Toxoplasmosis in a free ranging hairy dwarf porcupine (Sphiggurus spinosus) with a novel genotype

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Abstract

Toxoplasmosis is a zoonotic disease caused by the ubiquitous coccidia *Toxoplasma gondii*. Rodents play an important role in maintaining its life cycle, as they are one of the main diet sources for felids (wild and domestic), the unique definitive hosts. However, reports of toxoplasmosis in porcupines (Rodentia Order) are uncommon, with gaps concerning its pathophysiology. South America is the continent with the greatest genetic diversity of rodents and *T. gondii*. A free-ranging hairy dwarf porcupine was admitted to a wildlife rescue center with a history of trauma. During rehabilitation, the animal presented neurological symptoms (sporadic episodes of hind limbs paresis) and died five months later. The main findings during necropsy were brain congestion and severe incisor overgrowth associated with maxillary perforation. The histopathological exam showed moderate encephalitis, with variable-sized round cysts, positive for PAS stain and immunohistochemistry for *T. gondii*. Additionally, two cysts were observed in the medulla of the adrenal gland. Molecular techniques were performed to characterize the parasite load by qPCR (Cq=30) and the genotype by PCR-RFLP with 11 markers, which revealed a new genotype. This case adds to the body of knowledge in comparative pathology of Neotropical Rodentia and reports a new genotype circulating in South America.

1. INTRODUCTION

Toxoplasma gondii (T. gondii) may be the most successful parasite in the world, as it has a global distribution and can infect almost all warm-blooded animal species (Su et al., 2010; Dubey, 2010; Robert-Gangneux et al., 2012). Infections occur by ingestion or transplacentally (Dubey, 2010). Felids are their only definitive host (Vitaliano et al., 2014). Recent molecular studies have revealed a genotype diversity worldwide, with the largest genetic diversity in South America (Robert-Gangneux et al., 2012), specifically in Brazil (Pena et al., 2011), with reports of strains with resistance to sulfadiazine and pyrimethamine in human treatments (Bessa et al., 2021). Among the intermediate hosts, rodents (order Rodentia) play an important role in maintaining the life cycle of *T. gondii* , as they are one of the main food sources for wild felids (Galeh et al., 2021; Brito Jr et al., 2020; Horta et al., 2018; Gennari et al., 2015). Although histopathological lesions induced by *T. gondii* in rodents and other animals are suggestive of the disease, their morphological similarities with other apicomplexa require additional diagnostic tests, such as molecular and immunohistochemical exams (Harrison et al., 2007; Gardner, Payer et al., 1999).

South American porcupines belong to the Erethizontidae family. Their weight can reach up to 5 kg, and they

are known for their prehensile tail used to carry out their daily activities, mainly in trees. Their diets are mainly based on leaves, fruits, and bark (Roze, 2012). The hairy dwarf porcupine *Sphiggurus spinosus* (F. Cuvier, 1823) has a wide distribution in northeastern Argentina, southeastern Brazil, eastern Paraguay, and northern Uruguay (Barthelmess et al., 2016; Voss, 2015). This species is found in a wide range of Brazilian habitats, including the Cerrado, the Pantanal, and the Atlantic Forest. This species is categorized as Least Concern (LC) according to the red list of the International Union for Conservation of Nature (IUCN) and has been included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix III since 1976. Although the South American porcupines have a continental distribution due to their cryptic habits, the knowledge about their ecological and sanitary aspects is limited. This study reports the microscopic, immunohistochemical, and molecular features of toxoplasmosis in a free-ranging hairy dwarf porcupine in Brazil.

Among reports of toxoplasmosis in porcupines, Morales, Peña, and Dubey (1996) confirmed the first *T. gondii* in neotropical porcupines through histopathological and IHC analysis in a captive *Sphiggurus mexicanus* in Costa Rica. There are also reports in a *Hystrix cristata* (Harrison et al., 2007), three *Chaetomys subspinasus*from Brazil (Bezerra et al., 2015), and one *Erethizontidae* sp. in Germany (Fayyad et al., 2017).

