



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

Authors: Filoni, Claudia, Catão-Dias, José Luiz, Bay, Gert, Durigon, Edison Luiz, Jorge, Rodrigo Silva Pinto, et al.

Source: Journal of Wildlife Diseases, 42(2) : 470-477

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-42.2.470>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

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

Claudia Filoni,^{1,6} José Luiz Catão-Dias,^{1,2} Gert Bay,³ Edison Luiz Durigon,⁴ Rodrigo Silva Pinto Jorge,⁵ Hans Lutz,³ and Regina Hofmann-Lehmann^{3,1} Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Avenida Prof. Dr. Orlando Marques de Paiva 87, 05508-270, São Paulo, SP, Brazil; ² Fundação Parque Zoológico de São Paulo, Avenida Miguel Stéfano 4241, 04301-905, São Paulo, SP, Brazil; ³ Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Wintherturerstrasse 260, CH-8057 Zurich, Switzerland; ⁴ Laboratório de Virologia Clínica e Molecular, Departamento de Microbiologia, Universidade de São Paulo, Instituto de Ciências Biomédicas, Edifício Biomédicas II, Avenida Prof. Lineu Prestes, 1374, 05508-900, São Paulo, SP, Brazil; ⁵ Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Avenida Prof. Dr. Orlando Marques de Paiva 87, 05508-270, São Paulo, SP, Brazil; ⁶ Corresponding author (email: cfiloni@usp.br)

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 (Mur-ray et al., 1999; Daszak et al., 2000).

Data regarding viral infections in neotropical 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-

