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Prevalence of Feline Leukemia Virus and Feline Immunodeficiency Virus in Patients from Veterinary Centers in Three Colombian Cities

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Abstract: In Colombia, research on Feline Leukemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV) is incipient, despite being the viruses of greatest clinical and epidemiological importance in domestic cats, presenting a worldwide distribution but with very variable prevalence. To determine the molecular prevalence of FeLV and FIV and their associated factors in domestic cats treated in veterinary centers in three Colombian cities. A cross-sectional study with 1,708 felines evaluated for FeLV and 1,646 for FIV by qPCR was performed, following the ethical regulations for protection and research with animals. To guarantee methodological quality, selection and information bias control and confusion analysis with multivariate regression were performed. The prevalence of each virus was determined with its 95% confidence interval, statistically significant differences were identified using Chi-square test and adjusted odds ratios were calculated to determine the strength of the association in SPSS 29.0. The prevalence of FeLV was 11.8%, which presented a statistical difference with breed and age group, being higher in mixed breed (13.2%) and young adults (17.3%). The prevalence of FIV was 4.7% and this presented statistically significant differences according to breed, sex and age, being higher in mixed breed (5.1%), males (6.6%) and Seniors (9.1%). Two Colombian studies published on these viruses were carried out with ELISA or applying PCR to a small number of symptomatic animals, for this reason, this is the first study that shows a robust approximation of the molecular prevalence of these viruses in a passive epidemiological surveillance program. A high prevalence of both viruses was found and the subgroups with the highest occurrence of infection were identified. This is decisive to prioritize groups in clinical care, active epidemiological surveillance programs and etiological investigations.

Keywords: Feline Leukemia Virus, Feline Immunodeficiency Virus, Molecular Prevalence, Associated Factors, Colombia

Introduction

Feline Leukemia Virus (FeLV) belongs to the *Retroviridae* family, *Gammaretrovirus* genus. FeLV has A, B, C, AC, D and T subgroups; the first subgroup is the most infectious (Ramírez *et al*., 2016). Feline Immunodeficiency Virus (FIV) belongs to the *Retroviridae* family, *Lentivirus* genus; it has seven phylogenetic subtypes, A and B are widely distributed, while subtypes C, D, F and U-NZenv are found in lower proportion (Szilasi *et al*., 2019; Biondo *et al*., 2023). FeLV and FIV are considered the viruses of greatest clinical and epidemiological importance in domestic cats because of their shared transmission mechanisms, clinical spectrum, morbidity, mortality and impact on animal welfare (Moreno-García *et al*., 2022). These retroviruses have a high capacity for mutation and recombination, which favors a large population of viral subtypes with

different pathogenic capacities (Perharić *et al*., 2018; Canto-Valdés *et al*., 2019). FeLV has more pathogenic and lethality than FIV, FeLV-B subtype has been associated with lymphomas, FeLV-C with aplastic anemia and FeLV-T with immunodeficiencies (Ramírez *et al*., 2016; Hartmann, 2012; Chiu *et al*., 2018).

FIV in the acute phase produces transient fever, lymphadenopathy and lymphopenia, followed by the asymptomatic primary phase and progressive dysfunction of the immune system and chronic infections (Little *et al*., 2020). FeLV produces symptoms similar to FIV, in addition to opportunistic diseases such as stomatitis, dermatitis, gingivitis and neuropathological disorders (Seetaha *et al*., 2020). Added to these clinical factors, the diagnosis is realized with Enzyme-Linked Immunosorbent Assay (ELISA) for FeLV with the detection of p27 antigen and for FIV antibody against the p24 protein. Polymerase Chain Reaction (PCR) is also used in initial diagnosis, as a confirmatory test, or when is suspected false negative in ELISA (Sivagurunathan *et al*., 2018; Szilasi *et al*., 2021).

