

# ERRATUM: Retrospective study of retroviruses by immunoenzymatic test on cats in Grande Vitória (ES, Brazil) and associated neoplasms

Isabella Oliveira Almeida<sup>1</sup> , Mariana Arnoni Alves da Silva<sup>2</sup> , Bruna Voltolin de Sena<sup>3</sup> , Jeanne Saraiva da Paz<sup>1</sup> , Tamara de Almeida Jaretta<sup>4</sup> , Priscila Camargo Granadeiro Faria<sup>2</sup>  & Rodrigo dos Santos Horta<sup>5</sup> 

<sup>1</sup>Veterinarian, Programa de Pós-Graduação em Ciência Animal, Departamento de clínica e Cirurgia veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil.

<sup>2</sup>Veterinarian, Msc., Departamento de Medicina Veterinária, Universidade Vila Velha (UVV), Vila Velha, ES, Brazil.

<sup>3</sup>Veterinarian, MSc. Programa de Pós-Graduação em Ciência Animal, Departamento de clínica e Cirurgia veterinárias, Escola de Veterinária, UFMG, Belo Horizonte, MG, Brazil.

<sup>4</sup>Veterinarian, Msc., Autonomus, Vila Velha, ES, Brazil

<sup>5</sup>Veterinarian, Dsc., Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil.

Due to author's honest mistake the article "Retrospective study of retroviruses by immunoenzymatic test on cats in Grande Vitória (ES, Brazil) and associated neoplasms" (DOI <https://doi.org/10.29374/2527-2179.bjvm114220>), published in Brazilian Journal of Veterinary Medicine, published on vol. 43, e104220, 2021, was published with an error.

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## Abstract

Retroviruses are among the leading causes of death in domestic cats. Retroviruses associate with the host cell in a persistent and permanent way, leading to diverse clinical conditions. The feline leukemia virus (FeLV) is the most pathogenic retrovirus with the potential to cause both degenerative diseases and immunosuppression, as well as proliferative diseases, as its association with the cell may lead to a direct oncogenic effect. The feline immunodeficiency virus (FIV), in turn, can lead to the classic immunodeficiency syndrome, usually has a chronic, less aggressive course and has no direct oncogenic effect. The use of vaccines and control measures has resulted in a decrease in the prevalence of FeLV in the United States of America (USA) and Europe, however, in Brazil, statistics show prevalence rates above 50%. This study aimed to assess the prevalence of feline retroviruses, by immunoenzymatic assay testing, in the region of Grande Vitória, in Espírito Santo and also point out the frequency of neoplasms in these cats. A total of 388 cats were retrospectively evaluated (2014-2016). The prevalence of FIV was 2.3% and FeLV was 33.7%. Neoplasms were identified in the three cats seropositive for FIV and FeLV and in three cats infected only with FIV. Neoplasms were also found in 26.6% of cats that were seropositive only for FeLV, especially



mediastinal lymphoma. The high prevalence of FeLV demonstrated in this study highlights the need for establishing effective control measures, with emphasis on vaccination.

**Keywords:** epidemiology; feline retrovirus; cancer; ELISA.

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Retroviruses are among the leading causes of death in domestic cats. Retroviruses may associate with the host cell in a persistent and permanent way, leading to diverse clinical conditions. The feline leukemia virus (FeLV) is the most pathogenic retrovirus with the potential to cause both degenerative diseases and immunosuppression, as well as proliferative diseases, as its association with the cell may lead to a direct oncogenic effect. The feline immunodeficiency virus (FIV), in turn, can lead to the classic immunodeficiency syndrome, usually has a chronic, less aggressive course and has no direct oncogenic effect. The use of vaccines and control measures has resulted in a decrease in the prevalence of FeLV in the United States of America (USA) and Europe, however, in Brazil, prevalences may be above 50% in some regions. This study aimed to assess the prevalence of feline retroviruses, by immunoenzymatic assay testing, in the region of Grande Vitória, in Espírito Santo and also point out the frequency of neoplasms in these cats. A total of 388 cats were retrospectively evaluated (2014-2016). The prevalence of FIV was 2.3% and FeLV was 33.7%. Neoplasms were identified in the three cats seropositive for FIV and FeLV and in three cats infected only with FIV. Neoplasms were also found in 26.6% of cats that were seropositive only for FeLV, especially mediastinal lymphoma. The high prevalence of FeLV demonstrated in this study highlights the need for establishing effective control measures, with emphasis on vaccination.

