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DETECTION OF THE PRESENCE OF FELINE VIRAL LEUKEMIA VIRUS (FELV) USING THE PCR TECHNIQUE

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Abstract: The present study aimed to evaluate, using the Polymerase Chain Reaction (PCR) technique, the detection of the virus that causes Feline Viral Leukemia (FeLV) in blood samples from a group of 12 unvaccinated cats that lived together. In order to control the technique, 2 more animals were used that already had a positive result for FeLV in the SNAP FIV/FeLV rapid test (Combo IDEXX). Among the group of 12 cats, there were 4 males and 8 females with ages ranging from 7 months to 4 years of age, which were rescued from the wild in the municipality of São Gonçalo, Rio de Janeiro at different times. FeLV is a disease caused by a virus belonging to the Retroviridae family and its main route of elimination is through the saliva of contaminated felines. It has a high incidence in the clinical routine affecting domestic felines, being the most susceptible those that are free-living. Positive animals may be asymptomatic or show clinical signs such as the anorexia, apathy, anemia, stomatitis and even the appearance of lymphomas. For the spread of this disease, direct contact with a positive feline is necessary, which can be through the sharing of feeders and drinkers or through fights and licks, since the virus does not resist outside the host and is easily eliminated by cleaning products. . This disease has different forms of infection, including the abortive infection, in which the virus is eliminated by the immune system, the regressive form in which the virus spreads to monocytes and lymphocytes, and the progressive form where viral replication is intense, spread to bone marrow, epithelial and glandular tissues. PCR is able to detect the presence of the virus even in the regressive form of the infection, whereas rapid serological methods cannot. In view of this, it is important to carry out this routine diagnostic method for FeLV, since there are positive animals that are asymptomatic. The realization of this study allowed a reliable diagnosis from PCR, in which of these animals, the 2 animals already positive confirmed to be also in PCR, while the group of 12 that lived together had a negative result. Because it is considered one of the most serious diseases that affect cats and that has no cure, diagnosis is very important to prevent the spread of the disease to other healthy cats.
Keywords: Felines, felv, pcr.

INTRODUCTION

The virus that causes feline viral leukemia belongs to the family Retroviridae(FeLV) is an infectious agent that affects domestic cats with great incidence (*Felis catus*). It is considered a pathology of worldwide distribution and its involvement varies according to lifestyle and contact and interaction with other infected or not felines (Arjona et al., 2000).

Animals carrying FeLV may show nonspecific clinical signs such as the anorexia and apathy, as well as signs caused by direct immunosuppression such as the anemia, leukemia and lymphoma (Alves et al., 2015). The main route of elimination is saliva, which facilitates its spread, and the prevalence of this disease is higher in cats that live in high population density and in free-ranging, unneutered cats (Tilley & Smith Júnior, 2015).

The importance of reliable diagnoses is highlighted, including the PCR diagnostic method, Polymerase Chain Reaction, which consists of detecting viral nucleic acid sequences. This technique is highly sensitive, as there is amplification of the FeLV virus gene sequences, which facilitates its detection, and in addition, the method is highly specific for strains. Samples of blood, tissue, bone marrow and even saliva can be used to perform the same (Greene, 2015).

PCR is highly reliable when compared to rapid tests used in clinical routine, such as the

ELISA, in which it can detect the presence of the virus even in its latency phase (regressive form of infection) (Augusti, 2009).

The objective of this study was to evaluate the detection of the virus that causes feline viral leukemia (FeLV) through the PCR diagnostic technique in blood samples collected from a group of unvaccinated felines, since this pathology has a high incidence in the clinical routine and due to to its severity, there is a need for an accurate diagnosis.

MATERIAL AND METHODS

During the period from September to October 2021, blood samples were collected from 14 domestic felines, for the Brazilian short and unvaccinated. Of the 14 animals, two already had a positive result for FeLV in the rapid test SNAP FIV/FeLV (Combo IDEXX) and were used as a basis for the control of the technique, being an eight-year-old male and a three-year-old female. The 12 remaining cats were rescued from the streets in the period from 2019 to 2021 and started to live together. After the rescue, they remained with restricted access to the street in a residence located in the municipality of São Gonçalo, Rio de Janeiro. Among this group of animals were eight females and four neutered males, ranging in age from seven months to four years old. To carry out this study, the 14 cats were numbered from 1 to 14.

The collection of whole blood was performed with a 3mL syringe and 25x7 needle through venipuncture of the external jugular, in which a total volume of 1 ml was obtained. After collection, the blood was stored in tubes with EDTA (ethylenediaminetetraacetic acid) identified from numbers 1 to 14 as corresponding to each feline. Subsequently, the tubes with the blood samples were stored in a refrigerated and isothermal container so that they could be sent to the LabGen research laboratory at the University of Grande Rio Afya located in the municipality of Duque de Caxias.