The distribution of these retroviruses is worldwide and their prevalence can range between 2 and 31% (Lacerda *et al*., 2017). A study in Hungary with 335 cats reported a prevalence of 11.8% for FeLV and 9.9% for FIV using ELISA, results that increased to 17.3 and 13.1%, respectively, when using PCR; without statistically significant differences according to age, sex, castration status and vaccination (Szilasi *et al*., 2019). In Germany, a study with 17,462 cats reported a prevalence of FIV of 3.2% and 3.6 for FeLV using ELISA; no statistically significant differences according to age or number of cats in the household were found; however, there was higher risk in male and mixed breed cats (Gleich *et al*., 2009). In Greece, 435 cats were evaluated using ELISA, the seroprevalence was 3.9% for FeLV and 9.2% for FIV; advanced age, male sex and access to fresh air were the main factors associated with FIV (Kokkinaki *et al*., 2021). A study conducted in Lebanon using ELISA determined an FIV prevalence of 18.8% in 260 cats; a statistical association was only found with the symptoms of the patients (Khalife and Kassaa, 2023). Another study in Brazil with 274 felines obtained a prevalence of 28.4% for FeLV and 7.6% for FIV; the main associated factor was gender (higher in males) and no association was found with age, breed and accommodations (Biezus *et al*., 2019). In Colombia, research on these viruses is scarce, in Valle de Aburrá the prevalence of FeLV in 100 cats evaluated by PCR was 60% (Ortega *et al*., 2020) and 10.7% for FIV using ELISA in 1,728 cats (Molina, 2020). As indicated in other zoonotic pathogens, it is essential to study the effects of gender and age, because these variables influence immune response and the prevalence of pathogens (Barrak *et al*., 2021).

The evidence available has several implications: (i) Seroprevalence studies (mainly based on ELISA) or molecular prevalence (using PCR) demonstrate heterogeneity in the occurrence of infection, (ii) PCR increases the detection of cases, the molecular prevalence is of greater relevance to estimating the true population infected, (iii) In each population evaluated, there is high variability in the factors associated and (iv) In Colombia, the evidence is incipient and it is practically impossible to extrapolate the results of the few prevalence studies conducted in the country, given the diversity of feline populations and associated factors present in each locality. The Colombian studies published on these viruses were carried out with ELISA or applying PCR to a small number of symptomatic animals, for this reason, this is the first study that shows a robust approximation of the molecular prevalence of these viruses in a passive epidemiological surveillance program. This is relevant when considering that in Colombia there are no epidemiological surveillance programs for these viruses and their diagnosis is only based on the demand of the animal owners. The objective of this study was to determine the molecular prevalence of FeLV and FIV and the factors associated with these infections in domestic cats treated in veterinary centers in the main cities of Colombia.

Materials and Methods

Type of Study and Population

A cross-sectional study to estimate the molecular prevalence of 1,708 cats evaluated for FeLV and 1,646 for FIV, from Medellín, Bogotá and Barranquilla; screened in the TestMol laboratory, a biotechnology center specialized in the molecular diagnosis of infectious agents in animals, epidemiological monitoring of diseases in small and large species and development of research under the one-health approach. Patient samples are sent to the laboratory by the treating physicians or veterinary clinics, who manage the patient's clinical information. We worked with the entire institutional population registered in the laboratory databases during 2023; therefore, the sample size was not estimated. The feline population of the cities studied is close to one million, a sample size was calculated for a descriptive study with an infinite population using the following parameters: Maximum prevalence of 30%, sampling error of 3 and 95% confidence, obtaining a sample size of 896 animals. This shows that the number of felines included for both

viruses is sufficient for the type of study conducted. It is worth specifying that the difference in the sample size of each virus is due to the characteristics of the passive surveillance program that works with user demand.

Sample Collection

The veterinarian takes the sample (EDTA blood, swab, aspirate, secretions, fecal matter, CSF, abdominal fluid, urine and tissue) from the evaluated feline and sends it to the lab. Samples remain frozen until collected by laboratory assistants who evaluate whether the samples correspond to the requested test. The samples are transported to the laboratory to preserve the cold chain until the genetic material is extracted.

RNA Extraction and qPCR

The extraction was performed using the automated method with the Kingfisher™ Duo open extraction equipment (Thermo Fisher Scientific Inc.) and the MagMAX™ CORE M express-96 nucleic acid purification kit (Thermo Fisher Scientific, Waltham, MA, USA) for RNA extraction, according to the conditions established by the manufacturer. The concentration and quality of the genetic material obtained were quantified by spectrophotometry using NanoDrop equipment (A and E LAB).