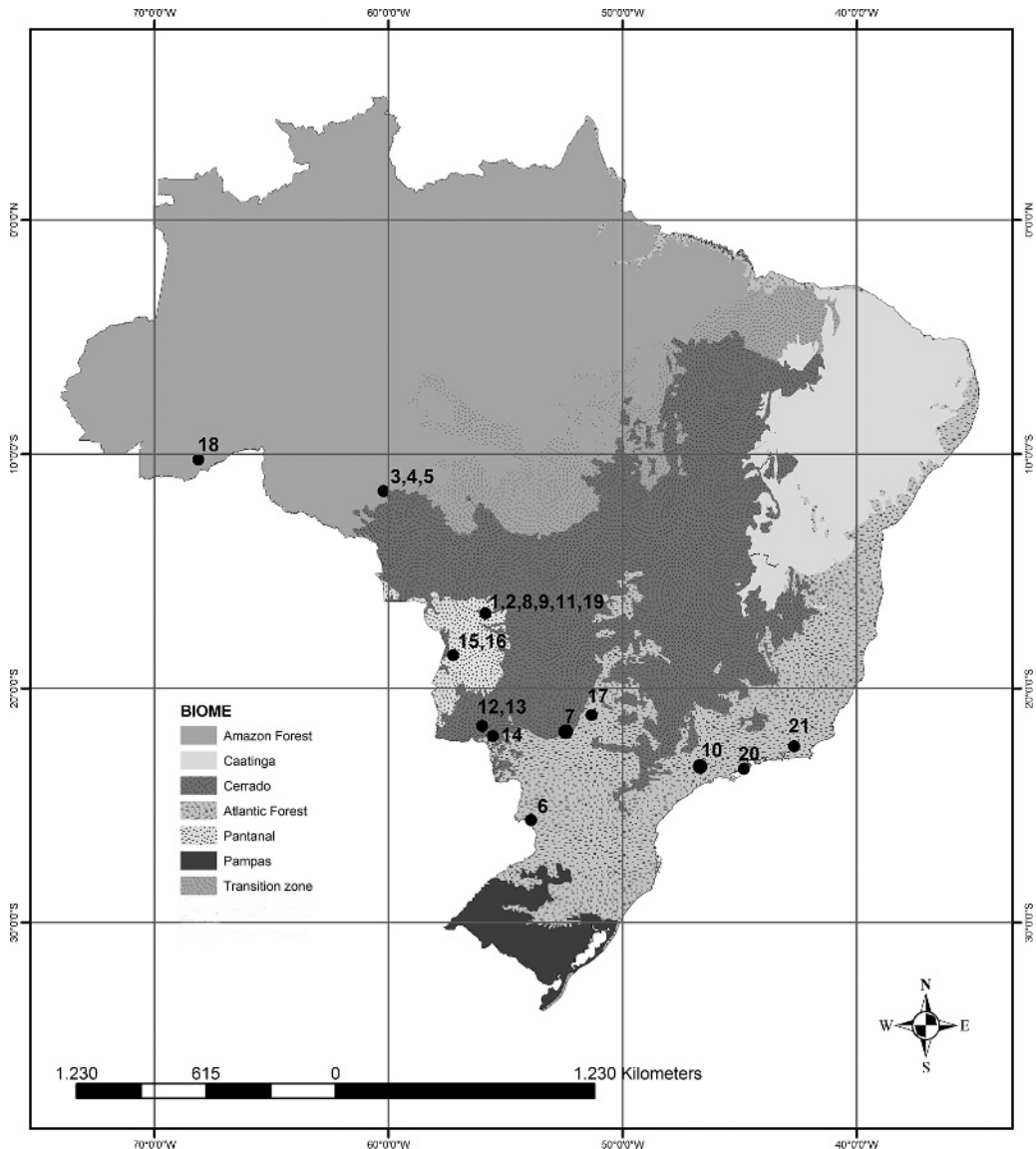


FIGURE 1. Map depicting the geographic areas within Brazil from which samples from 21 free-ranging felids were collected.

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 tigrinus* in Brazil.

Biome	Location ^a	Species ^b	n	Serologic results ^c										
				Indirect fluorescent antibody test					Western blot					
				FHV-1	FCV	FCoV	FPV	<i>Ehrlichia canis</i>	<i>Bartonella henselae</i>	FeLV	FIV	ELISA Indirect PLV		
Amazon Forest	VR	<i>P. concolor</i> (3,4,5)	3	2	N ^d	N	3	N	N	3	N	N	N	N
	RA	<i>L. pardalis</i> (18)	1	1	1	1	1	1	1	1	N	N	N	N
	BM	<i>P. concolor</i> (1,2,8,9,11,19)	6	N	2	N	N	2	N	5 ^e	1	1	1	2
Pantanal	CM	<i>P. concolor</i> (15,16)	2	N	N	N	2	N	1	N	N	N	N	N
	BaM	<i>P. concolor</i> (7)	1	N	N	N	N	1	1	N	N	N	N	N
Cerrado	JM	<i>P. concolor</i> (12,13)	2	N	N	N	2	N	2	N	N	N	N	N
	PM	<i>P. concolor</i> (14)	1	1	N	N	N	1	1	N	N	N	N	N
Atlantic Forest	PP	<i>P. concolor</i> (6)	1	1	1	N	N	1	1	N	N	N	N	N
	SS	<i>P. concolor</i> (10)	1	N	N	N	N	1	1	N	1	N	N	N
	IS	<i>P. concolor</i> (17)	1	1	N	N	1	1	1	1	N	N	N	N
	UP	<i>L. tigrinus</i> (20)	1	N	1	N	1	1	1	1	N	N	N	N
	NR	<i>L. tigrinus</i> (21)	1	N	1	N	1	1	1	1	N	N	N	N
TOTAL			21	6	6	100	40-640	20,480	64-1024	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); IS = 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 neotropical felids: most animals (76%) were seropositive for only one or two of the selected viruses. An exception was one Amazonian 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 neotropical 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.

The authors express their sincere appreciation to CENAP-IBAMA for the

