AKADÉMIAI KIADÓ

Acta Veterinaria Hungarica

68 (2020) 3, 285–288

DOI:
10.1556/004.2020.00049


CASE REPORT

# Fatal disseminated toxoplasmosis in a brown-throated sloth (*Bradypus variegatus)* from Northern Brazil – Case report

FABIANO JOSÉ FERREIRA DE SANT'ANA[1*], JULIANA DOS SANTOS BATISTA[2], GUILHERME REIS BLUME[1], LUCIANA SONNE[3] and CLAUDIO SEVERO LOMBARDO DE BARROS[4]

[1] Laboratório de Diagnóstico Patológico Veterinário, Universidade de Brasília (UnB), Brasília, Brazil

[2] Universidade Federal Rural da Amazônia (UFRA), Belém, Brazil

[3] Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

[4] Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Brazil

Received: March 5, 2020 • Accepted: July 16, 2020
Published online: October 30, 2020

**ABSTRACT**

The clinical and pathological findings of a case of fatal disseminated toxoplasmosis in a captive brown-throated sloth (*Bradypus variegatus*) from the northern region of Brazil are reported. Clinical signs were nonspecific and included apathy, prostration, dyspnoea, and loss of appetite. Treatment with penicillin was attempted, but the animal died within five days of the onset of clinical signs. Microscopically, there was acute inflammation in the liver, spleen, and lungs associated with necrosis and a few cysts and extracytoplasmic tachyzoites, with a morphology compatible with *Toxoplasma gondii*. Tissue sections were submitted for immunohistochemistry that confirmed *T. gondii* as the aetiological agent. To the authors' knowledge, this is the first report of toxoplasmosis in *B. variegatus*.

**KEYWORDS**

brown-throated sloth, *Bradypus variegatus*, *Toxoplasma gondii*, Northern Brazil

## INTRODUCTION

Toxoplasmosis is a cosmopolitan zoonosis caused by *Toxoplasma gondii*, a cyst-forming obligate intracellular coccidium. This organism belongs to phylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order Eimeriorina and family Sarcocystiidae (Hill et al., 2005). Domestic and wild cats are definitive hosts, but several vertebrate species can serve as intermediate hosts for *T. gondii*. The disease is described occasionally in wild animals and rarely in sloths (Túry et al., 2001). It is of great importance for human and animal health. Compromised fetal development resulting in malformations and abortion are well known in humans, and production losses due to abortion can be significant in livestock.

The life cycle of *T. gondii* in the intermediate hosts is complex and begins with the ingestion of sporulated oocysts excreted in the faeces of felids or with the ingestion of tissues of intermediate hosts that contain encysted bradyzoites or tachyzoites. In the infected animal, the sporozoites excyst, multiply in the intestinal epithelial cells and associated lymph nodes to form tachyzoites (endodyogeny), which can migrate and infect many tissues inducing necrosis and inflammation. In this phase, antibodies are produced and the invasiveness of the tachyzoites results in the formation of cysts containing numerous bradyzoites. Following the

*Corresponding author.
Tel.: +55 61 3468 7255.
E-mail: santanafjf@yahoo.com

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ingestion of tissue cysts by an intermediate host, bradyzoites will excyst and become tachyzoites (Dubey and Lappin, 2006).

Toxoplasmosis can cause a clinical or subclinical disease and the clinical signs may vary according to the severity of lesions in the organs, but may be mostly unspecific, including fever, lethargy, anorexia, ocular and nasal discharges, and respiratory distress (Hill et al., 2005). Although this disease has been described in other species of South American sloths, such as *Bradypus tridactylus* (Túry et al., 2001) and *Choloepus didactylus* (Shaw and Lainson, 1973), to the best of the authors' knowledge this is the first report of toxoplasmosis in *Bradypus variegatus*. The current study describes the anatomic pathology of fatal disseminated toxoplasmosis in a captive brown-throated sloth (*B. variegatus*) from the northern region of Brazil.

## CASE REPORT

A captive young, female brown-throated sloth (*B. variegatus*), from the 'Parque Zoobotânico Museu Paraense Emílio Goeldi', Belém county, Pará state, Northern Brazil, presented a history of large, round, alopecic cutaneous lesions, suggestive of mycotic infection. This sloth was found with its dam that also presented the same cutaneous alterations. Treatment was not performed and the animals were released in a park. After four months, both animals were localised again with some crusted cutaneous changes in the left elbow and hind legs. The dam was dead and the young sloth presented apathy, prostration, dyspnoea and loss of appetite, and was submitted for veterinary care. The animal was treated with daily dosages of enrofloxacin (Flotril 10%, MSD, São Paulo, Brazil) by nebulisation for five days and one 40,000 IU/kg dose of penicillin (Pentabiótico, Zoetis, São Paulo, Brazil). The treatment was unsuccessful and the sloth died naturally after five days.

