

Whisking away the veil: exploring feline immunodeficiency virus and feline leukaemia virus prevalence among shelter cats in Portugal



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Abstract

Feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) are important pathogens affecting feline hosts worldwide, compromising the health, well-being, quality of life, and lifespan of infected cats. This study explores the prevalence of these viral agents among shelter cats in Portugal, aiming to unravel their complexities and implications. The prevalence of FIV and FeLV was assessed by collecting blood samples ($n = 326$) from cats during medical routine check-ups at shelter admission and testing them with an immunochromatographic kit. The overall seroprevalence of FIV and FeLV was 15.3% (50/326, 95% confidence interval [CI]: 11.6–19.7). The proportion of cats positive to FIV, FeLV, and both FIV and FeLV was 9.8% (32/326, 95%

CI: 6.8–13.6), 2.1% (7/326, 95% CI: 0.9–4.4) and 3.4% (11/326, 95% CI: 1.7–6.0), respectively. The prevalence of FIV and both FeLV and FIV appeared to be higher in the adult population. No statistical association was found between sex, breed, hair, or municipality, and FeLV, FIV and both FeLV and FIV prevalence. This is the first report on retroviral prevalence in shelter cats in Portugal, which provides important information regarding FIV and FeLV among stray cats in Portugal, highlighting the need for surveillance of those viruses, improved compliance with vaccination programmes, and vigilant management strategies to limit and control these viruses in the feline population.

Key words: cats; FeLV; FIV; Portugal; shelter

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Introduction

Feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) are global sources of infectious diseases among feline populations (Little et al., 2020).

Recognised for its capacity to debilitate the immune system, FIV prompts an acquired immunodeficiency syndrome (AIDS)-like status that progressively diminishes its function, similar to the impact of the human immunodeficiency virus (HIV) on the human immune system (Burkhard and Dean, 2003; Elder et al., 2010). Feline immunodeficiency virus causes a lifelong infection, arising from integrating a DNA copy of the viral RNA into the cat genome (Westman et al., 2019). Transmission of FIV predominantly occurs through bite wounds, introducing virus-laden saliva and FIV-infected white blood cells (Westman et al., 2022). Though the risk is minimal among cohabiting cats that refrain from conflict, there is a residual possibility of infection (Little et al., 2020).

On the other hand, FeLV is known to cause diverse illnesses, coupled with the development of neoplasms, bone marrow disorders and immunosuppression (Canto-Valdés et al., 2023). FeLV primarily spreads via oronasal exposure to virus-laden secretions through close contact, both vertically (from infected queens to their kittens) and horizontally (among cohabiting or aggressive cats). Kittens face heightened susceptibility, while resistance to FeLV increases with age (Willett and Hosie, 2013; Sykes and Hartmann, 2014).

Such dynamics place shelters in considerable jeopardy for transmitting FIV and FeLV among cats (Munro et al., 2014).

In the United States of America (USA) alone, more than 100,000 cats in animal

shelters annually test positive for either FeLV or FIV, comprising around 5% of the shelter cat population (Dezubiria et al., 2023).

The seroprevalence unveiled in recent studies underscores the need for enhanced commitment to infection prevention and control protocols, specifically to testing and vaccination guidelines (Little et al., 2020).

Guidelines set forth by the Association of Shelter Veterinarians (ASV) and the American Association of Feline Practitioners (AAFP) state that all cats should be screened for retroviruses to optimise individual and population health (Association of Shelter Veterinarians, 2020).

It is important to presume that all cats entering shelters may harbour infections, regardless of their prior living conditions. Hence, avoiding housing untested cats together is recommended due to the ease with which retroviruses disseminate via bodily fluids, especially blood and saliva (Little et al., 2020).

Certain commercially available FIV antibody detection kits target the humoral response to epitopes of the FIV's matrix and capsid proteins (p15 and p24, respectively), while others centre on the FIV transmembrane glycoprotein (gp40). However, ascertaining FeLV infections through the detection of antibodies has proven unreliable due to the cat's inconsistent antibody response and the possibility of "abortive infections" (Westman et al., 2019), when virus clearance and clinical recovery occurs due to the development of an appropriate humoral and cell-mediated immune response (Westman et al., 2016). Consequently, all available FeLV point-of-care test kits instead identify the FeLV capsid antigen (p27) (Westman et al., 2019).

Although scant studies are available concerning these viruses in Portugal (Du-

arte et al., 2012; Vilhena et al., 2013), none have specifically targeted the shelter cat population.