samples as well as to all the researchers that collected them during their field projects throughout the country. We especially thank Rose Lilian Gasparini Morato and Otávio Borges Maia for having always been promptly helpful in conducting all legal paperwork requested, and Ronaldo Gonçalves Morato and Rogério Cunha de Paula for reporting sample origins and help in map figure preparation. We thank Dr. M. Meli, Dr. V. Cattori, Y. Ahmed, E. Gönçzi, T. Meili Prodan, B. Weibel, and N. Tschopp, Clinical Laboratory, Vetsuisse Faculty, University of Zurich, for excellent technical assistance. Laboratory work was performed using the logistics of the Centre for Clinical Studies at the Vetsuisse Faculty, University of Zurich. We are also indebted to Fundação Parque Zoológico de São Paulo, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES, Pró-Reitoria de Pós-Graduação from Universidade de São Paulo and the International Relations Office, University of Zurich, Switzerland. Regina Hofmann-Lehmann is the recipient of a professorship by the Swiss National Science Foundation (PP00B-102866). This study was conducted by Claudia Filoni as partial fulfillment of the requirements for a doctorate degree at Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo.

LITERATURE CITED

- BARKER, I. K., AND C. R. PARISH. 2001. Parvovirus infections. In *Infectious diseases of wild animals*, 3rd Edition, E. S. Williams and I. A. Barker (eds.). Iowa State University Press, Iowa, pp. 131–146.
- BROWN, E. W., S. MITHTHAPALA, AND S. J. O'BRIEN. 1993. Prevalence of exposure to feline immunodeficiency virus in exotic felid species. *Journal of Zoo and Wildlife Medicine* 24: 357–364.
- CARPENTER, M. A., E. W. BROWN, M. CULVER, W. E. JOHNSON, J. PECON-SLATTERY, D. BROUSSET, AND S. J. O'BRIEN. 1996. Genetic and phylogenetic divergence of feline immunodeficiency virus in the puma (*Puma concolor*). *Journal of Virology* 70: 6682–6693.
- CHANG, C. C., B. B. CHOMEL, R. W. KASTEN, V.

