



## Morphological and molecular diagnosis of diseases of free-ranging crab-eating foxes (*Cerdocyon thous*)

[Diagnóstico morfológico e molecular de doenças de cachorro-do-mato (*Cerdocyon thous*) de vida livre]

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### ABSTRACT

The aim of this study was to investigate the occurrence of diseases in free-ranging wild canids that were roadkill on highways in the State of Espírito Santo, Brazil. PCR tests were performed for the detection of *Brucella* sp., *Babesia* sp., *Rangelia* sp., and *Hepatozoon* sp. in the spleen. Morphological evaluation and identification of parasites was performed in the liver and lung. Twenty specimens of *C. thous* were necropsied at the Animal Pathology Sector of the Veterinary Hospital of the Universidade Federal do Espírito Santo. Tissue samples were processed for histopathological examination and polymerase chain reaction (PCR) analysis. There was no PCR amplification of genomic DNA sequences of *Brucella* sp., *Babesia* sp., *Rangelia* sp., and *Hepatozoon* sp. using DNA extracted from the spleen as template. Histologically, lesions associated with parasitism by *Platynosomum* sp. and *Angiostrongylus* sp. were observed in the liver and lung, respectively. This is the first report of *Platynosomum* sp. and *Angiostrongylus* sp. parasitism in *C. thous* in the state of Espírito Santo, Brazil. Therefore, this study demonstrated parasitism of crab-eating foxes by *Platynosomum* sp. and *Angiostrongylus* sp. Importantly, no evidence of infection with *Brucella* sp., *Babesia* sp., *Rangelia* sp., and *Hepatozoon* sp. was obtained by PCR analysis.

Keywords: wild canid, Histopathology, PCR

### RESUMO

O objetivo deste estudo foi investigar a ocorrência de doenças em canídeos silvestres de vida livre que foram atropelados em rodovias no estado do Espírito Santo, Brasil. Testes de PCR foram realizados para a detecção de *Brucella* sp., *Babesia* sp., *Rangelia* sp. e *Hepatozoon* sp. no baço. A avaliação morfológica e a identificação de parasitas foram realizadas no fígado e no pulmão. Vinte espécimes de *C. thous* foram necropsiados no Setor de Patologia Animal do Hospital Veterinário da Universidade Federal do Espírito Santo. Amostras de tecido foram processadas para exame histopatológico e análise de reação em cadeia da polimerase (PCR). Não houve amplificação por PCR das sequências de DNA genômico de *Brucella* sp., *Babesia* sp., *Rangelia* sp. e *Hepatozoon* sp. usando-se DNA extraído do baço como modelo. Histologicamente, lesões associadas ao parasitismo por *Platynosomum* sp. e *Angiostrongylus* sp. foram observadas no fígado e no pulmão, respectivamente. Este é o primeiro relato de *Platynosomum* sp. e *Angiostrongylus* sp. parasitismo em *C. thous* no estado do Espírito Santo, Brasil. Portanto, este estudo demonstrou parasitismo de cachorro-do-mato por *Platynosomum* sp. e *Angiostrongylus* sp. É importante destacar que não há evidências de infecção por *Brucella* sp., *Babesia* sp., *Rangelia* sp. e *Hepatozoon* sp. por análise de PCR.

Palavras-chave: canídeo silvestre, histopatologia, PCR

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## INTRODUCTION

Some of the diseases that affect wild animals may also affect human or domestic animal populations. Therefore, the diagnosis and study of these diseases may provide valuable knowledge on circulation of diseases among domestic and wild animals. The zoonoses with a wildlife reservoir constitute a public health issue worldwide. The importance of these diseases has been recognized, demanding attention to obtain data on natural occurrence of diseases and assessment of risk factors in certain ecosystems (Almeida *et al.*, 2018). The crab-eating fox (*Cerdocyon thous*) is a wild canid that, in addition to its natural broad feeding behavior, also have carcasses of domestic animals, processed foods, and human-generated food waste as food sources.

This situation favors the occurrence of infectious diseases that may affect domestic dogs since *C. thous* often lives near human habitats. This species has been suffering from loss of habitat, hunting, and trampling. They are considered one of the wild mammal species that suffer the most from the impact of roads and motor vehicles in the Brazilian territory (Beisigel *et al.*, 2013). Considering the scarcity of information on diseases affecting the crab-eating fox, the aim of this study was to investigate the occurrence of diseases in free-ranging crab-eating foxes that were road-killed on highways in the State of Espírito Santo, Brazil.