Therefore, this study endeavours to estimate the apparent prevalence of FIV and FeLV seropositive cats within an animal shelter in northeast Portugal.

Material and methods

Population and samples

This study was approved by the Ethics Committee of University of Trás-os-Montes e Alto Douro (UTAD) (process reference: Doc6-CE-UTAD-2022).

Blood was collected individually from each cat during routine medical check-ups upon admission. The sample was collected from the cephalic vein.

To detect FIV and FeLV, a kit based on an immunochromatographic technique (Uranotest® FeLV-FIV, Urano®vet, S.L, Barcelona, Spain) was used following the manufacturer's instructions. This test allows simultaneously qualitative detection of FIV antibody (gp40) and FeLV antigen (p27) in feline whole blood, serum, and plasma.

The Uranotest® FeLV-FIV reported a sensitivity of 94% versus viral isolation, a specificity of 99% versus viral isolation for FeLV, and a sensitivity of 96% versus Western Blot and a specificity of 98% versus Western Blot for FIV.

Data analysis

Statistical analysis was performed using JMP® 7.0 (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). Chi-square tests were used to compare proportions of positivity related to categorical independent variables and to establish significance within each independent variable (age group, sex, breed, hair, and municipality).

Cats were organised into three age groups: young (up to and including 11 months old), adult (12 to 83 months old), and senior (84 months and older).

Results

A total of 326 cats entering an official shelter were tested during the study period. The mean age was 15 months and there were 189 females (58.0%) and 137 males (42.0%). The sample population was sex-balanced (Tables 2, 3 and 4). All cats were sexually intact.

Cats came from the five municipalities of Terra Quente Transmontana: Mirandela (39.9%, $n = 130$), Alfândega da Fé (26.1%, $n = 85$), Carrazeda de Ansiães (12.6%, $n = 41$), Vila Flor (11.0%, $n = 36$) and Macedo de Cavaleiros (10.4%, $n = 34$). The 326 samples were collected from 2017 until 2023: 2017 (5.8%, $n = 19$), 2018 (23.3%, $n = 76$), 2019 (9.2%, $n = 30$), 2020 (12.0%, $n = 39$) and 2021 (26.7%, $n = 87$), 2022 (16.9%, $n = 55$) and 2023 (6.1%, $n = 20$) (Figure 1).

The overall seroprevalence of FIV and FeLV was 15.3% (50/326, 95% confidence interval (CI): 11.6–19.7) of the cats tested (Table 1). The proportion of cats positive to FIV, FeLV, and both FIV and FeLV was 9.8% (32/326, 95% CI: 6.8–13.6%), 2.1% (7/326, 95% CI: 0.9–4.4%) and 3.4% (11/326, 95% CI: 1.7–6.0%), respectively.

The seroprevalence of FIV based on different variables, age group, sex, breed, hair length, and municipality, is presented in Table 2.

Seroprevalence was statistically different among age groups, with a higher seroprevalence found in the adult group with 16.6% (26/157, 95% CI: 11.1–23.3), as opposed to 3.6% in young cats (6/169, 95% CI 1.3–7.6) (Table 2). No statistically significant differences were found between the categories of the independent