- ROMANO, AND N. TIETZE. 2001. Molecular evidence of *Bartonella* spp. in questing adult *Ixodes pacificus* ticks in California. *Journal of Clinical Microbiology* 39: 1221–1226.
- CHOMEL, B. B., R. W. KASTEN, K. FLOYD-HAWKINS, B. CHI, K. YAMAMOTO, J. ROBERTS-WILSON, A. N. GURFIELD, R. C. ABBOTT, N. C. PEDERSEN, AND J. E. KOEHLER. 1996. Experimental transmission of *Bartonella henselae* by the cat flea. *Journal of Clinical Microbiology* 34: 1952–1956.
- , H. J. BOULOUIS, AND E. B. BREITSCHWERDT. 2004a. Cat scratch and other zoonotic *Bartonella* infections. *Journal of the American Veterinary Medical Association* 224: 1270–1279.
- , Y. KIKUCHI, J. S. MARTENSON, M. E. ROELKE-PARKER, C. CHANG, R. W. KASTEN, J. E. FOLEY, J. LAUDRE, K. MURPHY, P. K. SWIFT, V. L. KRAMER, AND S. J. O'BRIEN. 2004b. Seroprevalence of *Bartonella* infection in American free-ranging and captive pumas (*Felis concolor*) and bobcats (*Lynx rufus*). *Veterinary Research* 35: 233–241.
- DANIELS, M. J., M. C. GOLDR, O. JARRETT, AND D. W. MACDONALD. 1999. Feline viruses in wildcats from Scotland. *Journal of Wildlife Diseases* 35: 121–124.
- DASZAK, P., A. A. CUNNINGHAM, AND A. D. HYATT. 2000. Emerging infectious diseases on wildlife—threats to biodiversity and human health. *Science* 287: 443–449.
- FILONI, C. F., 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. *Journal of Zoo and Wildlife Medicine* 34: 65–68.
- FROMONT, E., A. SAGER, F. LÉGER, F. BOURGUEMESTRE, E. JOUQUELET, P. STAHL, D. PONTIER, AND M. ARTOIS. 2000. Prevalence and pathogenicity of retroviruses in wildcats in France. *The Veterinary Record* 146: 317–319.
- GLAUS, T., R. OFMANN-LEHMANN, C. GREENE, B. GLAUS, C. WOLFENBERGER, AND H. LUTZ. 1997. Seroprevalence of *Bartonella henselae* infection and correlation with disease status in cats in Switzerland. *Journal of Clinical Microbiology* 35: 2883–2885.
- GUT, M., C. M. LEUTENEGGER, J. B. HUDER, N. C. PEDERSEN, AND H. LUTZ. 1999. One-tube fluorogenic reverse transcription-polymerase chain reaction for the quantification of feline coronavirus. *Journal of Virological Methods* 77: 37–46.
- HOFMANN-LEHMANN, R., E. HOLZNAGEL, A. AUBERT, P. OSSENT, M. REINACHER, AND H. LUTZ. 1995. Recombinant FeLV vaccine: Long-term protection and effect on course and outcome of FIV infection. *Veterinary Immunology and Immunopathology* 46: 127–137.
- , D. FEHR, M. GROB, M. ELGIZOLI, C. PACKER, J. MARTENSON, S. J. O'BRIEN, AND H. LUTZ. 1996. Prevalence of antibodies to feline parvovirus, calicivirus, herpesvirus, coronavirus, and immunodeficiency virus and of feline leukemia virus antigen and the interrelationship of these viral infections in free-ranging lions in East Africa. *Clinical and Diagnostic Laboratory Immunology* 3: 554–562.
- , J. B. HUDER, S. GRUBER, F. BORETTI, B. SIGRIST, AND H. LUTZ. 2001. Feline leukaemia provirus load during the course of experimental infection and in naturally infected cats. *Journal of General Virology* 82: 1589–1596.
- JESSUP, D. A., K. C. PETTAN, L. J. LOWENSTINE, AND N. C. PEDERSEN. 1993. Feline leukemia virus infection and renal spirochetosis in a free-ranging cougar (*Felis concolor*). *Journal of Zoo and Wildlife Medicine* 24: 73–79.
- KELLY, P. J., J. J. ROONEY, E. I. MARSTON, D. C. JONES, AND R. L. REGNER. 1998. *Bartonella henselae* isolated from cats in Zimbabwe. *Lancet* 351: 1706.
- KENNEDY-STOSKOPF, S. 1999. Emerging viral infections in large cats. In *Zoo and Wild Animal Medicine*, 4th Edition, M. E. Fowler and R. E. Miller (eds.). W. B. Saunders, Philadelphia, Pennsylvania, pp. 401–410.
- KUMMROW, M., M. L. MELI, M. HAESSIG, E. GOENCZI, A. POLAND, N. C. PEDERSEN, R. HOFMANN-LEHMANN, AND H. LUTZ. 2005. Feline coronavirus serotypes 1 and 2: Seroprevalence and association with disease in Switzerland. *Clinical and Diagnostic Laboratory Immunology* 12: 1209–1215.
- LABRUNA, M. B., R. S. P. JORGE, D. A. SANA, A. T. A. JÁCOMO, C. K. KASHIVAKURA, M. M. FURTADO, C. FERRO, S. A. PEREZ, L. SILVEIRA, T. S. SANTOS, JR., S. R. MARQUES, R. G. MORATO, A. NAVA, C. H. ADANIA, R. H. F. TEIXEIRA, A. A. B. GOMES, V. CONFORTI, F. C. C. AZEVEDO, C. S. PRADA, J. C. R. SILVA, A. F. BATISTA, M. F. V. MARVULO, R. L. G. MORATO, C. J. R. ALHO, A. PINTER, P. M. FERREIRA, F. FERREIRA, AND D. M. BARROS-BATTESTI. 2005. Ticks (Acari: Ixodida) on wild carnivores in Brazil. *Experimental and Applied Acarology* 36: 149–163.
- LEAL, E. S., AND A. P. RAVAZZOLO. 1998. Detecção do vírus da imunodeficiência felina (FIV) em felídeos selvagens pertencentes à Região Neotropical, através da técnica de reação em cadeia da polimerase (PCR). *A Hora Veterinária* 17: 57–60.
- LEUTENEGGER, C. M., R. HOFMANN-LEHMANN, C. RIOLS, M. LIBEREK, G. WOREL, P. LUPS, D. FEHR, M. HARTMANN, P. WEILENMANN, AND H. LUTZ. 1999a. Viral infections in free-living populations of the European wildcat. *Journal of Wildlife Diseases* 35: 678–686.
- , D. KLEIN, R. HOFMANN-LEHMANN, C. MISLIN, U. HUMMEL, J. BÖNI, F. BORETTI, W. H. GUENZBURG, AND H. LUTZ. 1999b. Rapid feline immunodeficiency virus provirus quantitation by