Initially, in the laboratory, the genetic material of each feline was extracted from whole blood using the Flexigene DNA extraction kit and protocol. After extraction, the samples were quantified in a spectrophotometer (Denovix) to measure the concentration and purity of the extracted DNA. The proper concentration was considered the highest possible with the purity for satisfactory results.

The Polymerase Chain Reaction (PCR) technique was performed in order to amplify the fragments of interest to evaluate the feline leukemia virus (FeLV). Reactions were performed using 1µL (50ng) of genomic DNA, 10 pmoles of each primer (Sense and Antisense) (Table 1), 1X Buffer, 1.5 mM MgCl2 (magnesium chloride), 0.2 mM of each nucleotide (dATP, dCTP, dTTP and dGTP), 1 U of the enzyme Taq DNA polymerase (Invitrogen) and sterile water, to reach a final volume of 25 µL. PCR was performed in a Veriti thermocycler (Thermo Fisher Scientific) and a cycle that included an initial denaturation of 95°C for 10 minutes was used for amplification of the FeLV gene, followed by 30 cycles at 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. The final extension was 72°C for 10 minutes.

Gene		Primer	Author
FeLV	F*	5' TTACTCAAGTAT GTTCCCATG 3'	Jackson et al., 1992
	R*	5' AGGTCGAACTC TGGTCAACT 3'	

* Founder and Reverse.

Table 1. Oligonucleotides used to perform PCR for FeLV by the LabGen laboratory at the University of Grande Rio Afya

Subsequently, the PCR products were submitted to electrophoresis in a 2% agarose gel to verify if the amplifications occurred correctly, visualized in a UV transluminator

(Biometrix) and documented by the photodocumenter (WizeDoc).

RESULTS AND DISCUSSION

The quantification and quality of the DNA of the present samples can be found in Table 2. It was possible to observe that the concentration of DNA varied from 10.23 ng/µL to 51.45 ng/µL and the quality of the DNA was presented in quantity satisfactory, which allowed the PCR to be performed.

Sample	DNA concentration (ng/µL)	DNA Purity 260/280nm
Animal 1	38,98	1,45
Animal 2	22,45	1,61
Animal 3	30,30	1,81
Animal 4	35,36	2,03
Animal 5	19,91	1,82
Animal 6	26,00	2,36
Animal 7	30,19	2,32
Animal 8	23,30	1,79
Animal 9	23,10	2,1
Animal 10	51,45	2,36
Animal 11	43,02	1,34
Animal 12	31,02	2,69
Animal 13	25,46	1,89
Animal 14	10,23	2,3

Table 2. Result of quantified genetic material from felines numbered 1 to 14 for diagnosis of FeLV.

After performing the electrophoresis in 2% agarose gel, positive and negative results were obtained. The animals number 2 and 14, used to control the technique and that already had a positive result in the rapid test SNAP FIV/FELV (Combo IDEXX), confirmed to be positive for FeLV in the PCR while the animals numbered 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 were negative. Both positives did not live with the rest of the animals.

In the figure below it is possible to observe the products of PCR amplification where the positive animals had a band and the negative ones had none. In the first well, there is the molecular weight marker, in the following wells, the animals numbered 1 to 14, in sequence, and finally the well with the negative control. Animals number 2 and 14 had a positive result and had the band at 460 PB (base pairs).

All cats in this study were rescued at different times by their respective owners. Cats number 2 and 14, positive for FeLV, already had clinical signs compatible with the disease and according to Little (2016) the animals may remain asymptomatic or show symptoms such as the anemia, chronic inflammatory diseases, lymphomas and even secondary and opportunistic infections.

