Heartworm (Dirofilaria immitis) disease in a Brazilian oncilla (Leopardus tigrinus)

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ABSTRACT.- Filoni C., Pena H.F.J., Gennari S.M., Cristo D.S., Torres L.N. & Catão-Dias J.L. 2009. Heartworm (Dirofilaria immitis) disease in a Brazilian oncilla (Leopardus tigrinus). Pesquisa Veterinária Brasileira 29(6):474-478. Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva 87, Cidade Universitária, São Paulo, SP 05508-270, Brazil. E-mail: claudiafiloni@triade.org.br

Heartworm disease is caused by the intravascular nematode Dirofilaria immitis, a pathogen of public health importance usually associated to domestic dogs and cats, and to a lesser extend to other mammal species. The oncilla (Leopardus tigrinus) is a threatened neotropic felid species that naturally occurs in Brazil. Here, we report the encounter of adult and larval stages of heartworms in a female specimen of L. tigrinus, probable of free-ranging origin, from Ubatuba, São Paulo, Brazil, which died showing clinical signals compatible with heartworm disease. This was the first reported case of D. immitis infection and associated disease in L. tigrinus, also suggesting that the oncilla acted as a definitive host for this parasite. The present findings confirmed D. immitis as a pathogenic agent for this felid species, thus supporting the recommendation for the inclusion of diagnostic testing for this pathogen in routine health screening procedures for captive and free-ranging oncillas in Brazil, especially in those localities where climate conditions support the occurrence of the parasite. Potential reservoirs as oncillas are established beyond the reach of veterinary care, thus representing a continuing risk for domestic animals and humans acquiring heartworm infection. We encourage further serologic and molecular studies aiming to establish D. immitis prevalences in L. tigrinus and other wild carnivores in the region of Ubatuba, as well as ecological and veterinary studies to access the role of this pathogen for the survival of this threatened felid species.

INDEX TERMS: Dirofilaria immitis, heartworm disease, Leopardus tigrinus, neotropic felid, Brazil.
em *L. tigrinus*, sugerindo também que o gato-do-mato-pequeno agiu como hospedeiro definitivo para este parasita. Estes achados confirmaram que *D. immitis* é um agente patogênico para esta espécie de felino, sugerindo a recomendação para a inclusão de testes diagnósticos para este patógeno em procedimentos rotineiros de avaliação da saúde para gatos-do-mato-pequenos mantidos em caçoeiro e de vida livre no Brasil, especialmente em localidades onde as condições climáticas permitam a ocorrência do parasita. Os gatos-do-mato-pequenos são potenciais reservatórios da *D. immitis*, mas vivem fora do alcance veterinário, representando desta forma um risco contínuo para animais domésticos e humanos. Encorajamos a realização de outros estudos epidemiológicos a fim de estabelecer as prevalências de infecção por *D. immitis* em *L. tigrinus* e outros carnívoros selvagens na região de Ubatuba, assim como estudos ecológicos e médicos veterinários para se acessar o papel deste patógeno para a sobrevivência desta espécie ameaçada de extinção.

**TERMOS DE INDEXAÇÃO:** Dirofilaria immitis, doença do verme do coração, *Leopardus tigrinus*, felídeo neotropical, Brasil.

**INTRODUCTION**

The oncilla (*Leopardus tigrinus*), also known as little spotted cat, is the smallest Brazilian felid species, resembling in size and body structure the domestic cat (*Felis catus*) (Nowell & Jackson 1996). The oncilla naturally occurs in a variety of habitats along the neotropic region; despite this, the species is considered near threatened by the World Conservation Union (IUCN 2007), vulnerable by the Brazilian National List of Threatened Species (Brasil 2008), and is listed in the restrictive Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2008). Only recently, important data regarding ecological, behavioral, genetic, and distribution aspects has been obtained for the species in Brazil (Oliveira 2008)

