









Pathological aspects of disseminated protothecosis-like infection in a dog

Adriano Lima Stelzer Bindaco¹ Halana do Carmo Silva¹ Carolina Magri Ferraz²
Fernando Luiz Tobias² Mayra Cunha Flecher² Igor Luiz Salardani Senhorello^{3*}

¹Laboratório de Anatomia Patológica Maylla Gava, Vila Velha, ES, Brasil.

²Universidade Vila Velha (UVV), Vila Velha, ES, Brasil.

³Faculdade de Medicina Veterinária, Universidade Estadual Paulista (UNESP), 16050-680, Araçatuba, SP, Brasil. E-mail: igor.salardani@unesp.br.

*Corresponding author.

ABSTRACT: Anatomopathological descriptions of infections caused by saprophytic algae in dogs are rare. This study reported the anatomopathological and microbiological findings suggestive of disseminated protothecosis in a dog residing in the state of Espírito Santo, Brazil. During necropsy, macroscopic alterations were observed in peripheral lymph nodes, heart, kidneys, pancreas, and lungs. Microbiological, cytological, and histopathological examinations were performed. Cytology and histopathology revealed piogranulomatous inflammation associated with sporangia and endospores, while microbiological culture suggested the growth of *Prototheca* spp. These anatomopathological and microbiological findings were crucial for diagnosing and documenting the first case of disseminated *Prototheca* spp. compatible infection in a dog in the state of Espírito Santo, Brazil.

Key words: algae, *Prototheca*, infection, canine, systemic lesions.

Aspectos patológicos de uma infecção disseminada semelhante à Prototecose em um cão

RESUMO: Descrições anatomopatológicas de infecções causadas por algas saprófitas em cães são raras. Este estudo relata os achados anatomopatológicos e microbiológicos sugestivos de prototecose disseminada em um cão residente no estado do Espírito Santo, Brasil. Durante a necropsia foram observadas alterações macroscópicas em linfonodos periféricos, coração, rins, pâncreas e pulmões. Foram realizados exames microbiológicos, citológicos e histopatológicos. A citologia e a histopatologia revelaram inflamação piogranulomatosa associada a esporângios e endósporos, enquanto a cultura microbiológica sugeriu o crescimento de *Prototheca* spp. Esses achados anatomopatológicos e microbiológicos foram cruciais para diagnosticar e documentar o primeiro caso de infecção disseminada compatível com *Prototheca* spp. estado do Espírito Santo, Brasil.

Palavras-chave: alga, *Prototheca*, infecção, canino, lesões sistêmicas.

Prototheca spp. are saprophytic unicellular algae, which inhabit humid environments rich in organic matter, such as soil, mud, sewage, and feces. Among species of the genus, *P. zopfii* and *P. wickerhamii* stand out for their pathogenicity and for causing zoonotic disease in humans and animals (CAMBOIM et al., 2010). In dogs, protothecosis may appear as either disseminated or as cutaneous, ocular, or enteric manifestations (SIQUEIRA et al., 2008). The present case describes the anatomopathological and microbiological findings suggestive of disseminated protothecosis in a dog, in the Southeastern region of Brazil, and provides important data on diagnosis and morphological characteristics of the agent and the resulting lesions.

The corpse of an 8-year-old mixed breed bitch (non-defined breed, NDB), residing in the city of Vila Velha, was sent to the Animal Pathology Laboratory of the Veterinary Hospital Professor Ricardo Alexandre Hippler at the Universidade Vila Velha (VH-UVV), in Vila Velha, Espírito Santo (ES), Brazil. The dog died naturally, and the owner reported that 45 days before the dog's death, it experienced an acute episode of diarrhea, followed by other clinical signs such as fever, apathy, reduced appetite, and nasal discharge, with a progressive worsening of the condition. It is also noteworthy that the dog was a house pet that lived in an apartment without foreign contact and with access to the street restricted to walks twice a day at scheduled times.

Anatomopathological examination showed generalized peripheral lymphadenomegaly and

presented diffuse pale pink to white parenchyma, with millimeter yellowish multifocal to coalescent nodules (Figure 1A). The heart presented pallor of the cardiac musculature and a irregular, flat and whitish multifocal to coalescent area (Figure 1B). The lung was hypocrepitant, with a focally extensive, firm and dark red area in the dorsal portion of the left caudal lobe, extending to the parenchyma. The pancreas had multifocal white dots. Both kidneys were diffusely pale with irregular white to yellow multifocal to coalescent area, in the cortical and medullary (Figure 1C).