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### \*Correspondence

Bruna Voltolin de Sena  
Universidade Vila Velha  
R. Viana, s/n - Boa Vista I  
CEP 29107-553 - Vila Velha (ES), Brasil  
E-mail: brunasena.vet@gmail.com

It should read:

### \*Correspondence

Rodrigo Dos Santos Horta  
Universidade Federal de Minas Gerais - UFMG  
Av. Antônio Carlos, 6627, Pampulha  
CEP 31270-901 - Belo Horizonte (MG), Brasil  
E-mail: rodrigohvet@gmail.com

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## Introduction

Infectious diseases are among the leading causes of death in domestic cats, especially in young animals (Egenvall et al., 2009). Feline retroviruses, such as the feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV), represent an important cause of mortality in domestic cats. These viruses widespread throughout the world and affect up to 50% of cats, depending on the geographic region, feline population, lifestyle, and diagnostic test (Rojko & Hardy, 1994).

Viruses belonging to the Retroviridae family are enveloped, and have two simple strands of RNA in their genome, in addition to two molecules of reverse transcriptase enzyme and two molecules of integrase enzyme. Reverse transcriptase is responsible for transforming the single strand of RNA into a double strand of proviral DNA, which is inserted in the host cell genome through integrase. After insertion of proviral DNA into the host's DNA of certain cells, the entire cell progeny will carry the genetic material of the virus (Little et al., 2020).

FIV, originally denominated T-lymphotropic feline lentivirus, was first described by Pedersen et al. (1987) through the identification of domestic cats with signs of immunodeficiency and without FeLV infection. FIV has been classified into five subtypes (A to E) and among lentiviruses, FIV is phylogenetically the most similar to HIV (Pedersen et al., 1987).

FeLV, on the other hand, was described in 1964 through the identification of viral particles linked to the lymphoblast membrane in a cat with lymphoma (Jarrett et al., 1964). Despite the existence of at least six subtypes: FeLV-A, FeLV-B, FeLV-C, FeLV-T and recently described FeLV-D (Ito et al., 2013) and FeLV-TG35 (Miyake et al., 2016), only FeLV-A can be transmitted horizontally between domestic cats, although it usually presents reduced pathogenicity if not recombined (Hartmann, 2012). The other subtypes are formed from FeLV-A mutations or recombination with endogenous proviral sequences, for example enFeLV, an endogenous sequence, analogous to FeLV, and acquired during the evolution of the domestic cat (Hartmann, 2012; Souza et al., 2002).

FIV infection has little impact on the cat population, with over 50% of infected animals remaining clinically asymptomatic for at least two years (Hartmann, 2012). However, FeLV is definitely more pathogenic and responsible for several clinical syndromes and up to 1/3 of deaths in this species in endemic regions (Rojko & Hardy, 1994). Cats with progressive FeLV infection have a median life expectancy of 2.4 years but up to 80% do not live more than three years (Little et al., 2020).

In endemic areas, the presence of neoplasms related to retroviruses in domestic cats is high. FIV infection may indirectly predispose the cat to the development of neoplasms due to immune and inflammatory dysfunction, increasing five times the risk of neoplasms. In this context, lymphomas and leukemias stand out, but also squamous cell carcinomas and possible fibrosarcomas and mast cell tumours (Hartmann 2012; Poli et al., 1994). In cats infected with FeLV, immune depletion also occurs, however, the risk of developing lymphomas and leukemias is higher in these animals. Cats infected with FeLV are 62 times more likely to develop neoplasms, which is related to the direct oncogenesis caused by this virus. FeLV is considered an oncovirus, as the insertion of proviral DNA into the DNA of feline cells may cause cell transformation and modification of gene transcription (Hartmann, 2012; Poli et al., 1994). Co-infection with FIV and FeLV may further increase the risk of cancer, as observed in a study conducted by Shelton et al. (1990), which demonstrated a risk 77 times greater of developing lymphoid neoplasms in those patients.

The purpose of this study was to evaluate the prevalence of feline retroviruses, using the immunoenzymatic test, in the region of Grande Vitória, in Espírito Santo and also point out the frequency of neoplasms in these cats.