Reverse transcription of the genetic material was performed using the onescript® first-strand cDNA synthesis kit (onescript® hot reverse transcriptase). The volumes of reagents (buffer, RT enzyme, random primers and dNTPs) were used for the reaction mixture recommended by the manufacturer to finally add the quantified RNA and process in the end-point thermocycler (TUV Rheinland of North America INC).

qPCR Amplification

The real-time PCR assay was performed with specific primers (Macrogen©, Korea) that were designed within the Unique region (U3) of the Long Terminal Repeat (LTR) of the virus for the detection of its genetic material. The assay was performed on the 4-channel Mic qPCR cycler equipment (Biomolecular Systems, Australia), with its own protocols standardized by the laboratory. Positive controls were provided by the TestMol laboratory and PCR-grade water was used as a negative control. For the internal extraction control, specific primers for cytochrome B genes in mammals were employed (Pfeiffer *et al*., 2004).

Bias Control

The samples were taken by veterinary doctors with experience in the care of domestic cats. Traceability and metrological assurance were guaranteed in the transportation and processing of the samples and the molecular tests were performed according to the recommendations of the manufacturer by trained and standardized personnel. The laboratory has positive and negative controls, blind and independent evaluation of all positive and negative results (these are selected randomly) and an internal and external quality control program.

Statistical Analyses

All variables were categorical, therefore the description was made with absolute frequencies (n) and relative frequencies (%). The prevalence of each virus was determined with a 95% confidence interval. The identification of statistical differences was carried out using the Pearson chi-square test for nominal variables and the trend chi-square test for ordinal variables. Adjusted odds ratios were calculated to determine the strength of the association and to control possible confounding variables, using a multivariate binary logistic regression, whose goodness of fit was determined with Hosmer-Lemeshow. The analyses were performed in SPSS 29.0, with p-values less than 0.05 considered statistically significant.

Results

In the population analyzed for FeLV, the highest proportions were observed in Medellín (73.5%), in mixed breeds (86.1%) and in young adults (40.3). For FIV, similar results were found with 74.8% in Medellín, 85.6% for mixed breed and 41.7% in young adults (Table 1).

The prevalence of FeLV was 11.8%, this proportion presented statistically significant differences according to breed and age group. Based on these variables, the subgroups with the highest prevalence of FeLV were the mixed breed (13.2%) and young adults (17.3%). The prevalence of FIV was 4.7%, this propotión was statistically different according to sex and breed, FIV was higher in males with a prevalence of 6.6% and mixed breed cats with a prevalence of 5.1% (Table 2).

In the multivariate regression, no confounding variables were found. In this analysis, the prevalence of FeLV in the mixed breed was 4.1 times that observed in purebred cats and in young adults, it was 2.1 times compared to puppies. The occurrence of FIV in mixed breed, male and senior felines was around 2.5 times that found in purebred, females and puppies, respectively (Table 3).

Table 1: Description of the study population according to demographic characteristics

		1,708 cats evaluated for FeLV % (n)	1,646 cats evaluated for FIV % (n)
City	Medellín	73.5 (1256)	74.8 (1231)
	Bogotá	23.0 (0393)	21.9 (0360)
	Barranquilla	03.5 (0059)	03.3 (0055)
Breed	Purebred	13.9 (0238)	14.4 (0237)
	Mixed	86.1 (1470)	85.6 (1409)
Sex	Female	50.4 (0858)	49.9 (0816)
	Male	49.6 (0843)	50.1 (0819)
Age group	Puppy	37.3 (0599)	36.5 (0581)
	Young adult	40.3 (0648)	41.7 (0664)
	Mature adult	17.4 (0279)	17.0 (0271)
	Senior	05.0 (0080)	04.8 (0077)

Table 2: General prevalence of FeLV and FIV and specific prevalence of each virus according to the demographic characteristics of the study population

		FeLV % (n)	FIV % (n)
Prevalence		11.8 (202)	4.7 (77)
95% CI		10.3-13.4	3.6-5.7
City	Medellín	12.3 (155)	4.2 (52)
	Bogotá	09.2 (36)	5.6 (20)
	Barranquilla	18.6 (11)	9.1 (05)
	p Pearson Chi^2	00.060	0.166
Breed	Purebred	03.4 (8)	2.1 (05)
	Mixed	13.2 (194)	5.1 (72)
	p Pearson Chi^2	<0.001**	0.043*
Sex	Female	12.0 (103)	2.8 (23)
	Male	11.6 (98)	6.6 (54)
	p Pearson Chi^2	00.8081	<0.001**
Age group	Puppy	08.3 (50)	4.0 (23)
	Young adult	17.3 (112)	4.7 (31)
	Mature adult	08.6 (24)	5.2 (14)
	Senior	07.5 (6)	9.1 (07)
	p Tendence Chi^2	<0.001**	0.085

