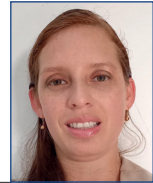


# Seroprevalence of feline leukaemia and feline immunodeficiency virus in domestic cats from Pereira, Risaralda, Colombia

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## Abstract

In Colombia, the increase in the population of domestic cats is directly related to outbreak diseases and associated with risks to domestic and wild animal health. Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are two retroviral agents representing the most significant cause of feline morbimortality. Since they can be easily transmitted between cats through body fluids, these viruses can cause neoplastic and non-neoplastic alterations, immunosuppression, non-specific chronic diseases, and infections by opportunistic microorganisms. In order to improve animal health status and prevent these diseases from spreading, a timely and reliable diagnosis is necessary to detect these infectious agents. However, due to factors such as the distribution, size, mobility, and growth of the population of domestic felines, the prevalence and possible risk factors associated with the presentation of these viruses are currently unknown. This research was a

descriptive cross-sectional study in which 100 domestic felines were sampled. The simultaneous diagnosis of the viruses was performed using whole blood samples for feline leukaemia and blood plasma for feline immunodeficiency and analysed by the Uranotest FeLV-FIV immunochromatographic dual method. The presence of infectious agents was correlated with study variables such as gender, age, origin, and feline habits. A prevalence of 14% for FIV and only 6% for FeLV was observed, with a 2% incidence of coinfection. Correlations were observed between infection and the age of the animals and their place of origin. The results allow us to know the current state of the spread of these infectious agents, which is essential for making decisions about animal health departmental prevention plans.

**Key words:** *diagnostic; immunoassay; prevalence; tumour; viral diseases*

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## Introduction

Feline leukaemia and feline immunodeficiency are the retroviruses with the most significant impact on the health of domestic cats and some wild felids worldwide (Pino et al., 2015). These pathogens generate persistent infections, which seriously affect the immune system of infected animals (Lagos-Lopez et al., 2018). These feline retroviruses have high recombination and mutation capacities, which implies that they have great relevance in the health of felines, not only because of their clinical manifestations but also because their diagnosis can be highly complex (Canto-Valdés et al., 2019).

The feline leukaemia virus (FeLV) is considered the virus that most generates clinical syndromes in cats. It has a high pathogenic potential, and despite the existence of its vaccine, there is high morbidity and mortality worldwide (Tique, 2009). It is a virus of the *Retroviridae* family, *Orthoretroviridae* subfamily, and *gammaretrovirus* genus (Nascimento et al., 2014). Three subgroups have been identified (A, B, C, and T variant of A), which can act alone or together to cause disease (Tique et al., 2009). FeLV-A is the most frequent subgroup and is responsible for transmission between animals; therefore, vaccination against subgroup A virus is the only way to induce immunity to said infection (Willett., 2013).

FeLV transmission occurs through close contact with infected cats through bites, open wounds, and the common use of litter boxes or food and water containers (Nascimento et al., 2014). However, other routes of transmission have been reported, such as grooming (Lagos-López et al., 2018), saliva, milk, urine, and faeces (Sykes et al., 2014), vertical transmission (Nascimento et al., 2014), various fomites (Willett et al., 2013), and blood

transfusions or needles (Calle-Restrepo, 2013). FeLV has no preference for animal breed, age, or sex. However, it is usually found more frequently in stray animals, and young cats between 1 to 3 years are usually the most susceptible (Tique et al., 2009). FeLV infection differs from cat to cat based on immunocompetence. However, the pathogenicity of the virus is related to its strain type, the virus subtype, and the viral load (Hartmann, 2011).

Feline immunodeficiency virus (FIV) belongs to the *Retroviridae* family, the *Lentivirinae* subfamily, and *lentivirus* genus (Tique et al., 2009). It can cause clinical signs similar to human immunodeficiency HIV (Long et al., 2021). Being a lentivirus, it requires a very long incubation time from the start of infection to the manifestation of clinical signs (Kokkinaki et al., 2021). Cats with this virus can live for long periods with constant medical check-ups and special handling by their owners and veterinarians (Lagos-López et al., 2018). This virus has a specific tropism for T lymphocytes, which causes a progressive disorder in the immune system until immunodeficiency. However, the ability to replicate in macrophages, monocytes, and dendritic cells like HIV has also been reported (Power et al., 2018).

