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EVALUATION OF THE CONCORDANCE LEVEL BETWEEN SEROLOGICAL AND MOLECULAR DIAGNOSIS OF FELINE IMMUNODEFICIENCY VIRUS AND FELINE LEUKEMIA VIRUS INFECTION - 605
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Introduction
Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are pathogenic viruses with clinical importance that are transmitted by contact between cats (Dunham et al. 2008). The acute phase of infection by FIV is characterized by a peak of viremia and this virus produces a persistent infection. With the development of antiviral immunity, circulating virus decreases to low levels as the host mounts an immune response to FIV (asymptomatic phase). In terminal stages of infection, that antiviral immune response wanes and viral loads again increase (Dunham et al. 2008, Sellon & Hartmann 2006). Only 30% of infected cats by FeLV exceeds the ability of the immune response to eliminate infection and the animals develop a persistent viremia (Dunham et al. 2008). FIV and FeLV infection are typically diagnosed by detection of FeLV antigens and antibodies against FIV by an enzyme-linked immunosorbent assay (ELISA) which are considerate in-clinic test systems (Arjona et al. 2007, Hartmann et al. 2007). Immunofluorescence tests are considered the “gold standards” for FeLV diagnosis (Dunham et al. 2008). Molecular diagnostic methods like polymerase chain reaction (PCR) for identification of the FIV are becoming more popular due to their advantages over serological methods, due the introduction of the FIV vaccine (Arjona et al. 2007, Sellon & Hartmann 2006). The aim of the present study was to evaluate the concordance level between ELISA and Nested-PCR tests for FIV infection diagnosis, and between ELISA and indirect immunofluorescence (IFI) tests for FeLV infection.

Materials and Methods
Samples from 141 cats with more six months old, independent of the sex or the breed, from clinic service of the Veterinary Hospital of College of Veterinary Medicine and Zootechny, University of São Paulo, Brazil, multiple-cat homes and animal shelters were evaluated.
for FIV and FeLV infection. The study was approved by Bioethic Commission of the College of Veterinary Medicine and Zootechy, University of São Paulo. Serum samples were used for anti-FIV antibodies were searched with ELISA (SNAP Combo® FeLV/FIV, Lab. IDEXX, EUA). For the detection of FIV, total DNA was purified from whole blood with a commercial kit (DNA IlustraTM Blood GenomicPrep Mini, GE Healthcare, UK), according to manufacturer’s instructions, FIV DNA was searched by Nested-PCR (Hohdatsu et al. 1998). FeLV p27 antigenemia was searched with ELISA (SNAP Combo® FeLV/FIV, Lab. IDEXX, EUA) and by IFI (VMRD Inc. Pullmann-Albion Road, Pullman, USA, Junqueira 2005), with serum and blood smear samples, respectively. To calculate the concordance level between tests, kappa (κ) value was used with a confidence level of 95%.

Results
Among the 141 cats, 33 (23.4%) samples were positive by ELISA for FIV infection and 30 (21.3%) by Nested-PCR for FIV. All positive cats for FIV by Nested-PCR test were also ELISA positive. However, there were three (2.1%) seropositive samples that resulted negative by Nested-PCR. On the other hand, the proviral genome was not detected in any seronegative cat. For FeLV infection, 35 (24.8%) samples were positive by ELISA test and 29 (20.6%) by IFI test. All cats positive for FeLV by IFI were also positive by ELISA assay. However, there were six (4.2%) samples ELISA-positive that resulted IFI negative. Between ELISA and Nested-PCR for FIV diagnosis κ value was 0.94 and between ELISA and IFI for FeLV diagnosis, was 0.88.

Discussion
An understanding of the type of test used, and viral component measured, is critical to allow correct interpretation of a test result (Dunham et al. 2008). No test is 100% accurate at all times under all conditions; results should be interpreted along with the patient’s health and risk factors (Levy et al. 2008). Among 33 FIV-positive by ELISA test resulted in this study, only three samples were negative by Nested-PCR. This difference may be due some factories, such as the marked variability of the viral genome of the FIV, or due to latent FIV cases in which there is no viremia (Bienzle at al. 2004, Steinríg & Klein 2003) or others aspects related with molecular technique, like the sensitivity of the primers. The advantage of the molecular diagnostic is the identification of the DNA proviral of the FIV, because the limitations of antibody detection for diagnosis of this infection are the presence of the antibodies generated by previous vaccination or maternally derived antibodies, and not seroconversion in early stage of the infection (Arjora et al. 2007, Dunham et al. 2008, Sellon & Hartmann 2006). The κ values observed in this study are considerate excellent concordance (Fleiss 2003), as reported by Arjora et al. 2007 between ELISA and Nested-PCR (κ = 0.87, n = 179) for diagnosis of FIV. ELISA methods detect free soluble FeLV p27 in plasma or serum, thus positive results may be reflective of transient or persistent viremia, whereas the IFI detects cell-associated p27 antigen primarily in neutrophils and platelets in the peripheral blood. IFI assays only becomes positive after infection of the bone marrow (Hartmann 2006). False-negative IFA results may occur in leukopenic cats and positive results in IFI for FeLV infection are likely to reflect persistent viremia (Levy et al. 2008), what may be explain the discrepancy of the results between ELISA and IFI observed in present study. Testing to identify infected cats is the mainstay of preventing transmission of the viruses (Bienzle et al. 2004). The retroviral status of all cats should be known because the serious health consequences of infection influence patient management both in illness and wellness care (Levy et al. 2008). Confirming positive test results is crucial, especially in asymptomatic cats (Dunham et al. 2008, Hartmann 2006, Sellon & Hartmann 2006) by using confirmatory tests.

Conclusion
High levels concordance between assays in this study evidence the quality diagnostic these tests. Ideally, ELISA assays for detect FIV and FeLV should be preferred screening tests, and Nested-PCR and IFI tests for FIV and FeLV, respectively, as confirmatory tests.

References

Keywords: feline immunodeficiency virus, feline leukemia virus, diagnosis