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Comparação entre os testes imunocromatográfico e reação em cadeia pela polimerase para diagnóstico de FIV e FeLV

Comparison between immunochromatographic tests and polymerase chain reaction for FIV and FeLV diagnosis

Comparación entre las pruebas inmunocromatográfica y la reacción en cadena de la polimerasa para el diagnóstico de FIV y FeLV

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Resumo

Os retrovírus responsáveis pela Imunodeficiência felina e Leucemia felina estão dispersos por todo o mundo, acometendo uma grande quantidade de animais. Essas doenças são de caráter vitalício e se manifestam quando o gato está imunossuprimido, trazendo inúmeras consequências a saúde destes. O diagnóstico correto e precoce auxilia no manejo, tanto dos animais infectados, quanto dos não infectados, no intuito de prolongar a vida desses felinos com qualidade. O presente trabalho tem como objetivo comparar dois diferentes testes para diagnóstico dessas enfermidades, a fim de discutir o motivo da discrepância entre os resultados, demonstrando os pontos positivos e negativos, e auxiliar o clínico na escolha do melhor método a ser usado em cada situação. Foram realizados os testes imunocromatográfico e por PCR em 66 animais residentes no município de Mineiros, Goiás, Brasil. Dentre esses, oito animais foram positivos para o teste imunocromatográfico, e cinco para o teste de PCR com diagnóstico positivo para FIV. Para FeLV, houve apenas um felino positivo para o ensaio imunocromatográfico e 38 positivos para o teste de PCR. Com esse resultado, a conclusão é que ambos os testes possuem suas limitações e deve-se considerar a patogenia do agente na escolha do melhor teste a ser realizado ou associá-los.

Palavras-chave: Gatos; Imunocromatografia; Imunodeficiência viral felina; Leucemia viral felina; PCR.

Abstract

The retroviruses responsible for feline immunodeficiency and feline leukemia are spread around the world, affecting a large number of animals. These diseases are lifelong and manifest when the cat is immunosuppressed, bringing numerous consequences to their health. The correct and early diagnosis helps in the management of both infected and uninfected animals in order to extend the life of these felines with quality. The present work aims to compare two different tests for the diagnosis of these diseases, in order to discuss the reason for the discrepancy between the results, to demonstrate the positive and negative points of

each one and to help the clinician to choose the best method to be used in each situation. The immunochromatographic test and PCR was performed in 66 animals living in the city of Mineiros, Goiás, Brazil. Among these, eight animals were positives in the immunochromatographic test and five in the PCR test for diagnosis of FIV. For FeLV, there were only one positive feline on immunochromatography and 38 positives on PCR. With this result, the conclusion is that both tests have their limitations and one should consider the pathogenesis of the agent in order perform the test correctly or associate them.

Keywords: Cats; Feline Viral Immunodeficiency; Feline Viral Leukemia; Immunochromatography; PCR.

Resumen

Los retrovirus responsables de la inmunodeficiencia felina y la leucemia felina se encuentran dispersos en todo el mundo, afectando un gran número de animales. Estas enfermedades duran toda la vida y se manifiestan cuando el gato está inmunodeprimido, lo que conlleva numerosas consecuencias para su salud. El diagnóstico correcto y temprano ayuda en el manejo de animales infectados y no infectados, a fin de prolongar la vida de estos gatos con calidad. El presente trabajo tiene como objetivo comparar dos pruebas diferentes para diagnosticar estas enfermedades, con el fin de discutir la razón de la discrepancia entre los resultados, demostrar los puntos positivos y negativos de cada uno y ayudar al médico veterinario a elegir el mejor método para cada situación. La prueba inmunocromatográfica y la PCR se realizaron en 66 animales residentes en la ciudad de Mineiros, Goiás, Brasil. Entre estos, ocho animales dieron positivo en la prueba de inmunocromatografía y cinco en la prueba de PCR para el diagnóstico de FIV. Para FeLV, solo hubo uno felino positivo en la inmunocromatografía y 38 positivos en la prueba de PCR. Con este resultado, la conclusión es que ambas pruebas tienen sus limitaciones y se debe considerar la patogénesis del agente al elegir la mejor prueba a realizar o asociarlas.

Palabras clave: Gato; Inmunocromatografía; Inmunodeficiencia viral felina; Leucemia viral felina; PCR.

1. Introduction

Disseminated worldwide, the feline immunodeficiency virus (FIV) and the feline leukemia virus (FeLV) are considered the most important in feline veterinary medicine

(Spada *et al.*, 2018). Both are retroviruses, that is, they have the ability to transform their RNA into DNA inside the animal cell, using the enzyme reverse transcriptase. They are also able to introduce their genetic material into the feline genome, in the form of proviral DNA (Del Barrio, 2016). For this reason, they are considered lifelong infections (Stavisky *et al.*, 2017).