Heartworm disease or dirofilariosis is caused by the arthropod transmitted filarid nematode *Dirofilaria immitis*. Domestic dogs (*Canis familiaris*) are the usual definitive host for heartworm. Several other mammal species, particularly carnivores, may become infected in endemic areas. These include domestic cats and wild felids, wild canids, sea lions, mustelids, otariids, rodents as muskrats, horses and, rarely, humans as incidental hosts (Anderson 2001). All these species may not be definitive hosts for *D. immitis* and the effects of this parasite on the health and ecology are still unclear for many of these species (Matsuda et al. 2003, Nelson et al. 2003). *D. immitis* has been detected in several species of captive wild felids. These included an African lion (*Panthera leo*) (Ruiz de Ibáñez et al. 2006), a snow leopard (*Uncia uncia*) (Murata et al. 2003), a leopard (*Panthera pardus*), a tiger (*Panthera tigris*), a flat-headed cat (*Prionailurus planiceps*), a leopard cat (*Prionailurus bengalensis*), and a clouded leopard (*Neofelis nebulosa*) (Zahedi et al. 1986). Among free-ranging felids, an ocelot (*L. pardalis*) from Texas, USA was reported harboring one adult heartworm in absence of detectable disease (Pence et al. 2003), and serum antibodies against this nematode have been detected in a North American puma (*Puma concolor*) (Paul-Murphy et al. 1994).

Heartworm has a tropical and subtropical distribution in southern Europe, Asia, Australia, and the Americas. Heartworm has been known to exist in the South America since the nineteenth century. However, canine heartworm prevalence is currently exhibiting a downward trend in all the Latin America. In Brazil, the national prevalence seems to have declined from about 8% in 1988 to 2% in 2001 (Labarthe et al. 2003, Labarthe & Guerrero 2005). The main reason for this reduction seemed to be the improvement of preventive veterinary care directed to domestic carnivores, as the populations of vectors have not declined (Labarthe & Guerrero 2005).

Adult heartworms usually live in pulmonary arteries and in the right chambers of the heart of their definitive hosts (Lister & Atwell 2008), but some have already described the parasite in the left cardiac chambers (Nelson et al. 2005). The female heartworms are viviparous, releasing microfilariae into the bloodstream. These are taken up by blood-sucking mosquitoes, where they develop to infective larvae and are inoculated into the dermis of the final host where, after several months of migration and maturation, reach the sites where they will live as adults (Simón et al. 2007, Litster & Atwell 2008). Pathological changes induced by heartworms are in part due to physical obstruction of the heart chambers, valves, and vessels. The immunopathology of infection is complex and the clinical manifestations largely depend on the immune response that is stimulated by the parasites (Simón et al. 2007). A pulmonary endarteritis develops, characterized by myointimal proliferations and obstructive fibrosis, which leads to pulmonary hypertension and congestive right heart disease (Robinson & Maxie 1993, Nelson et al. 2003, Simón et al. 2007). Surface proteins of *Wolbachia* bacterial endosymbiontics of heartworms are released, especially after death of the parasites, stimulating the host immune system and acting as important protagonists in the inflammatory pathology of heartworm disease (Simón et al. 2007). Dead adult heartworms cause tromboembolism, exacerbating the pulmonary hypertension and initiating a granulomatous reaction in the vessel wall that may extend to the pulmonary parenchyma. The death of adult heartworms can be associated with a peracute syndrome consisting of respiratory distress, ataxia, collapse, seizures, hemoptysis, and sudden death of the host (Nelson et al. 2005). In cats, severe complications can occur in the presence of even a small number of adult heartworms (Simón et al. 2007). Feline heartworm disease is characterized by strong vascular inflammatory reactions, frequent tromboembolisms, central nervous system and gastrointestinal involvement (Lister & Atwell 2008).

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The objective of this report was to present a case of heartworm disease in an oncilla from a coastal southeastern locality in Brazil.

MATERIALS AND METHODS
An adult female oncilla of unknown origin was attended by a veterinarian clinician in the town of Ubatuba (23° 26’ S, 45° 03’ W), São Paulo State, Brazil. Clinical exams revealed prostration, hypothermy (34-35°C), dyspnea, hemoptysis, and anisocory. Despite prompt veterinary care, the animal died within 24 hours. As the animal had been found sick in front of the clinic and there was none responsible for it, the governmental National Research Center for Carnivores Conservation (CENAP), an unity of Chico Mendes Institute Conservation of the Biodiversity (ICMBio), was notified accordingly (Process no. 02027007068/03-72). The carcass was then transported under refrigeration in 24 hours to the Laboratório de Patologia Comparada de Animais Selvagens (LAPCOM), Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade de São Paulo (USP). At FMVZ, the carcass was laterally radiographed and subsequently submitted to necropsy. Fragments of tissues were fixed in formalin, embedded in paraffin, routinely sectioned, and stained with hematoxylin and eosin (HE) in glass slides for histopathologic evaluation. The skin and skull were deposited at the Museum of Zoology of USP, and fragments of tissue were deposited at the Genome Resource Bank from CENAP and at FMVZ-USP.

