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MOLECULAR DIAGNOSIS OF FELINE LEUKEMIA VIRUS BY PCR IN FELINES TREATED AT A VETERINARY CLINIC

DIAGNÓSTICO MOLECULAR DO VÍRUS DA LEUCEMIA FELINA POR PCR EM FELINOS ATENDIDOS EM CLÍNICA VETERINÁRIA

DIAGNÓSTICO MOLECULAR DEL VIRUS DE LA LEUCEMIA FELINA MEDIANTE PCR EN FELINOS ATENDIDOS EN CLÍNICA VETERINARIA

Caroline Leal Gomes de Lima[1], Fabiana Batalha Knackfuss[2], Danielle Dutra Voigt[3], Tamara Silva[4], Mário dos Santos Filho[5], Thereza Christina de Vasconcelos[6]

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ABSTRACT

Feline leukemia virus (FeLV) is a clinically and epidemiologically important gammaretrovirus associated with immunosuppression, anemia and neoplasia in cats. This study aimed to estimate FeLV infection prevalence detected by polymerase chain reaction (PCR) and to explore potential risk factors in clinically healthy domestic cats attended at a veterinary clinic in Brazil. Whole-blood samples from 72 cats were collected for FeLV proviral DNA detection by PCR. Demographic and epidemiological variables (age, sex, neuter status, street access, rescue history and contact with FeLV-positive cats) were recorded. Odds ratios (OR) were calculated and Fisher's exact test was applied when appropriate ($p<0.05$). Seven of 72 cats (9.7%) were PCR-positive. Street access was the only factor associated with PCR positivity ($p=0.012$). These findings support PCR as a sensitive screening tool in asymptomatic cats and reinforce preventive actions such as routine testing, segregation of positives, vaccination of negatives and restriction of street access.

KEYWORDS: Felis silvestris catus. FeLV. PCR. Molecular diagnosis. Risk factors.

RESUMO

Objetivou-se estimar a prevalência de infecção pelo vírus da leucemia felina (FeLV) detectada por reação em cadeia da polimerase (PCR) e explorar fatores de risco em felinos domésticos clinicamente saudáveis atendidos em clínica veterinária no Brasil. Foram coletadas amostras de sangue de 72 gatos para detecção de DNA proviral de FeLV por PCR. Registraram-se variáveis demográficas e epidemiológicas (idade, sexo, estado reprodutivo, acesso à rua, histórico de resgate e contato com gatos FeLV-positivos). Odds ratios (OR) foram calculados e associações foram testadas pelo teste exato de Fisher ($p<0,05$). Sete de 72 gatos (9,7%) foram PCR-positivos. O

[1] Veterinarian. Works in the field of Food Inspection and physicochemical control of products of animal origin. Afya UNIGRANRIO, Duque de Caxias, Rio de Janeiro, Brazil.
[2] Animal Scientist. Professor and researcher in the field of animal genetics and breeding, with an interest in biostatistics. Afya UNIGRANRIO, Duque de Caxias, Rio de Janeiro, Brazil.
[3] Biomedical Scientist. Works as a researcher in the field of molecular genetics with an emphasis on the study of chronic-degenerative diseases. Afya UNIGRANRIO, Duque de Caxias, Rio de Janeiro, Brazil.
[4] Researcher in the field of molecular and cellular biology and human genetics. Afya UNIGRANRIO, Duque de Caxias, Rio de Janeiro, Brazil.
[5] Animal Scientist and Veterinarian. Professor and researcher in the field of small animal internal medicine and diagnostics, with work in veterinary cardiology and pneumology. Universidade de Vassouras, Vassouras, Rio de Janeiro, Brazil.
[6] Veterinarian. Professor and researcher with interests in biochemistry and pathology. Afya UNIGRANRIO, Duque de Caxias, Rio de Janeiro, Brazil.

