

Evaluation of a recombinant p24 antigen for the detection of Feline Immunodeficiency Virus-specific antibodies¹

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ABSTRACT.- Mazur C., Reis J.K.P., Leite R.C., Danelli M.G.M., Hagiwara M.K. & Medeiros M.A. 2010. **Evaluation of a recombinant p24 antigen for the detection of Feline Immunodeficiency Virus-specific antibodies.** *Pesquisa Veterinária Brasileira* 30(10):877-880. Laboratório de Vírus Veterinárias, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23890-000, Brazil. E-mail: mazur@ufrj.br

Feline Immunodeficiency Virus is a worldwide infection and is considered a significant pathogen. The diagnosis of FIV infections is mainly based on commercially available rapid tests that are highly expensive in Brazil, hence it is rarely performed in the country. Furthermore, lentiviruses grow slowly and poorly in tissue cultures, making the production of viral antigen by classic means and thus the establishment of FIV immunodiagnosis impracticable. In order to deal with this, recombinant DNA techniques were adopted to produce the protein p24, a viral capsid antigen. The protein's reactivity evaluation analyzed by Western blot indicated that this recombinant antigen can be a useful tool for the immunodiagnostic of FIV infections.

INDEX TERMS: FIV, feline immunodeficiency virus, cats.

RESUMO.- [Avaliação do antígeno recombinante p24 para a detecção de anticorpos específicos do Vírus da Imunodeficiência Felina.] O vírus da imunodeficiência felina tem distribuição mundial e é considerado um patógeno significativo. No Brasil, a prática diagnóstica é baseada principalmente em teste rápidos, importados e de custo elevado, disponíveis comercialmente. Devido ao seu custo proibitivo em nosso país, o diagnóstico da infecção pelo FIV é raramente realizado. Ademais, os lentivírus se multiplicam lenta e pobremente em cultura de células, o que torna a produção de antígeno por meios clássicos e o estabelecimento do imunodiagnóstico impraticável. Com o objetivo de lidar com

esta questão, técnicas de DNA recombinante foram utilizadas para produção de um antígeno do capsídeo viral, a proteína p24. A avaliação da reatividade realizada por *Western blot* indicou que este antígeno recombinante pode ser útil para o imunodiagnóstico de infecções pelo FIV.

TERMS DE INDEXAÇÃO: FIV, vírus da imunodeficiência felina, gatos.

INTRODUCTION

Feline Immunodeficiency Virus (FIV) is a lentivirus that causes a chronic and progressive immunodeficiency and is considered one of the main pathogens of domestic felines. Infected cats show a progressive depletion of CD4+ T-lymphocytes and immunological deficits which, after a long asymptomatic period, may lead to severe opportunistic infections followed by death, characterizing the clinical phase known as Feline Acquired Immunodeficiency Syndrome (FAIDS). Due to the similarity to HIV, FIV-infected cats have been used as experimental models for HIV/AIDS (De Rozières et al. 2004).

FIV is structured like other lentiviruses. Virions measure 80-100 nm in diameter and consist of an envelope, a nucleocapsid, a nucleoid and a matrix protein. Surface projections of 8 nm length are densely dispersed, small,

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inconspicuous spikes that cover evenly the surface. Each virion also contains two copies of the positive-stranded, polyadenylated RNA genome of about 9400 base pairs. The viral genome is divided in three genes, *gag*, *pol* and *env* that codify internal structural proteins, reverse transcriptase and other enzymes and envelope proteins, respectively. There are other several accessory genes (Olmsted et al. 1989, Bendinelli et al. 1995).

Naturally, the control of the disease is linked to the diagnosis of FIV infections, which relies mainly on the detection of specific antibodies which are considered indicators of the persistent infection. Most available FIV diagnostic tests detect serum, plasma, or whole blood antibodies to FIV. The American Association of Feline Practitioners recommends the use of antibody assays to determine the FIV status of all cats, regardless of age or health status, even with an increase of the vaccination practice (Levy et al. 2008).

