

## TOXOPLASMOSIS IN BLACK-FACED LION TAMARIN (*Leontopithecus caissara*)

### TOXOPLASMOSE EM MICO-LEÃO-DE-CARA-PRETA (*Leontopithecus caissara*)

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#### SUMMARY

Neotropical primates are susceptible to several parasitic diseases, the most relevant of which is toxoplasmosis. The black-faced lion tamarin is one of the most endangered callitrichid, with no more than 400 individuals in the wild. A young specimen of black-faced lion tamarin died acutely after being rescued and sent to the Sorocaba Zoological Park. This is the first report of acute fatal toxoplasmosis in *L. caissara* in Brazil.

**KEY-WORDS:** Protozoa. Neotropical primates. Infectious disease. Acute death.

#### RESUMO

Os primatas neotropicais são suscetíveis a inúmeras enfermidades parasitárias, sendo a mais relevante a toxoplasmose. O mico-leão-de-cara-preta é um dos calitriquídeos mais ameaçados no mundo, com aproximadamente 400 indivíduos em estado selvagem. Um exemplar de mico-leão-de-cara-preta, jovem veio à óbito de forma aguda após ser resgatado e enviado ao Zoológico do interior de São Paulo. Este é o primeiro relato de toxoplasmose aguda fatal em *L. caissara* no mundo.

**PALAVRAS-CHAVE:** Protozoário. Primata neotropical. Doença infecciosa. Morte aguda.

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## INTRODUCTION

There are currently four known species of lion tamarins: the golden lion tamarin (*Leontopithecus rosalia*, Linnaeus, 1766), the golden-headed lion tamarin (*L. chrysomelas*, Kuhl, 1820), the black lion tamarin (*L. chrysopygus*, Mikan, 1823) and the black-faced lion tamarin (*L. caissara*, Lorini & Persson, 1990) (RYLANDS et al., 2002).

The black-faced lion tamarin is an endangered species, classified as “Endangered” (EN) by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA), and as “Critically Endangered” (CR) by the International Union for Conservation of Nature (IUCN) (BRASIL, 2020; LUDWIG, 2021). One of the main threats to the species is the small population, which is related to the fragmentation and destruction of their natural habitat due to environmental degradation for agriculture and extractivism (NASCIMENTO et al., 2011). The total population of black-faced lion tamarins in the wild is estimated to be around 400 individuals and the species is protected in two conservation units: the Superagui National Park and the Jacupiranga Mosaic, located respectively in the Brazilian States of Paraná and São Paulo (LORINI et al., 1994). There is no record of this species in captivity in Brazil or in any zoological in the world.

Among the several diseases that afflict the neotropical primates living in captivity, the parasitic diseases caused by protozoa belonging to phylum *Apicomplexa* present a particularly high incidence. Toxoplasmosis is one of the most prevalent zoonoses throughout the world and may afflict most homeothermic vertebrates, including birds. It is a cosmopolitan infectious disease caused by *Toxoplasma gondii*, a coccidian protozoa that has been reported in neotropical primates, particularly in callitrichids, and leading to several deaths in zoos (EPIPHANIO et al., 2003; SANTOS et al., 2018; SALLES et al., 1998; PERTZ et al., 1998, EPIPHANIO et al., 2001).

In Brazil, the first reports of toxoplasmosis in non-human primates date back to the late 1990s and early 2000s, with several deaths recorded in zoos across the State of São Paulo, particularly of common woolly monkeys (*Lagothrix lagotricha*), southern muriquis (*Brachyteles arachnoides*) and lion tamarins (*Leontopithecus* sp.) (BOUER et al., 1999; EPIPHANIO et al., 2000; SANTOS et al., 2014). The course of the disease is variable, but in most clinical cases it evolves acutely, and the clinical signs are nonspecific or related to respiratory problems and apathy, which can make diagnosis difficult and lead to high mortality rates in Neotropical primate colonies, representing yet another threat to these species.

## CASE REPORT

An infant black-faced lion tamarin coming from illegal wildlife trade was confiscated by the Environmental Police of the State of São Paulo. The animal presented a history of inadequate feeding and incorrect handling, and was forwarded to the Municipal Zoological Park “Quinzinho de Barros” at the city of Sorocaba, State of São Paulo, Brazil. During the physical examination, the animal appeared to be in good health, weighting 205 grams, but after a few days it presented diarrhea, dyspnoea, seizures

and developed an acute fatal profile. The period between the rescue of the animal and the date of death was only three days.

