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Estimation of the prevalence of feline leukaemia virus in Astana, Kazakhstan

Gulzhan N. Yessembekova[1], Bolat B. Abdigulov[2], Anar S. Kyzeybayeva[1], Gulmira K. Myrzakayeva[1], Indira K. Akzhunusova[3]

[1]Faculty of Veterinary and Livestock Technology,
S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan,
[2]Laboratory of Applied Genetics, National Center for Biotechnology, Astana, Kazakhstan,
[3]Karaganda Scientific Research Veterinary Station, Karaganda, Kazakhstan

Corresponding author: Gulzhan N. Yessembekova: gulzhan_nk@mail.ru
Co-authors: (1: BA) abdigulovbolat3@gmail.com;(2: AK) anarsabirbaevna@mail.ru;
(3: GM) m.gumika@list.ru; (4: IA) akzhunusova76@mail.ru
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Abstract

Background and Aim. Feline leukemia virus (FeLV) is one of the most important retroviral pathogens in domestic cats, causing immunosuppression, anemia, lymphoma, and leukemia. FeLV exists in two forms: exogenous (exFeLV), which is infectious and horizontally transmitted, and endogenous (enFeLV), which is inherited as integrated proviral sequences. Differentiating between these forms is essential for accurate epidemiological assessment and diagnostics, since enFeLV sequences can interfere with molecular assays and lead to false-positive results. This study aimed to evaluate the prevalence of endogenous FeLV sequences in domestic cats in Astana and to determine whether exogenous FeLV was actively circulating in this population.

Materials and Methods. A total of 203 whole-blood samples from domestic cats were collected during routine veterinary examinations, and genomic DNA was extracted using a modified Kanai method. Two independent real-time PCR systems were employed: primers targeting the conserved *env* region to detect enFeLV, and primers specific to the U3 region of the long terminal repeat (LTR) to identify exFeLV. Amplifications were performed on the CFX96 Touch platform, and samples with quantification cycle (*Cq*) values <40 were interpreted as positive.

Results. Of the 203 analyzed samples, 197 (97%) were positive for enFeLV sequences, confirming the widespread presence of endogenous retroviral elements in the genome of cats, while no amplification was detected with U3-specific primers, indicating the absence of active exFeLV infection. The *Cq* values for enFeLV-positive samples ranged from 11.24 to 37.04, reflecting variability in proviral copy number among individuals. These findings demonstrate that enFeLV is nearly ubiquitous among domestic cats in Astana, while no evidence of exFeLV circulation was detected.

Conclusion. The results underscore the importance of using *U3-LTR* specific assays to reliably differentiate endogenous from exogenous forms of FeLV and to avoid false-positive diagnoses. Further studies should focus on monitoring potential recombination events between enFeLV and exFeLV, evaluating the expression of endogenous loci, and assessing their role in disease pathogenesis.

Keywords: Feline leukemia virus; cats; PCR; Exogenous forms (exFeLV); endogenous forms (enFeLV); sequencing.

Introduction

Feline leukemia retrovirus (FeLV) is one of the most significant infectious agents in domestic cats, causing immunosuppression, anemia, lymphoma, and leukemia [1-3]. FeLV is divided into exogenous and endogenous forms. Exogenous forms (exFeLV) are clinically relevant exogenous forms and are

transmitted horizontally, whereas endogenous forms (enFeLV) are viral sequences integrated into the genome of cats during evolution and inherited [1, 4-6].

Endogenous retroviruses, including enFeLV, are widespread in domestic cat populations and may vary in copy number and preservation levels [7-9]. Their importance lies not only in the evolutionary aspect, but also in the actual pathobiology: they can have both protective and pathogenic effects on the body. For example, enFeLV expression can compete with an exogenous virus for cellular receptors, reducing the effectiveness of infection [10]. On the other hand, there is a risk of recombination between enFeLV and exFeLV, leading to the emergence of new pathogenic subtypes such as FeLV-B and FeLV-D [4, 11-13]. These recombinant variants have a modified tropicity spectrum and are often associated with more severe clinical manifestations.

Epidemiological data indicate that most domestic cats contain between 8 and 12 copies of enFeLV in their genome, although the exact number may vary between populations and breeds [7]. It is noteworthy that animals with a high copy of enFeLV may have a lower severity of clinical manifestations when infected with exFeLV, presumably due to the formation of immune tolerance to viral proteins [5]. These observations confirm the complex nature of the interaction of endogenous and exogenous forms of the virus.

From the point of view of molecular diagnostics, the presence of enFeLV is a significant problem. When using non-specific primers, especially for gag and env sites, both exogenous and endogenous sequences are amplified, which can lead to false positive results [14]. This significantly complicates the interpretation of the results of PCR analysis and can lead to errors when screening clinically healthy animals, especially in the absence of obvious clinical signs.