Sterile swabs containing Stuart transport medium (Model 23010P, Absorve®) were used for sampling the lymph nodes for bacterial and fungal culture, in addition to imprinting the organs for cytopathological evaluation. Fragments of the heart, lungs, spleen, lymph nodes, intestines, pancreas, liver, kidney and brain were collected for histological evaluation. The microbiological exams were performed at the Veterinary Microbiology and Immunology Laboratory and the cytopathological

and histopathological exams at the Animal Pathology Laboratory of the VH-UVV.

Cytological slides were stained using Diff-Quik, and tissue fragments underwent routine histological, which were submitted to Hematoxylin and Eosin (HE), Periodic Acid- Schiff (PAS) and Grocott staining techniques. For bacterial isolation, swabs were cultured on Blood Agar Base (Kasvi®) added with 5% sheep blood, and MacConkey Agar (Kasvi®). Cultures were incubated in a bacteriological oven at 35- 37 °C for 24 to 72 hours. To evaluate the fungal growth, the swabs were inoculated in Sabouraud Dextrose Agar (Neogen®) and Mycosel Agar (BD®) and kept in an incubator at 25 – 28 °C, for 7 days.

Cytology revealed a pyogranulomatous inflammatory pattern associated with an abundant amount of round, blue capsulated structures of different sizes, sometimes containing internal septation, free in the background of the slide and the cytoplasm of macrophages, suggestive with sporangia and endospores of *Prototheca* spp. (Figure 1E).

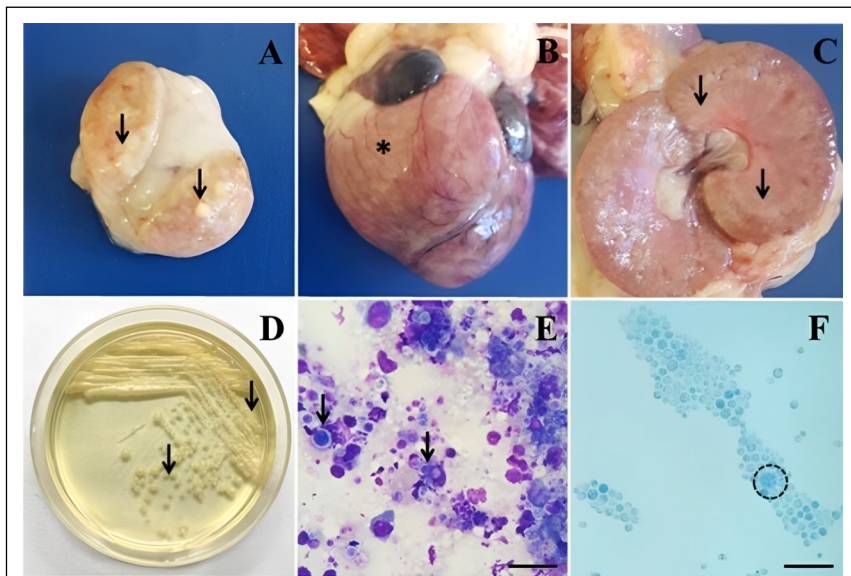


Figure 1 - Anatomopathological and microbiological findings suggestive of disseminated infection by *Prototheca* spp. in a dog. A. Superficial cervical lymph node showing loss of cortico-medullary delineation with delimited multifocal to coalescent area, yellowish-white colored (arrow). B. Heart with multifocal to coalescing irregular white area (asterisk) in pericardium and musculature. C. Kidneys with marked diffuse parenchymal pallor associated with a multifocal to coalescent irregular white area (arrow). D. Round, white to golden colonies compatible with *Prototheca* (Sabouraud-Dextrose Agar Culture Medium). E. Imprint cytology of the superficial cervical lymph node revealing round structures (arrows) inside macrophages and free in the background, compatible with *Prototheca* spp.. Diff-Quik stain, bar = 100.00 µm. F. Round and septate structure, compatible with *Prototheca* sporangia (arrow) – Cotton Blue Stain.

Microbiological results revealed a growth in Sabouraud-Dextrose agar and blood agar. In both media, the round-shaped colonies exhibited a white to golden color and mucoid appearance, however, on Sabouraud-Dextrose agar the colonies were larger, ranging from 3 and 6 mm in diameter (Figure 1D). Colonies were stained with cotton blue and Gram. Cotton blue staining followed by light microscopy evaluation revealed structures compatible with sporangia and endospores of *Prototheca* spp. (Figure 1F).

