

Helminthfauna of road-killed cougars (*Puma concolor*) from the Northeastern Region of São Paulo State, Brazil

Helminthofauna de onças pardas atropeladas (*Puma concolor*) da região Nordeste do Estado de São Paulo, Brasil

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Abstract

As the second-largest Neotropical carnivore, cougars (*Puma concolor*) are especially important for maintenance of the biodiversity and ecosystem health. Five wild adult cougars (*Puma concolor*), found roadkilled in highways in the Northeastern region of São Paulo, were evaluated in search for parasites. Ten species representing nine families were identified. The most prevalent helminths were *Uncinaria bidens*, *Lagochilascaris major*, *Spirometra* sp., and *Oncicola canis*, followed by *Cylicospirura subaequalis*, *Toxascaris leonina*, *Taenia omissa*, *Echinococcus* sp., *Filaroides* sp. and *Oncicola oncolica*. It is important to note that some helminths found in this study, such as *L. major*, *Spirometra* sp., *O. oncolica*, *O. canis*, *Echinococcus* sp., *T. leonina*, *C. subaequalis*, and *Filaroides* sp. are known to affect domestic carnivores, which may indicate interaction between wild and domestic hosts. This study represents a new host record for four of the species found in cougars, *U. bidens*, *L. major*, *O. canis*, and *Filaroides* sp., and new locality records for *U. bidens*, *T. leonina*, *C. subaequalis*, and *Filaroides* sp.

Keywords: Felidae, Endoparasites, Nematoda, Cestoda, neotropical region, wildlife.

Resumo

Como o segundo maior carnívoro neotropical, as onças-pardas são muito importantes para a manutenção da biodiversidade e a saúde do ecossistema. Cinco pumas adultos de vida livre (*Puma concolor*), mortos em rodovias na região Nordeste de São Paulo, foram avaliados quanto a presença de parasitas. Dez espécies, representando nove famílias foram identificadas. Os helmintos mais prevalentes foram *Uncinaria bidens*, *Lagochilascaris major*, *Spirometra* sp., e *Oncicola canis*, seguidos por *Cylicospirura subaequalis*, *Toxascaris leonina*, *Taenia omissa*, *Echinococcus* sp., *Filaroides* sp. e *Oncicola oncolica*. É importante salientar que alguns dos helmintos encontrados neste estudo, tais como *Lagochilascaris major*, *Spirometra* sp., *O. oncolica*, *Oncicola canis*, *Echinococcus* sp., *Toxascaris leonina*, *Cylicospirura subaequalis*, e *Filaroides* sp., afetam sabidamente carnívoros domésticos, o que pode indicar a interação entre hospedeiros selvagens e domésticos. Este estudo representa novos registros de hospedeiro para quatro espécies de helmintos, sendo elas *U. bidens*, *L. major*, *O. canis*, and *Filaroides* sp., além de novo registro de localidade para *U. bidens*, *T. leonina*, *C. subaequalis*, e *Filaroides* sp.

Palavras-Chave: Felidae, Endoparasitas, Nematoda, Cestoda, região neotropical, vida Selvagem.

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As the second-largest Neotropical carnivore, cougars (*Puma concolor*) are especially important for biodiversity maintenance and ecosystem health (Melo et al., 2016). These felids occupy extensive home ranges, with low tolerance to overlapping, they are often observed in low population density (Culver, 2010). Even though, cougars are frequently observed in anthropized areas, as they are tolerant to human presence (Knopf et al., 2014). Human actions, especially agriculture and timber exploitation, wildfires and roadkills, are important factors linked to the reduction of the cougar population in several areas (Azevedo et al., 2013). The Northeastern region of São Paulo State is covered by Cerrado savanna and Atlantic rainforest, both strongly affected by deforestation, resulting in a highly fragmented landscape, composed of some large stretches and several small patches of natural vegetation surrounded by plantations of sugarcane and eucalyptus, cattle farms, and roads and highways (Miotto et al., 2012).

The helminths parasites of cougars are known from studies developed in USA and a few South American countries (Waid & Pence, 1988; Rickard & Foreyt, 1992; Noronha et al., 2002; Foster et al., 2006; Vieira et al., 2008; Pinto et al., 2011; Aranda et al., 2013; Moleón et al., 2015; Gomez-Puerta et al., 2016). Two checklists group the data of these studies, listing ten species of nematodes, four cestodes, and three acanthocephalans (Vicente et al., 1997; Vieira et al., 2008). Even though, there is little information on parasites affecting cougars in São Paulo State, one of the Brazilian States most affected by human action, leading to the destruction and fragmentation of the original Atlantic rainforest cover. The close interface between wild and urban landscapes may increase parasite exchange between domestic and wild carnivores, as well as to humans. The aim of this study was to describe the parasites of roadkilled cougars collected in an anthropized area from São Paulo State, Brazil.

