

Detection of *Chlamydophila felis* and Feline Herpesvirus Type-1 in non-domestic felids in Brazil

Detecção de *Chlamydophila felis* e Herpesvirus felino tipo 1 em felídeo não doméstico no Brasil

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Abstract

Little is known about the occurrence of feline upper respiratory tract disease agents, namely Feline Herpesvirus type 1 (FHV-1) and *Chlamydophila felis*, and co-infection of these agents with Feline Immunodeficiency virus (FIV) and Feline Leukemia Virus (FeLV) in non-domestic felids in Brazil. Between 2009 and 2010, 72 conjunctival swab and serum samples were collected from eight non-domestic felid species (*Leopardus pardalis*, *Leopardus tigrinus*, *Panthera leo*, *Panthera tigris*, *Puma concolor*, *Puma yagouaroundi*, *Oncifelis colocolo*, and *Panthera onca*) maintained in captivity in Brazilian zoos. DNA extracted from conjunctival swabs were used in PCR assays for the detection of *Chlamydophila sp*, FHV-1, and retrovirus DNA, respectively. Antibodies to FIV and FeLV antigen were detected in non-domestic felid serum samples using a commercial ELISA kit. Antibodies to FIV were found only in five (6.9%) felids. No sampled non-domestic felid was positive for FeLV antigen detection. One (1.3%) out of 72 non-domestic felid conjunctival swab samples was positive for *Chlamydophila sp* and Feline Herpesvirus-1 in PCR. This felid was an ocelot and was negative for FIV and FeLV. The results of this survey showed the occurrence of co-infection with *C. felis* and FHV-1 in an ocelot (*Leopardus pardalis*) in Brazil.

Keywords: *Chlamydia*. Ocelot. PCR. Swab.

Resumo

Poucos trabalhos descrevem a ocorrência dos agentes do complexo respiratório felino, Herpesvírus Felino tipo 1 (FHV-1) e *Chlamydophila felis*, e a coinfeção com o vírus da imunodeficiência felina (FIV) e leucemia viral felina (FeLV) em felinos não domésticos no Brasil. Entre 2009 e 2010, 72 amostras de swab de conjuntiva e de soro foram coletados de oito espécies de felinos não domésticos (*Leopardus pardalis*, *Leopardus tigrinus*, *Panthera leo*, *Panthera tigris*, *Puma concolor*, *Puma yagouaroundi*, *Oncifelis colocolo*, and *Panthera onca*) mantidos em cativeiro em zoológicos brasileiros. O DNA foi extraído das amostras de swab de conjuntiva para detecção de *Chlamydophila sp* e FHV-1 pela PCR. Anticorpos para FIV e antígeno para FeLV foram determinados pelo kit comercial de ELISA. Anticorpos para FIV foram detectados em cinco felídeos (6,9%). Nenhuma amostra foi positiva para a presença de antígeno de FeLV. Um (1,3%) dos 72 felinos não domésticos apresentou fragmentos de DNA de *Chlamydophila sp* e FHV-1 pela PCR. Este felino era uma jaguatirica que não apresentou anticorpos para FIV e nem antígeno para FeLV. Estes resultados demonstram a ocorrência de coinfeção de *C. felis* e FHV-1 em uma jaguatirica (*Leopardus pardalis*) no Brasil.

Palavras-chave: *Chlamydia*. Jaguatirica. PCR. Swab.

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Introduction

Feline upper respiratory tract disease, caused by both feline herpesvirus type-1 (FHV-1) and *Chlamydophila felis*, is characterized by nonspecific clinical signs, such as conjunctivitis, nasal and ocular discharge, and sneezing, which often result in misdiagnosis and underestimation of the real

prevalence of these diseases. Laboratory diagnosis to identify the agent involved in the etiology of upper respiratory tract disease is critical to successful treatment and control (SYKES, 2005).