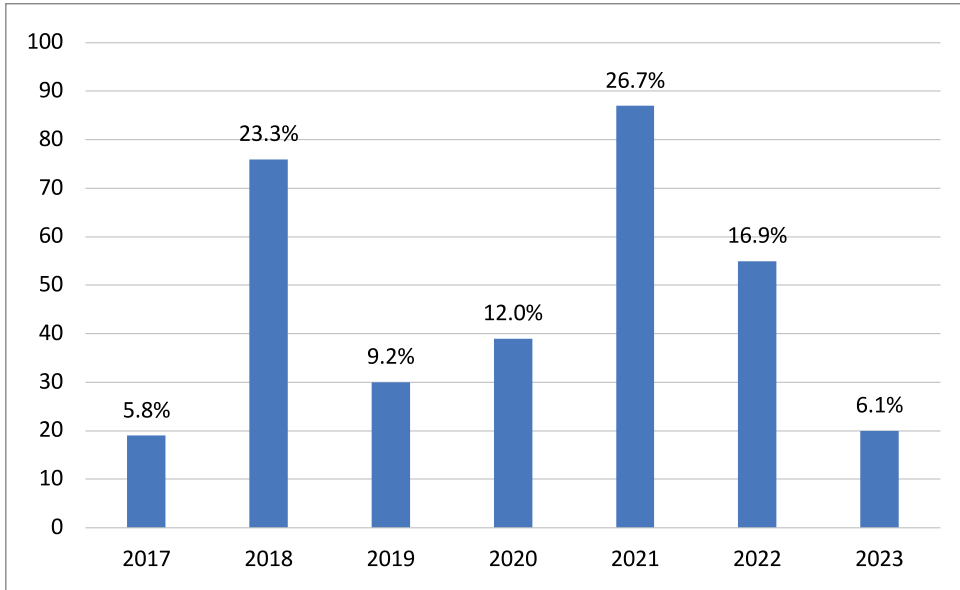


Figure 1. Number of cats tested yearly between 2017 and 2023

Table 1. Seroprevalence of FIV and FeLV in stray cats ($n=326$) between 2017 and 2023

Pathogen	Seropositive cats	Prevalence [%]	95% CI
FIV	32	9.8	6.8-13.6
FeLV	7	2.1	0.9-4.4
FIV and FeLV	11	3.4	1.7-6.0
Overall seroprevalence	50	15.3	11.6-19.7

variables sex, breed, hair length, and municipality (Table 2). The overall seroprevalence of FIV was 9.8% (11/326, 95% CI: 6.8–13.6).

Concerning FeLV seroprevalence, despite the higher seroprevalence in defined breed cats (7.7%; 1/13; 95% CI: 0.2–36.0) when compared to moggy cats (1.9%; 6/313, 95% CI: 0.7–4.1) and in short hair cats (2.3%; 7/311, 95% CI: 0.9–4.6) versus medium hair cats (0%; 0/15, 95% CI: 0.0–21.8), no statical association was found between FeLV and the independent variables of age group, sex, breed,

hair length, and municipality (Table 3). The overall seroprevalence of FeLV was 2.1% (7/326, 95% CI: 0.9–4.4).

Table 4 provides insights into the seroprevalence of both FIV and FeLV based on independent variables of age group, sex, breed, hair length, and municipality. The prevalence was higher in adult cats compared to young cats, i.e. 6.4% (10/157, 95% CI: 3.1–11.4) versus 0.6% (1/169, 95% CI: 0.0–3.3) respectively, but no significant differences were observed in FIV and FeLV seroprevalence based on sex,

Table 2. Seroprevalence of FIV by age group, sex, breed, hair length, and municipality

Variable	Title	Cats tested (n)	Relative distribution (%)	Positive (n)	Seropositive (%)	95% CI
Age group ($P < 0.001$)	Young	169	51.8	6	3.6	1.3-7.6
	Adult	157	48.2	26	16.6	11.1-23.3
Sex ($P = 0.336$)	Female	189	58.0	16	8.5	4.9-13.4
	Male	137	42.0	16	11.7	6.8-18.3
Breed ($P = 0.491$)	Defined	13	4.0	2	15.4	1.9-45.5
	Moggy	313	96.0	30	9.6	6.6-13.4
Hair ($P = 0.639$)	Medium	15	4.6	2	13.3	1.7-40.5
	Short	311	95.4	30	9.7	6.6-13.5
Municipality ($P = 0.503$)	Medium	130	40.0	11	8.5	4.3-14.6
	Small	196	60.0	21	10.7	6.8-15.9
Total	All	326	100	32	9.8	6.8-13.6

Table 3. Seroprevalence of FeLV by age group, sex, breed, hair, and municipality

Variable	Title	Cats tested (n)	Relative distribution (%)	Positive (n)	Seropositive (%)	95% CI
Age group ($P = 0.777$)	Young	169	51.8	4	2.4	0.7-6.0
	Adult	157	48.2	3	1.9	0.4-5.5
Sex ($P = 0.413$)	Female	189	58.0	3	1.6	0.3-4.6
	Male	137	42.0	4	2.9	0.8-7.3
Breed ($P = 0.159$)	Defined	13	4.0	1	7.7	0.2-36.0
	Moggy	313	96.0	6	1.9	0.7-4.1
Hair ($P = 0.557$)	Medium	15	4.6	0	0	0.0-21.8
	Short	311	95.4	7	2.3	0.9-4.6
Municipality ($P = 0.871$)	Medium	130	40.0	3	2.3	0.5-6.6
	Small	196	60.0	4	2.0	0.6-5.1
Total	All	326	100	7	2.1	0.9-4.4

Table 4. Seroprevalence of FIV and FeLV by age group, sex, breed, hair length, and municipality

