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e-ISSN:2706-6800
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Detection of Feline Leukemia virus p27 antigen by microELISA vs immunochromatographic tests in cats from Guayaquil.

Detección de antígeno p27 del virus de la Leucemia Felina por método de microELISA vs test inmunocromatográficos en gatos de Guayaquil

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Abstract: The domestic cat is one of the pets of choice, which leads to the evaluation of diagnostic methods for diseases such as feline viral leukemia or Vilef, where the efficacy and speed of diagnosis is important because it is a highly fatal disease. The aim of this study was to determine the percentage of sensitivity of two immunochromatography tests commonly used in veterinary offices to diagnose Vilef and to check their usefulness by making a comparison with the results of the microELISA method, as a reference technique. Using a quantitative, experimental, applied approach, the research was developed from the collection of blood sera from cats in a clinical laboratory in the city of Guayaquil, Ecuador. Forty blood sera were analyzed for detection of feline leukemia virus p27 antigen by the microELISA method and later with immunochromatography tests. The results determined that when reaching a kappa value range of 0.8, the tests present a very good concordance degree, resulting in a 93 % of sensitivity. Concluding that the tests evaluated are reliable and can be considered as tools for rapid diagnosis.

Keywords: Cats, p27 antigen, Feline Leukemia virus, microELISA, immunochromatographic

Resumen: El gato doméstico es uno de los animales de compañía de preferencia, lo que conduce a la evaluación de los métodos de diagnóstico de enfermedades que padece como La leucemia viral felina o Vilef, donde la eficacia y rapidez del diagnóstico es importante por ser una enfermedad altamente mortal. El presente estudio tuvo como objetivo determinar el porcentaje de sensibilidad de dos test de inmunocromatografía de uso común en consultorios veterinarios para diagnosticar Vilef y de comprobar su utilidad haciendo una comparativa con los resultados del método de microELISA, como técnica de referencia. Mediante un enfoque cuantitativo, experimental, de tipo aplicado, se desarrolló la

Published
Instituto Superior Tecnológico Edwards Deming. Quito - Ecuador

Periodicity
January - March
Vol. 1, Num. 24, 2025
pp. 42-52
http://centrosuragraria.com/index.php/revista

Dates of receipt
Received: July 02, 2024
Approved: November 04, 2024

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investigación, a partir de la recolección de sueros sanguíneos de gatos en un laboratorio clínico de la ciudad de Guayaquil, Ecuador. Se analizaron 40 sueros sanguíneos para detección de antígeno p27 del virus de leucemia felina por el método de microELISA y posteriormente con los test de inmunocromatografía. Los resultados determinaron que al alcanzar un rango de valor kappa de 0.8, los test presentan un grado concordancia muy bueno, resultando así mismo con un 93 % de sensibilidad. Concluyendo que los test evaluados son confiables y pueden ser considerados como herramientas para un diagnóstico rápido.

Palabras clave: Gatos, antígeno p27, virus de Leucemia Felina, microELISA, inmunocromatográficos

Introduction

Feline leukemia, or FIV, is one of the main viral threats of major recurrence in domestic cats. VileF is produced by a rotavirus, whose transmission is influenced by population density and exposure to the virus by the approach of infected cats with persistent viremic picture, being the main way of contagion is through saliva (Hwang et al., 2018; Perharic et al., 2018).

The severity of infection depends on factors such as the age of the cat and the strength of the immune system, determining the possible elimination or latency of the virus and the resulting VileF genotype (Aiyaranoi et al., 2018). In addition, of the four stages of the disease, which are: abortive, regressive, progressive and focal, the progressive phase is the one with the least favorable prognosis, where the age of the cat at the time of infection is a determining factor for clinical outcomes and the process of evolution of the disease (Calle-Restrepo et al., 2013).

The use of diagnostic tests for VileF has become essential for the determination of a positive patient. The diagnosis will depend on the stage of the disease, with p27 antigen detection being the first test to be performed, using methods such as microELISA and immunochromatography. The importance of a diagnosis lies in the speed of the data provided and the effectiveness of the results obtained. This allows an adequate management of infected and uninfected animals, in order to extend or prolong the days of these felines with a good quality of life (Campbell et al., 2020).