Chlamydophila felis, formerly *Chlamydia psittaci*, belongs to the *Chlamydiaceae* family, which comprises obligate intracellular bacteria whose cell wall resembles those of Gram-negative bacteria (ARTOIS; REMOND, 1994; ADAMS et al., 2010). Clinical signs produced by the infection are influenced by the age of the cat, its immunocompetence, affected tissues and virulence of the inoculum (RAMSEY, 2000). Transmission is thought to occur mainly by direct contact with infectious ocular secretions. Few reports have been published concerning the prevalence of *C. felis* among non-domestic felids, mainly in Brazil. Such reports include a study that found seroprevalence of 27% among wild cats in Europe (MILLÁN; RODRÍGUEZ, 2009), and the detection of *C. felis* in tissues (liver and kidney) of a fishing cat (*Felis viverrina*) in the Netherlands using the Direct Immunofluorescence Assay (KIK et al., 1997).

FHV-1, an Alpha-herpesvirus member of the family *Herpesviridae*, shows biological behavior similar to other herpesviruses, causing primary infection followed by latent infection without apparent clinical signs. During latency, the virus incubates in sensory ganglia, where no viral replication occurs (MAGGS, 2005). When reactivation takes place, susceptible animals may be infected by contact with agents excreted in nasal and ocular secretions (STILES, 2000). Although seropositivity to this agent has been reported among captive and free-ranging wild felids in Brazil, Europe, Asia, North America, and Africa, active virus detection has been infrequently observed in these animals (ARTOIS; REMOND, 1994; DANIELS et al., 1999; LEUTENEGGER et al., 1999; MURRAY et al., 1999; OSTROWSKI et al., 2003; BATISTA et al., 2005; FILONI et al., 2006; FILONI et al., 2011).

The co-infection of immunosuppressive retroviruses (such as Feline Immunodeficiency Virus – FIV– and Feline Leukemia Virus – FeLV) with *C. felis* and FHV-1 may increase the severity of clinical signs and excretion of the upper respiratory tract disease agent (REUBEL et al., 1992). Retroviral infections have been detected among free-ranging and captive non-domestic felids in Brazil by molecular and serological techniques (FILONI et al., 2003; BATISTA et al., 2005; FILONI et al., 2006; GUIMARÃES et al., 2009; FILONI et al., 2011).

The present study describes the occurrence of *C. felis*, FHV-1, FeLV and FIV infections in non-domestic felids in Brazil.

Materials and Methods

Sampling population and assays

From 2009 to 2010, 72 blood samples and conjunctival swabs were collected from non-domestic felid species, namely *Leopardus pardalis* (ocelot, n = 14), *Leopardus tigrinus* (little spotted cat, n = 24), *Panthera leo* (lion, n = 5), *Panthera tigris* (tiger, n = 2), *Puma concolor* (puma, n = 6), *Puma yagouaroundi* (jaguarondi, n = 16), *Oncifelis colocolo* (pampas cat, n = 1), *Panthera onca* (jaguar, n = 3), which were kept in captivity in ten Brazilian Zoos in different sites. Animals were immobilized using a combination of ketamine (10 mg/kg) and xylazine (1 mg/kg) (FILONI et al., 2003). Blood samples were collected by venipuncture. Serum samples were obtained after incubation at room temperature until clot formation, followed by a centrifugation. Sera were placed in 1.5 mL polypropylene tubes and stored at -18°C. A dry, sterile cotton swab that was moistened with tears or exudate was used to firmly swab the conjunctival sacs of both eyes. Swabs specimens were stored at -20°C until they were used in the PCR assays (SYKES et al., 1997) (Table 1).

