SCIENTIFIC ARTICLE

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

Estudo retrospectivo de retrovírus por teste imunoenzimático em gatos na Grande Vitória (ES, Brasil) e neoplasias associadas

BJVM
Brazilian Journal of Veterinary Medicine
p-ISSN 0100-2430
e-ISSN 2527-2179

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

[1]Veterinarian, Autonomus, Vila Velha, ES, Brazil
[2]Veterinarian, Msc., Departamento de Medicina Veterinária, Universidade Vila Velha (UVV), Vila Velha, ES, Brazil.
[3]Veterinarian, Msc., Autonomus, Vila Velha, ES, Brazil
[4]Veterinarian, Dsc., Departamento de Medicina Veterinária, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil.

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.

Resumo

As retroviroses encontram-se entre as principais causas de morte em gatos domésticos. Os retrovírus associam-se à célula hospedeira de forma persistente e permanente, levando a quadros clínicos diversos. O vírus da leucemia felina (FeLV) é oretrovírus mais patogênico, com potencial para ocasionar tanto doenças degenerativas e imunossupressão, quanto doenças proliferativas, pois sua associação à célula pode levar ao efeito oncogênico direto. Já o vírus da imunodeficiência felina (FIV), pode levar à clássica síndrome de imunodeficiência, costuma ter um curso crônico, menos agressivo e não possui efeito oncogênico direto. O uso de vacinas e de medidas de controle resultaram em redução da prevalência de FeLV nos Estados Unidos da América (EUA) e Europa, entretanto, no Brasil, determinadas regiões podem apresentar prevalências superiores a 50%. Esse estudo teve como objetivo avaliar a prevalência das retroviroses felinas, pelo teste de ensaio imunoenzimático, na região da Grande Vitória, no Espírito Santo, apontando ainda, a frequência de neoplasias nesses gatos. Um total de 388 gatos foram retrospectivamente avaliados (2014-2016). A prevalência de FIV foi de 2,3% e de FeLV 33,7%. Neoplasias foram identificadas nos três gatos sororeagentes para FIV e FeLV e em três gatos infectados apenas por FIV. Constatou-se a presença de neoplasias ainda em 26,6% dos gatos sororeagentes apenas para FeLV, com destaque para o linfoma na forma mediastinal. A elevada prevalência de FeLV demonstrada neste estudo aponta sobre a necessidade da instituição de medidas efetivas de controle, com destaque para a vacinação.

Palavras-chave: epidemiologia, retrovírus felinos, câncer, ELISA.

How to cite: Almeida, I. O., Silva, M. A. A., Sena, B. V., Paz, J. S., Jaretta, T. A., Faria, P. C. G., & Horta, R. S. (2021). Retrospective study of retroviruses by immunoenzymatic test on cats in Grande Vitória (ES, Brazil) and associated neoplasms. *Brazilian Journal of Veterinary Medicine*, *43*, e104220. https://doi.org/10.29374/2527-2179.bjvm114220

Received: July 19, 2020.
Accepted: November 06, 2020.

***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

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 integraseenzyme.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 carrythe 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 intofive 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 timesthe 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. Catsinfected 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 neoplasmsin 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 pointout the frequency of neoplasms in these cats.

Material and methods

Retrospectively,the results ofthe 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 catsand 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

establishing neoplasms and other associated comorbidities. Descriptive statistics was performed with the aid of Excel 2013 software version 15.0.4569.1504.

Results

A total of 388 cats were attended between 2014 and 2016 and subjected to the SNAP Triple Test. It was possible to identify the breed of 285 patients and 90% were mixed-breed cats. Regarding sex, 51% were male (n = 198) and 49% female (n = 190). Age was accessed in 244 cases and ranged from two months to 17 years old (3.4 ± 3.7 years).

Of the total of 388 cats, 137 were positive for retroviruses (35.3%), three concomitantly for FIV and FeLV (0.8%),being two males, six only for FIV (1.5%), being four of these males, and 128 for FeLV (33%) with 73 males. Therefore, the frequency of FIV was 2.3% and FeLV 33.7%. Among the cats positive for retroviruses (n = 137), 108 were mixed-breed cats (78.8%), seven were Siamese (5.1%), and there was also a Persian (0.7%) and a Maine Coon (0.7%). The breed was not described in the medical records of 20 cats (14.5%). Among the infected cats, males were slightly overrepresented (53.7%).

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 seroragent 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 of128 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.

Discussion

Among domestic species, the feline species is affected by the largest number of retroviruses, including endogenous and exogenous viral particles (Modiano et al., 2013). There are no local studies demonstrating the percentage of cats vaccinated for FeLV in Espírito Santo, but it is known that feline immunization is still far from desired, as observed in other regions of Brazil (Souza et al., 2002; Teixeira et al., 2007).