## MATERIAL AND METHODS

Twenty free-ranging crab-eating foxes (*Cerdocyon thous*) that were road killed between January and July 2018 and obtained from the Reserva Biológica de Sooretama (Sooretama, State of Espírito Santo, Brazil), Museu de Biologia Professor Mello Leitão (Santa Tereza, State of Espírito Santo, Brazil), Universidade Federal do Norte Fluminense Darcy Ribeiro (UENF; Campos dos Goytacazes, State of Rio de Janeiro, Brazil), and the Secretaria do Meio Ambiente de Marataízes (Marataízes, State of Espírito Santo, Brazil), were included in this study. This experimental protocol has been approved by the Ethics Committee on Animal Use of Universidade Federal do Espírito Santo (UFES) under protocol number 065/2017. All carcasses were kept frozen at the place of origin and sent to the Laboratório de Patologia Animal

do Hospital Veterinário da UFES, where they were subjected to necropsy.

Samples of the spleen, liver, and lungs (1 cm × 1 cm in duplicates) were stored at -80°C. Tissue samples were also fixed in 10% buffered formalin solution for histological processing, and 3 µm sections were stained with hematoxylin and eosin (HE). Liver and lung samples containing helminth-associated lesions were photo documented using a Dino Eye capture camera, and the slides containing parasites were selected. Then, the length and width of all the eggs and fragments of adult parasites were measured using the ImageJ 64-bit Java 1.8.0\_112 software and the mean value was obtained.

Frozen tissue samples were processed at the Laboratório de Patologia Molecular at the Universidade Federal de Minas Gerais (Belo Horizonte, State of Minas Gerais, Brazil) for DNA extraction according to the guanidine extraction protocol (Pitcher *et al.*, 1989). PCR for brucellosis was conducted as previously described by Bayliet *et al.* (1992), and PCR for hemoparasites (*Babesia* sp. *Rangelia* sp. and *Hepatozoon* sp.) was performed according to the protocol described by Harrus *et al.* (2011). PCR was performed using template DNA samples from 19 out of the 20 animals because samples from one animal had advanced degree of autolysis. Positive control for *Brucella* sp. was a previously confirmed case affecting the canine placenta, and positive control for piroplasmida was a confirmed case of *Theileria equi* in equine liver.

## RESULTS

Sixty per cent of the animals (12/20) originated from the Reserva Biológica de Sooretama (19°03'14.3"S 40°05'48.0"W), 30% (6/20) from the Museu de Biologia Professor Mello Leitão (19°56'08.9"S 40°35'58.3"W), 5% (1/20) from the Universidade Estadual do Norte Fluminense Darcy Ribeiro (21°45'49.0"S 41°17'15.1"W), and 5% (1/20) from the Secretaria de Meio Ambiente de Marataízes (21°02'23.8"S 40°49'18.2"W). Considering their sex, 70% (14/20) were males and 30% (6/20) females, whereas 95% (19/20) were adults, and 5% (1/20) juveniles.

One animal had evidence of traumatic lesions, including fractures that were inflicted by an attack from a domestic dog. PCR for *Brucella* sp.,

*Babesia* sp., *Rangelia* sp., and *Hepatozoon* sp. had negative results for all spleen samples. Microscopic evaluation of the liver revealed that 75% of the animals (15/20) had hepatic congestion, 65% (13/20) periductal fibrosis, 15% (3/20) biliary ductal proliferation, 5% (1/20) periductal hyperplasia and 5% (1/20) lymphocytic inflammatory infiltrate. In 30% (6/20) of the animals, helminths with morphologic features of trematodes were observed in the lumen of bile

ducts. Many trematodes with an oral sucker and eggs with a yellow to brown shell were observed within the organism (Gardiner and Poynton, 2006). Cross-sections of the parasites demonstrated an average length of 204.03  $\mu\text{m}$  and average width of 166.27  $\mu\text{m}$  of suckers. In addition, 17 egg fragments (measuring on average  $32.64 \pm 1.32 \mu\text{m}$  length and  $18.73 \pm 1.95 \mu\text{m}$  width) with thick brown shell were observed with miracidia in the interior (Figure 1).

