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First Evidence of Feline Herpesvirus, Calicivirus, Parvovirus, and *Ehrlichia* Exposure in Brazilian Free-ranging Felids

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ABSTRACT: Serum samples from 18 pumas (*Puma concolor*), one ocelot (*Leopardus pardalis*), and two little spotted cats (*Leopardus tigrinus*) collected from free-ranging animals in Brazil between 1998 and 2004 were tested by indirect immunofluorescence (IFA) for antibodies to feline herpesvirus 1 (FHV 1), calicivirus (FCV), coronavirus (FCoV), parvovirus (FPV), *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Bartonella henselae*. Serum samples also were tested, by Western blot and ELISA, for feline leukemia virus (FeLV) specific antibodies and antigen, respectively, by Western blot for antibodies to feline immunodeficiency virus (FIV), and by indirect ELISA for antibodies to puma lentivirus (PLV). Antibodies to FHV 1, FCV, FCoV, FPV, FeLV, FIV, PLV or related viruses, and to *B. henselae* were detected. Furthermore, high-titered antibodies to *E. canis* or a closely related agent were detected in a puma for the first time.

Key words: *Anaplasma*, *Bartonella*, *Ehrlichia*, feline viruses, free-ranging felids, *Leopardus tigrinus*, *Leopardus pardalis*, *Puma concolor*, serology.

Infections of nondomestic felids with viruses that are common in domestic carnivores have been reported for numerous species worldwide (Mochizuki et al., 1990; Olmsted et al., 1992; Paul-Murphy et al., 1994; Hofmann-Lehmann et al., 1996; Daniels et al., 1999; Leutenegger et al., 1999a; Fromont et al., 2000; Ostrowski et al., 2003). Although the implications of these infections on wild felid conservation are usually difficult to assess, it is broadly accepted that monitoring these infections is an important component for the management of endangered populations (Murray et al., 1999; Daszak et al., 2000).

Data regarding viral infections in neotropic Brazilian felids are sparse and have concentrated primarily on feline retroviruses. A feline immunodeficiency virus (FIV) *pol* gene from a Brazilian zoo puma (*Puma concolor*) has been sequenced (Carpenter et al., 1996) and antibodies to FIV have been reported from a Brazilian free-ranging puma (Brown et al., 1993). Additionally, FIV provirus has been reportedly detected in Brazilian jaguars (*Panthera onca*), pumas, jaguarundis (*Herpailurus yagouarondi*), ocelots (*Leopardus pardalis*), margays (*Leopardus wiedii*), pampas cat (*Oncifelis colocolo*), and little spotted cats (*Leopardus tigrinus*) (Leal and Ravazzolo, 1998). Although evidence of exposure to feline leukemia virus (FeLV) and FIV was not detected in a captive small neotropic felid population from São Paulo state in Brazil (Filoni et al., 2003), three captive neotropic felids were found to be FeLV viremic (one margay and two pampas cats) in a survey conducted in North American zoos (Kennedy-Stoskopf, 1999). Captive jaguars, pumas, margays, a pampas cat, and a free-ranging ocelot in Brazil were found FeLV positive on indirect immunofluorescence assays (IFA) (Schmitt et al., 2003).

In Brazil, antibodies to feline coronavirus (FCoV) were reported in captive jaguars, pumas, margays, a pampas cat, and a free-ranging ocelot (Schmitt et al., 2003). To the best of our knowledge, there are no published reports of FHV 1, FCV, and FPV infections in wild felids in Brazil.

Studies have demonstrated the exposure of a variety of free-ranging and captive felids to *Bartonella henselae*, including neotropic felids from North American zoos (Kelly et al., 1998; Yamamoto et al., 1998; Rotstein et al., 2000; Molia et al., 2004; Pretorius et al., 2004). An extensive serosurvey of pumas reported seropositive animals throughout most of the geographical range of the species (Chomel et al., 2004b); the overall seroprevalence in South American pumas was found to be 22.4%. To our knowledge, there is no previous report of *Ehrlichia canis* and *Anaplasma phagocytophilum* infections in captive or free-ranging neotropic Brazilian felids.