2. MATERIAL AND METHODS

An adult female hairy dwarf porcupine (*Sphiggurus spinosus* , F. Cuvier, 1823) was admitted to a wildlife rescue center with a history of trauma. It was found in a periurban area of the metropolitan region of São Paulo, between the Atlantic rainforest and densely urbanized area (Figure 1). The animal was kept under medical care for almost five months, and, during the rehabilitation, the animal presented sporadic episodes of bilateral hind limb paresis (Supplementary material 1). Days before death, a purulent discharge in the right nostril was observed. Necropsy was performed, and tissue fragments (brain, heart, lungs, liver, spleen, kidney, adrenal glands, small intestine, uterus, and urinary bladder) were collected and fixed in 10% neutral buffered formalin, and fresh brain and liver fragments were frozen at -20degC. The samples were referred to Adolfo Lutz Institute within the framework of the wildlife epidemiological and laboratorial surveillance project: potential for the emergence and re-emergence of infectious diseases (SES-PRC-2020/32339 - PROC. 28/2020) in order to detect potential zoonotic diseases in wildlife.

For histopathology, the tissues were processed routinely, embedded in paraffin-wax, sectioned at 4 μm-thick and stained with hematoxylin and eosin (H&E) and Periodic Acid of Schiff (PAS), for routine microscopic analysis. Immunohistochemical and molecular methods are fully described in the Technical appendix 1.

The address where the porcupine was found was recorded. We geocoded the address using Geographic Information System ArcGIS 10.2.2. and its online imagery basemaps only for location purposes.

3. RESULTS AND DISCUSSION

The main gross findings were: purulent discharge in the right nostril extending to the frontal sinus; dental overgrowth of the lower right incisor associated with a solution of continuity in the maxillary and the right infraorbital regions, and marked congestion of the cortical blood vessels in the brain. No lesions were observed in other organs. Histologically, mild to moderate multifocal non-suppurative encephalitis, characterized by mononuclear perivascular cuffs with two to four layers of lymphocytes, plasma cells and macrophages, associated with mild to moderate multifocal gliosis, occasional necrosis of neuropil, randomly in white and gray matter. Multifocal protozoan cysts ranging from 66,3x44,9 microns to 126,7x136,0 microns, PAS positive, were observed (Figure 2). Protozoan cysts showed immunolabeling for *T. gondii* at IHC (Figure 2). Two protozoan cysts were found in the medulla of the adrenal gland.

In rodents (mainly mice and rats) is hypothesized that in latent infections, there is a neuroanatomical tendency of parasite cysts to be more abundant in amygdalar structures, resulting in impaired learning and memory and behavioral abnormalities, associated with loss of fear from predators, which could favor the biological cycle of the *T. gondii* (Hari Dass et al., 2014; Boillat et al 2020). In this porcupine, we observed *T. gondii* cysts exclusively in the central nervous system and in the medulla of the adrenal gland, the latter

with a neuroectodermal origin. We believe that this case corresponds to a reactivation of latent infection due to the predominance of large cysts, rare inflammatory infiltrates around cystic structures, and absence of*Toxoplasma gondii* in other tissues, such as the liver and spleen.

Molecular confirmation through qPCR showed a moderate parasite load (Cq=30). In ToxoDB database platform, different genotypes (#1- #278) of *T. gondii* in human and animal samples are registered. This sample is likely a new genotype, with similarity to other known genotypes, since 9 out of 11 markers matched to ToxoDB PCR-RFLP genotypes #6, #80, #126 and #244 (Table 1). The genotype #6 (also known as BrI and Africa 1) is the major virulent lineage previously reported in Brazil, and it was previously identified in domestic and wild Brazilian animals like chickens, dogs, cats, sheep, goats, pigeons, and capybaras (Dubey et al., 2012; Costa et al., 2021; Barros et al., 2014; Yai et al., 2009). The genotype #6 is also observed in HIV-immunosuppressed humans in Brazil, with severe and diffuse cerebral toxoplasmosis (Ferreira et al., 2011). The genotypes #80 and #126 are described in Brazilian cats. Information about genotype #244 was not found in the literature**.** Beyond that, the only genotyping study on *Sphiggurus spinosus* , showed 4/7 markers similarity (Richini-Pereira et al., 2016).

