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SHORT COMMUNICATION
Cutaneous mycoflora and CD4:CD8 ratio of cats infected with feline immunodeficiency virus

Archivaldo Reche, Jr., DVM, MSc, PhD[1]*, **Alexandre GT Daniel** DVM[1], **Telma CP Lazaro Strauss** DVM[1], **Carlos P Taborda** Biomed Scientist, MSc, PhD[2], **Shirlei A Vieira Marques** Sci Lab Tech[2], **Kátia Haipek** DVM, MSc[1], **Lilian J Oliveira** DVM, MSc[1], **Janaína M Monteiro** DVM, MSc[1], **José R Kfoury, Jr.,** DVM, MSc, PhD[1]

[1]*Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil*
[2]*Instituto de Ciências Biomédicas, Departamento de Microbiologia, Universidade de São Paulo, São Paulo, SP, Brazil*

This study was designed to compare cutaneous mycoflora isolation and CD4+:CD8+ ratio in feline immunodeficiency virus (FIV)-infected cats with that in FIV-uninfected cats. Sixty cats were examined. Twenty-five were FIV-infected cats and 35 were FIV-uninfected cats. All 60 cats were FeLV-negative. Fungi were speciated and immunophenotyping of peripheral CD4+ and CD8+ T lymphocytes was performed. At least one fungal colony was isolated from 22/25 (88%) FIV-infected cats. Among the FIV-uninfected cats fungal colonies were recovered from 13/35 (37%) specimens. Dermatophytes were recovered from 2/25 (8%) FIV-infected cats (one *Microsporum gypseum*, one *Microsporum canis*) and 3/35 (8.5%) FIV-uninfected cats (*M gypseum*). *Malassezia* species was the most commonly isolated organism from both groups of cats (51.6%). *Malassezia* species was more commonly isolated from FIV-infected cats than FIV-uninfected cats (84% vs 28.6%). The CD4+ to CD8+ lymphocyte ratio for FIV-infected cats was significantly lower than the CD4+ to CD8+ ratio in the FIV-uninfected cats. The CD4+ to CD8+ lymphocyte ratio for FIV-infected cats with cutaneous overall fungal isolation was significantly lower than the CD4:CD8 lymphocyte ratio in the FIV-infected cats but without cutaneous fungal isolation. We can conclude that immunologic depletion due to retroviral infection might represent a risk factor to cutaneous fungal colonization in cats.

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Feline immunodeficiency virus (FIV) is a lentivirus that infects domestic and feral cat populations worldwide. Infected cats exhibit similar disease patterns as human immunodeficiency virus (HIV) infected patients, thus the cat represents an excellent comparative model for studying mainly the immunopathogenesis of HIV infection. Both FIV and HIV are associated with progressive immune suppression eventually leading to the development of AIDS. FIV and HIV are characterized by a gradual decline in CD4+ T lymphocytes and increased susceptibility to opportunistic pathogens.[1–3]

Superficial mycotic infections are common in patients infected with HIV. In communities where HIV infections are frequent, some of these dermatological presentations serve as markers of the stage of HIV infection[4] Preliminary studies have demonstrated that cats infected with FIV harbor a great diversity of fungi on skin and mucosae than non-infected cats.[5,6]

The purpose of the present study was to compare cutaneous mycoflora isolation and CD4+ to CD8+ lymphocytes ratio in FIV-infected cats with that in FIV-uninfected cats.

Sixty client-owned cats were examined. The owner's written consent was obtained for inclusion of the cat in the study. Twenty-nine cats were females and 31 were males. Cats ranged in age from 6 months to 14 years, with mean age of 4.8 years. All cats were domestic shorthairs. None of the 60 cats had clinical dermatologic problems at the moment of specimen collection.

*Corresponding author. Departamento de Clínica Médica, Faculdade de Medicina Veterinária e Zootecnia, Av Prof Dr Orlando Marques de Paiva, n87, CEP: 05508-270 São Paulo, Brazil. E-mail: valdorec@usp.br

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All the 60 cats were tested for retroviruses with a commercially available enzyme-linked immunosorbent assay (ELISA) test kit (Snap FIV antibody/FeLV antigens combo, Idexx Laboratories, Westbrook, ME, USA), using serum or plasma, according to the manufacturer's instructions. Based on serological testing results, cats were grouped in FIV-infected and FIV-uninfected. They were matched as closely as possible for age, sex, breed and lifestyle. All the 60 cats were from single cat households.