Some species of wild cats, such as the wild cat, lion, tiger, jaguar, and leopard, are also susceptible to certain strains of FIV (Tique et al., 2009). This virus has different subtypes (A, B, C, D, E and T), subtypes A and B being the most common (Erbeck et al., 2021). It mainly affects wild-type males (Pino et al., 2015). Other risk factors include age, which has a higher incidence in individuals between 5 and 10 years old (Hartmann et al., 2011). Some authors mention a higher susceptibility in mestizo individuals than in pure breeds (Rocha et al., 2014).

The primary transmission mechanism is horizontally through bites from infected cats since the virus is eliminated through saliva, with wild-type males having the highest incidence of FIV due to frequent territorial confrontations (Sacristán et al., 2021). In a shared environment where individuals do not present aggression, the transmission of this virus is uncommon, even if some felines are infected. Although vertical transmission is scarce, it can occur through the placenta, when the offspring passes through the channel calving, or when the calf consumes infected milk (Lagos-López et al., 2018). Sexual transmission is rare, although the virus can be isolated from the semen of infected cats (Little et al., 2020). FIV, which causes feline acquired immunodeficiency syndrome, currently has no preventive vaccine or treatment (Hartmann, 2012).

Immunosuppressive and debilitating conditions, such as the nonspecific clinical manifestations and the ease of transmission of FIV and FeLV, make the diagnosis of these diseases essential in feline clinical practice (Sobrinho et al., 2011). Both infections can cause immune-mediated diseases, development of neoplasms, neurological symptoms, cytopaenias, and the most characteristic sign of these are opportunistic infections of viral, bacterial, and fungal origin during the course of the diseases (Aiyaranoi et al., 2018).

For the diagnosis of FIV and FeLV, various serological tests have been developed to identify antibodies to FIV or the viral antigen in FeLV (Boenzli et al., 2014). Indirect Immunofluorescence (IIF) and ELISA tests are currently available for detecting specific antibodies, and Polymerase Chain Reaction (PCR) for detecting gene segments (Ortiz et al., 2011). Additionally, some rapid tests have been

designed, such as immunochromatographic kits from serum or whole blood, for detecting antibodies to the viral proteins p24, gp40, and p15 in FIV and viral antigen p27 in FeLV, these tests being the most used and recommended as detection tests for these pathologies due to their easy handling, rapid results, high sensitivity, and specificity (Calle-Restrepo et al., 2013). This study demonstrated the presence of these two infectious agents in domestic felines through immunochromatographic tests to know the prevalence of these diseases and the factors related to their presence in the city of Pereira Risaralda, Colombia.

## Materials and methods

### *Ethical statement*

This project was approved by the Animal Bioethics Committee of UNISARC (Corporación Universitaria Santa Rosa de Cabal) (Date: 20 April 2021)

### *Experimental design*

Location and cat sampling population:

A cross-sectional study was conducted out with the feline population in the Animal Welfare Mobile Unit programme of Ukumarí Biopark for a spaying campaign in Pereira city in 2021, and street felines cared for rescue foundations in the same city.

Qualified veterinarians took the samples with the prior informed consent of the cat owner, i.e., the person donating the cat blood sample. A survey was completed with basic information on the feline, such as name, age, procedence, socioeconomic stratum, sex, nutrition type, and habits (street or home).

A population of 100 cats was sampled with a level significance of 95%. Variables such as sex, age, habits, and socioeconomic stratum to which the feline

belongs were considered: one (low-low), two (low), and three (medium-low) from the city of Pereira (Caballero Méndez et al., 2022), that correspond to areas with limited economic resources, limiting their access to veterinary services, as possible associated risk factors. Regarding age, the felines were categorized into four age groups: juvenile or group 1 (< 1 year), adult or group 2 (1-6 years), old or group 3 (> 6 years), and group 4 for whose ages are not reported. The population number was defined based on other studies and prevalence reported for other studies (Tique et al., 2009; Massey et al., 2019). However, the sample size was smaller than the last report in this geographic zone (total felines: 338) (Santisteban-Arenas et al., 2021). The prevalence data in this study show the current epidemiology situation in zones of low economic status.

### **Animal sampling**

Some cats were anesthetized during sterilization surgery and other cats were immobilized to obtain blood samples. The cephalic or external jugular veins were located, and the vein puncture was performed with 24 needles; the sample was extracted by aspiration with a syringe, 1 mL venous blood was collected in purple-top BD Vacutainer pediatric tubes containing EDTA as an anticoagulant. The tubes were marked with a consecutive identification number assigned to each animal. The samples were stored at 4°C and transported for analysis to the Multifunctional Laboratory of the Health Sciences Faculty, Technological University of Pereira.