4. CONCLUSION

Herein, we describe the pathologic, IHC and genotyping features of toxoplasmosis in a free-ranging hairy dwarf porcupine. To the best of our knowledge, this is the first report describing the molecular, immunohistochemical, and pathological features of this condition in neotropical porcupines, including the report of a new genotype. It reinforces the importance of wildlife surveillance to detect zoonotic diseases, especially in urban areas.

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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. No ethical approval was required for this specific study.

References

Barthelmess, E. (2016). Family Erethizontidae (New World Porcupines). In Wilson, D.E., Lacher, T.E., & Mittermeier, R.A. (Eds.), *Handbook of Mammals of the World Volume 6. Lagomorphs and Rodents.* (pp.372-397). Lynx Edicions in association with Conservation International and IUCN.

Barros, L.D., Taroda, A., Zulpo, D.L., Cunha, I.A.L, Sammi, A.S., Cardim, S.T., Miura, A.C., Su, C., Machado, R.Z., Vidotto, O., & Garcia, J.L. (2014). Genetic characterization of Toxoplasma gondii isolates from eared doves (Zenaida auriculata) in Brazil. Revista Brasileira de Parasitologia Veterinária, 23(4). *doi* : 10.1590/S1984-29612014073 .

Bessa, L.G., Vitor, R.W.A, & Martins-Duarte, E.S. (2021). Toxoplasma gondii in South America: a differentiated pattern of spread, population structure and clinical manifestations. Parasitology Research, 120(9), 3065-3076. *doi* : 10.1007/s00436-021-07282-w.

Bezerra, R.A., Giné, G.A., Maciel, B.M., Gaiotto, F.A., & Albuquerque, G.R. (2015). Identification and genetic characterization of Toxoplasma gondii in free-ranging bristle-spined porcupine (Chaetomys subspinosus), a threatened arboreal mammal from the Brazilian Atlantic Forest. Parasites and vectors, 17(8), 277. *doi* : 10.1186/s13071-015-0882-6.

Boillat, M., Hammoudi, P. M., Dogga, S. K., Pagès, S., Goubran, M., Rodriguez, I., & Soldati-Favre, D. (2020). Neuroinflammation-associated aspecific manipulation of mouse predator fear by Toxoplasma gondii. Cell Reports, 30(2), 320-334. *doi* : 10.1016/j.celrep.2019.12.019.

Brito Junior, P.A., Rocha, J.M., Silva, C.A.D., Oliveira, P.M.V., Correia, J.E., Cruz, L.A.D., Sevá, A.D.P., Oliveira, T.V., Silva, A.V.D., Alvarez, M.R.D.V., & Albuquerque, G.R. (2020). Survey of anti-Toxoplasma gondii antibodies in wild mammals captured from Atlantic Forest fragments in Bahia, northeastern Brazil. Revista brasileira de parasitologia veterinaria, 29(4). *doi* : 10.1590/S1984-29612020083.

Costa, W.L.G.D., Barbosa, I.M.F.N., Prado, D.P.G.D., Domann, N., Rezende, H.H.A. (2021). A systematic review of Toxoplasma gondii genotypes in Gallus gallus domesticus worldwide: The focus is Brazil. Transboundary and emerging diseases. *doi* : 10.1111/tbed.14221.

Dubey J.P. (2010). Toxoplasmosis of Animals and Man. CRC Press.

Dubey, J.P., Hill, D.E., Rozeboom, D.W., Rajendran, C., Choudhary, S., Ferreira, L.R., Kwok, O.C.H., & Su, C. (2012). High prevalence and genotypes of Toxoplasma gondii isolated from organic pigs in northern USA. Veterinary Parasitology, 188(1-2):14-8. *doi* : 10.1016/j.vetpar.2012.03.008.