Table 1 – Number, species and zoo location of sampled non-domestic felids

| Species (Common name) | Location (n) of swab samples | Total number of samples |
|---|--|-------------------------|
| <i>Leopardus pardalis</i> (ocelot) | Am (2), IS (2), So (1), Bau (3), Ita (2), Pir (4) | 14 |
| <i>Leopardus tigrinus</i> (little spotted cat) | IS (2), So (14), SC (1), Ita (1), Pir (3), Cat (1), NO (2) | 24 |
| <i>Panthera onca</i> (jaguar) | Am (1), So (1), Bau (1), | 3 |
| <i>Puma concolor</i> (puma) | Rp (1), Am (2), So (1), Cat (2) | 6 |
| <i>Puma yagouaroundi</i> (jaguarondi) | IS (4), So (3), SC(2), Bau (3), Ita (3), Pir (1) | 16 |
| <i>Oncifelis colocolo</i> (pampas cat) | So(1) | 1 |
| <i>Panthera tigris</i> * (tiger) | So (1), Ita (1) | 2 |
| <i>Panthera leo</i> * (lion) | Am (1), So (1), Bau (3) | 5 |

Am = Americana zoo, Bau = Bauru zoo, Cat = Catanduva zoo, IS = Ilha Solteira zoo, Ita = Itatiba zoo, NO = Nova Odessa zoo, Pir = Piracicaba zoo, Rp = Ribeirão Preto, SC = São Carlos zoo, So = Sorocaba zoo

* Exotic felids

Samples were collected from institutions in different cities in the state of São Paulo, such as Americana (22°44'21"S, 47°19'53"W), Bauru (22°18'53"S, 49°03'38"W), Campinas (22°54'20"S, 47°03'39"W), Catanduva (21°08'16"S, 48°58'22"W), Ilha Solteira (20°25'58"S, 51°20'33"W), Itatiba (23°00'21"S, 46°50'20"W), Nova Odessa (22°46'39"S, 47°17'45"W), Piracicaba (22°43'31"S, 47°38'57"W), Ribeirão Preto (21°10'39"S, 47°48'37"W), São Carlos (22°01'03"S, 47°53'27"W) and Sorocaba (23°30'06"S, 47°27'29"W) (Table 1).

All procedures were performed with permission from the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) (license number 20012-1/2009), and following the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation. The study was approved by the Ethics Committee of the Faculty of Agricultural and Veterinary Sciences, UNESP Jaboticabal.

Serum assay

Commercially available immunoassay Snap™ Combo FeLV antigen/FIV antibody test kit (IDEXX Laboratories Inc., Westbrook, ME, USA) was used in the detection of FeLV antigen and antibodies against FIV, according to the manufacturer's recommendations.

Polymerase Chain Reaction

DNA extraction. Conjunctival swab samples were submitted to vortex for 2 min and then centrifuged at 20,000 x g for 30 min at 4°C (RASO et al., 2006). DNA was extracted from pellets using Genomic DNA from Tissue kit® (Macherey-Nagel GmbH & Co., Düren, Düren, German) according to the manufacturer's instructions.

PCR amplification and sequencing. Each sample of DNA extracted from conjunctival samples was used in PCR reactions for *Chlamydomophila* sp. and FHV-1.

Five microliters of DNA template were used in 25 µL reaction mixtures containing 10X PCR buffer, 1.0 mM MgCl₂, 0.2 mM deoxynucleotide triphosphate (dNTPs) mixture, 1.5 U Taq DNA Polymerase^{*} (Invitrogen, Carlsbad, CA, USA), and 1 µM of primers.

PCR reactions for *Chlamydomphila* sp. were performed using primers oligo420 (5'-CAGGATATCTTGTCTGGCTTTAA-3') and oligo422 (5'-GCAAGGATCGCAAGGATC-3'), which target the conserved region of the chlamydial major outer membrane protein gene (MOMP) and amplify a 260 base pair (bp) DNA fragment (BUXTON et al., 1996). PCR cycling conditions were 10 min at 94°C; 34 cycles at 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 4 min. Positive (*C. felis* vaccine - Fel-O-Vax LVK-IV^{*}; Fort Dodge, Fort Dodge, IA, USA), negative (cat swab sample negative for *Chlamydomphila* sp.) and no-template (autoclaved ultrapure water, Promega Corp., Fitchburg, WI, USA) controls were routinely included in each PCR reaction (SEKI et al., 2010).

