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Molecular Detection of *Cytauxzoon* spp. in Asymptomatic Brazilian Wild Captive Felids

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ABSTRACT: *Cytauxzoon* spp. DNA was detected for the first time in blood samples from asymptomatic Brazilian wild captive felids. In 2006, 72 EDTA blood samples from seven wild felids species: *Puma concolor* (puma), *Leopardus pardalis* (ocelot), *Puma yagouaroundi* (jaguarundi), *Leopardus wiedii* (margay), *Leopardus tigrinus* (little spotted cat), *Oncifelis colocolo* (pampas cat) and *Panthera onca* (jaguar) were analyzed using polymerase chain reaction to amplify the 18S rRNA gene segment in order to verify the presence of *Cytauxzoon* spp. DNA. Nine samples were positive: six ocelots, two pumas, and one jaguar. In Brazil, wild felids may be natural reservoirs for *Cytauxzoon* spp.

Key words: Brazil, *Cytauxzoon* spp., PCR, wild felids.

Cytauxzoonosis is a tick-borne disease caused by *Cytauxzoon felis*, a piroplasm belonging to the family Theileriidae. In vertebrates, the life cycle includes an intraerythrocytic phase and a tissue phase consisting of large schizonts that develop in macrophages and monocytes. The tissue phase is critical in the pathology that develops in acutely ill cats (Greene et al., 2006). In North America, bobcats (*Lynx rufus*) are considered the natural hosts of *C. felis*, and there is a high prevalence of *C. felis* in this species. Bobcats are generally able to survive the schizogenous phase of infection and develop a persistent subclinical parasitemia (Glenn et al., 1982; Blouin et al., 1984, 1987; Kocan and Blouin, 1985); however, a fatal case of cytauxzoonosis in a free-ranging bobcat has been reported (Nietfield and Pollock, 2002).

The Florida panther (*Puma concolor coryi*) is another possible host for *C. felis* (Butt et al., 1991; Rotstein et al., 1999). In addition, subclinical infection has also been demonstrated in the cougar (*Puma concolor stanleyana*; Rotstein et al., 1999), and *C. felis* DNA has also been reported from Iberian lynx (*Lynx pardinus*; Luaces et al., 2005; Millán, et al., 2007).

Domestic cats are considered as dead-end hosts and, in cats, *C. felis* causes an acute, highly fatal febrile disease (Hoskins, 1991). However, apparently healthy but infected free-roaming cats have been found, suggesting that domestic cats may be an additional host for *C. felis* (Meinkoth et al., 2000; Haber et al., 2007). *Cytauxzoon manul*, a closely related piroplasm, was reported parasitizing Pallas cats (*Otocolobus manul*) in Mongolia (Ketz-Riley et al., 2003; Reichard et al., 2005). When domestic cats were inoculated with Pallas cat blood that was infected with *C. manul*, they showed a parasitemia but did not present signs of the disease. Furthermore, the inoculation with *C. manul* did not provide protection against *C. felis* (Joyner et al., 2007).

The tick *Dermacentor variabilis* has been experimentally demonstrated as a vector of *C. felis* from bobcats to domestic cats (Blouin et al., 1984). Among wild felids, fatal cases of cytauxzoonosis were reported in a captive tiger (*Panthera tigris*; Garner et al., 1996), a bobcat (Nietfield and Pollock, 2002), and in captive-reared lions in Brazil (Peixoto et al., 2007). The present work aimed at assessing the presence of DNA of *C. felis* in Brazilian wild captive felids using 18S rRNA polymerase chain reaction (PCR).

In 2006, blood samples were collected (using the IBAMA [Brazilian Institute of Environment and Renewable Natural Resources] license number 02027.002943/2005) from 72 Brazilian wild felids belonging to the following species: *Puma concolor*

TABLE 1. The number and origin of each species of sampled Brazilian captive wild felids.

