

Occurrence of Feline Immunodeficiency Virus and Feline Leukemia Virus Infection in Cats

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Abstract: Problem statement: The objective of the present study was to evaluate occurrence of the Feline Immunodeficiency Virus (FIV) and the Feline Leukemia Virus (FeLV) infection in asymptomatic domestic cats in the metropolitan region of Belo Horizonte, Minas Gerais, using the Polymerase Chain Reaction (PCR) for FIV detection and SNAP Combo Plus for FeLV and FIV detection. **Approach:** Blood samples were collected from the jugular vein of 78 healthy cats, mixed breeds and ages and both sexes. Specific primers were designed for PCR to amplify a 244 bp fragment of FIV gag gene. **Results:** Five animals (6.41%) were positive by PCR and three animals (3.85%) were positive by SNAP Combo Plus for FIV and 14 animals were positive for FeLV (17.95%). **Conclusion:** These results suggest that there is a significant occurrence of asymptomatic infected animals which may serve as potential transmitters of FIV and FeLV.

Key words: Belo horizonte, Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV), Polymerase Chain Reaction (PCR), asymptomatic infected animals, potential transmitters, minas gerais, domestic cats, via vaginal, clinical history, serologic analysis

INTRODUCTION

The Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) belong to the family *Retroviridae*, they are distributed worldwide and induce persistent infections in domestic cats and other small felids (Hosie *et al.*, 1989; Pedersen *et al.*, 1987; Yamamoto *et al.*, 1989). Between 1.0-20.0% of domestic cats are infected with FIV in diverse geographic areas of the world, with an overall prevalence of infection between 1.0-15.0% in healthy cats and from 3.0-44.0% in sick cats (Bendinelli *et al.*, 1995; Courchamp and Pontier, 1994; Ueland and Lutz, 1992). FeLV infection prevalence rates vary from 1.0-38.0% (Hosie *et al.*, 1989).

FIV infection is characterized by an impairment of immune functions and a progressive depletion of CD4⁺ cells. The most common clinical signs of FeLV

infection are immune suppression, anemia and lymphoma (Hardy *et al.*, 1976). FIV and FeLV infected cats may remain asymptomatic for years; however, eventually succumb due to direct viral effects or, more commonly, to secondary infections resulting from virus-induced immunosuppression (Pedersen *et al.*, 1987; Bendinelli *et al.*, 1995; Hardy *et al.*, 1976; Johnson *et al.*, 1994; Norsworthy *et al.*, 1993; Okada *et al.*, 1994). The saliva of infected cats is the main form of transmission of FIV and FeLV. The transmucosal infection via vaginal and rectal route can also occur, as well as through milk (Hardy *et al.*, 1976; Bachmann *et al.*, 1997; Carpenter *et al.*, 1998). The rate of FIV and FeLV infection is influenced by gender (males greater than females due to roaming behavior); age (older cats are more likely to be infected) and health (sick cats are more likely to be infected, due to an impaired immune system) (Courchamp and Pontier, 1994).

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The methods currently used for detection of FIV infection in domestic cats include virus isolation, immunological tests for detection of specific antibodies and molecular tests for detection of viral genomic sequences (Barlough *et al.*, 1991; Deacon and Leah, 1989; Hohdatsu *et al.*, 1992; Hosie and Jarrett, 1990; Rimstad and Ueland, 1992). Diagnosis of FeLV infection is based on clinical history and viral p27 protein detection in plasma, serum, or saliva of allegedly positive animals. Indirect immunofluorescence (IFA) and immunoenzymatic assays are the most used diagnostic methods (Miyazawa, 2002) and PCR is currently being used (Herring *et al.*, 2001).

The objective of the present study was to evaluate the occurrence of the feline immunodeficiency virus and the feline leukemia virus infection in asymptomatic cats in the metropolitan region of Belo Horizonte, Minas Gerais using Polymerase Chain Reaction (PCR) for FIV detection along with SNAP Combo Plus (IDEXX®, Westbrook, ME, USA) for FeLV and FIV detection.

MATERIAL AND METHODS

Animals: Blood samples were collected from the jugular vein of 78 healthy cats from shelters and private owners, mixed breeds and ages and both sexes. Samples were collected using two sterile disposable vacuum collection tubes, one for serologic analysis and the other one containing anticoagulant (EDTA) for DNA extraction. Animals were clinically examined and clinical signs were documented. All procedures and protocols used in this study were approved by the Universidade Federal de Minas Gerais Animal Experimentation Ethics Committee (101/09). A sample from a positive cat tested by SNAP Combo Plus and by PCR was used as a positive control. PCR reaction with no template was used as a negative control.