PCR for FHV-1 detection was performed using primers 5'-GACGTGGTGAATTATCAGC-3' and 5'-CAACTAGATTTCCACCAGGA-3' based on the gene that encodes FHV-1 thymidine kinase and amplifies a 287bp DNA fragment (SYKES et al., 1997). PCR cycling conditions were 5 min at 95°C; 35 cycles at 90°C for 1 min, 56°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 4 min. Positive (Herpesvirus vaccine Fel-O-Vax LVK-IV^{*}, Fort Dodge, Iowa, USA), negative (cat swab sample negative for FHV-1) and no-template (autoclaved ultrapure water, Promega Corp., Fitchburg, WI, USA) controls were routinely included in each PCR reaction.

Positive amplicons were purified from agarose gels using Silica Bead DNA Gel Extraction Kit^{*} (Glen Burnie, MD, USA) according to the manufacturer's recommendations. Purified amplicons were submitted to sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyzer – Applied Biosystems^{*}/Life

Technologies Corporation, Carlsbad, CA, USA). Consensus sequences were obtained by means of the analysis of sense and antisense sequences using the CAP3 program¹. Comparisons with GenBank sequences were performed using the basic local alignment search tool (BLAST) (ALTSCHUL et al., 1990).

Results

Chlamydomphila sp. DNA was detected in one (1.4%) out of 72 non-domestic felid conjunctival swab samples. The same animal (an ocelot from Sorocaba zoo) was also positive for FHV-1 PCR. *Chlamydomphila* sp. and FHV-1 DNA sequences were deposited in GenBank database under accession numbers JQ677600 and JQ677601, respectively. Sequencing showed 95% similarity with FHV-1 (closest GenBank similarity: accession numbers FJ478159 and M26660), and 92% similarity with *C. felis* (closest GenBank similarity: accession number AP006861).

All sampled animals were negative for antigen to FeLV. Five felids (6.9%) showed antibodies to FIV (three lions from the Bauru zoo, one lion from the Sorocaba zoo and one ocelot from the Itatiba zoo (Table 1). The ocelot positive for *Chlamydomphila felis* and HVF-1 in PCR was negative for FIV and FeLV.

Discussion

Active infection to *C. felis* was confirmed by PCR and sequencing in just one ocelot. To the authors' knowledge, this study reports the first molecular detection of *C. felis* in a non-domestic felid species. Previously, direct diagnosis of *C. felis* has only been performed by Direct Immunofluorescence Assay in a fishing cat (KIK et al., 1997). In Brazil, *C. felis* was detected in domestic cats by PCR in the northeastern region of the state of São Paulo (SEKI et al., 2010) and in Osasco, also in the state of São Paulo (GONSALES et al., 2013).

¹ <http://mobyle.pasteur.fr/cgi-bin/MobylePortal/portal.py>

The ocelot positive for *C. felis* PCR also tested positive for FHV-1 PCR. In Brazil, low percentages of seropositivity and low antibody titers were found among non-domestic felids maintained in captivity, suggesting that FHV-1 does not circulate extensively among these animals (BATISTA et al., 2005). Antibodies to FHV-1 have also been detected among neotropical free-ranging wild felids in Brazil (FILONI et al., 2006). Recently, FHV-1 seroprevalence of 19% (12/63) was found in non-vaccinated captive non-domestic felids in Brazil (FILONI et al., 2011). The observed failure to isolate FHV in felines is not surprising, since this virus is maintained as latent infection followed by intermittent shedding in nasal and ocular secretions (GASKELL; DAWSON, 1995).

The contact between domestic and non-domestic felids is the most feasible way of FHV-1 and *C. felis* transmission, mainly among captive felids. Animals that live in groups may be more susceptible to close contact with infected animals; thus, the possibility of pathogen transmission may be higher. Non-domestic felids with solitary habits, like those sampled in our study, are less frequently in contact with many different animals, which may limit the possibility of pathogen transmission.