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
Lymphoma	Mediastinal	8	1	0	10	19
	Multicentric	0	0	1	1	2
	Intestinal	0	1	1	6	8
	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
Splenicmastocytoma		0	0	0	1	1
Tubular Carcinoma		0	0	0	1	1
Acutelymphoidleukemia		0	0	0	4	4

In this study, the rate of 35.3% of reagent tests for retroviruses is possiblylinked 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 testshowed 12.5% of FeLV infection (Hagiwara et al., 1997).

As shown in other studies, males are more affected by retroviruses, whichis 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 among126 FeLV positive cats, 67 were males and 58 were females, but with no statistical significance.

The FIV seropositive rate of 1.5% found in the present study was lower compared to other studies, such as in North America, where the prevalence of FIV in domiciled cats was 2.5% and ranged from 3.5-23% in semi-domiciled cats (Levy et al., 2006). Surveys perfomed in Europe revealed incidences of 2-6% in the north (Hosie et al., 1989), and 10% in the south (Bandecchi et al., 2006).

Regarding FIV infection, there might be a risk of false negative results in serological tests, in immunosuppressed cats, or in the initial stages of infection (Levy et al., 2008). Nevertheless, the incidence observed in this study was lower than what was obtained in similar studies using the same diagnostic method, as 11.7% in cats from shelters in São Paulo (Hagiwara et al., 1997) and 21% in feral cats in Rio de Janeiro (Mendes-de-Almeida et al., 2004) were positive. In Belo Horizonte, in 145 shelter cats, there was a prevalence of FIV infection found by PCR technique in peripheral blood of only 4.1% (Teixeira et al., 2007).

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 transmissionare 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 foundthroughPCR 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 especialized in felines.

Feline retroviruses are recognized for their direct and indirect carcinogenic effects, with a high prevalence of neoplasms in reactive cats in this study. Little is known about the genetic basis of the wide diversity of neoplasms induced by FIV, FeLV and their recombinants.

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 (Biezus et al., 2019).

Immunosuppression induced by FeLV results in co-infections by *Mycoplasma haemofelis*, also detected in this study, feline coronavirus, calicivirus and herpesvirus, *Cryptococcus neoformans* and *Toxoplasma gondii*, among others (Ravazzolo & Costa, 2007), and may also contribute to an indirect carcinogenic effect by reducing immunological surveillance (Hartmann, 2012; Ogilvie et al., 1988). Unexpectedly, six cats with FeLV persistent antigenemia also presented chronic feline gingival stomatitis, an exacerbated inflammatory response in the oral cavity of multifactorial origin (Souza et al., 2002) characterized by increased expression of pro-inflammatory cytokines in circulating lymphocytes (Quimby et al., 2008) due to chronic antigenic stimulation or immunological dysregulation (Hartmann, 2012). This condition is frequently associated with infection by calicivirus, but not retroviruses, since these promote a depletion of the inflammatory response. However, these may also favor infection by calicivirus (Hartmann, 2012).

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.

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). Regading 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.

Conclusions

The data of this study showed the high frequency of FeLV, in its progressive form (persistent antigenemia), in cats attended in the state of Espírito Santo, but low frequency of FIV by the immunoenzymatic test. Mediastinal lymphoma was often associated with FeLV diagnosis.

Ethics statement

Not applicable.

Financial support

No funding.

Conflict of interests

No conflict of interests declared concerning the publication.

Authors' contributions

All authors contributed equally to the development of this research and writing of the manuscript.

Availability of complementary results

No complementary results are available.

The study was carried out at Universidade Vila Velha – UVV, Vila Velha, ES, Brasil.

References

Almeida, N. R., Danelli, M. G. M., Silva, L. H. P., Hagiwara, M. K., & Mazur, C. (2012). Prevalence of feline leukemia virus infection in domestic cats in Rio de Janeiro. *Journal of Feline Medicine and Surgery*, *14*(8), 583-586. http://dx.doi.org/10.1177/1098612X12444693. PMid:22492347.

Bandecchi, P., Dell'omodarme, M., Magi, M., Palamidessi, A., & Prati, M. C. (2006). Feline leukaemia virus (FeLV) and feline immunodeficiency virus infections in cats in the Pisa district of Tuscany, and attempts to control FeLV infection in a colony of domestic cats by vaccination. *The Veterinary Record*, *158*(16), 555-557. http://dx.doi.org/10.1136/vr.158.16.555. PMid:16632529.