To minimize the risk of diagnostic errors, primer systems are used that target unique areas that are missing from enFeLV. One of these regions is the U3 LTR region, which is specific to exFeLV [4, 14]. The use of such markers makes it possible to reliably distinguish between endogenous and exogenous forms of the virus. This approach is critically important both for practical veterinary medicine (screening in kennels, during the sale and movement of animals), and for scientific research aimed at studying the prevalence and evolution of retroviruses in cats.

In the present study, a PCR analysis of 203 DNA samples of domestic cats was performed in order to assess the prevalence of endogenous FeLV sequences and exclude active exogenous infection. To improve the diagnostic accuracy, two primer systems were used, developed on the basis of modern publications [4, 14]. Additionally, data on the epidemiological situation in the region were taken into account, which made it possible to assess not only the infection rate, but also the potential risks of recombinant forms of the virus in the population.

Materials and Methods

Collecting samples

The samples for the study were collected in veterinary clinics in Astana. Whole blood taken from domestic cats during routine veterinary examinations was used as biological material. Sampling was carried out in vacuum tubes (vacutainers) containing the anticoagulant EDTA-K_2, a reagent widely used in diagnostic practice, as it effectively prevents blood clotting by chelating calcium ions, which allows samples to be kept in a stable state until the DNA isolation stage.

The use of EDTA-K2 also ensures minimal damage to blood cells and the preservation of nucleic acids, which is critically important when conducting molecular research, including PCR analysis. After sampling, the samples were transported to the laboratory under controlled conditions of temperature and storage time, which excluded degradation of the genetic material.

DNA isolation

DNA isolation was carried out according to the method described by *Kanai* et al. [15], with minor modifications that make it possible to efficiently extract genomic DNA even from clotted blood left after standard biochemical analyses. For isolation, 200 µl of a blood sample (including frozen and partially coagulated samples) was used, which were transferred to sterile 1.5 ml micro-samples containing 250 µl of a lysing buffer of the following composition: 720 mcg/ml of proteinase K, 150 mM NaCl, 50 mM EDTA and 2% SDS. The tubes were incubated at 60 °C for 3 hours with periodic stirring on a vortex to improve cell lysis and protein degradation. After incubation, 300 µl of 6 M NaCl and 600 µl of a mixture

of chloroform and isoamyl alcohol (24:1) were added to the lysate. The contents of the tubes were stirred on a vortex and centrifuged at 5000 rpm (≈1667 g) for 5 min. After phase separation, the aqueous phase containing nucleic acids was transferred to a new tube with 800 µl of isopropanol for DNA deposition. The samples were centrifuged at the same parameters, after which the resulting precipitate was washed with 70% ethanol, dried at room temperature, and eluted in 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The resulting genomic DNA preparations were used to set up PCR reactions.

PCR testing

Two independent sets of primers and temperature conditions were used for amplification, borrowed from the following publications:

1. The protocol is based on the methodology from the article by *Cavalcante* et al. [4]. A primer system specific to the LTR U3 region was used to detect exogenous FeLV.

Type	Name	Sequence
Forward primer	FeLV-U3-exo-f	5'-AACAGCAGAAGTTTCAAGGCC-3'
Reverse primer	FeLV-U3-exo-r	5'-TTATAGCAGAAAGCGCGCG-3'
Probe	FeLV-U3-probe	5'-FAM-CCAGCAGTCTCCAGGCTCCCCA-TAMRA-3'

Amplification was performed in a volume of 25 µl using HS qPCR (Biolabmix, Russia). The temperature regime consisted of 95 °C - 5 min of preliminary denaturation, followed by 40 cycles including: 95 °C - 15 sec, 60 °C - 1 min (annealing/elongation)

2. A protocol based on *Powers* et al. [14]. To determine the presence of endogenous copies of FeLV, a system of primers aimed at a conserved env site was used:

Type	Name	Sequence
Forward primer	enFeLV-F	5′-GTCTTATCCTAAGTCCACCGTTTA-3′
Reverse primer	enFeLV-R	5′-CTAGGCTCATCTCTAGGGTCTATC-3′
Probe	enFeLV-probe	FAM-5′-CCTGGCCCTAAGATGGGAATGGAAA- BHQ1-3′

The reaction was also carried out in 25 µl volume using the previously mentioned real-time PCR kit. The amplification conditions included: 95 °C - 5 min, 40 cycles, 95 °C - 5 sec, 60 °C - 15 sec.