Dubey, J.P., Ferreira, L.R., Alsaad, M., Verma, S.K., Alves, D.A., Holland, G.N., & McConkey, G.A. (2016). Experimental toxoplasmosis in rats induced orally with eleven strains of Toxoplasma gondii of seven genotypes: Tissue tropism, tissue cyst size, neural lesions, tissue cyst rupture without reactivation, and ocular lesions. Public Library of Science one, 11(5). *doi* : 10.1371/journal.pone.0156255.

Fayyad, A., Kummerfeld, M., Davina, I, Wohlsein, P., Beineke, A., Baumgärtner, W., & Puff, C. (2016). Fatal Systemic Toxoplasma gondii Infection in a Red Squirrel (Sciurus vulgaris), a Swinhoe's Striped Squirrel (Tamiops swinhoei) and a New World Porcupine (Erethizontidae sp.). Journal of comparative pathology, 154(2-3), 263-267. *doi* : 10.1016/j.jcpa.2016.02.002.

Galeh, T.M., Sarvi, S., Hosseini, S.A., & Daryani, A. (2021). Genetic diversity of Toxoplasma gondii isolates from rodents in the world: A systematic review. Transboundary and emerging diseases. *doi* : 10.1111/tbed.14096.

Gardner, C.H., Payer, R., & Dubey, J.P. (1999). An Atlas of Protozoan Parasites in Animal Tissues. American Registry of Path.

Gennari, S.M., Ogrzewalska, M.H., Soares, H.S., Saraiva, D.G., Pinter, A., Nieri-Bastos, F.A., Labruna, M.B., Szabó, M.P., & Dubey, J.P. (2015). Toxoplasma gondii antibodies in wild rodents and marsupials from the Atlantic Forest, state of São Paulo, Brazil. Revista brasileira de parasitologia veterinaria, 24(3), 379-382. *doi* : 10.1590/S1984-29612015045.

Hari Dass, S. A., & Vyas, A. (2014). Toxoplasma gondii infection reduces predator aversion in rats through epigenetic modulation in the host medial amygdala. Molecular ecology, 23(24), 6114-6122. *doi* : 10.1111/mec.12888.

Harrison, T.M., Moorman, J.B., Bolin, S.R., Grosjean, N.L., Lim, A., & Fitzgerald, S.D. (2007). Toxoplasma gondii in an African crested porcupine (Hystrix cristata). Journal of veterinary diagnostic investigation, 19(2), 191-194. *doi* : 10.1177/104063870701900210.

Horta, M.C., Guimarães, M.F., Arraes-Santos, A.I., Araujo, A.C., Dubey, J.P, Labruna, M.B., Gennari, S.M., & Pena, H.F.J. (2018). Detection of anti-Toxoplasma gondii antibodies in small wild mammals from preserved and non-preserved areas in the Caatinga biome, a semi-arid region of Northeast Brazil. Veterinary Parasitology: Regional Studies and Reports, 14:75-78. *doi* : 10.1016/j.vprsr.2018.08.007.

Khan, A., Fux, B., Su, C., Dubey, J.P., Darde, M.L. Ajioka, J.W., Rosenthal, B.M., & Sibley, L.D. (2007). Recent transcontinental sweep of Toxoplasma gondii driven by a single monomorphic chromosome. Proceedings of the National Academy of Sciences of the United States of America, 104, 14872–14877. *doi* : 10.1073/pnas.0702356104.

Lin, M.H., Chen, T.C., Kuo, T.T., Tseng, C.C., Tseng, C.P. (2000). Real-Time PCR for Quantitative Detection of Toxoplasma gondii. Journal of Clinical Microbiology, 38 (11), 4121–4125. *doi* : 10.1128/JCM.38.11.4121-4125.2000.

Morales, J.A., Peña, M.A., & Dubey, J.P. (1996). Disseminated toxoplasmosis in a captive porcupine (Coendou mexicanus) from Costa Rica. The Journal of parasitology, 82(1), 185-6. PMID: 8627495.