The aim of this study was to serologically survey free-ranging felids from Brazil to determine the extent of exposure to selected viruses (FHV 1, FCV, FCoV, FPV, FeLV, FIV, and the puma lentivirus, PLV) and hemoparasites (*E. canis, A. phagocytophilum*, and *B. henselae*).

The Genome Resource Bank from National Research Center for Carnivores Conservation—CENAP, unity of National Environmental Agency—IBAMA in Brazil provided serum samples, which were transported to Switzerland in full compliance with specific federal permits, like Convention on International Trade in Endangered Species—CITES (Permit Numbers 0112928BR and 1562/04), Genetic Heritage Management Council—CGEN and Agriculture Ministry (CSI 1530/2004), on dry ice as diagnostic specimens packed in compliance with IATA Packing Instruction 650. Samples were stored at CENAP at −80 C or in liquid nitrogen.

Blood samples were collected from 18 free-ranging pumas, one ocelot, and two little spotted cats between 1998 and 2004 from four biomes (Amazon Forest, Atlantic Forest, Cerrado, and Pantanal) that included 13 municipalities in the states of Rondônia, Acre, Mato Grosso, Mato Grosso do Sul, Paraná, São Paulo e Rio de Janeiro (Fig. 1, Table 1). Animals were captured with the use of trained dogs (for large felids) or live traps (small felids) and immobilized with the use of darts and a combination of tiletamine HCl and zolazepam HCl (Telazol®, Fort Dodge, Fort Dodge, Iowa, USA). At the time of sample collection, a complete physical examination was performed; animals were radio-instrumented and were released at the place of capture for further monitoring. One of the samples (serum from heart clot) was obtained from a necropsy performed on the little spotted cat from Ubatuba, São Paulo (Table 1, Fig. 1).

Antibody titers to FHV 1, FCV, FCoV, and FPV were determined in 21 serum samples by IFA as described (Hofmann-Lehmann et al., 1996). All sera were screened at a dilution of 1:20. For FHV 1, FCV, and FPV, positive and questionable results were titrated in a two-fold serial dilution starting at 1:20 until endpoint. For FCoV, positive samples were titrated using 1:25, 1:100, 1:400, and 1:1600 dilutions.

Exposure to *E. canis* and *A. phagocytophilum* or closely related agents was evaluated by IFA with the use of 1:80 dilutions of the serum samples and Mega Screen Fluoehrlichia c. slides (MegaCor Diagnostik GmbH, 6912 Hörbranz, Austria) or *E. equi* slides (VMRD, Inc. Pulman, Washington, USA). Serum samples from 20 animals were assayed for antibodies to *B. henselae* by IFA (Glaus et al., 1997). All sera were screened at dilutions of 1:64 and 1:128. Titers of ≥64 were considered positive. Positive serum samples were titrated until endpoint by twofold serial dilutions.

For quality control of the IFA slides, aliquots of the cell cultures (140 µl) or scrapings from the slides were tested for presence of unwanted antigens. Samples were incubated at 40 C for 10 min in 300 µl lysis buffer and total nucleic acid (TNA) was extracted with the use of the MagNA Pure LC instrument (Roche, Rotkreuz, Switzerland). The TNA samples were analyzed by real-time TaqMan poly-