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acesso à rua foi a única variável associada ao resultado positivo (p=0,012). Os achados reforçam a utilidade da PCR como ferramenta sensível para triagem em animais assintomáticos e sustentam medidas preventivas como testagem rotineira, isolamento de positivos, vacinação de negativos e restrição do acesso ao ambiente externo.

PALAVRAS-CHAVE: Felis silvestris catus. FeLV. PCR. Diagnóstico molecular. Fatores de risco.

RESUMEN
El virus de la leucemia felina (FeLV) es una infección viral de distribución mundial asociada a inmunosupresión, anemia y neoplasias en gatos. Este estudio tuvo como objetivo estimar la prevalencia de infección por FeLV detectada mediante reacción en cadena de la polimerasa (PCR) y explorar factores de riesgo en gatos domésticos clínicamente sanos atendidos en una clínica veterinaria en Brasil. Se recolectaron muestras de sangre total de 72 gatos para la detección de ADN proviral de FeLV por PCR. Se registraron variables demográficas y epidemiológicas (edad, sexo, castración/esterilización, acceso a la calle, antecedente de rescate y contacto con gatos FeLV-positivos). Se calcularon odds ratios (OR) y se aplicó la prueba exacta de Fisher cuando correspondía (p<0,05). Siete de 72 gatos (9,7%) fueron PCR-positivos. El acceso a la calle fue el único factor asociado a positividad (p=0,012). Los hallazgos respaldan la utilidad de la PCR como herramienta sensible de cribado en gatos asintomáticos y refuerzan acciones preventivas como testeo rutinario, aislamiento de positivos, vacunación de negativos y restricción del acceso externo.

PALABRAS CLAVE: Felis silvestris catus. FeLV. PCR. Diagnóstico molecular. Factores de riesgo.

INTRODUCTION

Feline leukemia virus (FeLV) is a worldwide viral infection of domestic and wild felids caused by an enveloped gammaretrovirus and associated with immunosuppression, anemia and FeLV-associated neoplasia, with prognosis varying according to infection outcome and clinical management.[1,2,3]

Transmission occurs mainly via close contact and saliva (e.g., mutual grooming, shared bowls) but can also occur through bite wounds and vertical transmission. Infected cats should be kept indoors, and risk-based testing and vaccination remain central preventive strategies in endemic areas.[3,4,5]

FeLV infection outcomes are commonly classified as abortive, regressive, progressive or focal/atypical. Point-of-care antigen tests (p27) are useful screening tools, but proviral DNA detection by PCR improves identification of regressive infections that may be antigen-negative, which is particularly relevant in clinically healthy cats.[2,4,6,7,8,9]

Given the clinical and epidemiological relevance of FeLV and the role of molecular methods in detecting proviral DNA, this study aimed to estimate FeLV prevalence by PCR in clinically healthy cats attended at a veterinary clinic in Brazil and to explore potential risk factors for PCR positivity.

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MATERIALS AND METHODS

The study was approved by the Ethics Committee on the Use of Animals (CEUA) of Universidade do Grande Rio (Afya UNIGRANRIO), Duque de Caxias, Rio de Janeiro, Brazil (protocol no.:054/2022.

Cats were attended at a veterinary clinic in Rio de Janeiro, Brazil, and enrolled by convenience as consecutive, client-owned patients presented for routine care. Written informed consent was obtained. Demographic and epidemiological variables (age, sex, neuter status, street access, rescue history and contact with FeLV-positive cats) were recorded.

Sample collection consisted of ~2 mL of venous blood obtained aseptically by jugular venipuncture and stored in EDTA tubes until processing. Genomic DNA was extracted using the FlexiGene DNA Kit (Qiagen®) following the manufacturer's instructions. DNA concentration and purity were assessed by spectrophotometry prior to PCR.

