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SURVEY OF FELINE LEUKEMIA VIRUS AND FELINE CORONAVIRUSES IN CAPTIVE NEOTROPICAL WILD FELIDS FROM SOUTHERN BRAZIL

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Abstract: A total of 57 captive neotropical felids (one *Leopardus geoffroyi*, 14 *Leopardus pardalis*, 17 *Leopardus wiedii*, 22 *Leopardus tigrinus*, and three *Puma yagouaroundi*) from the Itaipu Binacional Wildlife Research Center (Refúgio Bela Vista, Southern Brazil) were anesthetized for blood collection. Feces samples were available for 44 animals, including one *L. geoffroyi*, eight *L. pardalis*, 14 *L. wiedii*, 20 *L. tigrinus*, and one *P. yagouaroundi*. Total DNA and RNA were extracted from blood and feces, respectively, using commercial kits. Blood DNA samples were evaluated by polymerase chain reaction (PCR) for feline leukemia virus (FeLV) proviral DNA, whereas reverse transcriptase–PCR was run on fecal samples for detection of coronavirus RNA. None of the samples were positive for coronaviruses. A male *L. pardalis* and a female *L. tigrinus* were positive for FeLV proviral DNA, and identities of PCR products were confirmed by sequencing. This is the first evidence of FeLV proviral DNA in these species in Southern Brazil.

Key words: Feline coronavirus, feline leukemia virus, *Leopardus pardalis*, *Leopardus tigrinus*, neotropical cats.

BRIEF COMMUNICATION

Although Brazil hosts eight out of the 10 neotropical felid species, these cats are among the least studied in the world, and their health status is still poorly understood. Aspects related to disease occurrence in captive populations may provide valuable new insights for the development of conservation strategies for these species.

Two retroviruses, feline leukemia virus (FeLV) and feline immunodeficiency virus, are recog-

nized as causative agents of a variety of proliferative and degenerative diseases in the domestic cat. Although feline lentiviruses have been found in many species of wild felids,¹⁷ the reports of FeLV infection are less frequent. FeLV RNA, FeLV proviral DNA, and/or FeLV p27 antigen have been described in specimens of six captive neotropical felid species and in one free-ranging *Leopardus pardalis* specimen.^{4,13} In addition, serosurveys of neotropical felids indicate antibodies to FeLV in all neotropical species maintained in captivity, except *Panthera onca*, and antibodies to FeLV in two free-ranging *Panthera concolor*.⁴ Since serious clinical disease leading to death has been described in some wild felids,¹ FeLV infection is recognized as an emerging disease that may contribute to declines in these populations.

Feline coronavirus (FCoV) infections have also been described in wild felid species and are composed of two biotypes: the feline enteric coronavirus (FeCoV) and the feline infectious peritonitis virus (FIPV). Since FIPV originates from FeCoV by mutation within a persistently infected animal, FeCoV infection may be a risk factor for the development of infectious peritonitis. Outbreaks of FIPV infection have been described in nondomestic cats,^{6,9} and serologic evidence of FIPV infection was reported in

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several captive and free-ranging Brazilian felids.^{4,13}

Accordingly, this study evaluated blood and feces samples by polymerase chain reaction (PCR) for FeLV proviral DNA and reverse transcriptase-PCR (RT-PCR) for coronavirus RNA, respectively. A total of 57 captive felids, including one *Leopardus geoffroyi*, 14 *L. pardalis*, 17 *Leopardus wiedii*, 22 *Leopardus tigrinus*, and three *Puma yagouaroundi* from the Itaipu Binacional Wildlife Research Center (Refúgio Bela Vista, Southern Brazil) were sampled on three consecutive mornings in November 2006.

Itaipu Binacional Wildlife Research Center was chosen for this study for three main reasons. First, this center is a federal institution located near an environmentally protected area that includes three national parks from Argentina, Paraguay, and Brazil, and the wild-caught felids of this study are mainly from that region. Second, these felids have never been tested for FeLV or coronavirus infection. Third, these animals currently participate in a Brazilian reproduction program of wild felids, and continuous mating contact may enhance disease transmission among these specimens.

