkenheuer et al. (2003)
GAPDH R	CCA AAG TTG TCA TGG ATG ACC			

primer; and 1.25 U Taq DNA polymerase. In the first reaction, 5 µL of the sample DNA was added, and 2 µL of the amplification product from this reaction was used in the second reaction. Sterile ultrapure water was used to complete the final reaction volumes. The amplification protocol consisted of initial denaturation at 94 °C for 7 min, followed by 33 cycles at 94 °C for 55 s, 55.3 °C for 55 s in the first reaction and 59.5 °C for 55 s in the second reaction, 72 °C for 70 s, and a final extension at 72 °C for 7 min. The composition of the reactions and amplification protocols was adapted from a previously described report (Miyazawa & Jarrett, 1997).

For *GAPDH* gene amplification, final reaction volume of 25 µL was set up comprising of 10X buffer, 2.0 mM MgCl₂, 0.2 mM of each dNTP, 1.0 µM of each primer, 1.25 U Taq DNA polymerase, and 5 µL of sample DNA. The amplification protocol consisted of initial denaturation at 95 °C for 5 min, followed by 40 cycles at 94 °C for 30 s, 52 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. The composition of the reactions and amplification protocols was adapted from a previously described report (Birkenheuer et al., 2003).

Hematological and biochemical analysis

The CBC was performed on an automatic counter (ABX VET, Horiba™, Montpellier, France). Total plasma protein concentrations were determined using a manual clinical refractometer, and for specific leukometry blood smears stained with Giemsa stain (Merck S/A, Rio de Janeiro, Rio de Janeiro, Brazil) were performed and examined under an optical microscope at 100X magnification. The reference values used were according to the report by Jain (1993).

To determine the serum activity of the enzymes, levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltranspeptidase (GGT), and concentration of urea, creatinine, and bilirubin (total, direct and indirect) were assessed by the commercial kit Labtest® (Labtest, Lagoa Santa, Minas Gerais, Brazil) in the Bioplus 2000® biochemical analyzer (Bioplus Ltd., Barueri, São Paulo, Brazil) and the reference values were based on Kaneko et al. (1997).

Statistical analysis

To perform the bivariate analysis, the results of hematological and biochemical analyses of cats positive and negative for FIV or FeLV (outcome variables) were categorized into the following variables: has anemia (yes or no); leukopenia (yes or no); neutropenia (yes or no); lymphopenia (yes or no); thrombocytopenia (yes or no); hyperproteinemia (yes or no); increased ALT (yes or no); increased AST (yes or no); increased GGT (yes or no); increased urea (yes or no); increased creatinine (yes or no); increase in total bilirubin (yes or no); increase in indirect bilirubin (yes or

no), and increase in indirect bilirubin (yes or no). Frequency distribution was determined using the chi-square test with Yates correction or Fisher's exact test. The chance of occurrence (OR) of the bivariate analysis was calculated using association measures and a 95% confidence interval.

Results

Of the 200 cats evaluated in the present study using nested-PCR and commercial immunochromatographic test, 6% (12/200) and 3% (6/200) tested positive for FIV and FeLV, respectively. No cat was positive for both viruses.

Hematological and biochemical results were analyzed for possible associations with FIV positive status (Table 2). A greater proportion of FIV positive cats showed an increase in the concentration of bilirubins (total, direct, and indirect) in relation to FIV negative cats ($p < 0.05$) and showed a tendency toward hyperproteinemia.

Table 2. Contingency tables for hematological and biochemical variables categorized with FIV status.

VARIABLES	FIV				Odds ration	
	POSITIVES		NEGATIVES		CI 95%	p
	N	%	N	%		
ANEMIA						
YES (Packed cell volume < 24%)	3	25.0%	20	10.7%	2.8(0.6-11.2)	0.14
NO (Packed cell volume ≥ 24%)	9	75.0%	168	89.3%		
LEUKOPENIA						
YES (White blood cell < 5.500/mm ³)	0	0%	12	6.4%	*	0.46
NO (White blood cell ≥ 5.500/mm ³)	12	100%	176	93.6%		
NEUTROPENIA						
YES (Neutrophils < 2.500/mm ³)	0	0%	4	2.0%	*	0.77
NO (Neutrophils ≥ 2.500/mm ³)	12	100%	184	97.8%		
LYMPHOPENIA						
YES (Lymphocytes < 1.500/mm ³)	3	25.0%	25	13.3%	2.1(0.5-8.5)	0.22
NO (Lymphocytes ≥ 1.500/mm ³)	9	75.0%	163	86.7%		
THROMBOCYTOPENIA						
YES (Platelets < 200.000/mm ³)	5	41.7%	104	55.3%	0.5(0.1-1.8)	0.26
NO (Platelets ≥ 200.000/mm ³)	7	58.3%	84	44.7%		
HYPERPROTEINEMIA						
YES (Total Protein > 7,8g/dl)	5	41.7%	32	17.1%	3.4(1-11.6)	0.08
NO (Total Protein ≤ 7,8g/dl)	7	58.3%	156	82.9%		
INCREASED ALT						
YES (ALT > 83 U/L)	1	8.3%	8	4.3%	2(0.2-17.8)	0.43
NO (ALT ≤ 83 U/L)	11	95.7%	180	95.7%		
INCREASED AST						
YES (AST > 43 U/L)	4	33.3%	56	29.8%	1,1(0.3-4)	0.5
NO (AST ≤ 43 U/L)	8	66.7%	132	70.2%		
INCREASED GGT						
YES (GGT > 5,1 U/L)	0	0.0%	15	8.0%	*	0.38
NO (GGT ≤ 5,1 U/L)	12	100%	173	92.0%		

*Indeterminate.

