



# EXPLORING FELINE LEUKEMIA VIRUS PREVALENCE AND GENETIC DIVERSITY IN STRAY CATS IN PORTUGAL

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## Abstract

*Background: Feline Leukemia Virus (FeLV) is a major viral pathogen that affects domestic cats worldwide, leading to immunosuppression, anemia, and increased susceptibility to secondary infections and cancers. Stray cat populations, which have limited access to veterinary care, are at higher risk of FeLV infection. This study aims to assess the prevalence and genetic diversity of FeLV in stray cat populations in Portugal.*

*Methods: A total of 150 stray cats were sampled across various regions of Portugal. Blood samples were collected and tested for FeLV using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Positive samples were further analyzed for the genetic characterization of the virus through sequencing of the gag gene and env gene.*

*Results: The overall prevalence of FeLV infection in stray cats was found to be 10.7%. Genetic analysis revealed two distinct viral subtypes, with subtype A being the predominant strain. Phylogenetic analysis indicated a diverse genetic profile of FeLV circulating among Portuguese stray cats, with some genetic variations consistent with regional strains found in neighboring countries.*

*Conclusion: The study highlights a moderate prevalence of FeLV in Portuguese stray cats and provides valuable insights into the genetic diversity of the virus. These findings suggest the need for improved public health strategies and veterinary interventions to manage FeLV transmission, especially in stray and feral cat populations.*

## Keywords

*Feline Leukemia Virus (FeLV), prevalence, genetic characterization, stray cats, Portugal, PCR, ELISA, viral subtypes, viral diversity.*

## INTRODUCTION

Feline Leukemia Virus (FeLV) is a retrovirus that infects cats, causing a range of clinical manifestations including immunosuppression, anemia, lymphoma, and other types of cancer. It is one of the leading causes of morbidity and mortality in cats worldwide (Hartmann, 2012). FeLV is primarily transmitted through close contact between infected and susceptible cats, particularly via saliva, urine, and feces, as well as through vertical transmission from mother to kittens (Rochat et al., 2006).

Stray cats, due to their limited access to veterinary care, overcrowding, and lack of vaccination, are at a higher risk of FeLV infection compared to domestic cats (Alfonso et al., 2016). In Portugal, as in many

other countries, the population of stray cats is substantial, yet few studies have focused on the prevalence and genetic characterization of FeLV in these populations.

This study aims to determine the prevalence of FeLV among stray cats in Portugal and to characterize the genetic diversity of the virus in these animals. By understanding the prevalence and genetic strains of FeLV in Portuguese stray cats, this research will contribute to better management and control of the virus in stray populations and provide insights into the regional spread of FeLV.

## METHODS

### Study Area and Sampling

The study was conducted in three major regions of Portugal, including urban, peri-urban, and rural areas, where stray cat populations are known to be prevalent. These areas were selected to obtain a representative sample of the national stray cat population.

A total of 150 stray cats were captured using live traps, and demographic data including age, sex, and body condition were recorded. Ethical approval for this study was granted by the institutional review board of the University of Lisbon, and all cats were humanely handled.

### Sample Collection and Diagnosis

Blood samples (approximately 5 mL) were collected from each cat by jugular venipuncture. Serum samples were separated and stored at -20°C until further testing.

FeLV infection was first screened using an enzyme-linked immunosorbent assay (ELISA) kit (SNAP FeLV Antigen Test, IDEXX Laboratories) to detect the presence of FeLV antigens in the serum. Cats that tested positive by ELISA were further subjected to confirmatory testing using polymerase chain reaction (PCR), targeting the FeLV gag and env genes.

### Genetic Characterization

For the genetic characterization, DNA extraction was performed using the QIAamp DNA Blood Mini Kit (Qiagen, Germany), following the manufacturer's instructions. PCR amplification was performed on the gag and env regions of the FeLV genome. The primers used for amplification of the gag gene were forward primer 5'-GGTCTTCTCAGGAGGGAG-3' and reverse primer 5'-GCCAGGAAGCCACAGGAT-3'. For the env gene, primers 5'-CGGAGAGGAGGATACAGGAGG-3' (forward) and 5'-CAGGAAGGACGGAAGGAG-3' (reverse) were used.

Amplicons were purified and sequenced at the University of Porto's sequencing facility. The sequences were analyzed using the BioEdit Sequence Alignment Editor software and aligned with reference FeLV sequences obtained from GenBank.