PCR was performed to amplify FeLV proviral DNA. Each 25 µL reaction contained 1× PCR buffer, $MgCl_2$ (final 1.5 mM), dNTPs (final 0.2 mM each), 10 pmol of each primer (PF 5′-TTACTCAAGTATGTTCCCATG-3′ and PR 5′-AGGTCGAACTCTGGTCAACT-3′), 1 U of Taq DNA polymerase and 1 µL of genomic DNA (~50 ng), completed with nuclease-free water. Thermocycling was performed in a Veriti thermocycler (Thermo Fisher Scientific®) with initial denaturation at 95°C for 10 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 10 min.[10,11]

Each run included a known FeLV-positive control, a negative extraction control and a no-template control. Amplicons were resolved by electrophoresis on 2% agarose gel and visualized under UV transillumination. A sample was considered PCR-positive when a band at the expected size (288 bp) was present with valid positive and negative controls. Absence of the specific band in the presence of valid controls was interpreted as PCR-negative.[10,11]

To reduce the risk of cross-contamination, DNA extraction, reaction setup and post-amplification procedures were performed in physically separated areas using aerosol-resistant tips; negative controls were monitored in every run.

The sample size was defined by the study period and consecutive convenience enrollment of eligible cats; no a priori sample size calculation was performed. Bivariate analyses were conducted by calculating odds ratios (OR) and 95% confidence intervals (CI) for potential risk factors. Fisher's exact test (two-sided) was used for associations due to sparse data ($p<0.05$). When a cell count of zero occurred, a 0.5 continuity correction (Haldane–Anscombe) was applied to enable OR estimation; such estimates were interpreted cautiously due to instability and wide CIs. No multivariable model was fitted because of the low number of PCR-positive cats. An internal

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amplification control and analytical limit of detection were not established in this study, which should be considered a methodological limitation. Analyses were performed in SPSS 29.0.

RESULTS

A total of 72 clinically healthy cats were included, of which 7 (9.7%) were PCR-positive for FeLV. Age distribution is presented in Table 1; no cats were observed in the transitional month ranges between the predefined life-stage categories (25–35, 73–83, 121–131 and 169–179 months). Street access was the only exposure statistically associated with PCR positivity (Table 2). Because one cell was zero (no PCR-positive cat without street access), the OR estimate required continuity correction and yielded a wide 95% CI; thus, the magnitude of association should be interpreted with caution despite the significant Fisher's p-value.[12]

Table 1. Age distribution of sampled cats (n=72)

Age Range	Age (in months)	*n* sample	Percentage
Cub	2 a 6	18	25
Junior	7 a 24	28	39
Young	36 a 72	17	24
Adult	84 a 120	4	5
Sênior	132 a 168	4	6
Elderly	180 a 300	1	1

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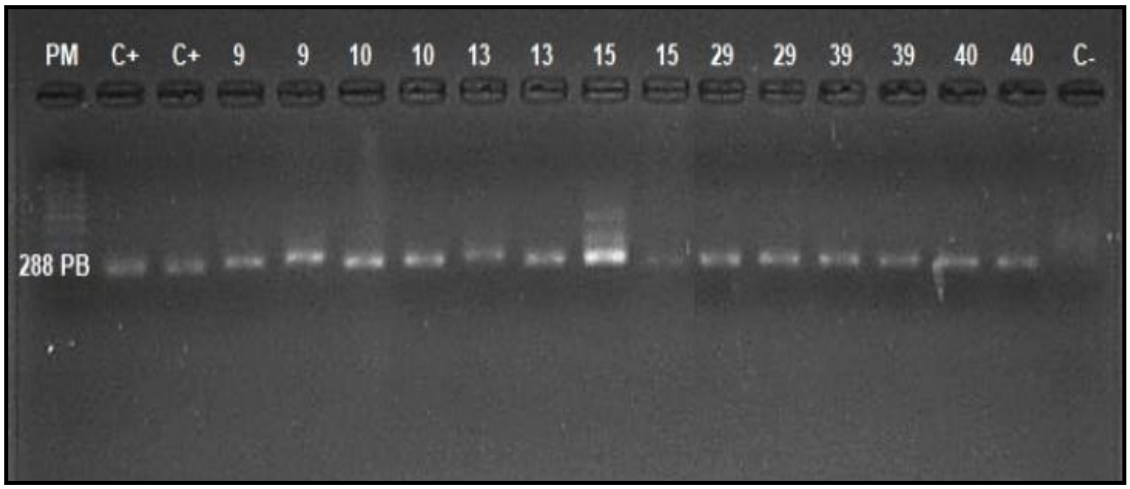
Table 2. Bivariate analysis of potential risk factors for FeLV PCR positivity (n=72)