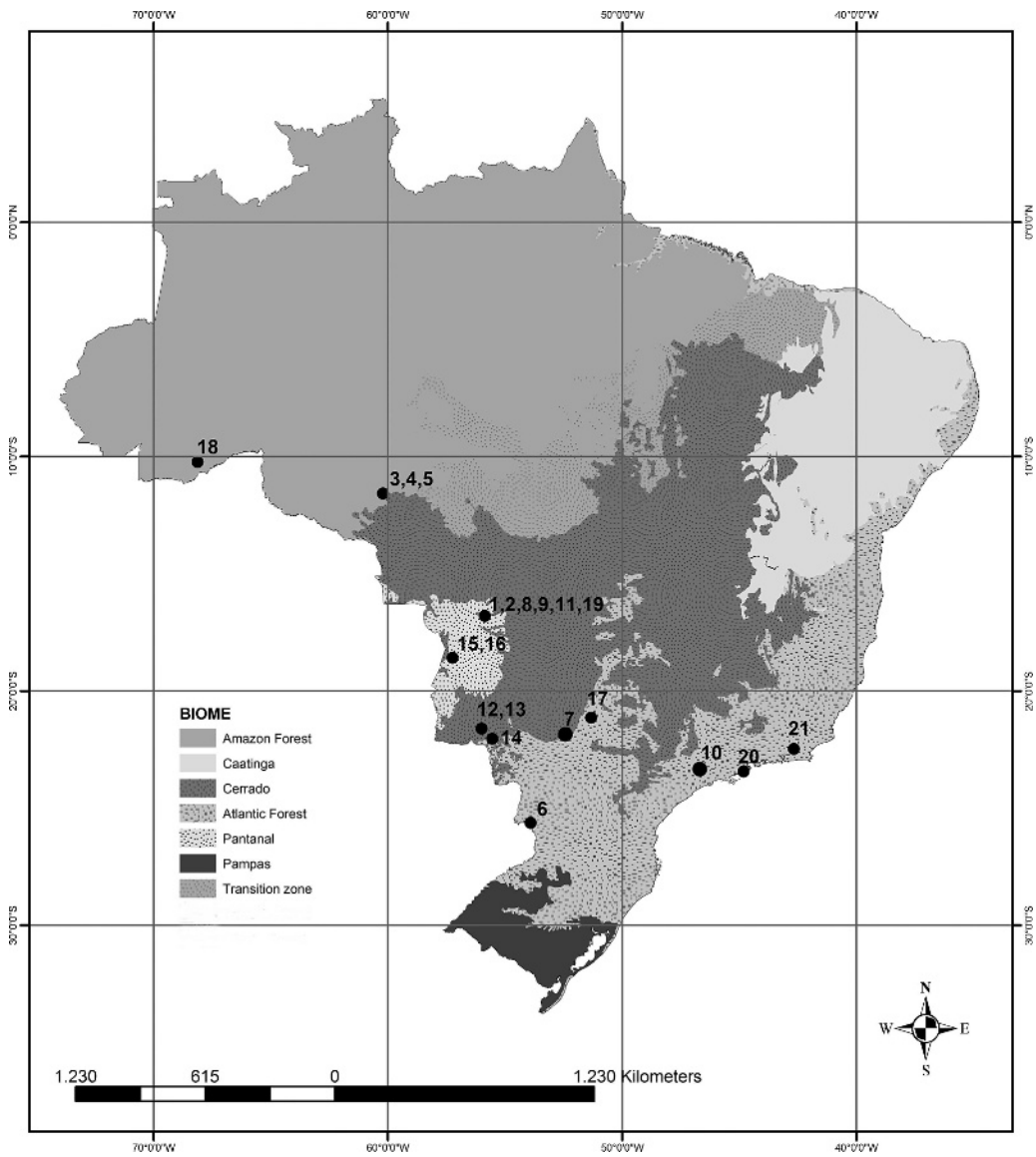

FIGURE 1. Map depicting the geographic areas within Brazil from which samples from 21 free-ranging felids were collected.

merase chain reaction (PCR) or reverse transcriptase (RT) PCR on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, California, USA) for the presence of the agents of interest: FIV (Leutenegger et al., 1999b), FeLV (Hofmann-Lehmann et al., 2001), FCoV (Gut et al., 1999), FHV 1 (Vogtlin et al., 2002), FPV (Meli et al., 2004), and FCV (Kummrow et al., 2005). No unwanted agents were detected in any antigen preparations.

All sera were examined for the presence of antibodies to FeLV by Western blot (Hofmann-Lehmann et al., 1995). Samples containing antibodies to gp70, p58, p27, p15, and p12 were judged positive. Additionally, the serum samples were

TABLE 1. Serologic test results for free-ranging *Puma concolor*, *Felis pardalis*, and *Felis tigrinis* in Brazil.