Thirty-four out of the 57 sampled felids (59.64%) were wild caught (one *L. geoffroyi*, seven *L. pardalis*, eight *L. wiedii*, 16 *L. tigrinus*, and two *P. yagouaroundi*), whereas 23 (40.36%) were captive-born in a zoo (seven *L. pardalis*, nine *L. wiedii*, six *L. tigrinus*, and one *P. yagouaroundi*). Animals were captured with nets, anesthetized, and collection procedures were performed according to the Brazilian Institute of Environment and Renewable Natural Resources Guidelines and Ethical Principles in Animal Research of the School of Veterinary Medicine of University of São Paulo. Blood samples were collected by venipuncture, placed into ethylenediaminetetraacetic acid tubes, and sent to the laboratory for a complete blood count (CBC), performed by manual method on a Neubauer's chamber,¹² and for PCR analysis. During blood sampling, all animals were weighed and evaluated for the presence of any serious clinical signs. Additionally, fresh fecal samples were collected from the ground of each of the 44 pens (26 animals were maintained as couples), resulting in one sample from *L. geoffroyi*, eight from *L. pardalis*, 14 from *L. wiedii*, 20 from *L. tigrinus*, and one from *P. yagouaroundi*.

All blood and feces samples were stored at -20°C and processed within a week. Commercial available kits were used to extract DNA from

blood samples (Genomic Prep Blood DNA Isolation Kit, GE Healthcare, Bucks 27-5236-01, United Kingdom) and RNA from feces samples (QIAamp Viral RNA Kit, Qiagen, Valencia, California 91355, USA), according to the manufacturer's protocols and to a method validated elsewhere,¹⁴ respectively.

To monitor for cross-contamination, ultrapure water was simultaneously extracted to feces and blood. A positive control (bovine coronavirus Kakegawa strain grown in HmLU-1 [hamster lung] cell line) was also simultaneously extracted to the feces to ensure correct extraction. Proper blood DNA extraction was ensured by PCR of housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using a primer set previously described.¹⁵ A blood DNA sample from a specific-pathogen-free domestic cat provided by Professor Regina Hofmann-Lehmann (University of Zurich, Switzerland) was used as the PCR positive control for the GAPDH reaction. The predicted 598-base pair (bp) products of GAPDH gene were then separated by electrophoresis in agarose gel containing ethidium bromide and photographed under ultraviolet light using an imaging system (Vilber Lourmat, Marne la Vallée, France).

A nested PCR was used to amplify a 601-bp fragment of the proviral LTR and *gag* genes regions of FeLV.¹¹ The outer and inner primer pairs of this reaction were based on the nucleotide sequences of the *gag* gene and the U3 region of long terminal repeat (LTR) of FeLV subgroup A. The LTR region is used to distinguish between exogenous and endogenous FeLV, avoiding false positives for exogenous infection.¹¹ As FeLV is presumed to be the same in domestic and nondomestic cats⁵ and because *gag* and LTR regions are conserved among different isolates of this virus, false negatives due to sequence variability at the primer sites are unlikely to occur.

A total of 200 ng of DNA sample was added in the first reaction, whereas in the second reaction, 1 μl of the first reaction product was used as template. Total DNA of a positive FeLV domestic cat sample, confirmed by immunofluorescence and proviral DNA sequencing, was provided by Professor Mitika Hagiwara from the University of São Paulo (Brazil) and was used as the positive control for PCR. The amplified products were separated by electrophoresis and photographed as described above. FeLV proviral DNA PCR products were then purified using a commercial kit (Wizard SV gel and PCR purification, Promega, Fitchburg, Wisconsin

Table 1. Partial results of the hemograms from feline leukemia virus–positive *Leopardus pardalis* and *Leopardus tigrinus* specimens.

Parameters	<i>Leopardus tigrinus</i>	<i>Leopardus pardalis</i>
Hematocrit (%) (reference range) ¹²	45 (37–44)	46 (33.4–44.8)
Erythrocytes ($\times 10^6/\text{mm}^3$) (reference range) ¹²	11.07 (5.48–7.96)	8.19 (4.90–6.22)
Hemoglobin (g/dl) (reference range) ¹²	16.26 (12.89–16.45)	16.6 (11.6–14.8)
Total plasma protein (g/dl) (reference range) ¹²	7.8 (5.8–7.6)	8.2 (7.0–8.2)

53711, USA) and bi-directionally sequenced at the Laboratory of DNA Repair (University of São Paulo, Brazil) to verify the identity of the amplified products.