Risk factors	Age Range	Positive	Negative	*ODDS Ratio*	CI= 95%	p-valor
Age (in months)	Cub to young	6	57	0,8421	0,0894	0,6515
	Adult to senior	1	8		7,9311	
Gender	Male	4	30	1,5556	0,3222	0,8769
	Female	3	35		7,5100	
Castrated	Yes	5	43	1,2791	0,2294	0,8882
	No	2	22		7,1320	
Street access	Yes	8	31	8,7742	1,0374	**0,0496***
	No	1	34		74,2122	
Rescued	Yes	8	55	1,4545	0,1635	0,8712
	No	1	10		12,9365	
Positive animal contact	Yes	4	18	3,4815	0,7082	0,2398
	No	3	47		17,1152	

Figure 1. Result of amplification in 1.5% Agarose gel bands after electrophoresis, in duplicate. (PM): Allelic scale. (288 PB): 288 base pairs. (C+): positive control. (C-): negative control



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DISCUSSION

The FeLV PCR positivity rate observed in clinically healthy cats reinforces the role of proviral DNA detection for identifying infection in cats without overt clinical signs, including regressive infections that may be missed by antigen-only screening. Differences in diagnostic approaches (PCR vs antigen-based assays) and population characteristics (owned cats vs shelter/rescue populations) likely contribute to the wide prevalence range reported across studies.[2,4,6,7,8,9,13,14,15]

Street access emerged as the only statistically associated exposure in this dataset. This finding is biologically plausible, as outdoor access increases contact networks, fighting/bite events and opportunities for exposure to infected cats. Accordingly, guidelines emphasize risk-based testing and prevention strategies for cats with outdoor lifestyles.[3,4,7,13,14,16]

However, the street-access OR estimate was imprecise, with a very wide 95% CI that included 1, reflecting sparse data and the need for continuity correction due to a zero cell. Therefore, the association should be interpreted as evidence of a possible relationship rather than as a stable estimate of effect size; larger samples would be required to refine precision and enable multivariable adjustment.

Discordant results between p27 antigen screening assays and proviral DNA PCR have been described, particularly in cats with concurrent hematologic disorders or in low-prevalence settings, supporting confirmatory PCR testing when antigen results are unexpected or when clinical suspicion persists.[9,16,17]

In addition, molecular studies from different regions, including Brazil and European stray-cat populations, have reported circulation of FeLV subgroups (e.g., FeLV-A and AB) and substantial genetic diversity, reinforcing the relevance of molecular surveillance and careful selection of conserved genomic targets for PCR-based assays.[14,15,18]

Limitations include the single-clinic, convenience sampling design (limited external validity), inclusion restricted to clinically healthy cats (possible selection bias), absence of an a priori sample size calculation, and the inability to perform multivariable analysis because of the low number of PCR-positive cases. In addition, an internal amplification control and an analytical limit of detection were not determined, which may affect interpretation in samples with low DNA quantity/quality. These aspects should be addressed in future multi-center studies with standardized diagnostic algorithms and larger sample sizes.

CONCLUSION

In clinically healthy cats attended at a veterinary clinic in Brazil, FeLV proviral DNA was detected by PCR in 9.7% of samples. Street access was the only factor statistically associated with PCR positivity, supporting preventive recommendations focused on restricting outdoor access, routine testing and risk-based vaccination.[3,4,5,16]

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