				Serologic results[c]								
				Indirect fluorescent antibody test						Western blot		
Biome	Location[a]	Species[b]	*n*	FHV-1	FCV	FCoV	FPV	*Ehrlichia canis*	*Bartonella henselae*	FeLV	FIV	ELISA Indirect PLV
Amazon Forest	VR	*P. concolor* (3,4,5)	3	2	N[d]	N	3	N	3	N	N	N
	RA	*L. pardalis* (18)	1	1	1	1	1	N	1	N	N	N
Pantanal	BM	*P. concolor* (1,2,8,9,11, 19)	6	N	2	N	N	1	5[e]	1	1	2
	CM	*P. concolor* (15,16)	2	N	N	N	2	N	1	N	N	N
Cerrado	BaM	*P. concolor* (7)	1	N	N	N	N	N	1	N	N	N
	JM	*P. concolor* (12,13)	2	N	N	N	2	N	2	N	N	N
	PM	*P. concolor* (14)	1	1	N	N	N	N	1	N	N	N
Atlantic Forest	PP	*P. concolor* (6)	1	1	1	N	N	N	1	N	N	N
	SS	*P. concolor* (10)	1	N	N	N	N	N	1	1	N	N
	l S	*P. concolor* (17)	1	1	N	N	1	N	1	N	N	N
	UP	*L. tigrinus* (20)	1	N	1	N	1	N	1	N	N	N
	NR	*L. tigrinus* (21)	1	N	1	N	N	N	1	N	N	N
TOTAL			21	6	6	1	10	1	19	2	1	2
Antibody titer (range)				20–160	20–40	100	40–640	20,480	64–1024			

[a] VR = Vilhena/ Rondônia (12°37′S, 60°07′W); RA = Rio Branco/Acre (09°52′S, 67°52′W); BM = Barão de Melgaço/ Mato Grosso (16°07′S, 55°52′W); CM = Corumbá/ Mato Grosso do Sul (19°03′S, 57°41′W); BaM = Bataguassu/Mato Grosso do Sul (21°41′S, 52°26′W); JM = Jardim/Mato Grosso do Sul 21°26′S, 56°11′W; PM = Ponta Porã/Mato Grosso do Sul (22°33′S, 55°41′W); PP = Parque Nacional do Iguaçu /Paraná (25°33′S, 54°33′W); SS = Serra da Cantareira/São Paulo (23°33′S, 46°41′W); lS = Ilha Solteira/São Paulo (20°26′S, 51°18′W); UP = Ubatuba/São Paulo (23°26′S, 45°03′W); Nova Friburgo/Rio de Janeiro (22°18′S 42°33′W).

[b] Individual animal identification number.

[c] Number of animals positive. All animals tested negative for antibodies to Anaplasma phagocytophium on IFA; all animals tested negative on Direct FeLV p27 ELISA.

[d] N = negative.

[e] No material was available from one puma for the *B. henselae* IFA.

tested for the presence of FeLV p27 as a measure for viremia by a sandwich ELISA (Lutz et al., 1983). One sample tested positive. However, upon retesting in the presence of mouse serum, it was determined that this represented a false positive; the positive reaction resulted from cross-linking of the monoclonal mouse anti-FeLV antibodies by feline antimouse antibodies and not by binding of the FeLV antigen. Antibodies to FIV were detected by Western blot (Lutz et al., 1980, 1988). Samples with antibodies to p24 and p15 were considered positive. Antibodies reactive to a specific puma lentivirus peptide were detected by an indirect ELISA (Van Vuuren et al., 2003).

Results of the serosurvey are summarized in Table 1. This is the first reported serologic evidence that FHV 1, FCV, FPV, and the obligate intracellular bacterium *E. canis* (or antigenically related agents) are present among free-ranging felids in Brazil. Antibodies to FCoV in a free-ranging ocelot, antibodies to FeLV, FIV, and PLV (or related lentiviruses) in free-ranging pumas, and antibodies to *B. henselae* in all three feline species also were detected. Antibody prevalence rates based on all 21 animals were as follows: *B. henselae* (95%); FPV (48%); FHV 1 and FCV (29%); FeLV and PLV (10%); and FCoV, FIV, and *E. canis* (5%). Antibodies to *A. phagocytophilum* were not detectable.