Biezus, G., Machado, G., Ferian, P. E., da Costa, U. M., Pereira, L. H. H. D. S., Withoeft, J. A., Nunes, I. A. C., Muller, T. R., de Cristo, T. G., & Casagrande, R. A. (2019). Prevalence of and factors associated with feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) in cats of the state of Santa Catarina, Brazil. *Comparative Immunology, Microbiology and Infectious Diseases*, *63*, 17-21. http://dx.doi.org/10.1016/j.cimid.2018.12.004. PMid:30961813.

Coelho, F. M., Maia, M. Q., Luppi, M. M., Costa, E. A., Luiz, A. P. M. F., Ribeiro, N. A., Bomfim, M. R. Q., Fonseca, F. G., & Resende, M. (2011). Ocorrência do vírus da leucemia felina em Feliscattus em Belo Horizonte. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *63*(3), 778-783. http://dx.doi.org/10.1590/S0102-09352011000300037.

Egenvall, A., Nødtvedt, A., Häggström, J., Ström Holst, B., Möller, L., & Bonnett, B. N. (2009). Mortality of Life-Insured Swedish cats during 1999-2006: Age, breed, sex, and diagnosis. *Journal of Veterinary Internal Medicine*, *23*(6), 1175-1183. http://dx.doi.org/10.1111/j.1939-1676.2009.0396.x. PMid:19780926.

Hagiwara, M. K., Reche, A., & Lucas, S. R. R. (1997). Estudo clínico da infecção de felinos pelo vírus da leucemia felina em São Paulo. *Revista Brasileira de Ciência Veterinária*, *4*(1), 35-38. http://dx.doi.org/10.4322/rbcv.2015.065.

Hartmann, K. (2012). Clinical aspects of feline retroviruses: a review. *Viruses*, *4*(11), 2684-2710. http://dx.doi.org/10.3390/v4112684. PMid:23202500.

Herring, E. S., Troy, G. C., Toth, T. E., Forrester, S. D., Weigt, L. A., & Herring, I. P. (2001). Detection of feline leukaemia virus in blood and bone marrow of cats with varying suspicion of latent infection. *Journal of Feline Medicine and Surgery*, *3*(3), 133-141. http://dx.doi.org/10.1053/jfms.2001.0132. PMid:11876630.

Hofmann-lehmann, R., Tandon, R., Boretti, F. S., Meli, M. L., Willi, B., Cattori, V., Gomes-Keller, M. A., Ossent, P., Golder, M. C., Flynn, J. N., & Lutz, H. (2006). Reassessment of feline leukaemia virus (FeLV) vaccines with novel sensitive molecular assays. *Vaccine*, *24*(8), 1087-1094. http://dx.doi.org/10.1016/j.vaccine.2005.09.010. PMid:16198454.

Hosie, M. J., Robertson, C., & Jarrett, O. (1989). Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. *The Veterinary Record*, *125*(11), 293-297. http://dx.doi.org/10.1136/vr.125.11.293. PMid:2554556.

Ito, J., Watanabe, S., Hiratsuka, T., Kuse, K., Odahara, Y., Ochi, H., Kawamura, M., & Nishigaki, K. (2013). Refrex-1, a soluble restriction factor against feline endogenous and exogenous retroviruses. *Journal of Virology*, *87*(22), 12029-12040. http://dx.doi.org/10.1128/JVI.01267-13. PMid:23966402.

Jarrett, W. F., Crawford, E. M., Martin, W. B., & Davie, F. (1964). A virus-like particle associated with leukaemia (lymphosarcoma). *Nature*, *202*(4932), 567-569. http://dx.doi.org/10.1038/202567a0. PMid:14195054.

Levy, J. K., Crawford, P. C., Kusuhara, H., Motokawa, K., Gemma, T., Watanabe, R., Arai, S., Bienzle, D., & Hohdatsu, T. (2008). Differentiation of feline immunodeficiency virus vaccination, infection, or vaccination and infection in cats. *Journal of Veterinary Internal Medicine*, *22*(2), 330-334. http://dx.doi.org/10.1111/j.1939-1676.2008.0078.x. PMid:18371028.

Levy, J. K., Scott, H. M., Lachtara, J. L., & Crawford, P. C. (2006). Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in north america and risk factors for seropositivity. *Journal of the American Veterinary Medical Association*, *228*(3), 371-376. http://dx.doi.org/10.2460/javma.228.3.371. PMid:16448357.

Little, S., Levy, J., Hartmann, K., Hofmann-Lehmann, R., Hosie, M., Olah, G., & Denis, K. S. (2020). AAFP feline retrovirus testing and management guidelines. *Journal of Feline Medicine and Surgery*, *22*(1), 5-30. http://dx.doi.org/10.1177/1098612X19895940. PMid:31916872.