The main route of transmission of retroviruses is through the saliva of the infected animal, although other fluids, such as urine, faeces and nasal secretions can also transmit (Munro *et al.*, 2014). FIV and FeLV infections are two of the most common causes for euthanasia, however, if the proper treatment and correct management is done, positive cats can live for a long time and with quality of life (Spada *et al.*, 2018). Both agents can cause depletion of the immune system, although FeLV is more related to regenerative anemias and the development of neoplasms (Westman *et al.*, 2016b). Infection with both retroviruses is also possible, and when it occurs, the animal tends to weaken more quickly and more severely (Arjona *et al.*, 2007; Spada *et al.*, 2018).

The fact that the diagnosis for retroviruses is not compulsory makes it difficult to establish their true prevalence in the feline population. Treatment is usually supportive. Although exist some anti-retroviral drugs that work in a more specific way, there is a need for more studies of the real benefits of these drugs (Alves *et al.*, 2015). The main test routinely used by veterinarians is the immunochromatographic test. Although the test is practical and relatively inexpensive, it has a limited sensitivity when compared to the polymerase chain reaction (PCR) (Arjona *et al.*, 2007).

This study aimed to test 66 felines from the city of Mineiros, Goiás, Brazil, through two different diagnostic tests: the immunochromatographic test and the PCR. The result of the two tests showed discrepancies between them, and the objective of this work is search to understand the reason for this difference, in addition to guide the veterinarian, so that each test is requested and interpreted correctly.

2. Methodology

For this experiment, 66 cats from the city of Mineiros, Goiás, Brazil, in the following geographical coordinates: 17°33’48.2’’S 52°33’10.3’’W. The animals was submit of blood puncture through the external jugular vein, followed by blood storage in the tubes containing

ethylenediamine tetra-acetic acid (EDTA). The material was subjected to the FIV Ac/FeLV Ag (Alere®) Test Kit immunochromatographic, following the manufacturer's instructions, by Lemos *et al*. (2019), to simultaneously detect feline immunodeficiey virus (FIV) IgG antibodies and feline leukemia virus (FeLV) antigens (p27 antigens) in whole blood, serum or feline plasma.

The same samples were submitted to PCR. The DNAs that use the molecular test were selected for the blood sample cells with EDTA, following the protocol of Watanabe *et al.* (2003). Subsequently, they were extracted using phenol (Sigma-Aldrich, P.A. – ACS, purity 99%) and chloroform (Sigma-Aldrich, P.A. – ACS, purity 99.8%), followed by precipitation using ethanol (Sigma-Aldrich, P.A. – ACS, purity 99.5%) and purified according to the protocol by Sambrook and Russell (2001). The genetic material used was submitted to PCR following the protocols of Lara *et al.* (2008) and Sheets *et al.* (1993), for FIV and FeLV, respectively. The method used in the FIV test was the nested PCR. The primers used to detect proviral DNA are described in the table below.

Table 1. Primers used in polymerase chain reactions in felines from the city of Mineiros, Goiás, Brazil.

Primer	**Sequences**
FeLV F	5’ TTT AAA CTA ACC AAT CCC CAC G 3’
FeLV R	5’ CCC CAA ATG AAA GAC CCC 3’
FIV F external	5’ AAT ATG ACT GTA TCT ACT GC 3’
FIV R external	5’ TTT TCT TCT AGA GTA CTT TCT GG 3’
FIV F internal	5’ TAT TCA AAC AGT AAA TGG AG 3’
FIV R internal	5’ CTG CTT GTT GTT CTT GAG TT 3’

Source: Lara *et al*. (2008) and Sheets *et al*. (1993).

The PCR reaction for FeLV amplifies the 240bp region, while for FIV amplifies the 658bp fragment in the first phase and 329bp in the second phase. The amplifications are visualized through electrophoresis (Prolab, DGH-12 – 90V).

This experiment was authorized by the Ethics Committee on Animal Use (CEUA), of the Centro Universitário de Mineiros (UNIFIMES), under protocol number 15/2017.

3. Results and Discussion

A general sample was 66 animals, being that 28 of them were females and 38 were males. 38 animals have less than one year old and 28 were considered adults. Only seven animals were definite breed, and all cats in this study were domiciled, but had access to the street.

The immunochromatographic test resulted in eight equal 12.12% animals positive for FIV and one equal 1.52% animals for FeLV. All FIV positive animals were mixed breed males and only one cat were considered adult. The feline FeLV positive was male, mixed breed and kitten.