In daily practice in veterinary care centers, immunochromatography tests are frequently used because of their rapid response time, with a result obtained in a few minutes, as opposed to laboratory tests such as the microELISA, which can take up to 72 hours. It is important to

mention that in spite of the time advantages obtained with a test, these can yield false negatives, while laboratory tests such as the microELISA test are more sensitive and specific.

Immunological tests use antigen to detect antibody or antibody to detect a pathogen antigen in a patient sample (Vazquez, 2020). Sensitivity to antigen or antibody detection is a factor that validates the performance of the laboratory test. The immunological microELISA test that detects the p27 antigen in plasma or serum has a high sensitivity and specificity, without considering vaccination, lactation or colostrum intake as interference (Muñoz, 2005; Palmero and Carballés, 2010; Calle et al., 2013; Esquivel, 2019). The present study aims to reflect the validity of the results, measuring the percentage of sensitivity of imunochromatography tests used in veterinary centers, detecting the p27 antigen for feline leukemia diagnosis, using the microELISA method as a reference test.

Methodology

The methodology applied in the present study presents a quantitative approach, experimental design, applied type, with correlational objectives, with experimental variables, diachronic transversal type, developed in a controlled environment during a limited period of time. The study was carried out from October 2022 to January 2023, 40 blood samples were chosen from cats received at the Diagnovet Clinical Veterinary Laboratory in the city of Guayaquil.

The larger samples were selected for further processing with the microELISA technique. Once this procedure was finished, they were analyzed with the two immucromatography tests of different laboratories of manufacture and of greater use in veterinary centers, which are represented as Test IC 1 and Test IC 2, for their identification. As described by Krecic, these two tests are the most requested in veterinary practice for the diagnosis of feline leukemia by detection of p27 antigen (Krecic et al., 2017).

In previous years the diagnosis of feline leukemia was made based on clinical symptoms, since there were very few guardians who authorized laboratory tests to determine whether their cat was positive or not to the

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disease, currently the ease of testing makes them become frequently used for diagnosis in veterinary centers. Campbell in his comparative study of immunochromatography tests establishes the importance of assessing the sensitivity of these tests, to ensure an effective diagnosis that helps in the management of infected animals and their treatment (Campbell, et al., 2020). Under this sense, all the points were examined and the necessary resources were gathered for the methodological structure to comply with the objectives set out in the research.

For the comparative evaluation of results, the 2x2 contingency table (Table 1) was used, taking the microELISA test as a reference and relating the results with the two results obtained with the serological tests, in this case the two immucromatography tests referred to. The degree of concordance between the two techniques was evaluated and then the kappa index or kappa value was calculated to obtain the final data, categorized according to the ranges of values in Table 2.

Table 1. *Cohen's 2x2 contingency table*

		Reference diagnostic tests		
		Positive	Negative	
Diagnostic tests evaluated	Positive	a	c	f1
	Negative	b	d	f2
		c1	c2	N

Source: Cohen 1960

$$\text{Observed concordance CO} = a + \frac{d}{N}$$

$$\text{Concordance due to chance CA} = f1 \times \frac{c1 + f2 \times c2}{N}$$

$$\text{Actual or non-random concordance CR} = CO - CA$$

Maximum match that can occur beyond chance MCMA = 1 - CA

$$\text{Kappa} = \frac{CR}{MCMA}$$

Source: Cohen 1960

Table 2. *Ranges of Kappa Values*

Kappa value ranges	Degree of agreement between tests
<0.00	No agreement
0.00-0.20	Insignificant
0.21-0.40	Under
0.41-0.60	Moderate
0.61-0.80	Good
0.81-1.00	Very good

Source: Cohen, 1960

$$\text{Sensitivity} = \frac{a}{a + b}$$

Source: Cohen, 1960

Results

Forty blood serum samples from cats received at the reference laboratory were analyzed and were subjected to the microElisa test at , resulting in 30 positive and 10 negative results. Subsequently, they were

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analyzed with the selected immunochromatography tests, resulting in 28 positive and 12 negative for Test IC 1 and 28 positive and 12 negative for Test IC 2, as shown in Table 3.