Species	Common name	Associação Mata Ciliar Jundiaí	Pedreira Zoo	Campinas Zoo	Ribeirão Preto Zoo	Brasília Zoo	No. of animals
Leopardus pardalis	Ocelot	24	0	0	2	3	29
Leopardus tigrinus	Little spotted cat	8	0	1	2	3	14
Leopardus wiedii	Margay	2	0	0	0	0	2
Oncifelis colocolo	Pampas cat	3	0	0	0	0	3
Panthera onca	Jaguar	1	4	1	2	1	9
Puma concolor	Puma	3	2	0	1	3	9
Puma yagouaroundi	Jaguarundi	6	0	0	0	0	6
TOTALS		47	6	2	7	10	72

(puma), *Leopardus pardalis* (ocelot), *Puma yagouaroundi* (jaguarundi), *Leopardus wiedii* (margay), *Leopardus tigrinus* (little spotted cat), *Oncifelis colocolo* (pampas cat) and *Panthera onca* (jaguar). These wild felids were maintained in captivity at the Associação Mata Ciliar (AMC) Jundiaí, São Paulo, Brazilian Center for Conservation of Neotropic Felids (23°11′11″S, 46°53′03″W) and at the Campinas (22°54′20″S, 47°03′39″W), Pedreira (22°44′31″S, 46°54′05″W), Ribeirão Preto (21°10′39″S, 47°48′37″W) and Brasília (15°50′16″S, 47°°42′48″W) Zoos (Table 1).

DNA was extracted from 200 µl of whole blood using the QIAamp DNA Blood Mini kit (QIAGEN, Valentia, California, USA) according to the manufacturer instructions. Each extracted DNA sample was used as a template in 50-µl reaction mixtures containing 10× polymerase chain reaction (PCR) buffer, 1.5 mM $MgCl_2$, 200 µM deoxynucleotide triphosphate (dNTPs) mixture, 25 pmol of each primer, and 1.25 U of DNA Taq polymerase (Invitrogen, Carlsbad, California, USA). Primer sequences, based on the 18S rRNA gene, were: 5′-GCGAATCG-CATTGCTTTATGCT-3′ and 5′-CCAAA-TGATACTCCGGAAAGAG-3′. The thermal cycling conditions consisted of an initial denaturation at 95 C for 5 min, followed by 40 amplification cycles (95 C for 45 sec, 59 C for 45 sec, and 72 C for 5 min) using a Perkin-Elmer™ model PT-200 gradient cycler (Perkin Elmer, Waltham, Massachusetts, USA; Birkenheuer et al., 2006). In each set of amplifications, both positive and negative controls were included. A confirmation sequence was obtained for positive amplicons. In order to prevent amplicon contamination, precautions taken included the use of disposable gloves and a "clean-to-dirty" flow of procedures. In addition, sample processing, DNA extraction, reaction setup, PCR amplification, and post-amplification processing were performed in separate rooms to avoid amplicon contamination. Positive samples were immediately sequenced bidirectionally. Comparisons with sequences deposited in GenBank were carried out using the basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov/Blast.cgi).

Only nine animals (13%) were found to be PCR positive: two ocelots, two pumas, and one jaguar from the Brasilia Zoo, and four ocelots from the Associação Mata Ciliar (Jundiaí, São Paulo; Fig. 1). The sequences were deposited in GenBank under accession numbers EU376525, EU376526, and EU376527. The *Cytauxzoon* spp. DNA found was 99% similar to that deposited in the GenBank from a Brazilian *Leopardus tigrinus* (accession no. DQ382277) and 98% similar to *C. felis* from North American cats (accession no. AF399930).

The prevalence observed in the present study was similar to that reported in lynx from Spain (Millán et al., 2007). Kocan and Blouin (1985) found piroplasms similar to *C. felis* in five out of 16 bobcats from Oklahoma, USA. A prevalence of

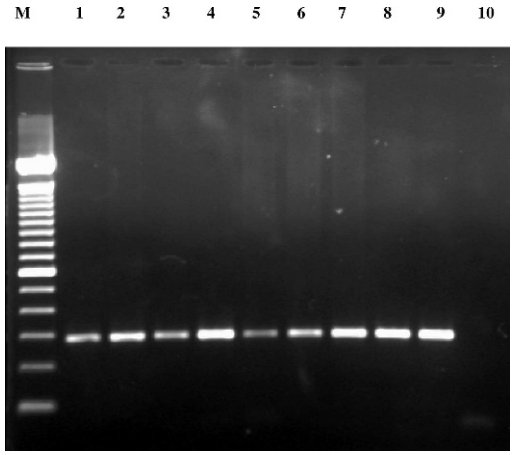

FIGURE 1. Agarose gel electrophoresis demonstration of *Cytauxzoon sp* PCR. Lane M: molecular standard size. Lane 1=jaguar positive sample (284 pb); Lane 2 through 7=ocelot positive samples; Lanes 8 and 9=positive puma samples; Lane 10=negative control.