Pena, H.F.J., Marvulo, M.F.V., Horta, M.C., Silva, M.A., Silva, J.C.R., Siqueira, D.B., Lima, P.A.C.P., Vitaliano, S.N., & Gennari, S.M. (2011). Isolation and genetic characterisation of Toxoplasma gondii from a red-handed howler monkey (Alouatta belzebul), a jaguarundi (Puma yagouaroundi), and a black-eared opossum (Didelphis aurita) from Brazil. Veterinary Parasitology, 175(3-4), 377–381. *doi* : 10.1016/j.vetpar.2010.10.015.

Richini-Pereira, V.B., Marson, P.M., Silva, R.C., & Langoni, H. (2016). Genotyping of Toxoplasma gondii and Sarcocystis spp. in road-killed wild mammals from the Central Western Region of the State of São Paulo, Brazil. Revista da Sociedade Brasileira de Medicina Tropical, 49(5):602-607. *doi* : 10.1590/0037-8682-0270-2016.

Robert-Gangneux, F., & Dardé, M.L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. Clinical Microbiology Reviews, 25(2), 264–296. *doi* : 10.1128/CMR.05013-11.

Roze, U. (2012). Porcupines: the animal answer guide. In The Animal Answer Guides: Q&A for the Curious Naturalist. John Hopkins University Press. *doi* :10.1086/678663.

Su, C., Shwab, E.K., Zhou, P., Zhu, X.Q., & Dubey, J.P. (2010). Moving towards an integrated approach to molecular detection and identification of Toxoplasma gondii. Parasitology, 137(1), 1–11. *doi* : 10.1017/S0031182009991065.

Vitaliano, S.N., Soares, H.S., Minervino, A.H.H., Santos, A.L.Q., Werther, K. Marvulo, M.F., Siqueira, D.B., Pena, H.F.J., Soares, R.M., Su, C., & Gennari, S.M. (2014). Genetic characterization of Toxoplasma gondii from Brazilian wildlife revealed abundant new genotypes. International Journal for Parasitology: Parasites and Wildlife, 3(3), 276–283. *doi* : 10.1016/j.ijppaw.2014.09.003.

Voss, R.S. (2015). Superfamily Erethizontoidea Bonaparte, 1845. In Patton, J.L., Pardiñas, U.F.J., & D'Elía, G. (Eds.), *Mammals of South America, Volume 2: Rodents* (pp. 786–805). The University of Chicago Press.

Yai, L.E., Ragozo, A.M., Soares, R.M., Pena, H.F., Su, C., & Gennari, S.M. (2009). Genetic diversity among capybara (Hydrochaeris hydrochaeris) isolates of Toxoplasma gondii from Brazil. Veterinary parasitology, 162(3-4), 332-7. *doi* : 10.1016/j.vetpar.2009.03.007.

Declaration of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

TABLES

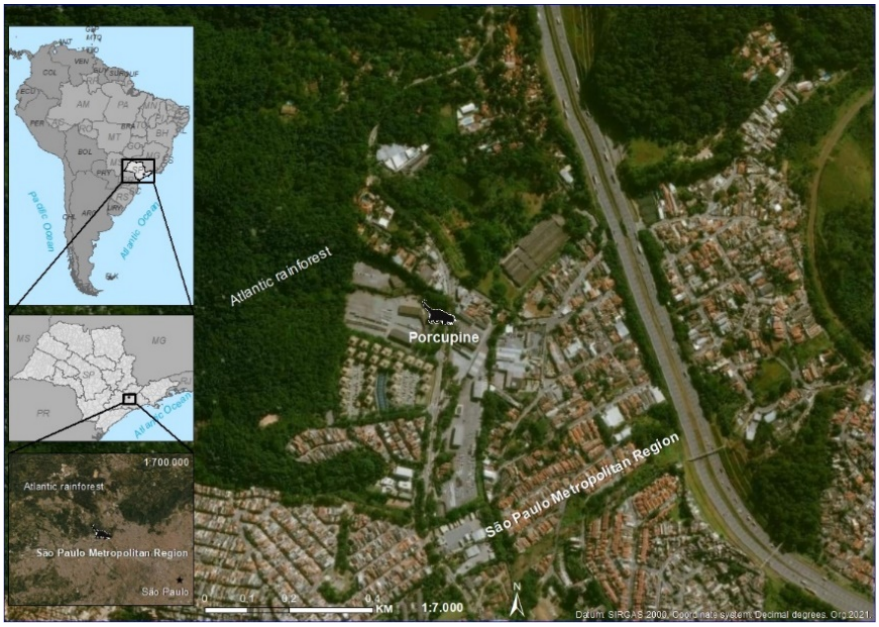
Table 1.