For coronavirus detection in fecal samples, total RNA was submitted to a pan-coronavirus RT-PCR³ using M-MLV reverse transcriptase for cDNA synthesis and *Taq* DNA polymerase for amplification of a 179-bp fragment of ORF1b of the *Coronavirus* genus, which, along with ORF1a, encodes the replicase complex of these viruses. This region presents highly conserved structure and function and is suitable to design primers allowing a broad-spectrum detection of genetically distant coronaviruses from groups 1, 2, or 3.³ False negatives or positives due to sequence variability are unlikely to occur. Thus, this reaction was used herein to identify feline coronavirus and also any uncharacterized variant in wild felids, following sequencing of the PCR products in the case of positive samples. Nucleic acid from the bovine coronavirus Kakegawa strain was used as a positive control for this reaction, and amplified products were also separated by electrophoresis and photographed as described above.

All animals showed weights that fell within the reference range,¹⁶ and serious clinical signs of disease were observed in only two animals: a *P. yagouaroundi* with a periorbital fistula due to a dental problem (Morales *et al.*, unpubl. data) and a *L. pardalis* with nonprogressive ataxia of the forelimbs.

Feces extraction positive and negative controls were positive and negative by coronavirus RT-PCR, respectively. All fecal samples from the wild felids were negative for coronaviruses by RT-PCR. Negative control of the blood extraction was negative in both GAPDH and FeLV reactions. A band of the predicted size for GAPDH gene was amplified from all blood samples. DNA for FeLV was identified in only two animals: an adult male *L. pardalis* and an adult female *L. tigrinus*; both were born in the zoo. PCR products showed over 99% nucleotide sequence identity

when aligned to the corresponding fragment of the FeLV complete genome of domestic cat (GenBank accession No. NC001940). Positive control was also sequenced to ensure quality control and showed a different nucleotide sequence from the tested wild felids.

CBCs were performed to evaluate the hematologic status of felids infected by FeLV or coronaviruses. Although hematologic abnormalities were observed in other animals, their description is not the aim of this study and, in general, they were not related to any serious clinical signs observed during sampling. Also, as a result of the variability and the lack of scientific support for reference intervals for neotropical felids, CBCs from these animals should be interpreted with caution. Although some reference intervals have been previously described¹² and used herein, felid species, chemical and physical restraint, and geographic localization could have interfered in the hematologic results of this study.

The hemogram from two FeLV-positive animals showed findings consistent with relative dehydration (Table 1). A mild lymphocytosis ($3,474/\text{mm}^3$ [reference range: $907\text{--}2,679/\text{mm}^3$]¹²) of unknown clinical significance was also noted in *L. tigrinus*. However, since dehydration and lymphocytosis were also observed in other 10 and 12 animals from this study, respectively, these data are unlikely to be associated with FeLV infection. Additionally, both animals showed weights that fell within the reference interval (*L. tigrinus*: 2.9 kg [reference range: 1.75–3.5 kg]¹⁶ and *L. pardalis*: 11.9 kg [reference range: 7–16 kg]¹⁶).

Interestingly, the above-mentioned nonprogressive ataxia of the forelimbs was observed in the FeLV-positive *L. pardalis*. This animal has had this alteration since its birth, and the etiology of the alteration is unknown. Although FeLV infection may lead to myelopathy in domestic cats, with alterations, including paresis, progressing to paralysis, abnormal vocalization, and hyperesthesia,² none of these clinical signs were

observed in this FeLV-infected wild felid. Moreover, this felid has produced a healthy offspring and neurologic signs have not progressed.

In order to detect actual infection and to prevent potential disease transmission during mating in this population, FeLV and coronavirus detection by PCR was preferred over FeLV p27 antigen and/or FeLV and coronavirus antibodies detection. FeLV regressive infection in domestic cats is represented by FeLV proviral DNA and absence of virus shedding.¹⁰ Animals are frequently negative by FeLV p27 antigen detection, plasma and cell-associated FeLV RNA, or viral culture, but are positive on proviral DNA PCR. These cats are at low risk of developing FeLV-associated diseases; however, reoccurrence of viremia and disease development has been observed.⁷ The outcome of regressive infection in wild felids is unknown. On the other hand, progressive infection is characterized by positive viremia and proviral DNA in domestic cats. Thus, in this study, FeLV proviral DNA was the preferred method to detect progressive or regressive infection. In this case, false-negative results may occur in cases of focal infection in certain tissues, such as spleen, lymph nodes, small intestine, or mammary glands, which is believed to be rare in domestic cats.¹⁰ Although coronavirus serology may detect previous exposure to the virus and sometimes actual infection, PCR is recognized as being more sensitive in the detection of viral shedding/infection.^{8,9}