DNA extraction: DNA was isolated from whole blood using the NucleoSpin® Blood Columns kit (Macherey-nagel, Neumann-Neander, Düren, Germany), following the manufacturers protocol. DNA samples were quantified in the NanoDrop (Thermo Fisher Scientific, Wilmington, Delaware, USA) and stored at 20°C for further analysis

Polymerase Chain Reaction (PCR) for FIV gag gene: Specific primers were designed for gel-based PCR using the sequences described by Hohdatsu *et al.* (1992) 5'-CTAGGAGGTGAGGAAGTTCA-3' (forward) and 5'-CTGCTTGTTGTTCTTGAGTT-3' (reverse) to amplify a FIV *gag* gene fragment of 244

bp. DNA was submitted to PCR in accordance with Hohdatsu *et al.* (1992) with some modifications as follows: an initial incubation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, extension at 72°C for 2 min and a 5 min final extension at 72°C. A 25 µL reaction consisted of 10X PCR-buffer (Invitrogen Life Technologies, Carlsbad, CA, USA), 3 mM of MgCl₂ (Invitrogen Life Technologies, Carlsbad, CA, USA), 1 mM each dNTP, 1 pmol of each primer and 1 unit of Taq-DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA) and 2µL of DNA (approximately 150 ng). PCR products were analyzed in a 8 % polyacrylamide gel stained by silver nitrate.

FIV and FeLV antibody test: Animal's serum samples were used to detect FIV and FeLV antibody, using SNAP Combo Plus kit (IDEXX®, Westbrook, ME, USA), following the manufacturers protocol with no modification.

RESULTS

Contingency tables demonstrate the distribution of PCR and SNAP Combo Plus results by gender (Table 1-3). No statistical evidence by the Chi Square test could differentiate infection percentage in males from females (p>0.05), for neither FIV nor FeLV.

Table 1: PCR results-occurrence of feline immunodeficiency virus infection in asymptomatic domestic cats in Belo Horizonte, MG

	Female (%)	Male (%)	Total (%)
Positive	(2.56) ^a	3 (3.85) ^a	5 (6.41)
Negative	35 (44.87) ^b	38 (48.72) ^b	73 (93.59)
Total	37 (47.43)	41 (52.57)	78 (100.0)

^{a, b}: Values with different lowercase letters in the same row differ statistically by Chi Square test (p<0.05)

Table 2: SNAP Combo Plus results-occurrence of feline immunodeficiency virus infection in asymptomatic domestic cats in Belo Horizonte, MG/Brazil

	Female (%)	Male (%)	Total (%)
Positive	0(0) ^a	3 (3.85) ^a	3 (3.85)
Negative	37 (47.43) ^b	38 (48.72) ^b	75 (93.15)
Total	37 (47.43)	41(52.57)	78 (100.0)

^{a, b}: Values with different lowercase letters in the same row differ statistically by Chi Square test (p<0.05)

Table 3: Occurrence of feline leukemia virus infection in asymptomatic domestic cats in Belo Horizonte, MG

	Female (%)	Male (%)	Total (%)
Positive	7 (8.97) ^a	7 (8.97) ^a	14(17.95)
Negative	30 (38.46) ^b	34 (43.59) ^b	64 (82.05)
Total	37 (47.44)	41 (52.56)	78 (100.0)

^{a, b}: Values with different lowercase letters in the same row differ statistically by Chi Square test (p<0.05)

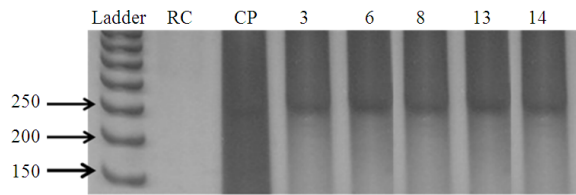


Fig. 1: 8% polyacrylamide eletrophoresis gel, stained by silver nitrate. Ladder-50 bp ladder; (RC) PCR reaction control; (PC) positive control; samples - 3, 6, 8, 13 and 14 are FIV positive (6.76%)

All infected animals were more than 2 years old and were asymptomatic at the time of the sample collections. PCR results revealed FIV *gag* gene detection in four cats (5.13%) (Fig. 1). In addition, SNAP Combo Plus results showed antibody detection against FIV in three (3.8%) cats, while for FeLV in eleven (14.10%). No animal was positive for both FIV and FeLV simultaneously considering results of FIV-PCR and FeLV SNAP, however, only one animal was positive for FIV and FeLV in the SNAP.