Mendes-de-Almeida, F., Faria, M. C. F., Branco, A. S., Serrão, M. L., Souza, A. M., Almosny, N., Charme, M., & Labarthe, N. (2004). Sanitary Conditions of a colony of urban feral cats (Felis catus, Linnaeus, 1758) in a Zoological Garden in Rio de Janeiro, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, *46*(5), 269-274. http://dx.doi.org/10.1590/S0036-46652004000500007. PMid:15517031.

Miyake, A., Watanabe, S., Hiratsuka, T., Ito, J., Ngo, M. H., Makundi, I., Kawasaki, J., Endo, Y., Tsujimoto, H., & Nishigaki, K. (2016). Novel feline leukemia virus interference group based on the ENV gene. *Journal of Virology*, *90*(9), 4832-4837. http://dx.doi.org/10.1128/JVI.03229-15. PMid:26889025.

Modiano, J. F., Henry, C. J., & Macy, D. W. (2013). The etiology of cancer. In S. J. Withrow, D. M. Vail & R. L. Page (Eds.), *Withrow and MacEwan's small animal clinical oncology* (5th ed., pp. 1-29). USA: Elsevier Saunders.

Ogilvie, G. K., Tompkins, M. B., & Tompkins, W. A. (1988). Clinical and immunologic aspects of FeLV-induced immunosuppression. *Veterinary Microbiology*, *17*(3), 287-296. http://dx.doi.org/10.1016/0378-1135(88)90070-3. PMid:2847393.

Pedersen, N. C., Ho, E. W., Brown, M. L., & Yamamoto, J. K. (1987). Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science*, *235*(4790), 790-793. http://dx.doi.org/10.1126/science.3643650. PMid:3643650.

Poli, A., Abramo, F., Baldinotti, F., Pistello, M., Da Prato, L., & Bendinelli, M. (1994). Malignant lymphoma associated with experimentally induced feline immunodeficiency virus infection. *Journal of Comparative Pathology*, *110*(4), 319-328. http://dx.doi.org/10.1016/S0021-9975(08)80309-X. PMid:7914523.

Quimby, J. M., Elston, T., Hawley, J., Brewer, M., Miller, A., & Lappin, M. R. (2008). Evaluation of the association of Bartonella species, feline herpesvirus 1, feline calicivirus, feline leukemia virus and feline immunodeficiency virus with chronic feline gingivostomatitis. *Journal of Feline Medicine and Surgery*, *10*(1), 66-72. http://dx.doi.org/10.1016/j.jfms.2007.05.007. PMid:17766156.

Ravazzolo, A. P. & Costa, U. (2007). Retroviroses. In Flores, E.F. (Ed.), *Virologia Veterinária* (pp. 811- 836). Santa Maria, Rio Grande do Sul: Editora da Universidade Federal de Santa Maria.

Rojko, J. L., & Hardy, W. D. (1994). Feline leukemia virus and other retroviruses. In R. G. Sherding, (Eds.), *The cat diseases and clinical management* (2nd ed., pp. 263-432). London: Churchill Livingstone.

Shelton, G. H., Grant, C. K., Cotter, S. M., Gardner, M. B., Hardy Junior, W. D., & DiGiacomo, R. F. (1990). Feline immunodeficiency virus and feline leukemia virus infection and their relationships to lymphoid malignancies in cats: a retrospective study (1968-1988). *Journal of Acquired Immune Deficiency Syndromes*, *3*(6), 623-630. PMid:2159993.

Souza, H. J. M., Teixeira, C. H. R., & Graça, R. F. S. (2002). Estudo epidemiológico de infecções pelo vírus da leucemia e/ou imunodeficiência felina, em gatos domésticos do município do Rio de Janeiro. *La Clinica Veterinaria*, *36*(36), 14-21.

Teixeira, B. M., Rajão, D. S., Haddad, J. P. A., Leite, R. C., & Reis, J. K. P. (2007). Ocorrência do vírus da imunodeficiência felina e do vírus da leucemia felina em gatos domésticos mantidos em abrigos no município de Belo Horizonte. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *59*(4), 939-947. http://dx.doi.org/10.1590/S0102-09352007000400019.

Westman, M. E., Malik, R., Hall, E., Sheehy, P. A., & Norris, J. M. (2015). Determining the feline immunodeficiency virus (FIV) status of FIV-vaccinated cats using point-of-care antibody kits. *Comparative Immunology, Microbiology and Infectious Diseases*, *42*, 43-52. http://dx.doi.org/10.1016/j.cimid.2015.07.004. PMid:26459979.

Yilmaz, H., Ilgaz, A., & Harbour, D. (2000). A prevalence of FIV and FeLV infections in cats in Istanbul. *Journal of Feline Medicine and Surgery*, *2*(1), 69-70. http://dx.doi.org/10.1053/jfms.2000.0066. PMid:11716594.