Table 3. *Results obtained in IC Test 1 and IC Test 2*

Variable	microElisa	%	Test IC 1	IC Test 2	%	Sig.
Positive	30	75	28	28	70	0.1573
Negative	10	25	12	12	30	
Total	40		40	40		

IC Test 1: Immunochromatography Test 1; IC Test 2: Immunochromatography Test 2

According to the results obtained, the contingency table (Table 4) was made and the formulas of observed concordance, concordance due to chance, real concordance or not due to chance, maximum concordance that can occur beyond chance were performed, reaching the Kappa value range of 0.8, determining that the degree of concordance between the diagnostic tests evaluated is very good, as can be seen in the following approach.

Table 4. Contingency table microELISA vs IC Test 1 and IC Test 2

		Reference diagnostic tests		
		+	-	
Diagnostic tests evaluated	+	28	0	28
	-	2	10	12
		30	10	40

Observed concordance CO = 28 + 10 = 0.95

$$\text{Concordance due to chance CA} = \frac{28 \times 30 + 10 \times 12}{N} = 0.6$$

$$\text{True concordance or not due to chance CR} = 0.95 - 0.6 = 0.35$$

$$\text{Maximum match that can occur beyond chance MCMA} = 1 - 0.6 = 0.40$$

$$\text{Kappa} = \frac{0.35}{0.40} = 0.8$$

With respect to the sensitivity percentage obtained based on Table 4 and applying the formula referred to above, the resulting value is 93 % out of 100.

$$\text{Sensitivity} = \frac{28}{28 + 2} = 0.93 \times 100 = 93\ \%.$$

There are no previous research reports detailing the degree of sensitivity of immunochromatography tests comparing the results with the microELISA reference test, because it is highly reliable and specific. (2020) on the diagnosis of ViLeF, a comparison is made between the PCR test as a reference test and the immunochromatography tests as tests to be evaluated, resulting from the total number of samples 39 positive by PCR and one positive by immunochromatography, concluding that the pathogenesis of the infectious agent must be taken into consideration to make a correct test and make associations with the clinical symptomatology in order not to make a wrong diagnosis.

Similarly a study conducted by Westman et al., (2017), focused on a comparison of the PCR technique with immunochromatography tests, determining false positives with gerontic cats being the ones that mostly obtained these results. He concluded that feline leukemia virus is a viral disease that is difficult to diagnose due to the complex relationship between the pathogen - feline host, evidencing in his research the unreliability of immunochromatography tests as diagnostic tests.

Evidently, the results obtained in this research are in contrast to the studies of Campbell and Westman in previous years, in the results that they present, there is little sensitivity in the serological tests for detection of p27 antigen with immunochromatography tests, unlike the

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current data where the degree of sensitivity in the same tests was very good. This differentiation is attributed to the improvements of the manufacturing laboratories in offering tests with rapid and better sensitivity, due to the demand for reliable diagnostic tests in veterinary centers that collaborate in the timely care of patients with serious or life-threatening diseases.

Conclusions

After the analysis of the 40 samples of blood serum of cats prescribed in the clinical veterinary laboratory Diagnovet of the city of Guayaquil, 30 positive and 10 negative results were obtained for the microElisa test. Unlike the results obtained with the selected immunochromatography tests, giving as a result for the IC 1 test 28 positive and 12 negative, and with the IC 2 test, 28 positive and 12 negative. In addition, the serological tests evaluated for the immunochromatography test, to detect p27 antigen for the feline leukemia virus, presented concordance with a range of Kappa value of 0.8 that evaluates them as very good and presenting a degree of sensitivity of 93%. Therefore, it is concluded that the immucromatographic tests used in this research are of very good reliability and sensitivity, which added to the rapidity with which the results are obtained, are a useful diagnostic tool for feline leukemia in veterinary centers. It was also evidenced the low probability of false negatives in the tests used in the study.

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