### **Sample processing**

In the laboratory, the qualitative detection of feline leukaemia virus antigens and feline immunodeficiency virus (FIV) antibodies was carried out by means of

joint immunochromatography. The test consists of two separate zones, first for detecting FeLV-antigen and second for detecting FIV-specific antibodies. Each zone consists of a rounded well where the sample is added (whole blood for feline leukaemia and blood plasma for feline immunodeficiency) and a results zone containing the T line (test line) and the C line (control line). The results were interpreted according to the manufacturer's instructions Uranotest FeLV-FIV. Sensitivity was 96% for FIV and 94% for FeLV, and specificity was 98% for FIV and 99% for FeLV.

### **Data analysis**

The frequency of animals infected with FIV and FeLV was determined, and a statistical description was made and variables such as origin, status, age group, genus, and sex correlated to determine possible risk factors. All data were subjected to normality and homoscedasticity tests before proceeding with the correlation analysis using the Chi-Square test and t-test ( $P < 0.05$ ). For all analyses, Statistical Analysis Software SAS was used.

## **Results**

The prevalence and possible risk factors of FeLV and FIV in Pereira city were determined. For this, blood samples of 100 domestic felines were analysed, of which 46% were females, and 54% were males (Table 1). We observed a higher prevalence for FIV of 14%, while for FeLV was only 6%, with a 2% rate of coinfection.

Regarding the sex variable, no correlation was observed between the male and female groups and the infections evaluated. Regarding the risk factors associated with FeLV, it was not possible to establish

**Table 1.** Feline leukaemia virus and feline immunodeficiency virus prevalence, in Pereira, Risaralda, Colombia, 2021

Virus	Females			Males			Total prevalence
	Positive	Negative	Total females	Positive	Negative	Total males	
FeLV	4	42	46	2	52	54	6%
FIV	6	40		8	46		14%

**Table 2.** Feline leukaemia virus and feline immunodeficiency virus prevalence, according to age in Pereira, Risaralda, Colombia, 2021

Age group	FeLV			FIV			Total
	Positive	Negative	Prev %	Positive	Negative	Prev %	
1 (<1 year)	1	32		1	32		33
2 (1–6 years)	4	49		9	44		53
3 (>6 years)	1	13		4	10		14
<b>Total</b>	6	94	6%	14	86	14%	100

a correlation between the presence of the infectious agent and any variable studied. However, for Feline Immunodeficiency Virus FIV, it was possible to establish important correlations from the perspective of epidemiological control, such as a correlation with socioeconomic stratum, since all animals infected with FIV came from stratum one (low-low) ( $P=0.0064$ ). Likewise, it was observed that young animals were the most affected by FIV, showing a negative correlation between age and disease frequency ( $P=0.0171$ ), with the youngest animals being the most diagnosed, with 72% of cases in animals between 0 and 6 years old (10 of 14 cases positives) (Table 2). Additionally, animals of advanced ages between 9 and 10 years were observed, related explicitly in one

of the sampled areas, with a positive correlation between the sampled area and their advanced age to be positive for FIV ( $P=0.0223$ ).

## Discussion

We observed a higher prevalence for FIV of 14%, while for FeLV was only 6%, with a 2% rate of coinfection. These results are similar to those reported in other countries such as the United States and Canada (Burling et al., 2017). In contrast, a Colombian report for Monteria city showed a lower prevalence of FIV (1.6%) but higher for FeLV (23.3%), with a similar prevalence for double infection (5%) (Tique et al., 2009). In Valle de Aburrá (Antioquia/Colombia), a prevalence

for FeLV of 21.89% was demonstrated for the viral antigen p27 (Molina, 2020), and a frequency for FIV was 10.71% detecting FIV-specific antibodies (anti-gp40) and the nuclear proteins p15, and p24 (Molina et al., 2013). For the department of Risaralda, a retrospective study was carried out between 2017 and 2018, obtaining a positivity incidence of 18.3% for FIV, 25.8% for FeLV, and a coinfection index of 8.2% (Santisteban-Arenas et al., 2021), demonstrating similar frequencies for FIV. However, in this study, the prevalence of FeLV was lower, similar to the coinfection rate. According to data from the Colombian Ministry of Health for 2019, Pereira city reported a population of approximately 43,331 domestic felines (MinSalud, 2019). Therefore, the current feline population is believed to be higher, and we can infer that the increase of these infectious agents can be proportional to the increase in this population.

This study results show that the sex variable showed no correlation with infections; however, studies have shown that seropositivity for FIV and FeLV is more frequent in males than females due to their free-roaming and more aggressive behaviour (Little et al., 2011).

