Indirect Western blot in the diagnosis of feline immunodeficiency virus

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RESUMO
O objetivo do presente estudo foi padronizar e estabelecer um Western blot utilizando o antígeno recombinante r-p24 no diagnóstico da imunodeficiência felina a vírus. Setenta e duas amostras com resultados positivos confirmados por SNAP Combo Plus e PCR, foram testadas a partir da técnica de Western blot utilizando 3,14 µg/cm da r-p24 (0,095 µg/animal), soro na diluição de 1:10 e 1:100 e conjugado na diluição de 1:1000. Todas as amostras (100%) tiveram seus resultados confirmados a partir do Western blot. Estes resultados indicam que o Western blot com a r-p24 é um excelente teste confirmatório e pode ser usado para o diagnóstico do FIV.

Palavras-chaves: imunodeficiência felina a vírus, padronização, Western blot, diagnóstico.

ABSTRACT
The aim of the present study was to standardize and establish a Western blot using recombinant antigen r-p24 for the diagnosis of feline immunodeficiency virus. Seventy-two samples with positive results confirmed by SNAP Combo Plus e PCR, were tested through Western blot using 3.14 µg/cm of r-p24 (0.095 µg/animal), serum dilution of 1:10 and 1:100, and conjugate dilution of 1:1000. All samples had the results confirmed by the Western blot. This results show that Western blot is a good confirmatory test for feline immunodeficiency virus infection.

Key words: feline immunodeficiency virus, standardization, Western blot, diagnosis

INTRODUCTION
Feline immunodeficiency virus (FIV) is a Lentivirus from the Retroviridae family that causes a chronic and progressive immunodeficiency. FIV is one of the most important feline pathogens (PEDERSEN et al., 1987) with a worldwide distribution and prevalence rate of 2.5 to 44%, and animal asymptomatic infection rate of 1 to 14% according to age, sex, geographic region, and exposure risk (HOHDATSU et al., 1998; FUCHS et al., 1994; YAMAMOTO et al., 1989).

The primary way of FIV natural transmission is the saliva through bites from infected animals. Additionally, intrauterus, perinatal, colostrum, milk, or semen transmission of seropositive animals may also occur (O’NEIL et al., 1995; HARTMANN et al., 1998). Clinical and...
laboratory diagnosis should be conjugated, since clinical signs of FIV can be mistaken for other pathologies. Serological and molecular methods, and viral isolation can be used (ALVES, 2010, HOSIE & JARRETT, 1990, EGBERINK et al., 1991, PANCINO et al., 1993, LOMBARDI et al., 1993, RIMSTAD & UELAND, 1992; DANDEKAR et al., 1992; HODATSU et al., 1992; BARLOUGH et al., 1991), nevertheless, highly sensitive tests would be very useful to detect such an infectious and contagious agent as FIV (MORTOLA, 2004, ALVES, 2010). Today’s number of domestic cats raised as pets grow each year in Brazil, making studies for the well being of these animals even more necessary. ALVES (2010) developed a highly sensitive ELISA, more accessible for FIV diagnosis in Brazil. However, Western blot procedure has been used as a confirmatory test for FIV, due to its higher sensitivity and specificity, when compared to ELISA and PCR (ALVES, 2010, HOSIE et al., 2009, MORTOLA, 2004).

The present study was aimed to the standardization and establishment of Western blot confirmatory diagnosis, using recombinant antigens from capsid protein p24, r-p24, based in the genomic sequence of FIV-B.

MATERIAL AND METHODS

Samples

This study is approved by CETEA (Ethics in Animal Experimentation Committee) of the Universidade Federal de Minas Gerais, under protocol number 101/09.

Seventy-two SNAP Combo Plus e PCR tested serum samples (positive and negative) were kindly donated by the Department of Clinical Veterinary of the Universidade de São Paulo-USP.