Each system was started independently. The reactions were performed on the CFX96 Touch platform (BioRad, USA), which allows multiplex analysis of up to 5 targets simultaneously in 96 samples. The fluorescence threshold was determined automatically, and Cq values <40 were interpreted as positive.

Results and Discussion

Of the 203 analyzed DNA samples from domestic cats, 197 (97%) showed a positive result of amplification using primers aimed at the conservative site of *env*. The data obtained confirm that endogenous FeLV sequences are almost universally distributed in the studied population. Such a high percentage of positive samples is consistent with the literature data, according to which most domestic cats contain from 8 to 12 copies of enFeLV in their genome. [7]. Thus, our results once again confirm that the endogenous retroviral FeLV sequences are a stable component of the *Felis catus* genome and are preserved in the vast majority of individuals, regardless of their clinical status.

At the same time, none of the studied samples demonstrated amplification using a system of primers specific to the LTR U3 region of exogenous FeLV. This indicates the absence of active circulation of replication-competent exogenous forms of the virus among the examined animals within this sample. Negative results for exFeLV may reflect a relatively low level of infection of the population with exogenous forms in the conditions of Astana city or the effectiveness of measures to prevent the spread of the virus among domestic cats. It is important to note that in shelters or large nurseries where animal crowding is higher, the detection rate of exFeLV is usually significantly higher [4, 5], this highlights the value of our data specifically for assessing the epidemiological situation in the urban population.

The *Cq* (cycle quantification) values equivalent to Ct (cycle threshold) recorded for positive samples ranged from 11.24 to 37.04, with an average value of 18.85. Lower *Cq* values reflect the high representation of endogenous FeLV copies in the genome of individual animals, which may be due to the

variability in the number of copies between individuals and breeds. On the contrary, values approaching cycle 37 indicate the presence of samples with a relatively low number of copies, which confirms the interindividual differences in the integration of enFeLV. Taken together, such data demonstrate significant genetic heterogeneity in the level of endogenous retroviral load, which may be important for the formation of different animal susceptibility to exogenous FeLV and for the variability of clinical manifestations of infection.

Thus, the study showed that in the studied sample of cats from Astana, endogenous copies of FeLV were detected in the vast majority of animals, while exogenous forms of the virus were not detected.

These results have not only local epidemiological significance, but also confirm the data of other authors on the widespread occurrence of enFeLV and the complexity of differential diagnosis between endogenous and exogenous forms. Considering that it is recombination between endogenous and exogenous sequences that can lead to the formation of new pathogenic subtypes (for example, FeLV-B and FeLV-D), further monitoring of the cat population using specific molecular markers is an important direction for veterinary practice and scientific research (Figure 1).

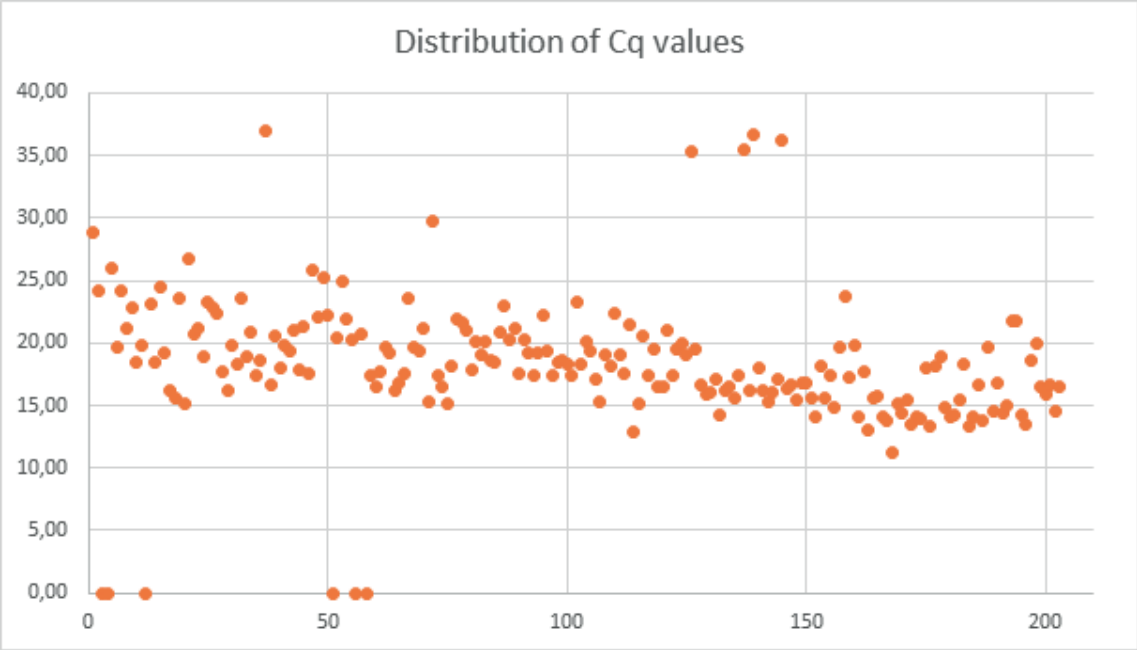

Figure 1 – Distribution of *Cq* values obtained by DNA amplification using primers to the enFeLV site. The X - axis shows the number of samples. Y- axis - displays the value of *Cq*