To the author's knowledge, this is the first study to survey FeLV proviral DNA and coronavirus infection in a captive neotropical felid population from Southern Brazil. Recently, FeLV proviral DNA was detected in four species of captive neotropical wild felids (*L. pardalis*, *P. onca*, *P. concolor*, and *P. yagouaroundi*) from a zoological garden in Minas Gerais State, Southeastern Brazil (Coelho et al., unpubl. data). In 2003, a study described FeLV p27 antigen in *L. pardalis*, *L. colocolo*, *L. weidii*, *P. concolor*, and *P. onca* in Southern Brazil.¹³ In an extended research study, 104 neotropical felids from Southeastern Brazilian zoological parks were tested for FeLV p27 antigen, and none of the animals showed positive results.⁵ This same research group continued to study neotropical cat populations and first detected FeLV RNA and proviral DNA in samples from captive Brazilian *P. yagouaroundi* specimens from São Paulo State, Southeastern Brazil, and showed antibodies against FeLV in specimens of all neotropical felids, except *P. onca*.⁴

In this present investigation, both FeLV-positive animals were born in the zoo, as were 21 other felids in this study. The wandering of feral cats around the pens of these wild felids is a serious problem that has been recognized by the veterinarian at this zoo. Thus, the possibility exists that domestic species may carry and transmit viruses to wild felids, and disease occurrence in this feral cat population should be investigated.

Recent studies have shown serologic evidence of FCoV in wild cats in Brazil, with higher incidence of FCoV infection in Brazilian nondomestic cats maintained in captivity, when compared to free-ranging animals.^{4,13} However, it was not possible to document coronavirus excretion in the feces of wild felids studied herein.^{4,13} These previous studies used indirect immunologic methods for detection of FCoV, which may only indicate contact with infectious agent rather than actual infection. A low number of excreted virus in feces, which may not be detected even in highly sensitive PCRs, may lead to false negatives. Nevertheless, given the rapid spread of coronavirus in high-density populations maintained in captivity, it is hypothesized that the wild felids in this study were probably free of coronavirus infection at the time of the sampling.

In conclusion, two out of 57 captive wild felids were infected with FeLV. Although the rate of infection is low, it was found in two different neotropical species. The possibility that FeLV may compromise the health and well-being of an already-endangered species and lead to declines in Brazilian wild cat populations cannot be ignored. These felids were not tested for the presence of viremia or antigenemia; however, neither of the positive animals will be used for reproduction anymore, as reoccurrence of viremia may occur even in regressive infection.⁷ Also, frequent FeLV testing will be a prerequisite to include a felid in the reproduction program of the Itaipu Binacional Wildlife Research Center. Contrastingly, coronavirus infection was not found among the wild felids that were surveyed. Since there is co-mingling with feral cats, continuous surveillance should be addressed to avoid the introduction of coronaviruses into this wild cat population. Although FeLV and coronaviruses have been described in different Brazilian wild felids populations,^{4,13} the frequency of these agents probably varies among different locations, and their detection is indispensable to prevent transmission and spread of diseases, especially in populations that are participating in a reproduction program.