Between the years 2016 and 2018, five road-killed cougars were referred to the Service of Wild Animal Pathology (SEPAS, FCAV/UNESP Jaboticabal). The highways where the animals were found run-over comprise four cities: Taquaritinga (21°24'21"S; 48°30'18"W), Matão (21°36'10"S; 48°21'57"W), Taquaral (21°04'19"S; 48°24'36"W), and Borborema (21°37'12"S; 49°04'26"W). After necropsy, the organs of each animal were individualized, slit open in metallic trays and thoroughly washed in tap water. The resultant material was sieved in metallic sieves (Tyler 100µm). The retained material was fixed and stored in 70% ethanol, in properly identified vials (organ, species, host sex, estimated age, and date of collection). The helminths were recovered after careful inspection of the contents under stereoscopic microscope. The taxonomic identification was based on morphological analyses of the parasites, which were prepared in temporary mounts. Taxonomic identifications were based on 10 individuals of each sex (dioecious), 10 adult individuals (monoecious) or the maximum number available. Images were obtained in Olympus BX51 microscope with digital camera, and then they were processed in Image Pro Plus 4.1 software. Morphologic and morphometric data were compared to previous published data (Lühe, 1910; Travassos, 1917; Machado Filho, 1964; Mueller, 1974; Waid & Pence, 1988; Vicente et al., 1997; Anderson et al., 2009; Gomez-Puerta et al., 2016). Subsequently, the descriptors of infection (prevalence, mean abundance, mean intensity and range of intensity) were calculated based on Bush et al. (1997). Vouchers were deposited in the Collection Oswaldo Cruz Institute (CHIOC/Fiocruz) and additional types were kept in the collection of the LabEPar, FCAV/Unesp.

A total of 344 adult helminths were found in the five necropsied animals, three adult males and two females, one adult and one young, all in good body condition. Ten different species were found: five nematodes, three cestodes and two acanthocephalans (Figure 1). All the cougars were infected by at least one species of helminth. Two to five species were observed simultaneously infecting the same host, with an average of two species per host. The descriptors of infection are presented in Table 1.

Human-induced environmental modification drives the emergence of diseases in humans and in domestic and wild animals (Nava et al., 2017). Animals carcasses found roadkilled can provide study materials for parasitological investigations and are regarded as advantageously available samples in eco-epidemiological studies (Richini-Pereira et al., 2010). Carnivores are known to harbor several pathogens, such as zoonotic helminths (Brown, 2004), in addition to which data found this study can be attributed to other animal species in the region through the circulation of pathogens between predator prey and the environment. The increase in wildlife-domestic carnivores-humans contact is of great concern due to the potential that this interaction represents for the emergence of diseases and the dissemination of parasites, especially in highly fragmented and anthropized areas such as the region studied (Daszak et al., 2001). It is important to note that some helminths found in this study, such as *L. major*, *Spirometra* sp., *O. onicola*, *O. canis*, *Echinococcus* sp., *T. leonina*, *C. subaequalis*, and *Filaroides* sp., are known to affect domestic carnivores (Dantas-Torres & Otranto, 2014), which may indicate that at some point, an interchange between these hosts may have occurred.

Considering the four valid species of the genus *Echinococcus* (Cestoda), two species, *E. vogeli* and *E. oligarthrus*, occur widely in the Neotropical region, using several carnivores as their definitive hosts (D'Alessandro & Rausch, 2008).