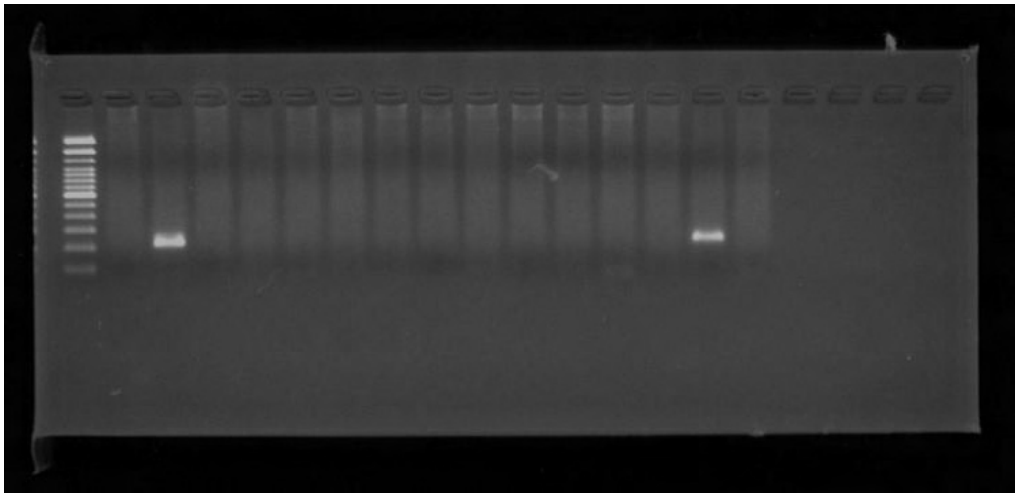

Figure 1. PCR result for FeLV after 2% agarose gel electrophoresis of felines numbered 1 to 14.

Animal number 2, an eight-year-old male, was rescued from the wild and soon after underwent the rapid test SNAP FIV/ FELV (Combo IDEXX) where he had his first positive result. The most susceptible to acquiring it are male cats and unneutered adults who have free access to the street and consequently come into conflict and interaction with others who may be positive, and also when there is an introduction of virus carriers to a group, in which they will share drinkers and feeders (Figueiredo & Araújo Júnior, 2011).

In the present study, all negative results are justifiable, since, with the exception of animals 2 and 14, the rest of the animals lived together, and as described by Donatelle et al. (2015), the spread of the disease in felines that live together is high since the main means of elimination and contagion between them is saliva through sharing water and food, in addition to contact with nasal secretions, urine and feces.

Animals number 1, 3, 4, 6, 8, 12 and 13 were rescued at 7 months of age at different times and were later castrated. Since then, they had no more contact with other cats and no longer had free access to the street, and as described by Levy et al. (2008) neutered cats and those with restricted access to the street have lower rates of contagion.

Animals number 5, 7, 9, 10 and 11 were rescued as adults, including males and females of different ages, and despite having had a free life with access, conflict and coexistence with other felines, none of them showed a positive result for FeLV. According to Coelho (2013), intimate and social contact with other felines increases the chances of possible horizontal transmission through licking or biting.

Although there are several ways of contagion, the susceptibility to acquiring this pathology varies depending on the immune system of each feline and also on the viral load to which it is exposed (Augusti, 2009). This may be a justification for the cats in this study that tested negative for FeLV despite having been free-living before rescue.

The number 14 animal, despite being positive for this disease, is stable, while the number 2 of the animal is in a serious condition, presenting a picture of anemia, in addition to the presence of tumors in the liver, spleen and kidneys. As this disease is considered one of the most serious diseases that affect felines, it is necessary to carry out a reliable and accurate diagnosis so that transmissions from an infected animal to a healthy animal are avoided (Levy et al., 2008). In addition to the horizontal transmission routes mentioned above, FeLV has the form of vertical transmission, which can be transplacentally or through breastfeeding (Medeiros et al., 2019).

The cats that tested negative lived in the same environment and shared feeders, drinkers and litter boxes. Despite the result, everyone will continue to have restricted access to the street and will not have access to other cats to avoid possible contamination, since according to Jericho et al. (2014), for FeLV transmission to occur, contact with a contaminated feline is necessary, as the virus is considered sensitive outside the host and in the environment it is easily eliminated by disinfectants, detergents and heat.

Positive animals number 2 and 14 were instructed to continue living in delimited environments with restricted access to contact with other felines. As described by Paula et al., (2014), this conduct is also intended as a form of protection against possible secondary infections that may compromise the health status of these animals, since FeLV weakens the host's immune system. In addition, positive felines must follow up with the veterinarian and periodically carry out complementary exams.

PCR is a highly sensitive diagnostic method that detects the presence of the virus contained in its nucleic acid sequences. Although rapid serological tests are the most routinely used, PCR is considered a confirmatory method when there are false-negative results in rapid tests that are incompatible with the animal's clinical condition and suspicion of FeLV (Matesco, 2014).

CONCLUSION

It can be concluded from this study that of the 14 cats tested, the 2 animals already positive in the rapid test confirmed to be positive for FeLV also in the PCR and that of the remaining 12, which lived together, had a negative result for this disease. Because it has a high incidence in the clinical routine affecting felines all over the world and because it is considered a serious disease that has no cure, a reliable diagnosis such as the PCR must be performed so that its spread can be avoided.

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