The lack of felids positive for FeLV (antigen or DNA) corroborated the results of a previous study that sampled captive neotropical small felids from Brazil, suggesting that retrovirus infection is not frequently found in these felid populations (FILONI et al., 2003). On the other hand, FeLV proviral DNA was detected in one ocelot and one little spotted cat from Itaipu Binational Wildlife Research Center, in southern Brazil (GUIMARÃES et al., 2009). Antibodies to FeLV were detected in two pumas among 18 free-ranging wild felids sampled in different geographic regions of Brazil (FILONI et al., 2006). Recently, FeLV seroprevalence of 2% (3/145) was detected in captive non-domestic felids in Brazil; additionally, FeLV antigen and DNA were detected in two other ocelots in the same study (FILONI et al., 2011). Reports of FeLV infection in non-domestic felids are rare and

predominantly found in captive animals (BRIGGS; OTT, 1986; CITINO, 1986). FeLV antigenemia was detected in non-domestic felids in Scotland, France, Switzerland, and Germany (MCORIST et al., 1991; ARTOIS; REMOND, 1994; DANIELS et al., 1999; LEUTENEGGER et al., 1999; FROMONT et al., 2000; MILLÁN; RODRÍGUEZ, 2009). FeLV is transmitted rapidly among young domestic felids via infected body fluids, such as during fighting or mating (MCORIST et al., 1991). These sort of interactions are probably uncommon among captive non-domestic felids and between wild and domestic cats in Brazil. Furthermore, captivity lifestyle decreases the risk of acquiring the virus in fighting. FeLV is more likely to appear as a sustained infection among some wild felid populations rather than as a result of occasional infection acquired from domestic cats (MCORIST et al., 1991).

In this study, antibodies to FIV were detected in six lions and one ocelot. In a previous study conducted in Brazil, antibodies to FIV were not found in captive neotropical small felids (FILONI et al., 2003). Also, this agent was frequently detected in blood samples from wild felids in Europe and Africa (MCORIST et al., 1991; OLMSTED et al., 1992; SPENCER et al., 1992; BROWN et al., 1993; DANIELS et al., 1999; LEUTENEGGER et al., 1999; FROMONT et al., 2000).

Furthermore, several studies have reported the absence of seropositivity to FIV among non-domestic felids around the world (OLMSTED et al., 1992; SPENCER et al., 1992; BROWN et al., 1993; ROELKE et al., 1993; PAUL-MURPHY et al., 1994; OSOFSKY et al., 1996; MUNSON et al., 2004; MILLÁN; RODRÍGUEZ, 2009; THALWITZER et al., 2010). Among 18 free-ranging Brazilian non-domestic felids, antibodies to FIV were detected in only one puma from the Pantanal biome (FILONI et al., 2006). Recently, FIV seroprevalence of 4.8% was detected among 145 non-domestic captive felids in Brazil (FILONI et al., 2011). The most common FIV transmission route is biting; therefore, a relative lack of social contact between non-domestic felids maintained in captivity may explain the absence

or low occurrence of FIV infection (DANIELS et al., 1999).

According to the literature, co-infection of immunosuppressive retroviruses (such as Feline Immunodeficiency Virus and Feline Leukemia Virus) with *C. felis* and HVF-1 may increase the severity of clinical signs and excretion of the upper respiratory tract disease agent (REUBEL et al., 1992). However, the ocelot positive for *C. felis* and HVF-1 by PCR was negative for FIV and FeLV.

Six of the seven existing species of Brazilian non-domestic felids, including ocelots (*Leopardus pardalis*), little spotted cats (*Leopardus tigrinus*), margays (*Leopardus wiedii*), pampas cats (*Oncifelis colocolo*), jaguars (*Panthera onca*), and pumas (*Puma concolor*) are endangered (www.ibama.gov.br). Knowledge of the occurrence of these agents in captive non-domestic felids may contribute to future ecological investigations regarding the epidemiology of these pathogens among free-ranging non-domestic felids and domestic cats in Brazil.

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Conclusion

The present paper describes the occurrence of *C. felis* and FHV-1 in non-domestic felid species maintained in captivity in Brazil. Multiple diagnostic protocols regarding the diagnosis of these infectious agents should be considered prior to translocating animals between zoos, reintroducing captive animals to the wild, or relocating them from densely to thinly populated areas.

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