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CASE REPORT

ACUTE TOXOPLASMOSIS IN A BROWN-HOWLER MONKEY (*Alouatta guariba clamitans*)

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ABSTRACT

Toxoplasmosis is a cosmopolitan disease caused by the obligatory intracellular protozoan *Toxoplasma gondii.* Non-human primates may be part of the parasite cycle, acting as intermediate hosts of *T. gondii,* and may die due to toxoplasmosis. Thus, this document aims to report a case of sudden death in a brown howler monkey (*Alouatta guariba clamitans*) attributed to toxoplasmosis, describing the macroscopic and microscopic lesions observed. The brown howler monkey was male, medium-sized, with a history of sudden death and clinical suspicion of yellow fever. At necropsy, poor nutritional status, mild jaundice, pulmonary edema with multifocal areas of hemorrhage, and marked splenomegaly were observed. Microscopy revealed the *foci* of lytic necrosis of hepatocytes, pericholangitis, periportal hepatitis, and macrovacuolar fatty degeneration; cysts with bradyzoites were found in the telencephalic cortex, liver, and spleen. Biological samples were sent for PCR detection of *T. gondii*, which tested positive for the technique. Cases of toxoplasmosis in non-human primates are relatively frequent, but the captivity situation presents a greater risk for these animals, in comparison to natural environments, increasing the risk of toxoplasmosis for them. In this context, preventive measures must be considered to reduce the chance of parasite transmission in zoos.

KEY WORDS: Non-human primates; pathology; toxoplasmosis; *Toxoplasma gondii.*

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INTRODUCTION

Toxoplasmosis is a disease of cosmopolitan distribution caused by the apicomplexan parasite *Toxoplasma gondii,* an obligatory intracellular protozoan, which can infect mammals and birds (Dubey, 2010). Domestic and wild felines are considered definitive hosts, participating in the enteroepithelial cycle, with the formation of infective oocysts, while non-felid mammals are considered intermediate and maintenance hosts, participating in the extra-intestinal cycle, with the formation of cysts with bradyzoites (Dubey et al., 2020). *T. gondii* has three main forms: sporozoites in oocysts of fecal material, bradyzoites in tissue cysts (slow multiplication and latency form), and tachyzoites (fast multiplication forms) (Dubey et al., 2012). The main source of infection is tissue containing cysts with bradyzoites, water, and food contaminated with sporulated oocysts. The oral route is considered the main mechanism of infection, although transplacental transmission also often occurs (Cubas et al., 2014; Greene, 2015).

Non-human primates may participate in the parasite cycle as intermediate hosts (Epiphanio et al., 2003). Primates from the genus *Alouatt*a are the most widely distributed New World monkeys, and *Alouatta guariba clamitans* (brown-howler monkey) is an endemic species in the Atlantic Forest in Brazil and Argentina (Meireles et al., 1999). Because of this wide distribution, howler monkeys are potential parasite carriers of great zoonotic importance. Therefore, this case report aimed to describe the macroscopic and microscopic lesions of acute toxoplasmosis in a brown-howler monkey (*A. g. clamitans*).

CASE REPORT

An adult male of brown-howler monkey (*A. g. clamitans*), not castrated and of medium size, was found dead in its enclosure in a zoo. Since the animal suddenly died, it was sent for necropsy under suspicion of yellow fever. The necropsy was performed according to King et al. (2013) and Simões et al. (2024). During the necropsy, the organs were evaluated and photographed. After this process, 1 cm^3 of fragments were collected and preserved in 10% buffered formalin for 24 to 48 hours. The samples were then dehydrated under increasing alcohol solutions, cleared using xylol, and then embedded in paraffin (Ribeiro et al., 2013). To provide the histological slides, the paraffin blocks were cut in a rotating microtome, 3 μm thick sections, which were placed on glass slides, deparaffinized, and stained with hematoxylin and eosin and observed under an optical microscope at 10× magnification.

As a complementary test, RT-qPCR was performed to detect DNA of *T. gondii* in liver, spleen, heart, and kidney samples. RT-qPCR was also

performed for yellow fever virus (YFV).

Macroscopically, on external examination, the corpse showed poor nutritional status (Figure 1A), mild jaundice of the oral, ocular, and preputial mucous membranes. Both lungs were not collapsed when the thorax was accessed and were shiny and moist (pulmonary edema). Additionally, the lungs were slightly reddish (hyperemia) and demonstrated multifocal areas of hemorrhage. The liver was markedly pale and demonstrated a slight accentuation of the lobular pattern (Figure 1B and 1C). There was a marked splenomegaly (Figure 1B) with a fleshy cut surface (Figure 1D). Visceral and subcutaneous fat were moderately yellow (jaundice).