Histopathological analysis revealed myocarditis, lymphadenitis, interstitial pneumonia, interstitial nephritis and pancreatitis (Figures 2A-E). All of the mentioned lesions presented a necrotizing pyogranulomatous inflammatory pattern associated with structures suggestive of intralésional sporangia and endospores of *Prototheca* spp. (6 to 20 µm in diameter), evidenced in PAS and Grocott stains (Figure 2F). A limitation of the present report was the lack of molecular confirmation of the agent. Despite repeated attempts to isolate DNA from paraffin-embedded material, these

efforts were unsuccessful, thereby hindering specie identification. However, the anatomopathological and microbiological findings as presented suggested a disseminated infection by *Prototheca* spp.

In dogs, protothecosis is classified as cutaneous, ocular, enteric, and/or disseminated. The disseminated manifestation of the infection is rare and occurs mainly in immunosuppressed individuals since the compromised immune system favors the dissemination of the algae (via hematogenous and lymphatic) to other organs (CAMBOIM et al., 2010). It was not possible to identify any previous disease that could have caused the immunosuppression of the patient in the present report.

In disseminated canine protothecosis, lesions such as myocarditis and nephritis (BERROCAL et al., 1997; SONNE et al., 2017; ARSENAULT et al., 2022; PRICE et al., 2023), lymphadenitis in the cervical (SUDMAN et al., 1973) and mesenteric lymph nodes (HOSAKA & HOSAKA, 2004; PRICE et al., 2023), and pneumonia

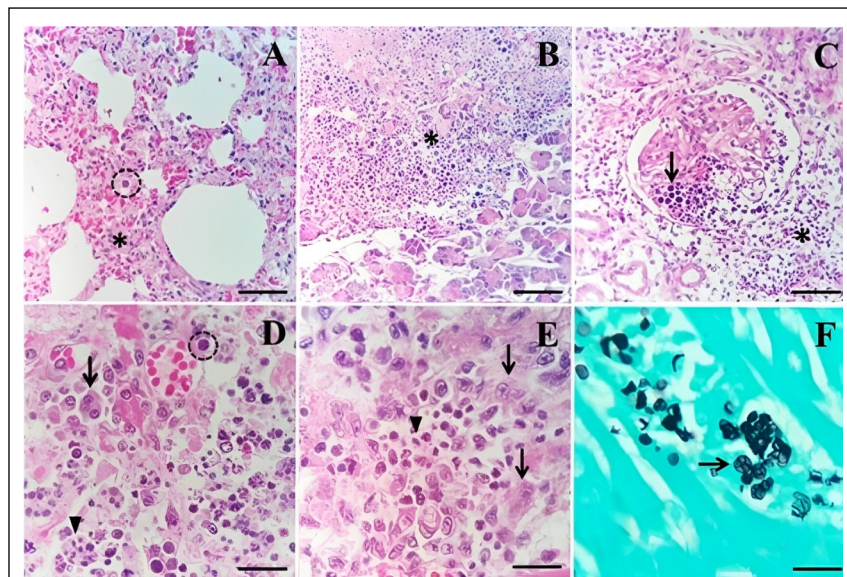


Figure 2 - Photomicroscopy of organs with disseminated infection similar to *Prototheca* spp. in a dog. A. Pyogranulomatous interstitial pneumonia (asterisk) associated with infection suggestive of *Prototheca* spp. (dotted circle). HE, bar = 100.00 µm. B. Pyogranulomatous pancreatitis with necrosis (asterisk). HE, bar = 100.00 µm. C. Pyogranulomatous interstitial nephritis, with glomerulus showing structures similar to sporangia and endospores of *Prototheca* spp. (asterisk). HE, bar = 100.00 µm. D. Diffuse necrotizing pyogranulomatous lymphadenitis, with multinucleated giant cell (arrow), and image suggestive *Prototheca* spp. free in tissue (dotted circle) and intracytoplasmic on macrophages (triangle). HE, bar = 50.00 µm. E. Necrotizing pyogranulomatous myocarditis, with degenerate neutrophils (triangle) and epithelioid macrophages (arrows). HE, bar = 50.00 µm. F. Structures compatible with sporangia of *Prototheca* spp. (arrow) in heart, showing internal septation in the Grocott stain. Bar = 50.00 µm.

(BUYUKMIHCI et al., 1975; SUDMAN et al., 1973) have also been reported. The findings of the present study confirmed the importance of the disseminated form of protothecosis in triggering systemic lesions in organs essential for body homeostasis.