Cutaneous specimens were obtained from the cat coat by rubbing the entire coat with a sterile piece of carpet.[7]

Culture was performed using Sabouraud's dextrose agar with 0.05% chloramphenicol (Chloramphenicol, Sigma Chemical Co, St Louis, MO, USA) and 0.4% cycloheximide (Chloramphenicol, Sigma Chemical Co, St Louis, MO, USA). Plates were incubated for 2 days at 27°C and observed daily. Pathogens were identified by cultural characteristics and microscopy[8,9]

An absolute white count was determined by the automated analyzer (ABC Vet, ABXT'S, France) and an absolute lymphocyte count, determined by staining blood films with Rosenfeld stain.[10] For the flow cytometry analysis, 200 µl of whole blood were incubated with FACS lysing solution (Becton Dickinson) for 15 min. Cells were washed once with FACS buffer (phosphate buffered saline (PBS) with 2% fetal bovine serum (FBS) and 0.1% sodium azide) and centrifuged ($500 \times g$) at 4°C for 5 min. The supernatant was removed, and the pellet was incubated with fluorescein isothiocyanate (FITC)-conjugated anti-feline CD4 (Serotec) or R-phycoerythrin (RPE)-conjugated anti-feline CD8 (Serotec, UK − www.serotec.co.uk) at room temperature for 20 min in a dark room. After the cells were washed three times, the number of stained cells was determined by counting 30,000 cells on fluorescence-activated cell sorter (FACS, Becton Dickinson). Lymphocytes were gated based on their specific forward and side-scatter characteristics.[11]

Data were analyzed using commercially available software (Flow Jo, Flow Cytometer Analysis Software, Tree Star, OR, USA).

The frequency with which a fungal specimen was isolated from each group (FIV-infected or -uninfected cats) was compared by using a Fisher's exact test. One-way ANOVA was used to compare CD4+ to CD8+ lymphocytes ratio and fungal status in both groups and Tukey−Kramer as multiple comparisons post test. Results are presented as means ± standard deviation (SD) where appropriate. A P value < 0.05 was considered significant.

In the FIV-infected group, 16 (64%) cats were males and nine (36%) females. Cats from this group ranged in age from 3 years to 9 years, with mean age of 5.4 years. Among the FIV-uninfected cats, 15 (43%) were males and 20 (57%) were females. Cats from this group ranged from 6 months to 14 years, with mean age of 5.1 years (Table 1). All cats were domestic shorthairs, neutered and indoor.

Coat fungi were isolated from 35/60 cats (58%). In one FIV-infected cat were isolated *Microsporum canis* and *Malassezia* species, for the remaining 34 cats a single fungal colony was isolated.

Fungal colonies were isolated from 22/25 (88%) FIV-infected cats. Among the FIV-uninfected cats, fungal colonies were recovered from 13/35 (37%) specimens. Dermatophytes were recovered from 2/25 (8%) of FIV-infected cats; these were speciated as *M canis* and *Microsporum gypseum*. Among the FIV-uninfected cats, *M gypseum* was isolated from 3/35 (8.5%) cats.

Malassezia species was the most commonly isolated organism from both groups of cats (51.6%). When comparing groups, *Malassezia* species was more commonly isolated from FIV-infected cats, compared with FIV-uninfected cats (84% vs 28.6%) ($P < 0.05$) (Table 1).

The CD4+ to CD8+ lymphocyte ratio for FIV-infected cats was significantly lower than the CD4+ to CD8+ ratio in the FIV-uninfected cats (0.328 ± 0.222 and 1.25 ± 0.507, respectively; $P < 0.01$). The CD4+ to CD8+ ratio for FIV-infected cats with cutaneous overall fungal isolation was significantly lower than the CD4+ to CD8+ ratio in the FIV-infected cats but without cutaneous fungal isolation (0.187 ± 0.044 and 0.683 ± 0.085, respectively; $P < 0.01$). The CD4+ to CD8+ ratio for FIV-infected cats with *Malassezia* species was also lower than CD4+ to CD8+ ratio in the FIV-infected cats but without *Malassezia* species (0.183 ± 0.047 and 0.617 ± 0.11, respectively; $P < 0.01$).

To the authors' knowledge, this is the first study to simultaneously examine cutaneous mycoflora and

Table 1. Total number, sex, age, diversity of cutaneous fungal genera isolated and CD4/CD8 ratio of 60 client-owned cats.