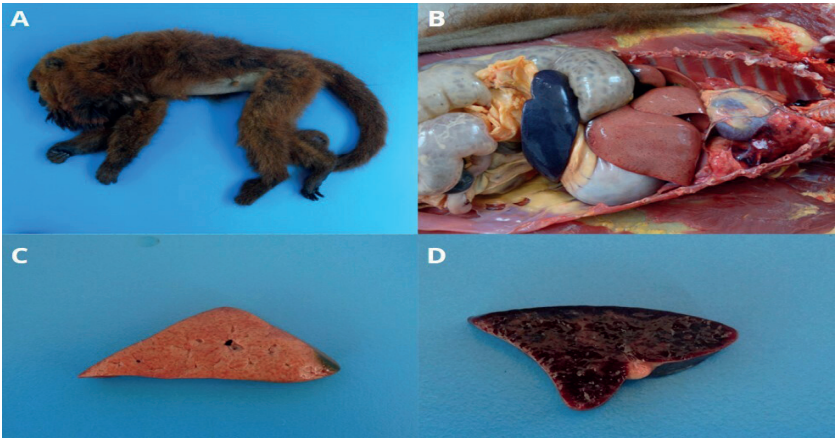

Figure 1. Macroscopic findings of toxoplasmosis in a brown-howler monkey (*Alouatta guariba clamitans*). A. Brown-howler monkey (*A. g. clamitans*) is in poor body condition. B. Panoramic view of the abdominal and thoracic cavities demonstrates splenomegaly and yellow liver discoloration. C. Liver cut surface with intense pallor and slight accentuation of the lobular pattern. D. Cut the surface of the spleen with a fleshy appearance.

On liver histology, there were multiple small random *foci* of lytic necrosis of hepatocytes and variable inflammation in the portal spaces (pericholangitis), consisting of mononuclear cells, mainly lymphocytes and plasma cells (Figure 2). In some of these portal spaces, the infiltrate was pronounced, exceeding the limiting plate and causing periportal hepatitis (Figure 2A). Other liver lesions included swollen hepatocytes with single, large, well-defined vacuoles (macrovacuolar fatty degeneration), large numbers of neutrophils in the sinusoids (neutrophilic leukocytostasis), Kupffer cell hypertrophy, and formation of Kupffer cell pseudogranulomas. In some necrotic *foci*, it was possible to verify the presence of cysts with bradyzoites and tachyzoites among cell debris and in the cytoplasm of Kupffer cells.

It was observed that the distension of the red pulp in the spleen was due to a large amount of blood (hyperemia) and hyperplasia of the white pulp. Microscopically, macrophages with tachyzoites and occasional cysts with bradyzoites in the spleen were observed. In addition to edema and hyperemia, an intense, multifocal neutrophilic inflammatory infiltrate in the lungs was associated with intralesional bacilli. In the brain, mainly in the telencephalic cortex and in the subcortical white matter, there were areas of multifocal gliosis specifically, close to capillaries (Figure 2B). In these areas, cysts containing bradyzoites and tachyzoites were observed. In the kidneys, there was a mild interstitial inflammatory infiltrate, consisting of lymphocytes and plasma cells. RT-qPCR amplified the 529 bp fragment of *T. gondii* genome, confirming the positivity for this agent. RT-qPCR was negative for YFV.

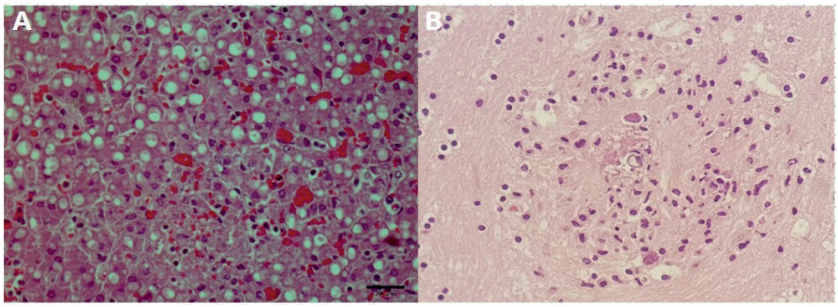

Figure 2. Histopathological findings of toxoplasmosis in a brown-howler monkey (*Alouatta guariba clamitans*). A. Liver: focus of random hepatic necrosis, swollen hepatocytes with single, large and well-defined vacuoles (macrovacuolar fatty degeneration) and cysts with bradyzoites (morphology compatible with *Toxoplasma gondii*). HE, mag. 40×. B. Brain (white matter): focal gliosis associated with cysts with bradyzoites (morphology compatible with *T. gondii*). HE, mag. 40×. HE: hematoxylin-eosin.