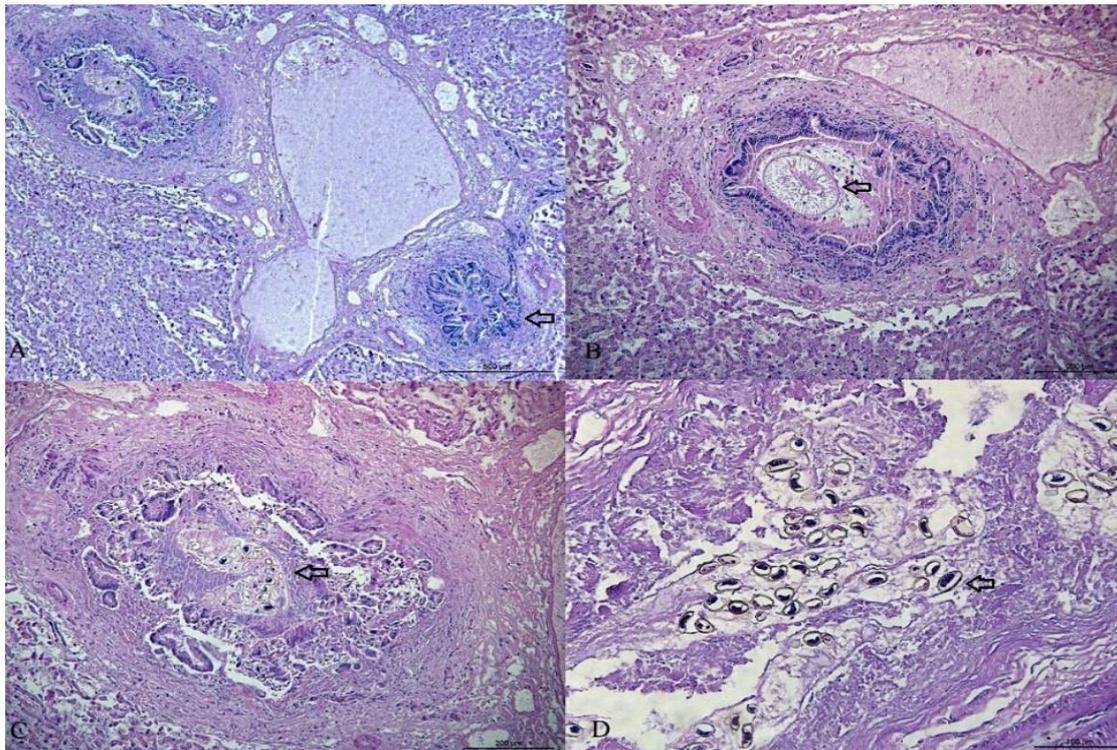


Figure 1. Adult male crab-eating-fox (*Cercopithecus thomasi*), liver: A) Periductal fibrosis, ductal proliferation, ductal hyperplasia (arrow), and an adult trematode within the bile duct (bar = 500  $\mu\text{m}$ ); B) Parasite sucker within the bile duct (arrow) with mild periductal lymphocytic inflammatory infiltrate (bar = 200  $\mu\text{m}$ ); C) Periductal fibrosis, proliferation of ducts (\*), and parasites with eggs (arrow) within the bile duct (bar = 200  $\mu\text{m}$ ); D) Fragments eggs of parasites with thick brown in the parenchyma (bar = 100  $\mu\text{m}$ ). Hematoxylin and eosin staining.

Microscopic evaluation of the lung revealed that 100% (20/20) of the animals had congestion, 95% (19/20) edema, 55% (11/20) hemorrhage, 15% (3/20) diffuse interstitial histiocytic inflammatory infiltrate, 5% (1/20) severe multifocal granulomatous pneumonia, and 5% (1/20) severe diffuse hemosiderosis. One animal (1/20) had nematode larvae within pulmonary alveoli. Sections of these parasites demonstrated elongated, spiral-shaped larvae, totaling six parasites, measuring on average  $176.21 \pm 9.14 \mu\text{m}$  in length and  $14.24 \pm 1.37 \mu\text{m}$  width. The larvae

were surrounded by a severe granulomatous inflammatory reaction with numerous macrophages, multinucleated giant Langhans cells, and marked diffuse hemosiderosis (Figure 2). Neither adult parasites nor egg were observed.