The *post mortem* examination highlighted paleness of the mucosae, the presence of white-reddish foam in the trachea, pulmonary congestion and edema (Figure 1A), enlarged mesenteric lymph nodes (Figure 1B) *a foto está invertida, ou seja, de cabeça para baixo* and hemorrhage in the terminal colon. Based on the macroscopic findings, the cause of death was determined to be respiratory failure. The histopathological analysis revealed moderate liver necrosis, atypical fibrous mononuclear interstitial pneumonia (Figure 1C) associated with vascular congestion, moderate atelectasis and emphysema, intestinal lymph node hyperplasia (MALT) (Figure 1D), and mesenteric lymphadenitis associated with pathogens compatible with tachyzoites (Figure 1E).

The diagnosis of toxoplasmosis was confirmed by Polymerase Chain Reaction (PCR) and immunohistochemistry (IHC), which enabled the visualization of the *T. gondii* tachyzoites inside the mononuclear cells (Figure 1F). For the IHC assay a polyclonal anti-*T. gondii* primary antibody (1:10000) (B103, Dako Corporation, Carpinteria, USA) was employed followed by a peroxidase-conjugated polymer detection system (Labvision, Thermo Fisher Scientific, MA, USA) (EPIPHANIO et al., 2003; SANTOS et al., 2018). Whole blood was used for the molecular analysis. DNA was extracted using a commercially-available kit (DNeasy Blood® & Tissue kit, Qiagen, USA) according to the manufacturer’s instructions. A nested PCR assay aimed at amplifying the 155pb fragment of gene B1 was employed for the molecular detection of *T. gondii* (SHL, 1999).

## DISCUSSION

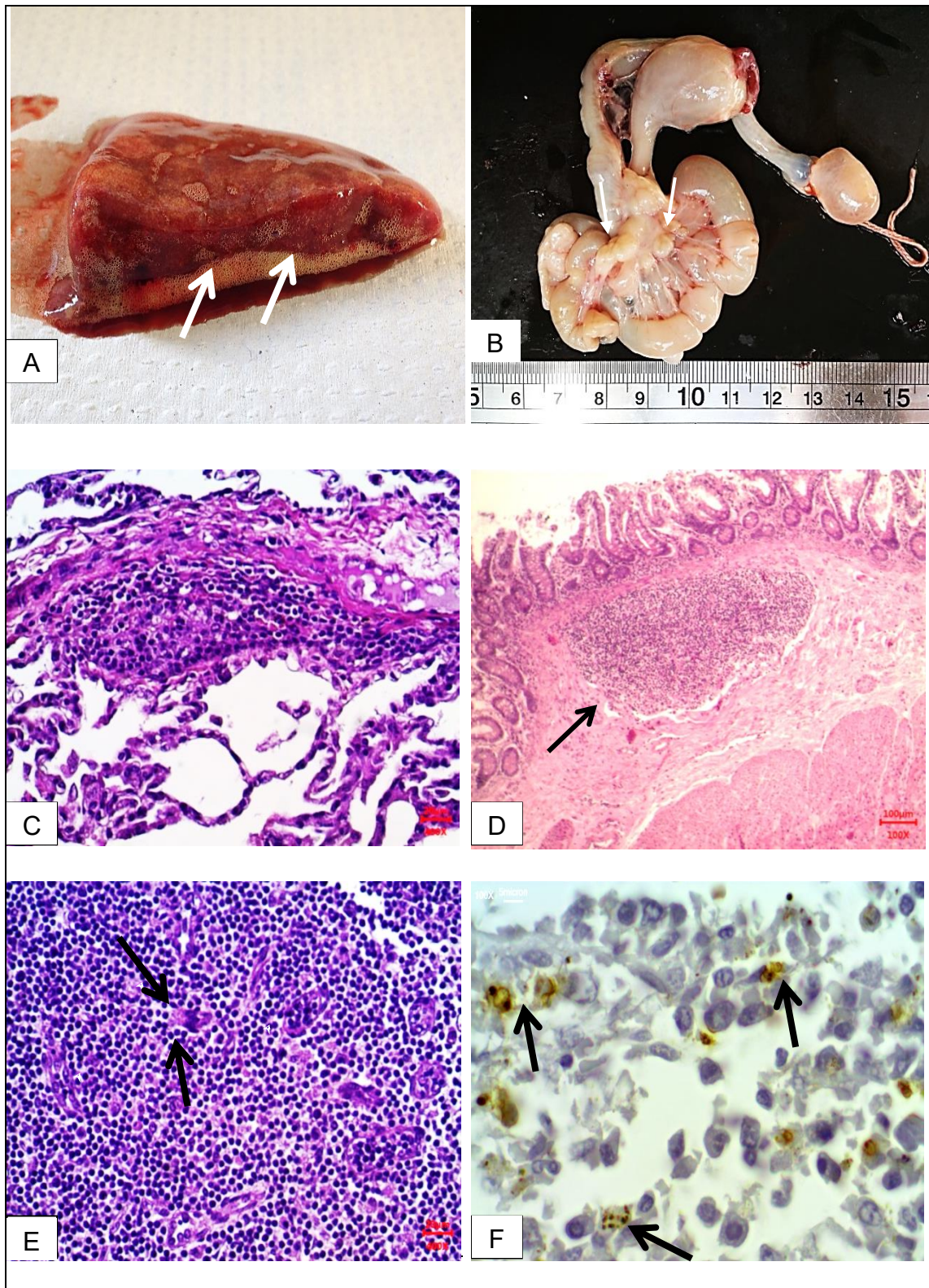
Toxoplasmosis is an acute fatal disease in neotropical primates caused by protozoa *T. gondii*. The disease may be transmitted congenitally, through the ingestion of food or water contaminated with sporulated oocysts, or through the ingestion of meat containing tissue cysts. Domestic and wild felines are considered definitive hosts for the protozoa and are the only hosts capable of excreting oocysts to the environment (DUBEY, 1996; DUBEY, 2009).