39% and 35% were found among Texas cougars and Florida panthers, respectively, in Florida, USA (Rotstein et al., 1999).

In Brazil, Mendes-de-Almeida et al. (2007) found piroplasms similar to *C. felis* in 59% of feral cats in a colony in Rio de Janeiro, Brazil. However, the first confirmed report of *C. felis* in South America was made by Peixoto et al. (2007) in a lion. The diagnosis was established by the finding of the characteristic schizonts in endothelial-associated macrophages from two lions with fatal cytauxzoonosis. The present work reports the first molecular detection of *C. felis* in Brazilian ocelots, pumas, and jaguars.

The nine parasitized wild felids were sampled in the summer and none had clinical signs of the disease. This finding was also observed by Millán et al. (2007) with Iberian lynxes. Although impossible to analyze in this study, the season may affect the prevalence of *Cytauxzoon* spp. in Brazilian wild felids due to the life cycle of the unknown tick vector in Brazil. It was not possible to determine how the wild felids became infected. Fleas (*Ctenocephalides canis*) were collected from animals from Jundiaí. No amplified product was found by *C. felis* PCR using DNA extracted from these fleas. In Brazil, Labruna et al. (2002) observed *Amblyomma cajennense*, *A. coelebs*, and nonidentified larva of *Amblyomma* spp. in free-roaming pumas; and *Rhipicephalus (Boophilus) microplus* and nonidentified immature instars of *Amblyomma* spp. on free-ranging jaguars. In the present study, ticks were not found parasitizing any of the study animals.

All the infected wild felids of the study were apparently healthy. Brazilian wild felids may be potential reservoirs for *C. felis* and, like bobcats (Glenn et al., 1982; Blouin et al., 1984; 1987; Kocan and Blouin, 1985) and Florida panthers (Butt et al., 1991; Rotstein et al., 1999) in the United States, Brazilian wild felids did not appear to be clinically infected; however, the possibility of disease in Brazilian wild felids cannot be discarded based solely on these limited observations. *Cytauxzoon felis* caused a fatal illness in a wild bobcat (Nietfeld and Pollock, 2002) and a tiger (Garner et al., 1996) in the United States and in two lions in Brazil (Peixoto et al., 2007). Disease risks may exist related to both translocations and reintroductions of wild felids and to interactions with domestic felids (Millán et al., 2007).

To the authors' knowledge, this is the first molecular detection of *Cytauxzoon* spp. in the Brazilian ocelot, puma, and jaguar. Future studies must focus on vector and natural reservoirs of *C. felis* among Brazilian wild and domestic cats, with an aim to elucidate the epidemiology of this disease among wild and domestic felids in Brazil.

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Jequitibás de Campinas, Bosque/Zoológico Municipal de Pedreira, and Bosque Municipal Fábio Barreto de Ribeirão Preto.

LITERATURE CITED

Birkenheuer, A. J., H. Marr, A. R. Alleman, M. G. Levy, and E. B. Breitschwerdt. 2006. Development and evaluation of a PCR assay for the detection of *Cytauxzoon felis* DNA in feline blood samples. Veterinary Parasitology 137: 144–149.

Blouin, E. F., A. A. Kocan, B. L. Glenn, and K. M. Kocan. 1984. Transmission of *Cytauxzoon felis* Kier, 1979 from bobcats, *Felis rufus* (Schreber), to domestic cats by *Dermacentor variabilis* (Say 1821). Journal of Wildlife Diseases 20: 241–242.

———, ———, and K. M. Kocan. 1987. Evidence of a limited schizogonous cycle for *Cytauxzoon felis* in bobcats following exposure to infected ticks. Journal of Wildlife Diseases 23: 499–501.

Butt, M. T., D. Bowman, M. C. Barr, and M. E. Roelke. 1991. Iatrogenic transmission of *Cytauxzoon felis* from a Florida panther (*Felis concolor coryi*) to a domestic cat. Journal of Wildlife Diseases 27: 342–347.

Garner, M. M., M. P. Lung, S. Citino, E. C. Greiner, J. W. Harvey, and B. L. Homer. 1996. Fatal cytauxzoonosis in a captive-reared white tiger (*Panthera tigris*). Veterinary Pathology 33: 82–86.

Glenn, B. L., R. E. Rolley, and A. A. Kocan. 1982. *Cytauxzoon*-like piroplasms in erythrocytes of wild-trapped bobcats in Oklahoma. Journal of American Veterinary Medical Association 181: 1251–1253.