RESULTS
Both in radiographic examination and at necropsy, no lesions compatible with trauma were detected. The most significant change observed at radiographic examination was cardiac enlargement compatible with congestive heart disease. At necropsy, the carcass showed satisfactory body condition and lactant status. The liver, kidneys, and lungs were congested. The right ventricle was enlarged and its walls were reduced in thickness with respect to the overall chamber dimensions, indicating eccentric hypertrophy. Additionally, four nematodes were present in cardiac chambers. The parasites were subsequently identified as adult *Dirofilaria immitis* filarids, according to their morphology under optical microscopy. These filarids were two males and one female at the right ventricle and a female at the left ventricle. Moderate presence of cestodes and nematodes was detected in small intestines. At histopathologic evaluation, lungs showed arteriolar fibromuscular proliferation, diffuse inter-alveolar fibrosis, perivascular eosinophytic inflammation, hemosiderosis, and presence of intravascular microfilaries in small arteries (Fig.1 and 2).

DISCUSSION
*Dirofilaria immitis* has already been described in several felid species (Zahedi et al. 1986, Paul-Murphy et al. 1994, Murata et al. 2003, Pence et al. 2003, Ruiz de Ibanez et al. 2006). However, most previous data of heartworm infection in nondomestic felids reported asymptomatic infections. Here we presented evidence of heartworm clinical disease in the oncilla. It is the first report of *D. immitis* infection in an oncilla and the first report that associated clinical disease to heartworm infection in a neotropic felid.

Gross and histopathologic findings were compatible with heartworm disease. The encounter of intravascular microfilariae, arteriolar fibromuscular proliferation, perivascular eosinophytic inflammation, and congestion in pulmonary tissue indicated the development of eosinophytic endoarteritis and secondary pulmonary hypertension. Pulmonary hypertension results in increased systolic pressure in right ventricle, leading to concentric cardiac hypertrophy that turns to eccentric hypertrophy in later stages (Van Vleet & Ferrans 1990), and to congestive right heart disease. The observed congestion on liver and kidneys are expected consequences of congestive right heart disease. The eccentric cardiac hypertrophy and the presence of inter-alveolar fibrosis suggested that the
heartworm disease in the oncilla had a chronic development. The animal seemed to have compensated the perturbations provoked by heartworm disease for an undetermined period of time before death.

The clinical signs could be explained by the pulmonary impairment and, possibly, also by heartworm antigens released in the bloodstream and/or microfilariae in brain vessels. It has been hypothesized that the sudden release of large quantities of heartworm antigens could result in systemic anaphylaxis (Litster & Atwell 2006, Litster & Atwell 2008). It was not possible to conclude that the oncilla died solely from complications of this disease as we did not know when and how the animal initiated to suffer from the acute life-threatening process. However, pregnancy, lactation, and handling may have further stressed the animal, worsening the physiopathologic imbalances caused by the heartworm infection.

It is broadly accepted that diseases are an increasing threat to wild felids (Nowell & Jackson 1996, Daszak et al. 2000, Funk et al. 2001). Unfortunately, available data on the occurrence of diseases in South American felid species is scarce, largely consisting of case reports and cross-sectional surveys, and usually are relied on small sample sizes (Furtado & Filoni 2008). Given this, the present report should be considered a start point to further evaluate the heartworm prevalences and its impact for the survival of this neotropical felid species.

CONCLUSIONS

This was the first report of Dirofilaria immitis infection in Leopardus tigrinus and account that associated clinical disease to D. immitis infection in a neotropical felid. This indicated that the parasite should be considered a pathogenic agent for this threatened felid species and diagnostic testing procedures for this pathogen should be included in routine health screening procedures for the species in Brazil, especially in those localities where climate conditions support the occurrence of the parasite.

The encounter of larval stages and adult male and female heartworms suggested that L. tigrinus acted as a definitive host for D. immitis and the species should be considered a potential wildlife reservoir for the parasite. The presence of wildlife reservoirs for D. immitis, similarly to stray dogs, implies in public health risks, even though preventive control programs have been shown efficient. On the other hand, potential microfilaremic reservoirs as oncillas and other wild carnivores are established beyond the reach of veterinary care, thus representing a continuing risk for domestic animals and humans acquiring heartworm infection. We encourage further epidemiologic studies aiming to establish D. immitis prevalences in L. tigrinus and other wild carnivores.

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