DISCUSSION

Toxoplasmosis is one of the causes of death in wildlife and captive primates, normally presenting itself as a fatal condition (Epiphanio et al., 2003; Casagrande et al., 2013). In captivity, *T. gondii* presents major circulation (compared to wildlife) as evidenced by the high frequency of serologically positive animals in this condition (Niehaus et al., 2020). In this sense, it is important to highlight this epidemiological characteristic, since the management practices in conservation centers (team hygiene, contaminated food and water), as well as proximity to intermediate and definitive hosts, may enhance the risk of infection to primates (Villar-Echarte et al., 2021).

Neotropical species (as well as marsupials) are considered more susceptible to toxoplasmosis when compared to those from the Old World. This pattern occurs following the primate's evolutionary history, especially due to the eating habits of American species that are primarily arboreal, reducing exposure to oocysts of *T. gondii* (Innes, 1997). Among the New World primate genera, there is also variable susceptibility. Callithrix and Leontopithecus present higher mortality when compared to those of the families Atelidae and Cebidae. Although the reason for this pattern is unclear, it is believed to be related to these species' behavior, natural history, and diet (Catão-Dias et al., 2013; Niehaus et al., 2020).

Deaths caused by *T. gondii* in monkeys are described, by some authors, as hyperacute (suddenly) without clinical signs and, or acutely, with few days of evolution, usually after presenting nonspecific clinical signs, such as malaise, hypothermia, anorexia and emesis (Casagrande et al., 2013; Villar-Echarte et al., 2021; Schiffler et al., 2023). The macroscopic lesions most commonly associated with toxoplasmosis in primates include pneumonia, pulmonary edema and congestion, hepatomegaly, splenomegaly, and mesenteric lymphadenitis (Epiphanio et al., 2003; Cedillo-Peláez et al., 2011; Fajardo et al., 2023). In this case report, all of these lesions were observed, besides the mesenteric lymphadenitis.

In histology, multifocal hepatic necrosis, necrotizing splenitis, lymphadenitis, interstitial pneumonia, enteritis, and encephalitis with *foci* of gliosis are commonly described (Schiffler et al., 2023). In this study, some of these alterations were observed, such as liver lytic necrosis of hepatocytes associated with variable inflammation in the portal spaces, swollen hepatocytes, and Kupffer cell hypertrophy. In the lung, an intense multifocal neutrophilic inflammatory infiltrate was observed, associated with intralesional bacilli, while in the telencephalic cortex and subcortical white matter, there were areas of multifocal gliosis.

Morphological diagnosis is performed by the observation of cysts containing bradyzoites and/or free tachyzoites in the affected tissues (Epiphanio et al., 2003; Cedillo-Peláez et al., 2011; Casagrande et al., 2013; Fajardo et al., 2023). Normally, the microscopic lesions will be associated with the rapid multiplication of tachyzoites in loco, causing focal necrosis, hemorrhages, and the presence of inflammatory aggregates (Dubey et al., 2012).

Immunohistochemistry and PCR are confirmatory tests for toxoplasmosis, especially because histology is limited in visualizing parasitic structures (Santos, 2022). In this case report, the PCR result confirmed the infection by *T. gondii.*

Cases of toxoplasmosis in non-human primates are relatively frequent, leading to pathological manifestations and alterations. The main pathological changes observed in this case report were, macroscopically, poor nutritional condition, jaundice, pulmonary edema concomitantly with hyperemia, and

multifocal areas of hemorrhage. The liver was markedly pale and with a slight accentuation of the lobular pattern, while in the spleen, there was a marked splenomegaly. Microscopically, an inflammatory infiltrate was observed in the liver, lungs, brain, spleen, and kidneys. Cysts containing bradyzoites or free tissue tachyzoites were observed in these organs.

CONFLICT OF INTEREST

The authors declare no conflict of interest to disclose.

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