Variable	Title	Cats tested (n)	Relative distribution (%)	Positive (n)	Seropositive (%)	95% CI
Age group ($P = 0.004$)	Young	169	51.8	1	0.6	0.0–3.3
	Adult	157	48.2	10	6.4	3.1–11.4
Sex ($P = 0.392$)	Female	189	58.0	5	2.7	0.9–6.1
	Male	137	42.0	6	4.4	1.6–9.3
Breed ($P = 0.492$)	Defined	13	4.0	0	0	0.0–24.7
	Moggy	313	96.0	11	3.5	1.8–36.2
Hair length ($P = 0.470$)	Medium	15	4.6	1	6.7	0.2–32.0
	Short	311	95.4	10	3.2	1.6–5.8
Municipality ($P = 0.701$)	Medium	130	40.0	5	3.9	1.3–8.8
	Small	196	60.0	6	3.1	1.1–6.5
Total	All	326	100	11	3.4	1.7–6.0

breed, hair length, or municipality (Table 4). The overall seroprevalence of FIV and FeLV was 3.4% (11/326, 95% CI: 1.7–6.0).

Discussion

To the best of the authors' knowledge, this study is the most extensive investigation conducted in Portugal concerning the prevalence of FIV and FeLV among shelter cats. It offers insights that shed light on the epidemiology of these viral infections within this specific population.

Regarding prevalence comparison to previous studies in Portugal, our findings reveal intriguing variations. A study focused on cats from northern and central Portugal reported prevalences of 17.1% (20/117) for FIV and 8.5% (10/117) for FeLV, with FIV and FeLV status considered as independent variables (Vilhena et al., 2013). An earlier investigation within

continental Portugal, conducted on wild-cats and feral stray cats, demonstrated 26% positivity for FeLV and 0% for FIV. In comparison, feral stray cats exhibited a 33.3% (6/18) prevalence for FeLV (Duarte et al., 2012). However, the latter study's opportunistic sampling could have introduced bias, potentially contributing to the high FeLV frequency observed.

Our data portrays the presence of FeLV, FIV, or FeLV and FIV in some stray cats. Remarkably, FIV showed a notably higher prevalence of 9.8%, whereas FeLV exhibited a prevalence of 2.1%. The prevalence of FIV and FeLV combined was 3.4%. Combining all three categories (FeLV, FIV, and FeLV and FIV), an overall seroprevalence of 15.3% was observed.

Concerning variability and global prevalence, our findings chart a distinctive path from previously documented data, both on a global and local scale.

Research spanning the globe highlights a relatively subdued prevalence of these viruses. Notably, FIV prevalence spans from 2.5% to 7.5% in North America, 15% in Australia, and spans from 3.2% to 8.3% across Europe (Szilasi et al., 2019). While Portugal aligns with the European range, the prevalence of FIV among cats entering shelters appears elevated at 9.8%, while FeLV prevalence slightly tapers to 2.1%. These disparities spotlight the health concerns looming over shelter and domestic feline populations.

Examining the global landscape, our results find parallels and divergences among various regions. A study involving 180 healthy cats in the Madrid metropolitan area, Spain, revealed positive values of 15.6% for FeLV, 8.3% for FIV, and 1.1% for both FeLV and FIV (Arjona et al., 2000). Meanwhile, a study involving shelter cats on Newfoundland, Canada, identified 6.2% seropositivity for FeLV and 2.2% for FIV (Munro et al., 2014). Notably, a pan-European study on FeLV infection indicated an overall FeLV viraemia prevalence of 2.3% among cats visiting veterinary facilities, with 10.4% being shelter and rescue cats. Noteworthy peaks occurred in Portugal (8.8%), Hungary, Italy, and Malta, with prevalence values between 5.7% and 8.8% (Studer et al., 2019). In Hungary, a 2019 study on 335 domestic cats reported 13.1% positivity for FeLV and 15.2% for FIV via an enzyme-linked immunosorbent assay (ELISA) (Szilasi et al., 2019), although it did not encompass stray or shelter animals.

In Ireland, a study by ELISA test kit reported 3.28% seropositivity for FeLV and 10.38% for FIV among 183 domestic cats (Szilasi et al., 2021). Meanwhile, an Australian study from 2011 to 2013 reported a shelter cat prevalence of 6% for FIV (124/2151; 95% CI 4.8-6.8) and 1% for FeLV (22/2241; 95% CI: 0.6-1.5). FIV and

FeLV prevalence was not determined due to constraints with the summary search function (Westman et al., 2016).