## DISCUSSION

The occurrence and reports of hepatic and pulmonary parasites in wild canids are extremely rare, and this is the first record of *Platynosomum* sp. and *Angiostrongylus* sp. in *C. thomasi* in the state

of Espírito Santo, Brazil. Infections with these parasites have been found in domestic dogs and cats, but in wild canids (especially crab-eating fox), there are just a few reports in Minas Gerais, Brazil. In this study, morphological features of the

hepatic helminths allowed identification as trematodes. The most common trematodes in wild canids belong to the family Dicrocoeliidae, including *Platynosomum* sp. and *Athesmia heterolecithodes* (Duarte, 2007).

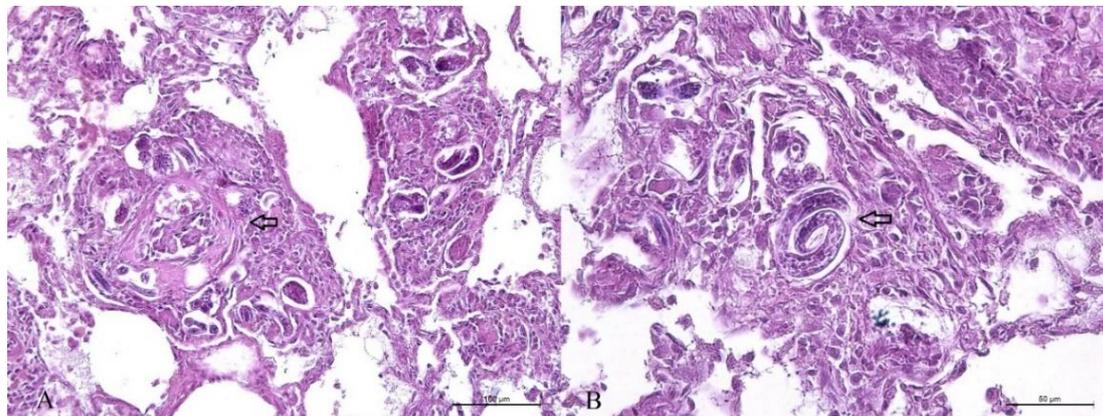


Figure 2. Adult male crab-eating-fox (*Cerdocyon thous*), lung: A) Granulomatous pneumonia (arrow) with intralesional nematode larvae (bar = 100 µm); B) Lung with elongated and spiral nematode larvae (bar = 50 µm). Hematoxylin and eosin staining.

According to Duarte (2007) there are few and subtle differences between *Platynosomum* and *Athesmia*, such as the more elongated and larger body and the intracecal ventral sucker of *Athesmia*. However, the absence of fresh adult parasites for morphometric analysis makes this differentiation difficult. Therefore, epidemiological aspects should be considered. There are no reports of *A. heterolecithodes* in Espírito Santo, Brazil, but *Platynosomum* sp. has already been reported in domestic cats (Gava et al., 2015; Campos et al., 2018). In addition, there are reports of infection of canids with *Platynosomum* sp., as described by Duarte (2007), in Juiz de Fora, MG, Brazil. Curi (2010) eggs of *Platynosomum* sp. in *C. thous* and in *Chrysocyon brachyurus* (maned wolf), by fecal examination in the region of Serra do Cipó - MG, Brazil.

Hepatic lesions observed in this study, including periductal fibrosis, periductal hyperplasia, ductal proliferation, presence of eggs, and intraductal adult parasites, were compatible with biliary parasitic diseases, such as *Platynosomum* sp. in cats (Ramos et al., 2017) and lesions caused by *Fasciola hepatica* and *Capillaria hepatica*. However, the histological characteristics of these two last parasites are quite different (Duarte, 2007). In relation to the pulmonary lesions found in the animals of this study, the most evident changes such as congestion, edema, and

hemorrhage may also be associated with traumas resulting from running over. However, one of the cases had a multifocal granulomatous pneumonia associated with parasite larvae. Ferreira Júnior et al. (2017) found that multifocal to coalescing granulomatous arteritis in *C. thous* was associated with the presence of parasites measuring 100–200 µm that were identified as *Angiostrongylus vasorum*. In this study, larvae found in the lung had sizes compatible with the findings of Ferreira Júnior et al. (2017) and therefore it was morphologically compatible with *A. vasorum*.