New World Primates (NWP) are considered highly susceptible to the disease, while Old World Primates (OWP) are more resistant to clinical toxoplasmosis. This may be explained by the arboreal habits presented by NWP and the low contact with the feces of felines in ancestral times, similarly to the Australian marsupials that evolved without such contact (CASAGRANDE et al. 2013).

Afflicted animals usually present non-specific clinical signs such as apathy, prostration, high fever, inappetence, dyspnea and serosanguinous nasal discharge. However, some animals do not manifest any clinical sign and die suddenly. The main macroscopic injuries observed are pulmonary congestion and edema, hepatitis and lymphadenitis. The most common histopathological findings are multifocal necrotic hepatitis, lymphadenitis, pneumonia and gastrointestinal disorders associated with the presence of *T. gondii* tachyzoites in most affected organs (CATÃO-DIAS et al., 2013).

In this case report, the characteristic injuries described in the literature were not evident, but the enlarged lymph nodes and pulmonary congestion were the main findings of the necropsy, directing the study towards toxoplasmosis. Further laboratory assays were necessary to

confirm the diagnosis, including PCR and IHC, which confirmed the cause of death to be toxoplasmosis. In this case report was unable to determine the origin of *T. gondii*.



**Figure 1.** A) Diffuse congestion and severe aerated content in lung tissue; B) Hyperplasia of mesenteric lymph nodes (arrows); C) Mononuclear necrofibrinous bronchiolitis. Bar=20µm; D) Gall hyperplasia in the small intestine (arrows); Bar=10µm. E) Necrofibrinous mesenteric lymphadenitis associated with forms compatible with tachyzoites (arrows). Bar=20µm. F) Mesentery, *L. caissara*. Note several tachyzoites inside the mononuclear cells. Immunohistochemistry stain (brownish) with *T. gondii* antibodies. Bar=5µm.

Currently there are no records of *L. caissara* specimens being kept in any zoo or breeding center around the world. The scientific studies and research regarding the species are based mostly on biological characteristics, such as behavior, genetic analysis, ecology and feeding habits, and there are no scientific studies in the field of veterinary medicine dealing with the species. Therefore, this is the first case report of fatal toxoplasmosis in black-faced lion tamarins.

Considering the high susceptibility of New World monkeys, this case report emphasizes the importance of the *post mortem* examination in the diagnosis of infectious diseases, which allows control and prophylactic measures to be adopted to avoid the spread of disease across these animals. Another important point is the establishment of a quarantine program for wild animals arriving at zoos, since

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the individuals may be incubating several pathogens without any clinical manifestations of disease and may, in many cases, serve as a source of infection for other animals.

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## ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Ethical approval was not required because no animals were used for research in this study.

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