Furthermore, the present study unveils a lower seropositivity for FIV and FeLV among shelter cats, with 9.8% (32/326) and 2.1% (7/326) testing positive, respectively. Notably, these values stand slightly above the outcomes of studies conducted in Canada (Munro et al., 2014) and Australia (Westman et al., 2016). Additionally, FIV prevalence in the present study surpasses the findings in Spain (Arjona et al., 2000).

Collectively, global investigations have unveiled a relatively low prevalence of these viruses, *i.e.*, FIV and FeLV. In comparison, FeLV prevalence varies between 2.3% and 7.5% in North America, just 2% in Australia, with a broader range of 3.6% to 15.6% in Europe (Szilasi et al., 2019). Within this global context, our data spotlights a potentially higher FIV prevalence (9.8%) and marginally lower FeLV prevalence (2.1%) among cats entering shelter facilities in Portugal, intensifying concerns for the health and welfare of both shelter and domestic feline populations.

Looking at potential factors and limitations, these prevalence divergences could be attributed to various factors, including variations in population density, sanitation, cat management practices, and socioeconomic conditions (Ludwick and Clymer, 2019; Munro et al., 2014). Areas with lower purchasing power parity have higher FeLV and FIV values than areas with higher purchasing power. Reasons to justify this difference may be a higher number of feral cats and cat colonies in lower-income economies, where there is less focus on animal welfare and control programmes (Ludwick and Clymer, 2019). Thus, the discrepancies observed across studies hint at the complex

interplay of these factors on viral spread.

The accuracy of immunochromatography tests is similar to that of the ELISA regarding diagnostic sensitivity and specificity (Hartmann et al., 2007; Pinches et al., 2007).

The true FeLV prevalence value might be lower than reported in the present study due to possible false positive results. Ideally, all positive results should be confirmed, and regressive FeLV infections with negative results should be identified using FeLV polymerase chain reaction testing (Westman et al., 2019).

Regarding risk factors analysis, no statistical difference was found between sex and seropositivity for FIV, FeLV or both FIV and FeLV, a circumstance which is contrary to others studies that found male cats to be more at risk of both viruses, especially FIV (Munro et al., 2014), or that male cats were significantly more likely to be FIV-infected (Westman et al., 2016).

Older cats were more likely to be positive for FIV or both FIV and FeLV in accordance with previous reports (Munro et al., 2014). Another study only found an association between age and risk of infection for FIV and for cats older than 3 years of age, but no association for FeLV infection (Westman et al., 2016). The same study reported that age, sex, and neutering status in males were important risk factors for FIV, but not for FeLV in domestic cats. Other authors found that being adult, male (intact or not) and having access outdoors were risk factors with a more significant effect on FIV seropositivity, while clinical illness was a stronger risk factor for FeLV seropositivity (Chhetri et al., 2015). In the present study, only age appears to impact FIV seroprevalence, not resembling those findings that associate male intact cats with a higher risk of FIV infection due to

fighting (Westman et al., 2016). Conversely, young cats are considered more likely to be infected by FeLV (Lutz et al., 2009), which contradicts our results, which found no association with age. The cats of the present study were stray, caught and taken to the shelter. As they were all free-roaming, unneutered and apparently healthy, it was impossible to determine the impacts of outdoor access, neutering and health status on FIV and/or FeLV seropositivity.

Considering vaccination programmes and eradication efforts, shelter admission screening for FIV and FeLV should be performed to help manage individual or group housing and limit the transmission of those viruses (Möstl et al., 2013).

FeLV infection status should be screened for all cats, including vaccinated ones, once the point of care test detects viral antigen vaccination against FeLV and does not interfere with testing (Little et al., 2020). This is extremely important in the shelter's context due to the unknown history of most cats entering those facilities.

Vaccination and testing programmes are of utmost importance to limit and try to control FeLV infection in domestic cats (Gleich et al., 2009), indicating the need to screen and vaccinate stray and feral populations to control the virus circulation where those groups are important.

However, even though more than 40 years have passed since the first commercial FeLV vaccine was introduced in the USA in 1984 (Lewis et al., 1981), seroprevalences for FeLV continue to be of concern, indicating that the virus exists and is circulating among stray cats entering shelters in Portugal, thus remaining a concern around the world. There is a need to improve FeLV vaccination.