Table 2. Continued...

VARIABLES	FIV				Odds ration	
	POSITIVES		NEGATIVES		CI 95%	p
	N	%	N	%		
INCREASED UREA						
YES (Urea > 64 mg/dl)	0	0.0%	4	2.1%	*	0.77
NO (Urea ≤ 64 mg/dl)	12	100%	184	97.9%		
INCREASED CREATININE						
YES (Creatinine > 1,8 mg/dl)	2	16.7%	42	22.3%	0.69(0.1-3.2)	0.48
NO (Creatinine ≤ 1,8 mg/dl)	10	83.3%	146	77.7%		
INCREASED IN TOTAL BILIRUBIN						
YES (Total Bilirubin > 0.6 mg/dl)	8	66.6%	60	32.0%	4.2(1.2-14.7)	0.01
NO (Total Bilirubin ≤ 0.6 mg/dl)	4	33.4%	128	68.0%		
INCREASED DIRECT BILIRUBIN						
YES (Direct Bilirubin > 0.3 mg/dl)	2	16.7%	2	1.1%	18,6(2.3-146)	0.01
NO (Direct Bilirubin ≤ 0.3 mg/dl)	10	83.3%	186	98.9%		
INCREASED INDIRECT BILIRUBIN						
YES (Indirect Bilirubin > 0.5 mg/dl)	9	75.0%	68	36.2%	5.2(1.3-20.2)	0.009
NO (Indirect Bilirubin ≤ 0.5 mg/dl)	3	25.0%	120	63.8%		

*Indeterminate.

Due to the low number of FeLV positive cats, it was not possible to perform a statistical analysis, only a descriptive analysis. Of the six positive animals, none had anemia, leukopenia, neutropenia, lymphopenia, and three (50%) had thrombocytopenia. Among biochemical parameters, two cats showed an increase in serum ALT (17.6%) and AST (17.6%), respectively, whereas none of the cats presented hyperproteinemia and increased concentrations of urea and creatinine. Furthermore, two cats presented an increase in total and indirect bilirubin concentrations (33.3%).

Discussion

To the best of our knowledge, this is the first study in Northeastern Brazil to assess hematological and biochemical parameters in cats naturally infected with FIV/FeLV. These retroviruses are associated with a variety of laboratory findings related to decreased animal immunity (Gleich & Hartmann, 2009; Teixeira et al., 2019).

The presence of anemic conditions has already been well described in FIV positive cats due to the inflammatory process triggered by the virus, which blocks the uptake of iron (Shelton et al. 1990) as well as in cats infected with FeLV-C subtype, leading to secondary infections and neoplastic cell formation (Gleich & Hartmann, 2009; Hartmann, 2011; Cristo et al., 2019). In our study, we observed a low risk for the development of hematological changes for both viruses. We believe that this is due to the stage of infection (subclinical) presented by these animals at the time of collection. Collado et al. (2012) observed that FeLV positive cats with clinical signs were more likely to have lower total red blood cell counts than those without signs.

There was no statistical association between the leukogram results and retroviral infection, reinforcing the hypothesis of the absence of bone marrow involvement. Changes in the total leukocyte count are related to the reduction in the number of circulating neutrophils, mainly in the acute phase of infections and later in the immunosuppression phase, due to insufficient myelopoiesis (Shelton et al., 1990; Costa et al., 2017; Turinelli & Gavazza, 2018). Lymphopenia is related to the onset of immunosuppression, with a decrease in the number of CD4 and CD8 cells (Hofmann-Lehmann et al., 1997; Novo et al., 2016; Little et al., 2020).

Regarding biochemical changes, we observed a tendency towards hyperproteinemia in FIV positive cats. Hartmann (2011) and Liem et al. (2013) reported an increase in plasma globulins in cats as a result of the polyclonal expansion of B lymphocytes in response to FIV. Hyperbilirubinemia is associated with the presence of FIV and has already been described in cases of immune-mediated hemolytic anemia in cats facing infectious diseases, including those caused by retroviruses (Kohn et al., 2006; Tasker et al., 2010). However, in the present study, the occurrence of hemolysis in an immune-mediated manner did not cause anemia in cats.

The results of the present study suggest that FIV/FelV positive cats without clinical signs selected for cross-sectional studies may not initially show laboratory alterations compatible with immunosuppression. We believe that the hyperbilirubinemia presented by FIV positive cats should be evaluated with caution, and further studies are needed to assess the role of FIV in animal metabolism.

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