An ELISA of immobilized labeled antigens that bind to antibodies is the only available FIV test for commercial or in-practice laboratories in North America and other countries, including Brazil. These tests come in kit format for in-practice use, or in a microwell format for laboratory use. This ELISA is based on detection of antibodies against the viral nucleocapsid protein p24 and a positive or negative result depends on a chromogenic reaction relative to a positive control (O'Connor et al. 1989, Mermer et al. 1992). The p24 is the major nucleocapsid protein encoded by the *gag* gene. Antibody detection against p24 is commonly used in FIV infection diagnosis due to its high conservation and antigenic characteristics (Calzolari et al. 1995, Mermer et al. 1992, Reid et al. 1992). Western blot is also available and is often recommended for confirmatory testing of ELISA positive samples (Barr 1996, Levy et al. 2008).

Nevertheless, routine and research diagnosis for FIV infections still remain problematic in some parts of the world such as Brazil. Despite its large worldwide diffusion and application, the ELISA based rapid tests usage still depends on imported kits with highly restrictive costs in Brazil. Moreover, any immunodiagnostic technique depends on the antigen source and in the case of lentiviruses on viral cultivation in cell cultures, which is expensive and time-consuming. Perhaps, these factors are the greatest obstacles for the availability of FIV diagnosis in our country.

The production of viral proteins by recombinant bacteria or other biological systems has been used for different purposes as an alternative technology to classic methods of protein purification from cultivated virus. Such alternatives are of special interest for viruses that are hardly grown in cell cultures such as lentiviruses. Basically, the desired coding region is ligated in a DNA vehicle as a bacterial plasmid and this construct is inserted in a appropriate biological system to express the recombinant protein, like bacteria. Cultures of recombinant bacteria represent a practical source of the heterologous protein.

With the aim to contribute to FIV diagnosis in Brazil, a recombinant antigen produced by *Escherichia coli* was

developed and its reactivity against sera samples of naturally infected and non-infected cats was evaluated by Western blot (WB), the gold standard test for the diagnosis of FIV and several virus infections.

MATERIALS AND METHODS

Cloning procedures. A DNA sample obtained from the peripheral blood mononuclear cells (PBMC) of a naturally FIV-infected cat was used successfully in order to amplify the p24 coding region by the PCR. The primer's design was based on the complete FIV B genomic sequence (Genbank/nucleotide database accession number M59418) and the characteristics of the chosen expression vector (pET100/D-TOPO[®], Invitrogen) (5'-CACCATGCCTATTCAAACAGTAAATGGAG-3', forward and 5'-CAAGAGTTGCATTTTATATCCTGGTGAGCC-3', reverse). The obtained amplicon was inserted in the plasmid expression vector following the manufacturer's recommendations. The correct insertion of the p24 coding region in the recombinant plasmid was confirmed by nucleotide sequencing.

Recombinant protein production. After the transformation of the recombinant plasmid by electroporation, the recombinant protein p24 (r-p24) was over expressed in the selected recombinant bacterial clone induced with 1M IPTG (isopropyl-D-thiogalactopyranoside) in a shaker incubator at 37°C for 4 hours. The purification based on the poly-histidine tail added to the recombinant protein was performed by immobilized metal affinity in nickel-charged resin (IMAC). The purified r-p24 was characterized by polyacrylamide gel electrophoresis (SDS-PAGE) that showed a protein of approximately 30 kDa and by WB with anti-histidine monoclonal antibodies.

Western blot assay. WB assays were conducted on a nitrocellulose membrane with about 1.8µg r-p24 protein/cm. For the evaluation of the r-p24 reactivity, 30 seronegative and



Fig.1. Western blot performed with the r-p24. The strips contain 500ng of the purified r-p24 (30 kDa) incubated with a negative (1) and positive (2) control serum diluted at 1:50.