	Total number	Age (years)	*M canis*	*M gypseum*	*Malassezia* species	CD4/CD8 (Mean ± SD)
FIV-infected	25 (16 Mc and 9 Fs)	3−9 (mean 5.4)	01 (4%)	01 (4%)	21 (84%)	0.328 ± 0.222
FIV-uninfected	35 (15 Mc and 20 Fs)	6 months−14 (mean 5.1)	0	3 (8.5%)	10 (28.6%)	1.25 ± 0.507

Mc = male castrated, Fs = female spayed.

CD4+ to CD8+ lymphocyte ratio in cats naturally infected with FIV. In previous studies only the coat and mucosae mycoflora of FIV-infected cats were evaluated.[5,6]

In the present study, yeast of the genus *Malassezia* species was isolated from coat specimens of 51.6% of all cats. The highly sensitive method for malassezia culture used in this study might have contributed to *Malassezia* species isolation.[9] *Malassezia* species consists of lipophilic yeasts, which are components of the cutaneous microflora of many warm-blood animals including humans, dogs and cats.[12] Many of the *Malassezia* species have been associated with various systemic and cutaneous diseases in humans. Experimental infections with *Malassezia* species suggest that certain patients, such as those with seborrheic dermatitis, have a predisposition to this condition.[13] Therefore, other host factors, in addition to obvious immunosuppression, may be related to the pathogenesis of this condition.[14–16] *Malassezia* species yeast infection also represents a clinical concern in dogs and cats dermatology.[17] In cats *Malassezia* species overgrowth has been described as a marker of serious, underlying diseases, such as retrovirus infection and neoplasia.[18] Recently, *Malassezia* species overgrowth was described in cats with allergic dermatitis and may represent a secondary cutaneous problem in allergic cats.[19]

In the present study, the CD4+ to CD8+ lymphocyte ratio for FIV-infected cats was significantly lower than the CD4+ to CD8+ ratio in the FIV-uninfected cats, as previously related.[3,20–23] In addition, the CD4+ to CD8+ ratio for FIV-infected cats with cutaneous *Malassezia* species isolation was significantly lower than the CD4+ to CD8+ ratio in the FIV-infected cats but without cutaneous fungal isolation. Considering that the response of the host to the yeast includes non-specific defense mechanisms as well as cell-mediated defense mechanisms, the immunity disruption induced in the FIV-infected cats may underlie yeast colonization. This hypothesis is supported by an observed lower CD4:CD8 in FIV-infected cats with positive fungal culture.

Malassezia species dermatitis is rarely diagnosed in cats and is frequently associated to other dermatologic problems such as atopic dermatitis and food reaction.[17,19] In this study, none of the 31 cats with *Malassezia* species colonization had any skin lesions which lead us to suggest that *Malassezia* species solely does not cause dermatitis even in FIV-infected cats. However, once any other underling skin problem is present, *Malassezia* species may overgrow and dermatitis can occur. Due to the fact that *Malassezia* species colonization was a common finding in the FIV-infected cats, it shall be considered that FIV-infected cats may have higher risk to develop *Malassezia* species dermatitis than FIV-uninfected cats.

Dermatophytes did not represent a common cutaneous pathogen in the present study. *M canis* and *M gypseum* were recovered from a small percentage of cats, two were FIV-infected cats and three were FIV-uninfected cats. The FIV-infected cat from which *M canis* and *Malassezia* species was recovered had the lowest CD4+ to CD8+ lymphocyte ratio (0.106). The reason for a low detection of dermatophytes in the present report is not clear. Lack of sensitivity is unlikely, as this study used established methodologies.[8] Considering that only domestic shorthair cats were used, it is possible this population represents cats with low frequency of dermatophyte colonization compared to long-haired cats. Further, single cat households have a lower risk of dermatophyte transmission.[24]

Finally, cats in this study were exclusively indoor cats, representing a population with decreased potential risk of cutaneous fungal colonization and transmission.[24]

We can conclude that immunologic dysfunction due to FIV infection might represent a risk factor to cutaneous *Malassezia* species colonization in cats.