- polymerase chain reaction using the TaqMan® fluorogenic real-time detection system. *Journal of Virological Methods* 75: 105–116.
- LINARDI, P. M., AND L. R. GUIMARÃES. 2000. Sifonápteros do Brasil. Museu de Zoologia USP/FAPESP, São Paulo, São Paulo, Brasil, 291 pp.
- LUTZ, H., N. PEDERSEN, J. HIGGINS, U. HÜBSCHER, F. A. TROY, AND G. H. THEILEN. 1980. Humoral immune reactivity to feline leukemia virus and associated antigens in cats naturally infected with feline leukemia virus. *Cancer Research* 40: 3642–3651.
- , ———, R. DURBIN, AND G. H. THEILEN. 1983. Monoclonal antibodies to three epitopic regions of feline leukemia virus p27 and their use in enzyme-linked immunosorbent assay of p27. *Journal of Immunological Methods* 56: 209–220.
- , P. ARNOLD, U. HUBSCHER, H. EGBERINK, N. PEDERSEN, AND M. C. HORZINEK. 1988. Specificity assessment of feline T-lymphotropic lentivirus serology. *Zentralblatt Veterinarmedizin Riehe B* 35: 773–778.
- MELI, M., A. KIPAR, C. MULLER, K. JENAL, E. GONCZI, N. BOREL, D. GUNN-MOORE, S. CHALMERS, F. LIN, M. REINACHER, AND H. LUTZ. 2004. High viral loads despite absence of clinical and pathological findings in cats experimentally infected with feline coronavirus (FCoV) type I and in naturally FCoV-infected cats. *Journal of Feline Medicine and Surgery* 6: 69–81.
- MOCHIZUKI, M., M. AKUZAWA, AND H. NAGATOMO. 1990. Serological survey of the Iriomote cat (*Felis iriomotensis*) in Japan. *Journal of Wildlife Diseases* 26: 236–245.
- MOLIA, S., B. B. CHOMEL, R. W. KASTEN, C. LEUTENEGGER, B. R. STEELE, L. MARKER, J. S. MARTENSON, D. F. KEET, R. G. BENGIS, R. P. PETERSON, L. MUNSON, AND S. J. O'BRIEN. 2004. Prevalence of *Bartonella* infection in wild African lions (*Panthera leo*) and cheetahs (*Acinonyx jubatus*). *Veterinary Microbiology* 100: 31–41.
- MURRAY, D. L., C. A. KAPKE, J. F. EVERMANN, AND T. K. FULLER. 1999. Infectious disease and the conservation of free-ranging large carnivores. *Animal Conservation* 2: 241–254.
- OLMSTED, R. A., R. LANGLEY, M. E. ROELKE, R. M. GOEKEN, D. ADGER-JOHNSON, J. GOFF, J. P. ALBERT, C. PACKER, M. K. LAURENSEN, T. M. CARO, L. SCHEEPERS, D. E. WILDT, M. BUSH, J. S. MARTENSON, AND S. J. O'BRIEN. 1992. Worldwide prevalence of lentivirus infection in wild feline species: Epidemiologic and phylogenetic aspects. *Journal of Virology* 66: 608–6018.
- OSTROWSKI, S., M. VAN VUUREN, D. M. LENAIN, AND A. DURAND. 2003. A serologic survey of wild felids from Central West Saudi Arabia. *Journal of Wildlife Diseases* 39: 696–701.
- PAUL-MURPHY, J., T. WORK, D. HUNTER, E. MCFIE, AND D. FJELLINE. 1994. Serologic survey and serum biochemical reference ranges of the free-ranging mountain lion (*Felis concolor*) in California. *Journal of Wildlife Diseases* 30: 205–215.
- PRETORIUS, A. M., J. M. KUYL, D. R. ISHERWOOD, AND R. J. BIRTLES. 2004. *Bartonella henselae* in African Lion, South Africa. *Emerging Infectious Diseases* 10: 2257–2258.
- ROTSTEIN, D. S., S. K. TAYLOR, J. BRADLEY, AND E. B. BREITSCHWERDT. 2000. Prevalence of *Bartonella henselae* antibody in Florida panther. *Journal of Wildlife Diseases* 36: 157–160.
- SCHMITT, A. C., D. REISCHAK, C. L. CAVLAC, C. H. L. MONFORTE, F. T. COUTO, A. B. P. F. ALMEIDA, D. G. G. SANTOS, L. SOUZA, C. ALVES, AND K. VECCHI. 2003. Infecção pelos vírus da leucemia felina e da peritonite infecciosa felina em felídeo selvagem de vida livre e de cativeiro da região do Pantanal matogrossense. *Acta Scientiae Veterinariae* 31: 185–188.
- SCHOULS, L., I. VAN DE POL, S. G. T. RIJPKEMA, AND C. S. SCHOT. 1999. Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. *Journal of Clinical Microbiology* 37: 2215–2222.
- SLEEMAN, J. M., J. M. KEANE, J. S. JOHNSON, R. J. BROWN, AND S. VANDE WOUDE. 2001. Feline leukemia in a captive bobcat. *Journal of Wildlife Diseases* 37: 194–200.
- VAN VUUREN, M., E. STYLIANIDES, S. A. KANIA, E. E. ZUCKERMAN, AND W. D. HARDY, JR. 2003. Evaluation of an indirect enzyme-linked immunosorbent assay for the detection of feline lentivirus-reactive antibodies in wild felids, employing a puma lentivirus-derived synthetic peptide antigen. *Onderstepoort Journal of Veterinary Research* 70: 1–6.
- VOGTLIN, A., C. FRAEFEL, S. ALBINI, C. M. LEUTENEGGER, E. SCHRANER, B. SPIESS, H. LUTZ, AND M. ACKERMANN. 2002. Quantification of feline herpesvirus 1 DNA in ocular fluid samples of clinically diseased cats by real-time Taqman PCR. *Journal of Clinical Microbiology* 40: 519–523.
- YAMAMOTO, K., B. B. CHOMEL, L. J. LOWENSTINE, Y. KIKUCHI, L. G. PHILIPS, B. C. BARR, P. K. SWIFT, K. R. JONES, S. P. RILEY, R. W. KASTEN, J. E. FOLEY, AND N. C. PEDERSEN. 1998. *Bartonella henselae* antibody prevalence in free-ranging and captive wild felids from California. *Journal of Wildlife Diseases* 34: 56–63.

Received for publication 24 March 2005.