Regarding the risk factors associated with FeLV, it was not possible to establish a correlation between the presence of the infectious agent and any variable studied. However, for FIV, it was possible to establish important correlations from the point of view of perspective epidemiological control, such as a correlation with socioeconomic stratum since all animals infected with FIV came from stratum one (low-low) ( $P=0.0064$ ). Some reports correlate low socioeconomic status closely with the incidence of FIV in domestic felines (Tran et al., 2019). Factors such as living conditions, aggressive behaviour, outdoor access, cohabitation with other

cats, mixed breeds, and coinfection with any of these agents are risk factors associated with the presentation of these infectious agents (Tique et al., 2009). Studies have reported that an outdoor lifestyle is a predisposing factor for FIV and FeLV infection (Chhetri et al., 2015), this condition allow that these viruses are mainly transmitted through bodily fluids or bites (Biezus et al., 2019).

This study showed that young animals were the most affected by FIV, though cats of advanced ages between 9 and 10 years were also diagnosed as positive, in one of the sampled areas. These results are congruent as reported by several studies where it is shown that elderly felines are more susceptible to FIV because the incubation period is longer. They can remain asymptomatic for a long time (Hartmann et al., 2012; Biezus et al., 2019) and a FIV-infected cat may survive several years because the disease may take longer to develop after infection and is more severe with increasing age (Collado et al., 2012). Therefore, prompt intervention in this sampled area is necessary to prevent the virus's spread to healthy felines.

The dual immunotest used in this study showed similar detection rates to other diagnostic techniques. Data sensibility near 100% allow for identification of true positives for both viruses, while the data specificity near 100% allows for identification of true negatives. This dual immunotest is the most used test in clinical diagnostic routines (Lacharoje et al., 2021). There are currently other technical, more sensible, and specific tests, such as PCR, ELISA, immunofluorescence, and culture, that allow for the detection of the antigen or viral DNA. However, these techniques are more expensive and are frequently unavailable to diagnostic laboratories routine (Levy et al., 2017).

## Conclusions

The prevalence obtained for the infectious agents FIV and FeLV in this study constitutes an overview of the current situation of these pathogens in the domestic feline population in the city of Pereira, Risaralda (Colombia), and a cause for concern due to the immunosuppressive potential caused by these pathogens accompanied by the ease of their transmission between domestic felines. Therefore, it is necessary to establish health and educational measures that allow for timely detection, control, prevention, and vaccination to mitigate the negative impacts on the public health of companion animals.

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## Seroprevalencija virusa mačje leukemije i mačje imunodeficijencije u kućnih mačaka iz Pereire, Risaralda, Kolumbija

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U Kolumbiji je povećanje populacije kućnih mačaka izravno povezano s izbijanjem bolesti i s rizicima za zdravlje domaćih i divljih životinja. Virus mačje leukemije (FeLV) i virus mačje imunodeficijencije (FIV) dva su retrovirusna uzročnika koji predstavljaju najznačajniji uzrok morbiditeta mačaka. S obzirom da se lako prenose između mačaka putem tjelesnih tekućina, ovi virusi mogu prouzročiti neoplastične i ne-neoplastične promjene, imunosupresiju, nespecifične kronične bolesti i infekcije oportunističkim mikroorganizmima. Da bi se poboljšao status zdravlja životinja i spriječilo širenje ovih bolesti, potrebna je pravovremena i pouzdana dijagnoza da bi se otkrio uzročnik ovih infekcija. Međutim, čimbenici poput distribucije, veličine, mobilnosti i rasta populacije domaćih mačaka uzrok su trenutnog nepoznavanja prevalencije i mogućih faktora rizika povezanih s prezentacijom ovih virusa. Ovo istraživanje bilo

je opisna usporedna studija u kojoj je uzorkovano 100 domaćih mačaka. Istovremena je dijagnoza virusa obavljena na uzorcima pune krvi za leukemiju mačaka i na plazmi za imunodeficijenciju mačaka, a analiza je obavljena pomoću Uranotest FeLV-FIV imunokromatografske dvostruke metode. Prisutnost uzročnika infekcije povezana je s varijablama studije poput spola, dobi, podrijetla i navika mačaka. Otkrivena je prevalencija od 14 % za FIV i tek 6 % za FeLV. Uz to, 2 % koinfekcije, a zamijećene su i veze između dobi životinja i mjesta njihovog podrijetla. Rezultati istraživanja daju nam uvid u trenutno stanje raširenosti spomenutih uzročnika infekcija. Ove informacije su ključne za donošenje odluka o preventivnim planovima odjela za zdravlje životinja.

**Ključne riječi:** dijagnoza, imunološko određivanje, prevalencija, tumor, virusne bolesti