Greene, C. E., J. Meinkoth, and A. A. Kocan. 2006. Cytauxzoonosis. *In* Infectious diseases of dog, cat, 3rd Edition, C. E. Greene (ed.). Saunders Elsevier, St. Louis, Missouri, pp. 722–733.

Haber, M. D., M. D. Tucker, H. S. Marr, J. K. Levy, J. Burgess, M. R. Lappin, and A. J. Birkenheuer. 2007. The detection of *Cytauxzoon felis* in apparently healthy free-roaming cats in the USA. Veterinary Parasitology 146: 316–320.

Hoskins, J. D. 1991. Canine haemobartonellosis, canine hepatozoonosis and feline cytauxzoonosis. Veterinary Clinics of North America: Small Animal Practice 21: 129–140.

Joyner, P. H., M. V. Reichard, J. H. Meinkoth, V. E. Milne, A. W. Confer, A. A. Kocan, and J. P. Hoover. 2007. Experimental infection of domestic cats (*Felis domesticus*) with *Cytauxzoon manul* from Pallas' cats (*Otocolobus manul*). Veterinary Parasitology 146: 302–306.

Ketz-Riley, C. J., M. V. Reichard, R. A. Van den Bussche, J. P. Hoover, J. Meinkoth, and A. A. Kocan. 2003. An intraerythrocytic small piroplasm in wild-caught Pallas' cats (*Otocolobus manul*) from Mongolia. Journal of Wildlife Diseases 39: 424–430.

Kocan, A. A., and E. F. Blouin. 1985. Hematologic and serum chemical values for free-ranging bobcats, *Felis rufus* (Schreber) with reference to animals with natural infections of *Cytauxzoon felis* Kier, 1979. Journal of Wildlife Diseases 21: 190–192.

Labruna, M. B., C. D. De Paula, T. F. Lima, and D. A. Sana. 2002. Ticks (Acari: Ixodidae) on wild animals from the Port–Primavera Hydroeletric Power Station area, Brazil. Memórias do Instituto Oswaldo Cruz 97: 1113–1136.

Luaces, I., E. Aguirre, M. Garcia-Montijano, J. Velarde, M. A. Tesouro, C. Sánchez, M. Galka, P. Fernández, and A. Sainz. 2005. First report of an intra-erythrocytic small piroplasm in wild Iberian lynx (*Lynx pardinus*). Journal of Wildlife Diseases 41: 810–815.

Meinkoth, J., A. A. Kocan, L. Whitworth, G. Murphy, J. C. Fox, and J. P. Woods. 2000. Cats surviving natural infection with *Cytauxzoon felis*: 18 cases (1997–1998). Journal of Veterinary Internal Medicine 14: 521–525.

Mendes-De-Almeida, F., N. Labarthe, J. Guerrero, M. C. F. Faria, A. S. Branco, C. D. Pereira, J. D. Barreira, and M. J. S. Pereira. 2007. Follow-up of the health conditions of an urban colony of free-roaming cats (*Felis catus* Linnaeus, 1758) in the city of Rio de Janeiro. Veterinary Parasitology 147: 209–211.

Millán, J., V. Naranjo, A. Rodríguez, J. M. P. La Lastra, A. J. Mangold, and J. La Fuente. 2007. Prevalence of infection and 18S rRNA gene sequences of *Cytauxzoon* species in Iberian lynx (*Lynx pardinus*) in Spain. Parasitology 134: 995–1001.

Nietfield, J. C., and C. Pollock. 2002. Fatal cytauxzoonosis in a free-ranging bobcat (*Lynx rufus*). Journal of Wildlife Diseases 38: 607–610.

Peixoto, P. V., C. O. Soares, A. Scofield, C. D. Santiago, T. N. Frana, and S. S. Barros. 2007. Fatal cytauxzoonosis in captive-reared lions in Brazil. Veterinary Parasitology 145: 383–387.

Reichard, M. V., R. A. Van den Bussche, J. Meinkoth, J. P. Hoover, and A. A. Kocan. 2005. A new species of *Cytauxzoon* from Pallas' cats caught in Mongolia and comments on the systematics and taxonomy of piroplasmids. Journal of Parasitology 91: 420–426.

Rotstein, D. S., S. K. Taylor, J. W. Harvey, and J. Bean. 1999. Haematologic effects of cytauxzoonosis in Florida panthers and Texas cougars in Florida. Journal of Wildlife Diseases 35: 613–617.

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