*p<0.05. **p<0.01

Table 3: Multivariate logistic regression models for FeLV and FIV

	B	Error	Wald	Sig.	OR (95%CI)
FeLV					
Breed (mixed/purebred)	1.42	0.425	11.213	<0.001**	4.1 (1.8-9.5)**
Age group			24.107	<0.001**	
Young adult/puppy	0.74	0.182	16.518	<0.001**	2.01 (1.5-3.0)**
Mature adult/puppy	-0.07	0.261	00.077	0.782	0.93 (0.6-1.6)
Senior/puppy	-0.11	0.452	00.062	0.804	0.89 (0.4-2.2)
p Hosmer-Lemeshow = 0.715					
FIV					
Breed (mixed/purebred)	0.96	0.047	04.013	0.042*	2.6 (1.1-6.6)**
Sex (male/female)	0.89	0.026	12.011	<0.001**	2.5 (1.5-4.1)**
Age group			04.060	0.204	
Young adult/puppy	0.16	0.028	00.320	0.572	1.2 (0.7-2.0)
Mature adult/puppy	0.20	0.035	00.320	0.572	1.2 (0.6-2.4)
Senior/puppy	0.97	0.046	04.055	0.033*	2.6 (1.1-6.5)*
p Hosmer-lemeshow = 0.918					

*p<0.05. **p<0.01

Discussion

In this study, a molecular prevalence of FeLV of 11.8% was found in 1,708 cats and 4.7% for FIV in 1,646 cats. Previous studies have reported a prevalence of FeLV of 12% in Malaysia (Sivagurunathan *et al*., 2018; Bande *et al*., 2012); and a prevalence for FIV of 4.3% in Canada (Sprißler *et al*., 2022), 5.8% in Thailand (Levy *et al*., 2006) and 5.8% in Brazil (Biezus *et al*., 2019). These proportions showed similar endemic levels in different populations of cats institutionalized or cared for in veterinary clinics. In addition, it is important to keep in mind that some of these studies were conducted with ELISA tests, indicating that the diagnosis made with highly sensitive and reliable tests is pertinent in these contexts (Fig. 1).

Despite the above, some previous studies have reported a higher prevalence for both viruses. FeLV prevalence of 59.4% was recorded in Colombia (Ortega *et al*., 2020), 22.3% in Brazil (Biezus *et al*., 2019) and 13.84% in Lebanon (Khalife and Kassaa, 2023); while FIV prevalence of 10% has been reported in Malaysia (Sivagurunathan *et al*., 2018), 18.8% in Lebanon (Biezus *et al*., 2019) and 31.3% in peninsular Malaysia (Bande *et al*., 2012). These differences can be attributed to several factors: Some studies included cats with obvious signs of disease, stray cats, use of ELISA tests with specificity problems (which can increase the proportion of false positives), design and validation of the tests (considering the viral genetic diversity in the nucleotide sequence can cause primer binding failure), among other clinical aspects. For example, in FeLV, it must be considered that in regressive phases of the virus, there is no viremia and latency occur in the bone marrow, making these individuals positive for the Provirus (PCR) but negative for the FeLV antigen (ELISA) (Hartmann, 2017); or FeLV and FIV are not found in blood but appear in other secretions such as milk, semen, vaginal, or nasal (Canto-Valdés *et al*., 2019). In addition, epidemiological aspects can be added that explain variations in endemic levels, as well as the use of diagnostic tests suitable for certain phases of infection, which accounts for the complexity and importance of this type of estimate in each country of interest.

The results corroborate the importance of qPCR for diagnosis and epidemiological surveillance, depending on the phase of infection. In suspected cases of FeLV, qPCR would be the most appropriate test because it allows animals to be detected in latent stages of infection without detectable viremia or circulating antigens, which generate false negatives using ELISA (Galdo Novo *et al*., 2016). In FIV, the use of ELISA to detect antibodies could generate false positives in young animals with maternal antibodies or false negatives because the antibody response is usually misleadingly low during the first 2-4 weeks of infection (Wilkes *et al*., 2015; Swango, 1991).