Our results indicate that co-infection with the studied viral pathogens is not common in the Brazilian neotropic felids: most animals (76%) were seropositive for only one or two of the selected viruses. An exception was one Amazonic ocelot seropositive for four of the selected viruses. The high prevalence of antibodies to parvoviruses (48%) may relate to the environmental stability of these nonenveloped viruses; free-ranging wild felids may be exposed in areas contaminated by feces, even if they are solitary, widely dispersed, and at low density (Barker and Parish, 2001). In contrast, FCoV do not persist in the environment and this may explain the single FCoV antibody positive animal that was detected in this study. However, even though FHV 1 and FCV do not persist well in the environment and require close contact to be transmitted, seropositive results to both of these viruses were relatively common (29%) in this survey. This situation is similar to that of free-ranging lions in Africa, where high antibody prevalence rates to FHV 1 have been reported (Hofmann-Lehmann et al., 1996).

The finding of antibodies to retroviruses addresses the need for additional monitoring. Two animals had antibodies to FeLV, but FeLV antigen was not detected. It is possible that these animals had undergone regressive FeLV infection and mounted specific immune responses. The finding of seropositive animals nonetheless may be of concern, because FeLV is potentially pathogenic (Jessup et al., 1993; Fromont et al., 2000; Sleeman et al., 2001) and domestic cats (*Felis catus*) have been implicated as a source of FeLV infection to nondomestic felids (Jessup et al., 1993; Sleeman et al., 2001). One puma was found to be FIV seropositive. This animal and a second puma also had antibodies to PLV (Table 1). To determine the nature and origin of these viruses, sequence analysis would be necessary; however, material suitable for these analyses was unavailable.

Antibodies to *E. canis* were detected in one puma. Although the titer was high, interpretation of this single positive is difficult. It could represent evidence of an *E. canis* infection or antibodies could have resulted from an infection with a related *Ehrlichia* species, such as *E. chaffeensis* or *E. ewingii*. Ticks are common in the area (Barão de Melgaço, Mato Grosso) where this animal was sampled and numerous tick species including *Amblyomma ovale, A. parvum, A. cajennense, Boophilus microplus*, and *Ixodes aragaoi* have been reported from pumas (Labruna et al., 2005).

A very high prevalence of antibodies to *B. henselae* (95%), which causes cat scratch disease in humans (Chomel et al., 2004a) was detected. Brazilian free-ranging felids may be a reservoir for *B. henselae*, and zoo guidelines and wildlife management programs in Brazil should further emphasize recommendations for safe handling of animals. *Bartonella henselae* is mainly transmitted via cat fleas (*Ctenocephalides felis*) in domestic cats (Chomel et al., 1996) and is potentially transmissible among domestic cats and wild felids (Chomel et al., 2004b). In addition to fleas, ticks from the genus *Ixodes* have been suggested as potential vectors for *Bartonella* spp. (Schouls et al., 1999; Chang et al., 2001; Chomel et al., 2004a). Free-ranging felids are considered as hosts for *C. felis* (Linardi and Guimarães, 2000) and ticks such as *Ixodes aragaoi* have been collected and identified parasitizing a puma (Labruna et al., 2005).

According to the physical examinations at the moment of sampling there were no signs of the infections or related diseases in the animals. The single necropsied little spotted cat was in very good body condition with the exception of heavy parasitosis, including *Dirofilaria immitis*. At present the significance of these pathogens to the overall health of these wild felid populations is unknown. Since the infectious agents investigated in the present study are common domestic carnivore's pathogens, it is not possible to determine whether these agents have been historically associated with neotropic felid populations or have been introduced by interactions with domestic carnivores. Isolation and molecular characterization of these pathogens, both in domestic and wild carnivores, would be helpful to answer this question and may provide important baseline data to develop effective programs aimed at infectious disease prevention and mitigation.

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