30 seropositive cat serum samples, tested previously by PCR for *gag* gene and ELISA were used. Basically, this assay was carried out using cat serum samples diluted 1/50 in milk TBS-T (4% non-fat milk in Tris-tween buffered saline) and horseradish peroxidase-conjugated anti-cat rabbit immunoglobulin (Fig.1).

Statistical analysis. The chi-square test was chosen to evaluate the agreement between the WB versus PCR and ELISA results. The number of control sera samples, 30 positive e 30 negative, was adopted based on chi-square analysis needs.

RESULTS

The WB assays indicated an agreement of 100% with the previously performed PCR and ELISA tests, what means that all the 30 positive sera reacted with the r-p24 and that unspecific reactions were not observed in any of the 30 negative sera samples. This result made the chi-square analysis dispensable.

DISCUSSION

Pedersen et al. (1987) described the isolation of a T-lymphotropic virus with the characteristics of a lentivirus from pet cats in Davis, California. Since then, it was evident that the virus is of substantial veterinary importance. In addition, the FIV infection exposes felines to opportunistic and pathogenic infectious agents, including some zoonotic ones such as toxoplasmas, bartonellas and even possibly, avian pandemic influenza virus (H5N1) (Lappin et al. 1992, Ueno et al. 1996).

FIV infections in cats can be diagnosed by different techniques: based on virus isolation from a peripheral blood lymphocyte culture, demonstration of specific antibodies or molecularly, using the PCR. Virus isolation is technically demanding, expensive, and takes several weeks, hence it hardly can be considered for routine diagnose purposes.

PCR is frequently used to detect, quantify, and assess genetic variations of infectious agents but FIV is difficult to detect by PCR, since gene sequences differ by as much as 20% among field isolates (Bachmann et al. 1997). Moreover, the amount of virus in blood is low during the clinically silent phase of infection, when most testing is performed (Pedersen et al. 1989). These aspects bring the use of nested PCR approaches, which increase possibilities for false positive results.

FIV antibodies can be detected by the immunofluorescence assay using FIV-infected lymphocytes, ELISA, Western blot and radioimmunoprecipitation assays. Anyway, with the exception of PCR, these techniques depend on the antigen production. Considering the difficulties in obtaining lentivirus antigens from cell cultures, recombinant DNA technology is a simple, economic and available system to produce viral antigens.

FIV diagnostic techniques can be mostly found in the ELISA format, microwell plates and lateral flow systems (rapid tests) that are commercially available. Rapid tests are the most frequently used at veterinary clinics and animal shelters around the world, due to its simplicity and low time consumption (Crawford & Levy 2007). However, even

considering these advantages, the actual cost of these tests showed to be impeditive in countries where it is imported. The microwell ELISA used in veterinary laboratories has been described as having high sensitivity and specificity (Barr et al. 1991). False negative results are rare and mostly due to acute infection prior to the generation of specific antibodies. False positive results have been attributed to poor technique and nonspecific reactivity against tissue culture components following vaccination. Based on these test characteristics, it was recommended that a positive in-practice ELISA in a healthy cat should be confirmed with a Western blot (Barr 1996).

There is a great veterinary interest in a large scale FIV epidemiologic survey in Brazil but the high costs of the rapid test and the difficulty to obtain the viral antigen by classical ways to establish immunodiagnostic techniques may explain the availability of just a few epidemiologic studies found in Brazil and its low numbers of surveyed animals (Teixeira et al. 2007, Caxito 2003, Hagiwara et al. 1987, Laral et al. 2008, Souza et al. 2002). The obtained recombinant bacteria represent a good source for viral antigen production in order to establish alternative diagnosis methods.

CONCLUSION

The results of the WB assays show that r-p24 can be suitable for the detection of FIV antibodies. The simple and inexpensive production of the r-p24 favors the establishment of FIV immunodiagnostic techniques for epidemiologic and clinical applications of this important animal disease.

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