LITERATURE CITED

1. Brown, M. A., M. W. Cunningham, A. L. Roca, J. L. Troyer, W. E. Johnson, and S. J. O'Brien. 2008. Genetic characterization of feline leukemia virus from Florida Panthers. *Emerg. Infect. Dis.* 14: 252–259.
2. Carmichael, K. P., D. Bienzle, and J. J. McDonnell. 2002. Feline leukemia virus–associated myelopathy in cats. *Vet. Pathol.* 39: 536–545.
3. Escutenaire, S., N. Mohamed, M. Isaksson, P. Thorén, B. Klingeborn, S. Belák, M. Berg, and J. Blomberg. 2007. SYBR Green real-time reverse transcription–polymerase chain reaction assay for the generic detection of coronaviruses. *Arch. Virol.* 152: 41–58.
4. Filoni, C. 2006. Exposição de Felídeos a Agentes Infeciosos Seleccionados. Ph.D. Dissertation, Univ. São Paulo, São Paulo, Brazil.
5. Filoni, C., C. H. Adania, E. L. Durigon, and J. L. Catão-Dias. 2003. Serosurvey for feline leukemia virus and lentiviruses in captive small neotropical felids in São Paulo State, Brazil. *J. Zoo Wildl. Med.* 34: 65–68.
6. Heeney, J. L., J. F. Evermann, A. J. McKeirnan, L. Marker-Kraus, M. E. Roelke, M. Bush, D. E. Wildt, D. G. Meltzer, L. Colly, J. Lukas, V. J. Manton, T. Caro, and S. J. O'Brien. 1990. Prevalence and implications of feline coronavirus infections of captive and free-ranging cheetahs (*Acinonyx jubatus*). *J. Virol.* 64: 1964–1972.
7. Hofmann-Lehmann, R., V. Cattori, R. Tandon, F. S. Boretti, M. L. Meli, B. Riond, and H. Lutz. 2008. How molecular methods change our views of FeLV infection and vaccination. *Vet. Immunol. Immunopathol.* 123: 119–123.
8. Kennedy, M., S. Citino, T. Dolorico, A. H. McNabb, A. S. Moffat, and S. Kania. 2001. Detection of feline coronavirus infection in captive cheetahs (*Acinonyx jubatus*) by polymerase chain reaction. *J. Zoo Wildl. Med.* 32: 25–30.
9. Kennedy, M., S. Citino, A. H. McNabb, A. S. Moffat, K. Gertz, and S. Kania. 2002. Detection of feline coronavirus in captive *Felidae* in the USA. *J. Vet. Diagn. Invest.* 14: 520–522.
10. Levy, J., C. Crawford, K. Hartmann, R. Hofmann-Lehmann, S. Little, E. Sundahl, and V. Thayer. 2008. American Association of Feline Practitioners' feline retrovirus management guidelines. *J. Feline Med. Surg.* 10: 300–316.
11. Miyazawa, T., and O. Jarrett. 1997. Feline leukaemia virus proviral DNA detected by polymerase chain reaction in antigenaemic but non-viraemic ("discordant") cats. *Arch. Virol.* 142: 323–332.
12. Santos, L. C. 1999. Valores de referência para exames laboratoriais. In: Santos, L. C. (ed.). *Laboratório Ambiental*. Editora Univ. Edunioeste, Cascavel, Brazil. Pp. 233–306.
13. Schmitt, A. C., D. Reischak, C. L. Cavlac, C. H. L. Monfortes, F. T. Couto, A. B. P. F. Almeida, D. G. G. Santos, L. Souza, C. Alves, and K. Vecchi. 2003. Feline leukaemia and feline peritonitis virus infection in captive and wildcats from Mato Grosso swamp region. *Acta Sci. Vet.* 31: 185–188.
14. Schreier, E., F. Döring, and U. Künkel. 2000. Molecular epidemiology of outbreaks of gastroenteritis associated with small round structure in Germany in 1997/98. *Arch. Virol.* 145: 443–453.
15. Shi, Y., and P. Roy-Burman. 2000. A novel truncated env gene isolated from a feline leukemia virus–induced thymic lymphosarcoma. *J. Virol.* 74: 1451–1456.
16. Silva, J. C. R., and C. H. Adania. 2006. Carnívora—*Felidae* (onça, suçuarana, jaguatirica, gato-do-mato). In: Cubas Z. S., J. C. R. Silva, and J. L. Catão-Dias. (eds.). *Tratado de Animais Selvagens*. Editora Roca, São Paulo, Brazil. Pp. 505–546.
17. Troyer, J. L., J. Pecon-Slattey, M. E. Roelke, W. Johnson, S. VandeWoude, N. Vazquez-Salat, M. Brown, L. Frank, R. Woodroffe, C. Winterbach, H. Winterbach, G. Hemson, M. Bush, K. A. Alexander, E. Revilla, and S. J. O'Brien. 2005. Seroprevalence and genomic divergence of circulating strains of feline immunodeficiency virus among *Felidae* and *Hyaenidae* species. *J. Virol.* 79: 8282–8294.

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