The results of this study confirmed the widespread occurrence of endogenous FeLV retrovirus among cats, with complete absence of signs of exogenous infection based on amplification of the U3 region. This is completely consistent with previous observations, according to which enFeLV is present in the genome of almost all domestic cats and varies in the number of copies depending on individual and population characteristics [7, 16]. The data obtained indicate that the detected amplification signals when using common primers to env reflect the presence of an embedded provirus, rather than an active exogenous infection. This result highlights the need to use highly specific diagnostic systems focused on unique areas missing from enFeLV, such as the LTR U3 region, which is especially critical when examining animals in populations with low morbidity [14, 17].

In addition to their purely diagnostic significance, endogenous FeLV retroviruses are also of interest in a broader biological context. They serve as a model for studying viral evolution, as well as interactions between the virus and the host. In particular, the ability of enFeLV to recombine with exogenous forms of the virus has been confirmed in a number of molecular studies [4, 9, 11, 18]. Such recombination events can lead to the formation of new pathogenic subtypes, including FeLV-B and FeLV-D, with an altered spectrum of cellular tropicity and a more severe clinical course. In addition, the transcriptional

activity of individual endogenous loci can influence the expression of host genes, the functioning of the immune system, and even the formation of individual resistance or susceptibility to infection [5, 10, 19].

The practical significance of enFeLV is also evident in the context of prevention. According to current European veterinary guidelines, FeLV vaccination should be combined with mandatory testing of animals for exogenous forms using U3-specific systems [20]. This minimizes the risk of false positive results due to endogenous copies and increases the reliability of epidemiological control. This approach is especially important in shelters and nurseries, where crowded conditions increase the likelihood of retrovirus activation due to stressful factors [21-23].

Thus, the results of this study not only confirm the almost universal presence of enFeLV in the genome of domestic cats, but also emphasize the urgency of the problem of differential diagnosis of endogenous and exogenous forms of the virus. The absence of amplification by U3 primers, combined with the high frequency of positive reactions to the conserved env site, demonstrates the importance of choosing the right molecular targets for PCR assays. In a broader sense, the findings complement the existing evidence base on enFeLV and highlight the need for further research aimed at: 1. evaluation of the expression of endogenous sequences; 2. study of their recombination potential; 3. Epidemiological monitoring of the circulation of exogenous FeLV in various cat populations. These areas may be of critical importance not only for veterinary practice and infection prevention, but also for understanding the role of endogenous retroviruses in the mammalian genome.

Conclusion

Analysis of 203 DNA samples from domestic cats showed the almost ubiquitous presence of endogenous FeLV sequences in the complete absence of signs of exogenous infection. This result emphasizes that endogenous forms of retrovirus are preserved in the animal genome as an inherited element and reflect the features of the evolutionary interaction of the virus and the host. The absence of amplification of the U3 region indicates that there is no active circulation of exogenous forms of the virus in the studied population.

The data obtained confirm the key role of differential diagnosis in detecting FeLV. The use of PCR with primers aimed at conservative sites (for example, env), without taking into account the endogenous origin of the sequences, can lead to false positive results and incorrect epidemiological conclusions. In conditions of low incidence of exogenous forms, this is especially critical, since a diagnostic error can affect the tactics of treatment, vaccination, and animal movement control.

The use of primers specific to the unique regions of the exogenous virus (in particular, the LTR U3 region) is a prerequisite for reliable detection of active infection. This approach is already reflected in international guidelines for testing and vaccination of cats and should be considered as a standard for the molecular diagnosis of FeLV.

Future research in this area should focus not only on improving diagnostic systems, but also on a deeper study of the role of enFeLV in the pathogenesis of diseases. Of particular interest are the issues of expression of endogenous loci, their potential involvement in recombination with exogenous forms, and their effect on the animal's immune response. Such data is necessary both to develop strategies for the prevention and control of infections in cats, and to understand the general patterns of interaction between retroviruses and the mammalian genome.

Authors' Contributions

GY, BA: supervision, conceptualisation, writing - original draft preparation, writing - review and editing; AK, GM, and IA: methodology, validation and formal analysis. All authors have read and agreed to the final version of the manuscript.

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