## Material and methods

Retrospectively, the results of the immunoenzymatic assay tests performed on 388 cats treated at a University Veterinary Hospital and at a Private Practice specialized in feline care in Grande Vitória, Espírito Santo (Brazil), between 2014 and 2016, were analyzed. Indoor and outdoor cats, with and without clinical disease, were included. The test used in both institutions was the SNAP FIV/FeLV Combo Test from IDEXX® (São Paulo/SP), however, whole blood was used for the tests of 232 cats and serum (after centrifugation) for the tests of 156 cats. The medical records of 285 cats were recovered and analyzed for characterization of the studied population and for

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Viruses belonging to the Retroviridae family are enveloped, and have two simple strands of RNA in their genome, in addition to two molecules of reverse transcriptase enzyme and two molecules of integrase enzyme. Reverse transcriptase is responsible for transforming the single strand of RNA into a double strand of proviral DNA, which is inserted in the host cell genome

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In endemic areas, the presence of neoplasms related to retroviruses in domestic cats is high. FIV infection may indirectly predispose the cat to the development of neoplasms due to immune and inflammatory dysfunction, increasing five times the risk of neoplasms. In this context, lymphomas and leukemias stand out, but also squamous cell carcinomas and possible fibrosarcomas and mast cell tumours (Hartmann 2012; Poli et al., 1994). In cats infected with FeLV, immune depletion also occurs, however, the risk of developing lymphomas and leukemias is higher in these animals. Cats infected with FeLV are 62 times more likely to develop neoplasms, which is related to the direct oncogenesis caused by this virus. FeLV is considered an oncovirus, as the insertion of proviral DNA into the DNA of feline cells may cause cell transformation and modification of gene transcription (Hartmann, 2012; Poli et al., 1994). Co-infection with FIV and FeLV may further increase the risk of cancer, as observed in a study conducted by Shelton et al. (1990), which demonstrated a risk 77 times greater of developing lymphoid neoplasms in those patients.

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Neoplasms were recognized, as shown in Table 1, in only eight of 251 animals non-seroreagent for FIV and FeLV (3.2%). However, all cats seroreagent for the two retroviruses presented neoplasms (3/3), and in those animals reagents only for FIV, neoplasms were observed in three (3/6). For cats with a positive result in the SNAP FIV/FeLV Combo Test only for FeLV, neoplasms were found in 34 of 128 animals (26.6%). Among cats infected only with FeLV, 19.5% (25/128) died before or during treatment due to the neoplasm, although it was not possible to specify the survival and disease-free interval.

Also in this study, feline chronic gingival stomatitis (n = 9, with six seroreagents for FeLV), hemotropic mycoplasmosis (n = 7, four seroreagents for FeLV), feline respiratory complex (n = 3, non-seroreagent), chronic kidney disease (n = 3, non-seroreagent), otitis (n = 2, non-seroreagent) and inflammatory bowel disease (n = 1, non-seroreagent) were diagnosed.

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**Table 1.** Frequency of neoplasms in cats submitted to the SNAP FIV / FeLV IDEXX test between 2014 and 2016 in Grande Vitória - ES.

Diagnosis	Non-reagent (n = 251)	Reagent for FIV and FeLV (n = 3)	Reagent only for FIV (n = 6)	Reagent only for FeLV (n = 128)	Total
Mediastinal	8	1	0	10	19
Multicentric	0	0	1	1	2
Intestinal	0	1	1	6	8
Lymphoma Mesenteric Lymph Node	0	0	0	1	1
Spinal	0	1	1	5	7
Renal	0	0	0	4	4
Unspecified retrobulbar	0	0	0	1	1
Splenic mastocytoma	0	0	0	1	1
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Acute lymphoid leukemia	0	0	0	4	4

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In this study, the rate of 35.3% of reagent tests for retroviruses is possibly linked to the lack of feline population control, outdoor access, feline behavioral characteristics, among them, hygiene and aggressiveness, in addition to low adherence to vaccination (Biezus et al., 2019). Healthy and sick cats were evaluated together in this work, however, studies that included only clinically sick cats revealed increased prevalences (Hagiwara et al., 1997). In a study carried out in Santa Catarina, SC, with 275 cats, using the ELISA test, FeLV was detected in 22.3% of cats. When separately evaluated, the incidence was 9.9% in healthy cats and 28.4% in sick patients (Biezus et al., 2019).