In the Brazilian territory, *Angiostrongylus* sp. had been described in *C. thous* in the state of Paraná (Duarte, 2007). In São Paulo, *Angiostrongylus* sp. was diagnosed by histopathology (Ferreira Júnior et al., 2017). These studies further support the interpretation that *Angiostrongylus* sp. was the pulmonary parasite identified in this study. Oliveira-Filho et al. (2012) also had negative results when searching for *Brucella* sp. in the crab-eating foxes. In contrast, Dorneles et al. (2014) diagnosed the *B. abortus* infection in five *C. thous*, while Hayashi (2013) performed PCR in 41 crab-eating foxes from Parque Nacional da Emas region, Brazil, but did not find any positive sample for *Brucella* sp. These negative results for *Brucella* sp. in crab-eating foxes, including those in the present study, despite using molecular tests that are highly sensitive and specific, indicates a

low frequency of *Brucella* sp. infection in wild canid populations. However, the general eating habit of the crab-eating fox, which can sometimes feed on aborted fetuses, placental tissues, and farm animal carcasses, indicates a potential role of the crab-eating foxes in the transmission of brucellosis, which needs to be better clarified.

*Babesia* sp., *Rangelia* sp., and *Hepatozoon* sp. are commonly detected in domestic animals of different species in several regions of Brazil, using various diagnostic techniques. However, in this study, DNA sequences of these pathogens were not detected. However, in a study in the Brazilian Pantanal, 78 *C. thous* were tested for *Babesia* sp. by ELISA and PCR techniques with 42 seroreactive but only one animal that was PCR positive (Sousa *et al.*, 2018). Conversely, in the highway ES-060 between the municipalities of Guarapari and Vila Velha, Almeida *et al.* (2013) diagnosed *Babesia* sp. in 10 of the 58 *C. thous* tested by PCR. The same authors used animal samples from regions similar to the present study and found a prevalence of 17.24%. However, the same authors revealed that in the sampled period (2004–2009), all positive animals showed concomitant infestation by ticks. In the present study, in addition to having collected material in later years, none of the animals had ectoparasites, which may explain the negative results for *Babesia* sp.

When rangelioidosis is considered, the results obtained in the present study are similar to those of Almeida *et al.* (2013) and Sousa *et al.* (2018), who also used PCR in their studies, in which even animals with ticks were negative for *Rangelia* sp. In contrast, *Rangelia* sp. was diagnosed in crab-eating foxes by PCR in São Paulo and Rio Grande do Sul, including free-ranging and captive animals. In that particular study, 13 were free-ranging, and 46.2% were positive (Soares *et al.*, 2014). In the same region, the *Rangelia vitalii* was diagnosed by PCR in one out of two animals (Fredo *et al.*, 2015). The high prevalence of rangelioidosis observed in the states of São Paulo and Rio Grande do Sul can be explained by the fact that those are endemic regions for this hemoparasite (Soares *et al.*, 2014).

Hepatozoonosis is a relatively common disease with a high prevalence in crab-eating foxes in different regions of Brazil. In the Brazilian Pantanal region, of the 78 specimens tested, 91%

were positive (Sousa *et al.*, 2018); in Espírito Santo, of the 58 animals from ES-060 between the cities of Vila Velha and Guarapari, 50% were positive (Almeida *et al.*, 2013) and in Rio Grande do Sul, five of eight *C. thous* tested were positive for *Hepatozoon* sp. The absence of ectoparasites in this study can justify the negative results for *Babesia* sp., *Rangelia* sp., and *Hepatozoon* sp. because all these pathogens are transmitted by ticks.

## CONCLUSIONS

This work represents the first reported cases of *Platynosomum* sp. and *Angiostrongylus* sp. parasitism in free-ranging *C. thous* in the State of Espírito Santo, Brazil. Importantly, there was no molecular evidence of *Brucella* sp., *Babesia* sp., *Rangelia* sp., or *Hepatozoon* sp. infection in these animals.

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