Grossly, pulmonary hyperaemia and oedema and multifocal whitish spots in the liver were observed. Furthermore, there were mild cutaneous lesions including alopecia and scabs in the left elbow and hind limbs. Samples of liver, kidney, heart, lung, ovary, uterus, and spleen were collected, fixed in 10% buffered formalin for 48 h, processed routinely for histopathological evaluation and stained with haematoxylin and eosin, periodic acid–Schiff (PAS) and Grocott methenamine silver (GMS). Immunohistochemical (IHC) staining was performed using a biotin-peroxidase system, with labelling of the biotinylated secondary antibody (kit LSAB-HRP, K0690 DakoCytomation) and diaminobenzidine as the chromogen. Antigen retrieval was performed by treatment with 0.1% trypsin followed by microwave. To block the endogenous peroxidase activity, the slides were incubated in a solution of $H_2O_2$ (10%) in methyl alcohol. The reagents were applied manually, with an overnight incubation for the anti-*T. gondii* polyclonal primary antibody (caprine origin G245, VMRD, Pullman, WA, USA) (1:1,000 dilution) and the chromogen (Romulin AEC, Biocare Medical) was applied for 5 min. The IHC sections were counterstained using Harris haematoxylin. The positive control consisted of samples of bovine fetal brain previously tested (Antoniassi et al., 2013). For the negative controls, the primary antibodies were replaced with normal serum (Ultra V Block, NeoMarkers Inc., Fremont, CA, USA).

Microscopically, there were some random areas of necrosis and inflammation in the liver, spleen, and lungs (Fig. 1A). In these areas, there were some tachyzoites and extra and intracellular hypereosinophilic oval protozoal cysts which measured approximately 20 × 15 µm, had a thin wall and numerous 2–3 µm, elongate bradyzoites (Fig. 1A–B). There was mild portal lymphoplasmacytic infiltration in the liver. The pulmonary interalveolar septa were thick and showed a moderate infiltration of macrophages, plasma cells and lymphocytes (interstitial pneumonia) (Fig. 1B). Furthermore, moderate hyperaemia and oedema were noted. Additional protozoal cysts were localised in the kidney interstitium without causing morphological changes. There was severe lymphoid hyperplasia in the spleen. PAS and Grocott stains did not reveal mycotic structures in the affected organs. The protozoal cysts were strongly positive and confirmed as *T. gondii* by IHC examination in all affected organs (Fig. 1C–D).

Sloths belong to the order Edentata (suborder Xenarthra). These animals inhabit tropical forest areas of Central and South America (Wetzel, 1985). There is little scientific information about diseases that affect free-living sloths, but their nutritional, digestive, and respiratory disorders correspond to the main clinical conditions diagnosed in the species found in captivity (Diniz and Oliveira, 1999). Infections by *Leishmania shawi* (Lainson et al., 1989), *Histoplasma* (Lainson and Shaw, 1975), *Trypanosoma rangeli* (Miles et al., 1983), *Salmonella* (Loureiro, 1985), *Eimeria* spp. (Lainson and Shaw, 1982), and *Pneumocystis* (Lainson and Shaw, 1975) have been confirmed to infect free-living sloths in Brazil. Young sloths seem to be more predisposed to many of these diseases, particularly when they are maintained in captivity (Diniz and Oliveira, 1999). In the current study, the affected animal was young. It is possible that the cutaneous lesions clinically observed, compatible with mycotic aetiology, had predisposed the animal to toxoplasmosis. Unfortunately, after the first attendance, the animal and its dam were released before treatment because there was a risk of death in captivity and cutaneous samples were not collected for mycological examination.

A serological study performed in other species of sloth (*Choleopus didactylus*), analysing samples of 50 animals in French Guiana (which is geographically near the Brazilian Amazon region), showed that all were seronegative for *T. gondii* (Carme et al., 2002). These results suggest that this infection seems to be uncommon in these animals. On the other hand, additional serological studies show that in this Brazilian region, toxoplasmosis occurs with high frequency in non-human primates (49.2% of 179 tested samples), mainly in New World monkeys and in domesticated monkeys living in human homes as pets (Minervino et al., 2017). A similar investigation showed a high seroprevalence of *T. gondii* antibodies in many species of neotropical felids