Western blot

To standardize the Western blot, the recombinant antigens produced based in the genomic sequence of FIV-B MAZUR et al. (2010), from capsid protein p24, r-p24 were used. Electrophoresis of 3.14 µg/cm of r-p24 (0.095 µg/animal) diluted in buffer 2X with 2-mercaptoetanol (Merck, Darmstadt, Germany), was performed in 12.5% and 5% polyacrylamide gels. After electrophoresis for 70 minutes at 110 volts in Tris-Glycine 1X buffer (Tris 25 mM, Glycine 0.25 mM, SDS 0.1% and distilled water q.s.p 1 L), gel products were transferred to a 0.45 µm HATF nitrocellulose membrane (Millipore Indústria e Comércio LTDA, SP, Brasil). Transference was done in 1x buffer (Tris 25 mM, glycine 192 mM, SDS 0.02%, methanol 20% and distilled water q.s.p 1 L), at 280 milliamperes (mA) for 120 minutes. The transferred membrane was cut in single 5 mm wide stripes and placed into incubation wells with a final volume of 250 µl for immunobloting reactions. Stripes were submerged in blocking solution (TBS 1X - Tris Buffered Saline (Tris 20 mM, NaCl 150 mM, in distilled water q.s.p 1 L, pH 7.5 with de 5% skim powder milk) with serum samples at 1: 10 and 1:100 dilution for 1 hour, at room temperature, under shaking. The reaction was washed 3 times with TBS 1X + 0.05% Tween 20. HRP Goat anti-Cat IgG Fc conjugated (Immunology Consultants Lab, Newberg, OR, USA) was added at 1:1000 for hour, at room temperature, under plataform shaking and then washed 3 times with TBS 1X + 0.05% Tween 20. Visualization reaction was developed with 250 µl DAB (Sigma Aldrich, VA, USA) according to manufacturer’s protocol and reaction was stopped with distilled water. Stripes were photographed at the end.

RESULTS

The antigen r-p24 at concentration 4 µg/cm (0.095 µg/animal) was reactive in the Western blot when used with FIV infected cat serum diluted at 1:10 e 1:100 and with the conjugate at 1:1000 (Fig. 1). All samples (100%) had the same results using Western blot.
DISCUSSION

FIV is an important cat pathogen and its prevalence varies among geographic locations (HOSIE et al., 2009). It is of great risk that FIV infected asymptomatic cats disseminate the virus therefore it is necessary highly sensitive tests to prevent that.

The identification tests for FIV infected cats stands as a mainstay for preventing transmission of the disease. The definitive diagnostical approach method for FIV would be virus isolation from blood lymphocytes, however too cumbersome and expensive for routine usage. Nevertheless, most available FIV tests do not detect antibodies to FIV in serum, plasma, or whole blood, since viral antigens are below the threshold of detection in the circulation of infected cats and depends on the stage of disease (BIENZLE et al., 2004; LEVY et al., 2001).

ALVAREZ et al. (2007) and THORN et al. (1987) showed that Western blot is a good confirmatory test for equine infectious anemia (EIAV) and HIV. Therefore, the aim of the present study was to standardize and establish a Western blot using recombinant antigen r-p24 for the diagnosis of feline immunodeficiency virus. Our samples with positive results confirmed by SNAP Combo Plus e PCR, were tested through Western blot using r-p24 (0.095 μg/animal), serum dilution of 1:10 and 1:100, and conjugate dilution of 1:1000. All samples had the results confirmed by the Western blot. Immune-enzymatic tests for FIV diagnosis with recombinant antigens are widely used (CALZOLARI et al., 2005, AVRAMÉAS et al., 1993; ROSATI et al., 2004; MAZUR et al., 2010; ALVES, 2010). LEVY et al. (2001) and ALVES (2010) recommended the use of screening tests for FIV infection such as enzyme-linked immunosorbent assays (ELISA) or other immunochromatographic tests, which are available in several formats for use in veterinary practice or diagnostic laboratories.

HOSIE & JARRET (1990) showed that serological tests can create false positive results due to low specificity. Hence, diagnosis must be associated with specific findings. FIV positive animals with infection risk should be confirmed through specific methods. Besides that false negative results are more common at the disease terminal stage, due to immunosuppression (TEIXEIRA et al., 2010).

When necessary, Western blot is the technique of choice for confirmatory for FIV diagnosis (MORTOLA et al., 2004; HOSIE et al., 2009; ALVES, 2010; SAND et al., 2010). The present Western blot confirmed 100% of the results in this study, showing high sensitivity and specificity.

CONCLUSION

The present Western blot, using recombinant antigen r-p24, demonstrated to be a highly sensitive and specific technique for the FIV diagnosis, making the confirmatory diagnosis less expensive including its wide potential usage as a common method.
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