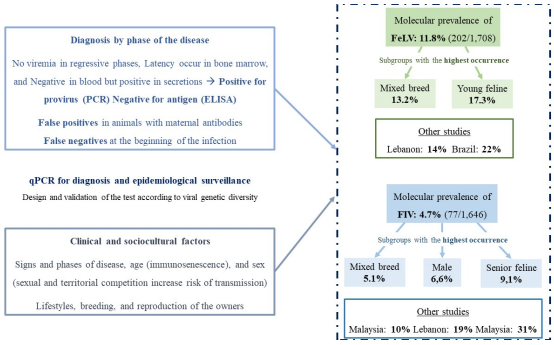

Fig. 1: Graphical synthesis of results and discussion

According to breed, studies from Brazil and Malaysia reported a greater prevalence of FeLV in mixed breeds, whereas in FIV evidence was not consistent if a greater proportion of cases was found in purebred (Sivagurunathan *et al*., 2018; Chiu *et al*., 2018). This implies challenges for subsequent studies, given that some authors have explained the differences by breed based on the lifestyles, breeding and reproduction of the owners. Particularly in purebreds, they tend to share narrower spaces and seek their reproduction without prior medical evaluations (which could include viral detection), among other practices that must be explored in depth in subsequent investigations, given that they were not reported in the medical charts used in this investigation. Genetic hypotheses have also been generated, since some breeds may have a greater predisposition or susceptibility to these viruses and mixed breeds have greater genetic diversity and are exposed to varied environments that can affect the immune response.

Regarding sex, similar to this study, research from Lebanon and Malaysia reported a higher prevalence of FIV in males. This higher prevalence in males has been described in several investigations, especially in non-neutered individuals who have access to the outside and due to sexual and territorial competition, they generate fights with a high risk of transmission (Costa *et al*., 2017). In turn, the differences between the current study and the findings from Lebanon and Malaysia for the distribution of FeLV according to sex could be explained by the epidemiological and clinical particularities of each population, which should be improved in the completion and registration of medical charts for further studies.

Based on age, higher prevalence was observed in senior cats, which can be explained by immunosenescence, specifically by alterations in the innate and adaptive immune response due to aging, which can increase susceptibility to the virus, since age is a risk factor for the presence of these retroviruses in felines (Ramírez *et al*., 2016).

The main limitations of this study are the cross-sectional design, which results in exploratory and non-

etiological statistical associations; and limitations in the completion of the medical chart which prevents delving into some clinical and epidemiological factors that could be useful for the analysis of these infections. The fact that Colombia does not have an active epidemiological surveillance program for these viruses restricts research to the data available in clinics, which are limited. This situation does not allow us to delve deeper into immunological, clinical and epidemiological explanations for the molecular prevalence found and to identify the reasons behind regional or methodological differences. The main value of this research is found in the large sample size and the use of molecular diagnosis for a more precise evaluation of the presence of these viral agents and is one of the few studies in Colombia with reports on FeLV and FIV.

Conclusion

A high prevalence of FeLV and FIV was found and the feline population and subgroups with the highest occurrence of each infection were identified, which is decisive for prioritizing groups in clinical care, epidemiological surveillance programs and in the design of subsequent etiological investigations. This research demonstrated the circulation of both viruses in the main cities of Colombia and provides initial molecular prevalences to establish endemic levels in passive epidemiological surveillance programs, which is key to guiding public policies for animal health or concrete actions such as the promotion of vaccination and early and massive screening.

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Author's Contributions

All authors contributed equally to the process of data analysis and preparation of the article; conceptualization, data curation, formal analysis, investigation, methodology, visualization, written reviewed and edited.

All authors are responsible for all aspects of the manuscript ensuring the accuracy of the paper and have read and agreed to the published version of the manuscript.

Ethics

This research did not manipulate patients, a study was conducted with a secondary source of the results of the qPCR tests performed in the laboratory. The use of each patient's information for research was supported by signing an informed consent by the guardians. All data provided were authorized by the laboratory. The information included epidemiological and demographic data, sex, age, breed and presence or absence of the virus. A confidentiality agreement was established and signed for the information provided by TESTMOL. Veterinary Doctors performed the sampling in compliance with all the professional ethics protocols in Colombia for the handling of animals in the veterinary medical practice under law 576 of 2000 and law 84 of 1989. Additionally, no personal information was collected from the owners of the animals. This approach adheres to ethical principles, ensuring that the privacy and confidentiality of individuals are respected.

Conflict of Interest

None of the authors declare conflict of interest.

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