SAG1	5-3SAG2	alt.SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
I?	I	I?	III	I?	II	u-1	III	I	u-1	I

Multilocus genotyping of *Toxoplasma gondii* strain from a hairy dwarf porcupine (*Sphiggurus spinosus)* by PCR-RFLP analysis. New genotype, with comparison to strains indexed into TOXO-DB.

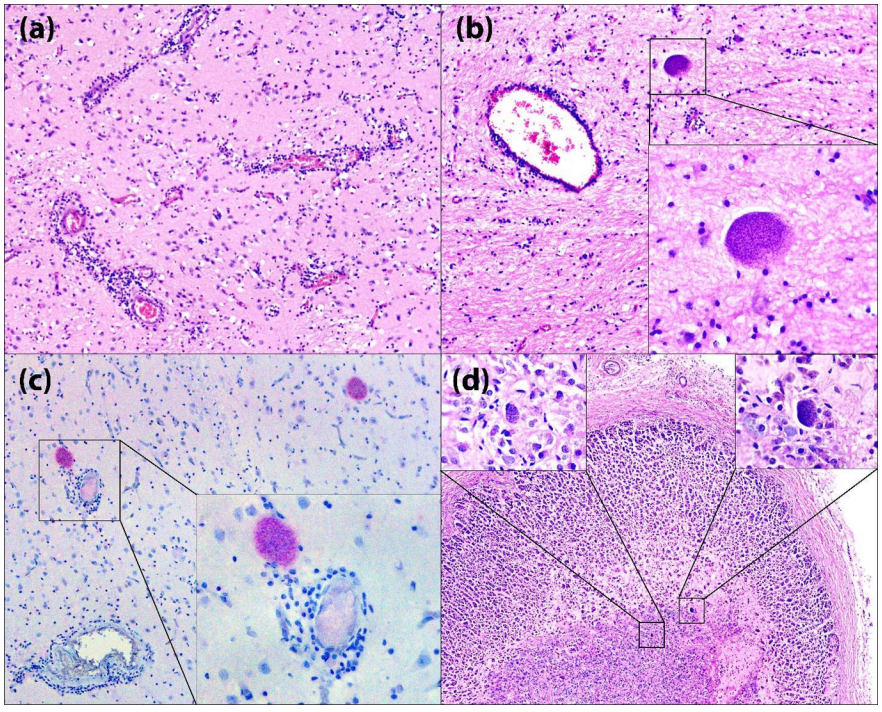
FIGURES

Figure 1. Location of the porcupine



The animal was found alive near a wildlife corridor of the Atlantic rainforest in the Metropolitan Region of São Paulo.

Figure 2. Histopathological and immunohitochemical findings



Histopathological analysis of *Toxoplasma gondii* infection in brain of a hairy dwarf porcupine. (a) Mononuclear cells infiltrating into the brain (encephalitis) and around a vessel, brain, H&E; (b) *Toxoplasma. gondii* tissue cyst in the middle of the inflammation, brain, H&E. (c) *Toxoplasma. gondii* tissue cyst next to a vascular cuffing with mononuclear cells infiltration, brain, IHC. (d) *Toxoplasma gondii* cysts in medullary, adrenal gland, H&E.