The same initial samples were submitted to PCR and the results found were five equal 7.58% and 38 equal 57.58% animals positive for FIV and FeLV, respectively. Among the FIVs positive, four equal 80% were kittens and only one equal 20% adult. There were only one equal 20% female and one equal 20% feline of definite breed among them. Should be noted that only three cats were considered FIV positive in both tests. Two animals were positive only by PCR and three animals were positive only by immunochromatography. For FeLV, the only feline positive by immunochromatography was also positive in PCR. Of the 38 FeLV animals diagnosed, 22 equal 57.89% were kittens and 16 equal 42.11% were adults. There were 23 equal 60.53% males and 15 equal 39.47% females, 35 equal 92.11% were mixed breed animals and three equal 7.89% definite breed animals.

It is observed in Table 2, the occurrence in city of Mineiros, Goiás, Brazil, for FIV in the immunochromatographic test was 12.12%, and for FeLV it was 1.52%, values consistent with the study by Stavisky *et al.* (2017) in the United Kingdom, in which the prevalence for FIV and FeLV was 11.4% and 3%, respectively, in the first shelter analyzed. In the second shelter, the occurrence found was lower, being 3% (FIV) and 0% (FeLV), using the same method. In the experiment done by Westman *et al.* (2016b) with the rapid test, in Australia, 15% of the animals were FIV positives and 2% FeLV positives. Teixeira *et al.* (2019) found in the state from Ceará, Brazil 6.1% of animals positives for FIV by immunochromatography. For Poffo *et al.* (2017), with the same method, in Cuiabá, Mato Grosso, Brazil, the value

found for FeLV was 4.5%, being considered a little higher than the value found in the present study.

Poffo *et al.* (2017) found 11.5% of their animals positives for FIV by PCR test, a frequency slightly higher than that reported in Mineiros, Goiás, Brazil. Bisol (2016) found in their animals tested by PCR for both infections, 3.38% positives for FIV and 9.6% positives for FeLV, values quite different from those found in this study.

Table 2. Results obtained by immunochromatography and PCR for diagnosis of FIV and FeLV in cats of Mineiros city, Goiás state.

Group	FIV Positive				FeLV Positive			
	Immunochroma-tography		Polymerase Chain Reaction		Immunochroma-tography		Polymerase Chain Reaction	
Total	8	12.12%	5	7.58%	1	1.52%	38	57.57%
Age								
Kittens	7	10.6%	4	6.06%	1	1.52%	22	33.33%
Adults	1	1.52%	1	1.52%	0	0%	16	24.24%
Gender								
Male	8	12.12%	4	6.06%	1	1.52%	23	34.85%
Female	0	0%	1	1.52%	0	0%	15	22.72%
Breed								
Definite	0	0%	1	1.52%	0	0%	3	4.55%
Mixed	8	12.12%	4	6.06%	1	1.52%	35	53.02%

Three cats were positive in both tests. Two positives only in PCR and three positives only in immunochromatography. The positive cat in immunochromatography was also positive by PCR.

According to Silva *et al.* (2014), Spada *et al.* (2018) and Teixeira *et al.* (2019), males match in most animals positive for FIV, a fact consistent with this research. Stavisky *et al.* (2017) also found a greater number of adult animals being positive for this infection, contrary to the result found in this study. This may be due to the fact that the present study includes

more kittens than adults in general sample. The present study confirmed that of Hagiwara and Reche-Júnior (2016), which describes the presence of FeLV being more common in young cats.

Part of the positives results for FIV were inconsistent between the two methods. Among eight positives animals in the rapid test, there were five negatives in the PCR. The five discordant animals can be false positives in immunochromatography or false negatives in PCR. False positives for FIV can occur in the rapid test of cats under the age of six months, since there is the possibility of maternal antibodies circulating in the blood, requiring a confirmatory test after these animals are over six months of age (Arjona *et al.*, 2007; Spada *et al.*, 2018). Among the five animals positive in immunochromatography and negative in PCR, four were kittens. The ideal in this case was to wait a few months and repeat the immunochromatography. On the other hand, the truly positives results for FIV in immunochromatography and negative in PCR may indicate that the animal has a very low circulating viral load, not having enough proviral DNA to be detected by the molecular method (Nichols *et al.*, 2017). It is important to note that the FIV has various subtypes and, therefore, there may be flaws in the PCR test, due to the fact that primers used do not detect all of them (Arjona *et al.*, 2008).