Vaccination against FIV is only available in Australia, New Zealand, and Japan

(Bęczkowski & Beatty, 2022). As there is currently no FIV vaccine on the market in Portugal, despite its reclassification as a non-core vaccine by the World Small Animal Veterinary Association in the 2016 guidelines (Day et al., 2016), the risk of false positive results is of no concern. However, more studies about the use of this vaccination in those countries should be performed to better understand its role in the protection and lifespan of cats, since the literature reports contradictory effectiveness results (Day et al., 2016).

Studying potential differences in the seroprevalences of FeLV and FIV between domestic and shelter cat populations is crucial for improving vaccination programmes and potentially eradicating the viruses. This can lead to more efficient protocols and better outcomes.

Future research should encompass the variations in seroprevalence between domestic and shelter cat populations, as this is pivotal for refining vaccination strategies and potentially eradicating these viruses. As we strive to devise efficient protocols and optimal outcomes, future research must delve deeper into the underlying causes of these variations, ultimately contributing to the global endeavour of safeguarding feline health.

Conclusions

This is the first report of an investigation on retroviral prevalence in shelter cats in Portugal and provides important information regarding FIV and FeLV among stray cats in this country. Our study of FIV and FeLV prevalence among shelter cats in Portugal brings new insights for both local and global feline health management. The observed variations in prevalence values regarding the existing literature emphasise the dynamics underlying these

viral infections. Notably, while our results align with the prevailing global trends of relatively low FIV and FeLV prevalence, they underscore the importance of addressing specific regional disparities and their potential implications for feline welfare.

Our findings indicate that we must continue to improve our understanding of the complex relationship between viral prevalence, regional disparities, and feline welfare. The disparity between FIV and FeLV seroprevalence values among communities highlights the multifaceted factors requiring further study. Looking at the complexities of seroprevalence, this study corroborates the need for greater compliance with vaccination protocols, comprehensive testing, and strategic management approaches to ensure the health and well-being of shelter and domestic cats. We can improve the health and lifespan of cats by taking a more personalised and unified approach to managing feline viruses. By understanding the prevalence of these viruses in local communities, we can also improve the health of cats in shelters and homes. This will contribute to global efforts to minimise the impact of these viruses and control them. In conclusion, this study provides vital insight into the dynamics of FIV and FeLV prevalence among shelter cats in Portugal. As we navigate the intricacies of viral transmission and feline wellbeing, these findings provide a basis for refining preventive measures and strategic interventions both locally and globally.

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Razotkrivanje stanja: istraživanje prevalencije virusa mačje imunodeficijencije i virusa mačje leukemije među mačkama iz skloništa u Portugalu

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Virus mačje imunodeficijencije (FIV) i virus mačje leukemije (FeLV) važni su patogeni koji pogađaju mačke diljem svijeta, ugrožavajući zdravlje, dobrobit, kvalitetu života i dugovječnost inficiranih mačaka. Ova studija istražuje prevalenciju tih virusnih uzročnika među mačkama iz skloništa u Portugalu, s ciljem otkrivanja njihove kompleksnosti i implikacija. Prevalencija FIV i FeLV procijenjena je prikupljanjem uzoraka krvi ($n = 326$) mačaka tijekom rutinskih zdravstvenih pregleda pri zaprimanju u sklonište i ispitujući ih imunokromatografskim kompletom. Sveukupna seroprevalencija FIV i FeLV bila je 15,3 % (50/326, 95% interval pouzdanosti [CI]: 11,6–19,7). Udio mačaka pozitivnih na FIV, FeLV te na FIV i FeLV zajedno bio je 9,8% (32/326,

95% CI: 6,8-13,6), 2,1% (7/326, 95% CI: 0,9-4,4), odnosno 3,4% (11/326, 95% CI: 1,7–6,0). Prevalencija FIV te FeLV i FIV zajedno bila je veća u odrasloj populaciji. Nije otkrivena statistička veza između spola, pasmine, dlake ili općine i prevalencije FeLV, FIV i FeLV i FIV zajedno. Ovo je prvi izvještaj o retrovirusnoj prevalenciji u mačaka iz skloništa u Portugalu koji otkriva važne informacije u svezi FIV i FeLV među mačkama lualicama u Portugalu, naglašavajući potrebu za nadziranjem tih virusa, većom sukladnošću s programima cijepljenja te strategijama opreznog upravljanja da bi se ograničili i kontrolirali ti virusi u populaciji mačaka.

Ključne riječi: mačke, FeLV, FIV, Portugal, sklonište