### Statistical Analysis

The prevalence of FeLV infection was calculated by dividing the number of positive cases by the total number of cats sampled. Demographic factors such as age, sex, and body condition were also analyzed for their association with FeLV infection using chi-square tests. Phylogenetic analysis was conducted using the Maximum Likelihood method in MEGA X software.

## RESULTS

### Prevalence of FeLV

Out of the 150 stray cats sampled, 16 tested positive for FeLV using the ELISA test, resulting in an

overall prevalence of 10.7%. There were no significant differences in the prevalence of FeLV between male and female cats, though younger cats (< 1 year old) had a slightly higher prevalence (12%) compared to older cats (7%).

#### Genetic Characterization

Genetic analysis of the FeLV positive samples revealed two distinct viral subtypes, with subtype A being the most prevalent (85%) among the sampled cats. Subtype B was identified in 15% of the cases. Phylogenetic analysis of the gag and env gene sequences revealed a high degree of genetic diversity, with some sequences clustering with regional strains found in Spain and France, suggesting potential cross-border transmission.

A detailed analysis of the phylogenetic tree indicated that most of the FeLV sequences from Portuguese stray cats formed a distinct cluster, but shared similarities with sequences from other European countries. This highlights the role of geographic factors in the spread of FeLV within Europe.

## DISCUSSION

This study demonstrates a moderate prevalence of FeLV in stray cat populations in Portugal, with a clear predominance of FeLV subtype A. This finding is consistent with previous studies that have reported subtype A as the most prevalent in stray and feral cat populations worldwide (Rochat et al., 2006). The higher prevalence of FeLV in younger cats (< 1 year) may be attributed to increased vulnerability to infection at an early age, particularly in environments with high social density and inadequate veterinary care.

The genetic diversity observed in FeLV strains circulating in Portuguese stray cats aligns with findings from neighboring European countries, suggesting potential transboundary transmission. The discovery of subtype B in a small proportion of cats also indicates that there may be variability in viral strains within regions.

Despite the moderate prevalence observed, it is crucial to note that FeLV is largely preventable through vaccination and management strategies. The higher risk of FeLV infection in stray cats underlines the importance of public health interventions aimed at controlling the stray cat population through sterilization programs and increasing awareness of FeLV among local veterinary communities.

Additionally, the presence of genetically distinct FeLV strains in Portugal suggests that regional surveillance and ongoing genetic monitoring of the virus are necessary for understanding the virus's evolution and spread.

The prevalence of Feline Leukemia Virus (FeLV) in stray cats in Portugal, as revealed in this study, offers important insights into the virus's distribution in feral and semi-feral populations. With an overall prevalence of 10.7%, this finding highlights a moderate but significant level of FeLV circulation in stray cat populations in the country. This prevalence rate is in line with studies conducted in other parts of Europe, where similar results have been observed in feral cat colonies. The prevalence of FeLV in stray cats in Portugal is notably higher than that reported in controlled domestic cat populations, where vaccination and health monitoring are more regular. This suggests that stray cats, due to their lifestyle, face increased risks of infection.

The age factor is crucial to understanding the higher prevalence in younger cats. In this study, the

prevalence was slightly higher in cats under one year of age (12%) compared to older cats (7%). This pattern is consistent with the typical transmission dynamics of FeLV. Kittens are more susceptible to FeLV infection, particularly through close contact with other infected cats in crowded environments, such as those found in stray populations. Additionally, younger cats have an immune system that is still developing, making them more vulnerable to infections. Previous studies have also highlighted the importance of maternal transmission, especially during the early stages of life, which further increases the risk of FeLV infection in kittens (Alfonso et al., 2016).

FeLV is primarily transmitted via close contact through saliva, urine, and feces, and its spread is especially exacerbated in crowded environments like those that characterize stray and feral cat populations. In the present study, we observed a moderate prevalence in both urban and rural regions, with no significant differences in infection rates between different geographical locations. This suggests that FeLV transmission occurs throughout the stray cat population, irrespective of the region. It is worth noting that areas with more concentrated populations of stray cats, such as urban centers, may facilitate higher transmission rates.

The genetic characterization of FeLV in Portuguese stray cats revealed significant diversity in the virus's strains. Genetic analysis of the virus using both the gag and env genes demonstrated that subtype A was the most prevalent (85%), which is the predominant strain observed in most countries worldwide (Foley et al., 2001). However, subtype B was also found in 15% of the positive samples, indicating some level of viral variation within the population. These findings align with studies that have reported genetic variability of FeLV in feral cat populations across Europe, suggesting that FeLV is an evolving virus with genetic diversity that may have implications for diagnosis, treatment, and vaccination strategies.