References

1. Lang W, Perkins H, Anderson RE, Royce R, Jewell N, Winkelstein Jr W,. Patterns of T lymphocyte changes with human immunodeficiency virus infection: from seroconversion to the development of AIDS. *J Acquir Immune Defic Syndr* 1989; **2**: 63–9.
2. McChesney MB, Fujinami RS, Lerche NW, Marx PA, Oldstone MB. Virus-induced immunosuppression: infection of peripheral blood mononuclear cells and suppression of immunoglobulin synthesis during natural measles virus infection of rhesus monkeys. *J Infect Dis* 1989; **159**: 757–60.
3. Barlough JE, Ackley CD, George JW, et al. Acquired immune dysfunction in cats with experimentally induced feline immunodeficiency virus infection: comparison of short-term and long-term infections. *J Acquir Immune Defic Syndr* 1991; **4**: 219–27.
4. Aly R, Berger T. Common superficial fungal infections in patients with AIDS. *Clin Infect Dis* 1996; **22**: 128–32.
5. Mancianti F, Giannelli C, Bendinelli M, Poli A. Mycological findings in feline immunodeficiency virus-infected cats. *J Med Vet Mycol* 1992; **30**: 257–9.
6. Sierra P, Guillot J, Jacob H, Bussiéras S, Chermette R. Fungal flora on cutaneous and mucosal surfaces of cats infected with feline immunodeficiency virus or feline leukemia virus. *Am J Vet Res* 2000; **61**: 158–61.
7. Mariat F, Adam-Campos C. La technique Du carré de tapis, mÈthode simple de prÈlÊvement dans les mycoses superficielles. *Ann Inst Pasteur* 1967; **113**: 666–8.
8. Gambale W, Larsson CE, Moritami MM, Correa B, Paula CR. Dermatophytes and other fungi of the haircoat of cats without dermatophytosis in the city of São Paulo, Brazil. *Feline Pract* 1993; **21**: 29–33.
9. Guillot J, Breugnot C, de Barros M, Chermette R. Usefulness of modified Dixon's medium for quantitative culture of *Malassezia* species from canine skin. *J Vet Diagn Invest* 1998; **10**: 384–6.
10. Birgel EH. Hematologia Clínica Veterinária. Conferencia anual da sicuedade Oaulista de Medicine. São Paulo, Anais: Sociedade Paulista de Medicina Veterinária, 1982: 50.
11. Byrne KM, Kim HW, Chew BP, Reinhart GA, Hayek MG. A standardized gating technique for the generation of flow cytometry data for normal canine and normal feline

blood lymphocytes. *Vet Immunol Immunopathol* 2000; **73**: 167–82.
12. Midgley G. The diversity of Pityrosporum (Malassezia) yeasts in vivo and in vitro. *Mycopathologia* 1989; **106**: 143–53.
13. Goodfield MJ, Saihan EM, Crowley J. Experimental folliculitis with *Pityrosporum orbiculare*: the influence of host response. *Acta Derm Venereol* 1987; **67**: 445–7.
14. Schechtman RC, Midgley G, Hay RJ. HIV disease and Malassezia yeasts: a quantitative study of patients presenting with seborrhoeic dermatitis. *Br J Dermatol* 1995; **133**: 694–8.
15. Wagner DK, Sohnle PG. Cutaneous defenses against dermatophytes and yeasts. *Clin Microbiol Rev* 1995; **8**: 317–35.
16. Pechère M, Saurat JH. Malassezia yeast density in HIV-positive individuals. *Br J Dermatol* 1997; **136**: 138–9.
17. Nardoni S, Corazza M, Mancianti F. Diagnostic and clinical features of animal malasseziosis. *Parassitologia* 2008; **50**: 81–3.
18. Perrins N, Gaudiano F, Bond R. Carriage of *Malassezia* spp yeasts in cats with diabetes mellitus, hyperthyroidism and neoplasia. *Med Mycol* 2007; **45**: 541–6.
19. Ordeix L, Galeotti F, Scarampella F, et al. *Malassezia* spp. overgrowth in allergic cats. *Vet Dermatol* 2007; **18**: 316–23.
20. Novotney C, English RV, Housman J, Davidson MG, Nasisse MP, Jeng CR. Lymphocyte population changes in cats naturally infected with feline immunodeficiency virus. *AIDS* 1990; **4**: 1213–8.
21. Walker C, Canfield PJ, Love DN. Analysis of leucocytes and lymphocyte subsets for different clinical stages of naturally acquired feline immunodeficiency virus infection. *Vet Immunol Immunopathol* 1994; **44**: 1–12.
22. Bendinelli M, Pistello M, Lombardi S, et al. Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen. *Clin Microbiol Rev* 1995; **8**: 87–112.
23. Hofmann-Lehmann R, Holznagel E, Ossent P, Lutz H. Parameters of disease progression in long-term experimental feline retrovirus (feline immunodeficiency virus and feline leukemia virus) infections: hematology, clinical chemistry, and lymphocyte subsets. *Clin Diagn Lab Immunol* 1997; **4**: 33–42.
24. Scott DW, Miller WH, Griffin CE. Muller & Kirk's small animal dermatology. 6th edn. Philadelphia: WB Saunders, 2000.