In contrast, of the five animals positives for PCR, two of them had negatives results in immunochromatography, they may correspond to false negative effects in the rapid test. In immunochromatography, the risk of false results can occur in animals in immunocompromised situations, especially in the terminal phase or at the beginning of the infection, while the body has not yet produced antibodies (Arjona *et al.*, 2007; Spada *et al.*, 2018). Frankenfeld *et al.* (2019) also says in his study that the decreased sensibility of immunochromatography is possible due to travel with pets in several countries, inserting new subtypes undetectable by this method.

Arjona *et al.* (2007), in turn, found in his work for diagnosis of FIV, 20 positives samples in the PCR. However, in addition to these 20, another five samples were positive in immunochromatography. In this case, the Western Blot test was performed to clarify the disagreement between the results of the other two tests, showing that the five positives surplus results in the immunochromatography consisted of false positives.

Although Western blot is considered the gold standard for the serological diagnosis of FIV infection, as it has high specificity, it does not differentiate vaccine antibodies, presenting

only 54% specificity when vaccinated animals are tested (Pedersen; Barlough, 1991; Levy *et al.*, 2006; Hosie *et al.*, 2009). However, it is still widely used to assess the sensitivity and specificity of commercial and developing tests, using recombinant antigens p24 and p17 from FIV (Hartmann *et al.*, 2007).

For FeLV, the immunochromatography searches for the antigen, which normally circulates in the blood for two or three weeks after the feline was exposed to the agent (Alves *et al.*, 2015). More recent methods of diagnosing FeLV, such as PCR can find the genetic material of the agent in the host's DNA (Arjona *et al.*, 2007; Westman *et al.*, 2016a). The result of the diagnostic tests for FeLV is related to the phase of the pathogenesis of the agents. The rapid test should be used as a screening. The performance of immunochromatography together with PCR is an important tool to define the prognosis of the animal (Figueiredo; Araújo-Júnior, 2011).

In this case, we had only one positive animal through immunochromatography, and it was diagnosed positively on PCR. Cats with positive results from both tests are classified as progressive infection (Figueiredo; Araújo-Júnior, 2011). In abortion infection, both the immunochromatographic test and the PCR will be negative, as there will be no viremia or insertion of the provirus in the animal cell (Hartmann, 2012).

If the cat has a regressive infection, the viremia will become smaller and the immunochromatographic test will be unable to detect, despite the fact that FeLV is positive (Alves *et al.*, 2015), what happened to 37 animals of that type study, consisting of false negative results. This usually occurs because the animal organism eliminates the virus from the circulation, but not before it inserts its genetic material into predilection cells, such as bone marrow (Arjona *et al.*, 2007; Figueiredo; Araújo-Júnior, 2011; Spada *et al.*, 2018). Since the rapid test is able to detect only the antigen, there is no interference from maternal antigens circulating in the blood of the felines or vaccines, and there is no minimum age to test the puppies (Alves *et al.*, 2015).

False positive animals for FeLV, have already been reported in studies that compared the ALERE immunochromatographic tests, which was used in this study and the SNAP®, with the SNAP® showing a greater number of false positive results and the ALERE test showed greater specificity (Westman *et al.*, 2016a; Medeiros *et al.*, 2019).

Also according to Alves *et al.* (2015), it is believed that animals negatives in the rapid test and positives in the detection of FeLV by PCR possibly will not be disseminators of the agent and will hardly develop the disease due to the low circulating viral load.

The PCR false negative for both infections can occur due to the wrong manipulation of the sample during the test, which can damage the DNA (Arjona *et al.*, 2008).

The comparative result between the immunochromatographic and FeLV PCR tests had the same result as the experiment by Arjona *et al.* (2007), which showed that of 64 animals that had been positive for FeLV through PCR, 25% of them were negative in the immunochromatography test.

In the present study, no positive animals were detected in immunochromatography and negative in PCR. In the study by Arjona *et al.* (2007), 6 animals presented this diagnosis, and in the study by Bisol (2016) there were 10 animals. The first justification proposed is that the infection is in its initial stage, where the antigen exists in the bloodstream, but there is still no presence of the provirus installed in the host's genetic material, configuring the different result. The second possibility is focal infection, where only a few specific tissues have proviral DNA. The third option is the false negative result in the rapid test (Arjona *et al.*, 2007).

4. Final Considerations

Due to the high occurrence of retroviruses, diagnostic tests are necessary, preferably in all felines, so that there is segregation between infected and non-infected animals, aiming at prevention and, in the long term, a decrease in the incidence of these agents in the population. feline. It is important to note that both immunochromatography and PCR have their limitations, justifying the need to take into account the clinical signs presented and the epidemiological data of the place. The present study demonstrated the importance of the association between two methods for better interpretation and reduction in the number of diagnostic failures, in addition to helping to establish the prognosis of positive cats.

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