DISCUSSION

In the present study occurrences of 6.41% (PCR) and 3.85 % (SNAP) for FIV and 17.95% (SNAP) for FeLV infection were found in Belo Horizonte's metropolitan area. These results were different from the results found for FIV in Sao Paulo (14.7%) (Lara *et al.*, 2008), Rio Grande do Sul (37.5%) (Caldas *et al.*, 2000), Rio de Janeiro (20.2%) (Souza *et al.*, 2002) and Minas Gerais (2.7%) (Caxito *et al.*, 2006). Results found for FeLV were similar to those found in different Brazilian states. Reche *et al.* (1997) and Souza *et al.* (2002) found 8 and 17% of positive cats in Sao Paulo and Rio de Janeiro, respectively (Souza *et al.*, 2002; Reche *et al.*, 1997). The variability of serological and molecular diagnosis of feline immunodeficiency virus infection have been reported lately in others researches, in agreement with our study (Bienzle *et al.*, 2004; Mortola *et al.*, 2004). Seronegative samples with PCR positive results may be also found in cats living in close contact with FIV infected seropositive animals, becoming infected without developing antibodies or disease (Dandekar *et al.*, 1992). However, such cats will usually seroconvert weeks to months later (Hosie *et al.*, 2009).

FIV infection rate of 6.41% found in the present study in asymptomatic animals differ from the rate of 1.47% found by Caxito *et al.* (2006), in healthy animals in the same state, Minas Gerais. Authors also found an infection rate of 13.95% in sick cats, which confirms

other data that demonstrate a higher susceptibility of sick animals to FIV infection (Hosie *et al.*, 1989; Ishida *et al.*, 1989). Minas Gerais and Sao Paulo states revealed that FIV infection was more prevalent in male (4.38% and 20.3%, respectively) than in female cats (0.90 and 9.7%, respectively) (Caxito *et al.*, 2006). However, in present results FIV and FeLV infection rate in male and female could not be differentiated, in agreement with similar results described in Rio de Janeiro (Souza *et al.*, 2002; Teixeira *et al.*, 2007).

CONCLUSION

Additionally, FIV and FeLV infection in housed cats was more elevated than in sheltered cats and these results are in accordance with the results found by Caxito *et al.* (2006) and Lara *et al.* (2008) in which housed cats (3.05% and 23.1%, respectively) had a higher FIV infection percentage comparing to street cats (1.11%, 5.9%, respectively). However, high FIV and FeLV infection rates are associated to cats with free street access, which frequently show more aggressive behavior (Hosie *et al.*, 1989; Hosie and Jarrett, 1990; Ishida *et al.*, 1989; Bandecchi *et al.*, 1992). According to animals age, 3.85 and 14.10% of FIV and FeLV infected cats were more than two years old, which are in the age group with the higher infection incidence. Hosie *et al.* (1989), showed that infection frequently occurs after the first year of age and its prevalence is higher in elderly cats. Serological studies showed the presence of anti-FIV antibodies in Sao Paulo (20.5%) (Reche *et al.*, 1997) and Rio de Janeiro (16.7 and 21.0%) state (Souza *et al.*, 2002; Reche *et al.*, 1997; Mendes-de-Almeida *et al.*, 2004). The higher number of anti-FIV cats in Sao Paulo and Rio de Janeiro comparing to Belo Horizonte, it's probably because serological methods are less specific and can generate false positive results (Hosie and Jarrett, 1990). However, obtaining a reliable diagnosis is important because FIV is a transmissible and infectious disease. Serological tests have limitations, so confirmation of positive ELISA results by western blot should be recommended (Hosie *et al.*, 2009).

Epidemiological studies of FIV and FeLV infection are essential for prevention of virus dissemination in domestic cats. The diagnosis is an important tool to determine the environment of FIV and FeLV circulation and positive cats should be segregate from uninfected ones. The early diagnosis of FIV and FeLV infection is also important for its zoonotic aspect, since immunosuppressed animals are likely to eliminate larger quantities of pathogens (O'Neil *et al.*, 1991), such as *Toxoplasma gondii* (Witt *et al.*, 1989), or *Cryptococcus neoformans* (Malik *et al.*, 1992). In

conclusion, FIV and FeLV accurate diagnosis at an early stage for the identification of subclinically infected cats, that contribute to the diffusion of disease, is required to quarantine any infected animals and to prevent the spread of the virus.

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