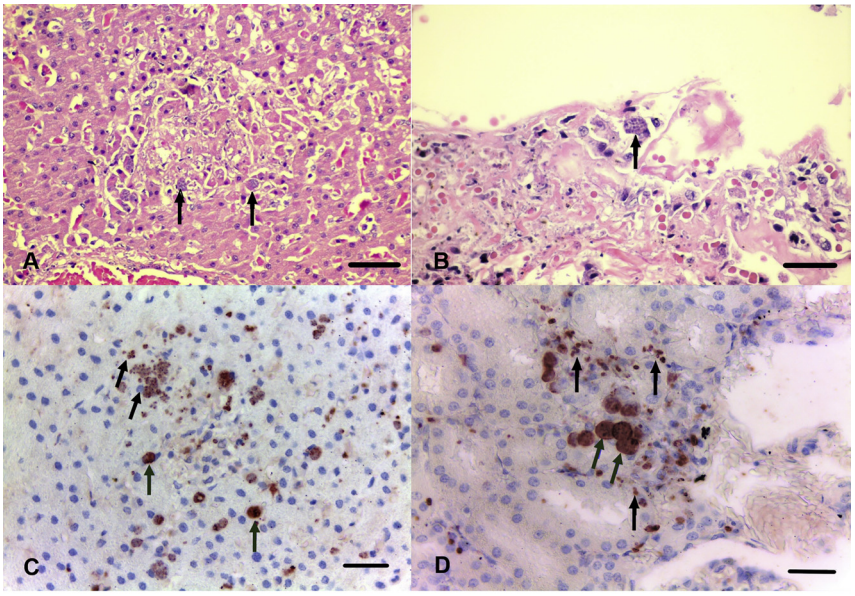


*Fig. 1. A.* Liver. Random area of coagulative necrosis with intralesional cysts of *Toxoplasma gondii* (arrows). Haematoxylin and eosin (HE), Bar = 85 µm. *B.* Lung. There is interstitial pneumonia characterised by marked expansion of the alveolar septa with necrosis of pneumocytes, cell debris, deposition of hyaline membranes, few macrophages and cyst of *T. gondii* (arrow). HE, Bar = 70 µm. *C.* Liver. Many positive tachyzoites (black arrows) and cysts containing bradyzoites (green arrows) are observed. Immunohistochemistry (IHC), Bar = 70 µm. *D.* Kidney. There are numerous tachyzoites (black arrows) and cysts containing bradyzoites (green arrows). IHC, Bar = 70 µm

sampled in zoos and at private breeders from other Brazilian regions (Silva et al., 2001). There are no similar studies conducted with native Brazilian sloths.

Based on the anatomopathological findings of the current case, the cause of death was attributed to disseminated toxoplasmosis because the microscopic findings, including inflammatory infiltrate and necrosis usually associated with protozoa, were detected in many organs such as the liver, spleen, lung, and kidney. Very similar lesions were described in other species of domestic and wild animals infected by *T. gondii* (Sallés et al., 1997; Díaz-Ayala et al., 2016; Fayyad et al., 2016). Additional lesions have been reported in animals infected by *T. gondii*, such as myocarditis, lymphadenitis, meningoencephalitis, myelitis, adrenalitis, and ophthalmitis (Dubey et al., 1990; Dubey and Carpenter, 1993; Herder et al., 2015; Díaz-Ayala et al., 2016; Fayyad et al., 2016). In some cases, granulomatous inflammation can also be observed in these affected tissues (Ochoa-Amaya et al., 2012). Toxoplasmosis can present as a systemic, neurological and/or reproductive disease. Disseminated toxoplasmosis has already been described in other species of sloth (*B. tridactylus*) (Túry et al., 2001). In this previous report and in the current case, both animals were kept in captivity, and it was not possible to define the source of infection. In both cases, it is possible that the infection had occurred in wildlife and the stress associated with captivity contributed to the pathogenicity and progression of the protozoosis (Diniz and Oliveira, 1999). It has been proposed for wild species, in some areas free of domestic cats, that infection occurs through the consumption of oocysts in the faeces of wild felids (Carme et al., 2002; Garcia-Bocanegra et al., 2010).

*Toxoplasma gondii* must be differentiated mainly from *Neospora caninum*, usually by IHC, although its differentiation from other protozoa such as *Sarcocystis neurona*, *Leishmania* spp. and *Trypanosoma* spp. is also important (Gardiner et al., 1998). Distinction is based upon the location, type and severity of lesions, parasite morphology, IHC and molecular techniques. The morphological aspects and strong immunoreactivity to an antibody specific to *T. gondii* were crucial for identification of the protozoan as the aetiological agent of the disseminated lesions in the current case. Additionally, serology and isolation can also be used for diagnosis (Dubey, 2010).

Although *T. gondii* is capable of infecting all warm-blooded animals including humans and domestic as well as wild animals, there are only few descriptions of disseminated toxoplasmosis in wild animals from Brazil, especially in sloths. Further epidemiologic, pathological and molecular studies are necessary to determine the real prevalence, pathogenesis and specific route of infection of *T. gondii* in sloths of Brazil.

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