This research also demonstrated a high frequency of FeLV, in Grande Vitória, ES, similar to the results obtained in Belo Horizonte, MG, also by the immunoenzymatic test of IDEXX®, with 32.5% prevalence (Teixeira et al., 2007). Using the same test, in Rio de Janeiro, RJ, a reagent result was obtained in 20.3% of tested cats, both domiciled and with outdoor access, according to the inclusion criteria of the present study (Souza et al., 2002). In a study conducted in São Paulo, with 298 cats, whose tutors sought veterinary assistance, the Dya System immunoenzymatic test showed 12.5% of FeLV infection (Hagiwara et al., 1997).

As shown in other studies, males are more affected by retroviruses, which is consistent with the sexual predisposition found in this study. In a study conducted in Istanbul, 30.6% of the tested males were positive, while only 14.8% of females tested positive for FeLV. Likewise, when tested for FIV, the rates of 8.2 and 3.7% of positive animals were found, among males and females, respectively (Yilmaz et al., 2000). This is possibly due to the greater propensity to aggressiveness of males, mainly due to territorialism (Biezus et al., 2019). However, sex may not represent a true risk factor according to Almeida et al. (2012), who showed that among 126 FeLV positive cats, 67 were males and 58 were females, but with no statistical significance.

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A limiting factor for the diagnostic technique employed in this study is that the majority (up to 60%) of cats infected with FeLV evolve to the regressive form of the infection, a category in which the viral antigen is not detected, which may underestimate the prevalence of the infection. In these cases, PCR is recommended, using oligonucleotide primers for flanking sequences of the DNA provirus, different from those presented in the enFeLV (Herring et al., 2001). However, in cats with the regressive form of the infection, the development of a disease related to FeLV or

even its transmission are unlikely (Hartmann, 2012) and the chosen test had good applicability in the studied population.

PCR also allows early detection of the virus in cases of recent infections (Westman et al., 2015). In the study conducted by Herring et al. (2001), a similar sensitivity for the investigation of FeLV was found through PCR in suspiciously regressive cats both in samples of peripheral blood and bone marrow. In Belo Horizonte, MG, a study with 1072 cats showed the proviral DNA of FeLV by PCR in samples of peripheral blood from 507 animals (47.5%), reaching 68.1% in the northern region of the city and showing greater prevalence in the group aged between one and three years old (Coelho et al., 2011). It is noteworthy that, in the two studies conducted in Belo Horizonte, the population included cats from shelters, in which the prevalence of FeLV is presumably higher (Coelho et al., 2011; Teixeira et al., 2007). In addition to the difference between the diagnostic techniques used in the aforementioned research and in the present study, the population was also different, with the evaluated animals being attended at a Veterinary Hospital and a Private Practice specialized in felines.

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In this study, 48 (35.0%) of the 137 cats seropositive for retroviruses presented neoplasms and 48 of these (85.4%) developed lymphomas. In cats infected with FIV and FeLV simultaneously, the risk of neoplasms increases by up to 77 times, mainly lymphomas, especially in its mediastinal form (Hartmann, 2012). Corroborating the results found, all cats with FIV and FeLV simultaneously developed lymphoma, however, they presented the spinal, multicentric and alimentary forms. The occurrence of lymphoma in 21% of cats positive for FeLV was similar to the study in Santa Catarina, which showed a rate of 17.24% of lymphoma in sick cats. Still in the same study, regarding cats diagnosed with leukemia, the rate of 3% of this study was lower than the percentage of 6.9% found among cats with FeLV (Biezu et al., 2019).

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Despite the inaccuracy of some information about the clinical condition of the patients, the unfavorable prognosis of those who develop neoplasms associated with FeLV was evidenced, as found by Hartmann (2012). The limitations of this study were also the elaboration of an epidemiological profile with cats attended at only two institutions in Espírito Santo and it was not possible to ensure that all attended cats were subjected to immunoenzymatic testing. The impossibility of performing PCR in peripheral blood may have resulted in an underestimation of FIV and FeLV infections.

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Moreover, vaccination for retroviruses is not included in the group of mandatory vaccines in the national scenario. Vaccination for FIV is not available in Brazil and is controversial, considering the high viral variability and the possibility of positive results in the immunoenzymatic test, especially when the diagnostic test is based on the detection of antibodies against p15 (Westman et al., 2015). Regarding FeLV, the evidence of high prevalence in domestic cats suggests the need for implementation of this vaccine on an emergency basis as a method of prophylaxis, especially in cats with outdoor access or those that live with cats with such access. As evidenced by Hofmann Lehmann et al. (2006), felines vaccinated against FeLV mostly remain negative in immunoenzymatic tests, but positive for proviral DNA until three years after the after the last vaccination.

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