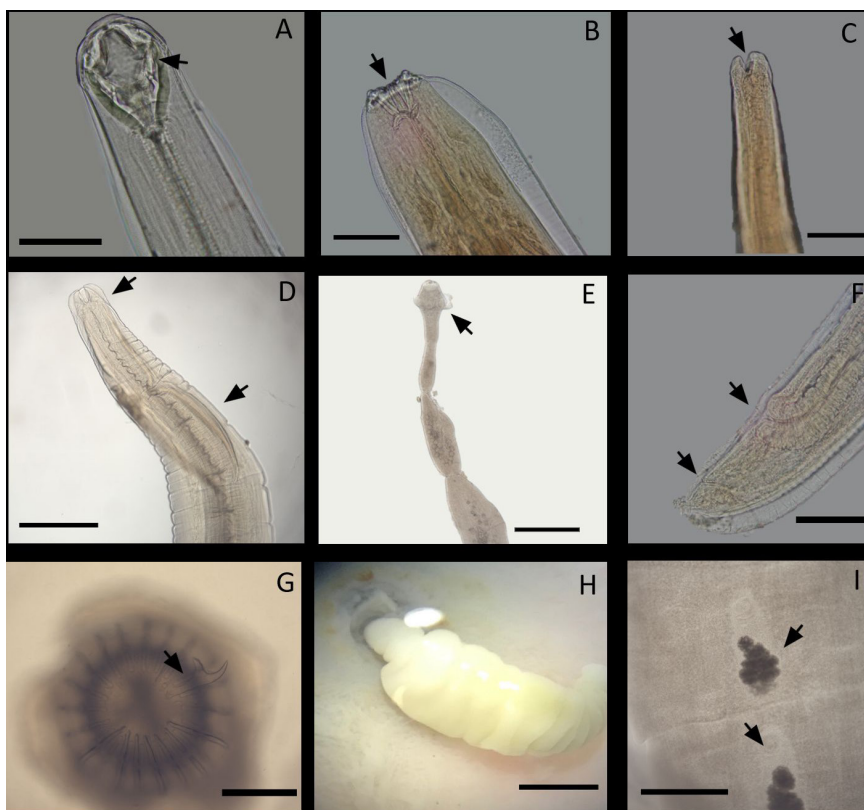


Figure 1. Helminths found in cougars road-killed in the Northeast Region of the State of São Paulo, Brazil. A) *Uncinaria bidens*, oral capsule provided with plates. Scale: 300µm B) *Cylicospirura subaequalis*, anterior region, presence of bifid teeth. Scale: 300µm C) *Lagochilascaris major*, presence of three lips, interlabial groove and absence of cervical wings. Scale: 300µm. D) *Toxascaris leonina*, anterior region with elongated cervical wings, three small lips. Scale: 500µm. E) *Echinococcus* sp. pyriform scolex with four suction cups, Scale: 500µm. F) *Filaroides* sp. Female, caudal extremity, lateral view. Scale: 300µm. G) *Taenia omissa*, rostellum armed with two rows of hooks. Scale: 300µm. H) *Oncicola oncocola* and *Oncicola canis*, parasites attached to the intestinal mucosa. I) *Spirometra* sp., mature proglotides with spiral-shaped uterus and genital pore. Scale: 500µm. Light microscopy.

Table 1. Descriptors of helminth infection observed on road-killed wild cougars (*Puma concolor*) collected in Northeastern region São Paulo State, between the years 2016 and 2018.

Helminths	Habitat	Prevalence (%)	Abundance	Mean and Range of Intensity	Total of parasites
Nematoda					
<i>Cylicospirura subaequalis</i> (CHIOC 38752)	Stomach	20	6	30**	30
<i>Lagochilascaris major</i> (CHIOC 38754)	SI*	40	4	10 (8-12)	20
<i>Toxascaris leonina</i> (CHIOC 38755)	SI	20	2,4	12**	12
<i>Uncinaria bidens</i> (CHIOC 38756)	SI	60	35,6	59,3 (2-111)	178
<i>Filaroides</i> sp. (CHIOC 38753)	Lung	20	2	10**	10
Cestoda					
<i>Spirometra</i> sp. (CHIOC 38759)	SI	40	10,4	26(20-32)	52
<i>Taenia omissa</i> (CHIOC 38760)	SI	20	0,2	1**	1
<i>Echinococcus</i> sp. (CHIOC 38758)	SI	20	0,8	4**	4
Acanthocephala					
<i>Oncicola oncocola</i> (CHIOC 38762)	SI	20	3,4	17**	17
<i>Oncicola canis</i> (CHIOC 38761)	SI	40	4	10(1- 19)	20

*Small intestine; ** Range of intensity N/A.

The cougars are the type-host for *E. oligarthrus* (Lühe, 1910). In the State of São Paulo, there are two human cases of infection by metacestodes of this parasite reported at the Ribeirão Preto Medical School Hospital (UHFMRP) (Meneghelli et al., 1992), the same region of the present study. As cougars are frequently registered close to rural settlements and urban areas in the studied region, the risk of human infection must be considered, especially regarding people with close contact to forest remnants and riparian forests, where these animals live.

The anthropic action may increase the contact between domestic and wild animals, as well as between humans and wildlife. New epidemiological situations may be resultant of these interactions and monitoring of wildlife diseases may provide valuable information (Daszak et al., 2001). The occurrence of helminths such as *L. major*, *Spirometra* sp., *O. oncocola*, *O. canis*, *Echinococcus* sp., *T. leonina*, *C. subaequalis*, and *Filaroides* sp. which are known to affect domestic carnivores, may indicate the permutation between wild and domestic hosts, in addition to the study representing new host records for four of the species found in cougars, *U. bidens*, *L. major*, *O. canis*, and *Filaroides* sp.; and also new locality records for *U. bidens*, *T. leonina*, *C. subaequalis*, and *Filaroides* sp.

Our results contribute to monitoring the health of this cougar population and combined with other ecological, behavioral and parasitic data, will help guide conservation actions to maintain a viable cougar population in that region as well as for public health.

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