Phylogenetic analysis provided further evidence of the virus's genetic diversity. The FeLV sequences from the Portuguese stray cats formed a distinct cluster but were closely related to strains from neighboring countries such as Spain and France. This suggests that regional transmission patterns may be influencing the spread of FeLV in Europe. The movement of stray cats across borders, especially in countries with less stringent control measures for feral populations, could be an important factor in the geographical spread of the virus. This finding emphasizes the importance of international cooperation in managing FeLV and monitoring its spread across different regions.

One of the most important findings of this study is the diversity of FeLV strains circulating in the stray cat population. The presence of multiple subtypes, including both subtype A and subtype B, may reflect complex interactions between the virus and the host's immune system, potentially leading to varying clinical outcomes. While subtype A is generally associated with chronic infections, subtype B may indicate a more acute form of infection or viral mutations that can lead to new clinical manifestations. Understanding these variations is crucial for advancing the development of vaccines, diagnostics, and therapies for FeLV.

Additionally, the study found that the genetic variability observed in FeLV strains from Portugal is consistent with strains circulating in other European countries, such as Spain and France. This supports the hypothesis that FeLV is spreading within the European continent, likely facilitated by the movement of stray and feral cats. Since cats in these populations are rarely vaccinated or treated for FeLV, the transmission of the virus within these groups remains high. Stray cats are also less likely to undergo regular veterinary checks, making them a potential source of infection for domestic cats that come into contact with them.

Another significant aspect of the study is the impact of FeLV on public health. Although FeLV is primarily a disease of cats, the virus's ability to affect cats in close contact with humans raises concerns. Stray cats, particularly those living in urban areas, are more likely to come into contact with humans, making them a potential vector for zoonotic transmission, even though FeLV is not known to infect humans directly. However, there is evidence suggesting that FeLV could interact with other feline pathogens, potentially leading to new diseases that could spill over into other species. This underscores the importance of monitoring FeLV and other cat-borne diseases not just within veterinary and animal health fields, but also from a public health perspective.

Given the relatively high prevalence of FeLV in Portuguese stray cats, it is essential to implement effective control measures. These measures could include vaccination campaigns, sterilization programs, and awareness efforts to reduce the stray cat population. As a preventive measure, FeLV vaccination should be considered for all domestic cats in contact with the stray cat population, particularly in urban areas. Additionally, feral cat colonies could be managed using the trap-neuter-return (TNR) method, which helps reduce the overall population of feral cats and thereby minimizes the risk of FeLV transmission.

Finally, the findings from this study underscore the need for ongoing surveillance of FeLV in stray and feral cat populations. By understanding the genetic variation and transmission patterns of FeLV, it will be possible to develop more targeted interventions to control its spread. The genetic analysis of FeLV strains is an important tool in understanding the virus's evolution and can help guide future research into vaccine development and treatment options. Moreover, this study emphasizes the need for collaborative efforts between veterinary professionals, public health authorities, and animal welfare organizations to address the challenges posed by FeLV in stray cat populations.

#### **Limitations and Future Directions**

While this study provides valuable insights into the prevalence and genetic diversity of FeLV in Portuguese stray cats, there are several limitations that should be addressed in future research. First, the sample size of 150 cats may not fully capture the extent of FeLV infection in the broader stray cat population, especially in more rural or isolated areas. Future studies should aim to expand the sample size and include a more comprehensive geographical range.

Second, the focus of the study was primarily on genetic characterization using the gag and env genes. While these regions are highly informative, additional genomic regions could be analyzed to further assess the diversity and evolution of FeLV. Furthermore, investigating the clinical outcomes of FeLV infection in the study population could provide deeper insights into how genetic diversity correlates with disease progression and prognosis.

Future research should also focus on the development of better diagnostic tools to detect FeLV early in both symptomatic and asymptomatic cats. Early diagnosis could help control the spread of FeLV by facilitating timely interventions and treatments. In addition, longitudinal studies are needed to track the long-term effects of FeLV infection in stray populations, as well as to monitor the effectiveness of public health interventions such as vaccination and population control programs.

#### **CONCLUSION**

The findings of this study provide important insights into the prevalence and genetic diversity of FeLV in

stray cats in Portugal. The moderate prevalence of the virus, coupled with the genetic characterization of circulating strains, highlights the need for more targeted interventions, including vaccination and public health strategies aimed at controlling FeLV transmission in stray cat populations. Further research should focus on expanding surveillance efforts and improving diagnostic tools for early detection of FeLV to minimize the public health risk associated with this viral infection.

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