Considering that *Prototheca* spp. inhabits soil, mud, sewage, trees and feces (human and animals), and that contamination occurs by ingestion or contact with non-intact skin or mucous membranes (CAMBOIM et al., 2010), it is suggested that the animal acquired the algae from the environment, given that it had access to the street for walks. It is noteworthy that the urban environment presents several substrates for the development of algae. In addition, the city of Vila Velha, where the animal lived, is subjected to frequent flooding in periods of rain, which may favor the presence of algae in the environment. It is also a coastal region, which according to a previous study may increase the chances of infection (STENNER et al., 2007). However, the anatomopathological examination of small and large intestines showed no significant changes, making it difficult to understand this pathogenesis. The diarrhea presented by the animal may be associated with chronic pancreatitis and is not necessarily related to the algae infection in the intestines. Intestinal lesions were also not found in other reports of disseminated protothecosis (BERROCAL et al., 1997; TYLER et al., 1980).

At the microscopic level, structures suggestive of algae were observed in all affected tissues amid pyogranulomatous inflammation, a characteristic pattern in *Prototheca* spp. infections (SONNE et al., 2017; WESSELOWSKI et al., 2022). However, the isolation and identification of the microorganism through microbiological culture is the method of choice to confirm the diagnosis (SIQUEIRA et al., 2008). Colony growth and morphology on Sabouraud-Dextrose agar and blood agar, as well as microscopy using cotton blue stains, are essential for the microbiological identification of *Prototheca* spp. (CAMBOIM et al., 2010). According to previous studies, *Prototheca bovis* (formerly *P. zopfii* genotype 2) and *Prototheca wickerhamii* are the most involved in pathogenic infections in humans and animals (STENNER et al., 2007). In the epidemiological context, it is important to identify the molecular character of the agent. However, all anatomopathological and microbiological characteristics support the suggestive diagnosis of disseminated protothecosis, a rare condition that is seldom reported in Brazil and marks the first occurrence documented in the state of Espírito Santo.

ACKNOWLEDGEMENTS

The authors acknowledge the Laboratório de Bacteriologia, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

We authors of the article entitled "Disseminated protothecosis in a dog: A case report" declared, for all due purposes, the project that gave rise to the present data of the same has not been submitted for evaluation to the Ethics Committee of the University /Research Institute "Universidade Vila Velha (UVV)", but we are aware of the content of the Brazilian resolutions of the National Council for Control of Animal Experimentation – CONCEA "<http://www.mct.gov.br/index.php/content/view/310553.html>" if it involves animals. Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

REFERENCES

- ARSENAULT, A. C. et al. Protothecosis and *Toxoplasma gondii* co-infection in a dog from Nova Scotia, Canada. **The Canadian Veterinary Journal**, v.63, n.11, p.1114, 2022. Available from: <<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9559031/>>. Accessed: Dec. 20, 2023.
- BERROCAL, A. et al. Systemic protothecosis in a dog: pathological description of a case. **Archivos de Medicina Veterinaria**, v.29, p.307-312, 1997. Available from: <<http://dx.doi.org/10.4067/S0301-732X1997000200017>>. Accessed: Nov. 17, 2020. doi: 10.4067/S0301-732X1997000200017.
- BUYUKMIHCI, N. C. et al. Protothecosis with ocular involvement in a dog. **Journal of the American Veterinary Medical Association**, v.167, n.2, p.158-161, 1975. Available from: <<https://escholarship.org/uc/item/7756q4s2>>. Accessed: Nov. 12, 2020.
- CAMBOIM, E. K. A. et al. Protothecosis: an emergent disease. **Pesquisa Veterinária Brasileira**, v.30, p.142-146, 2010. Available from: <<http://dx.doi.org/10.1590/S0100-736X2010000100015>>. Accessed: Nov. 15, 2020. doi: 10.1590/S0100-736X2010000100015.
- HOSAKA, S.; HOSAKA, M. A case report of canine protothecosis. **Journal of the American Animal Hospital Association**, v.5, p.593-597, 2004. Available from: <<https://doi.org/10.1292/jvms.66.593>>. Accessed: Nov. 23, 2020. doi: 10.1292/jvms.66.593.

PRICE, P. S. A. et al. Protothecosis in four dogs in New Zealand. **New Zealand Veterinary Journal**, v.71, n.6, p.321-328, 2023. Available from: <<https://doi.org/10.1080/00480169.2023.2248066>>. Accessed: Dec. 20, 2023.

SIQUEIRA, A. K. et al. Protothecosis in companion animals and aspects of the disease in human. **Ciência Rural**, v.38, p.1794-1804, 2008. Available from: <<https://doi.org/10.1590/S0103-84782008000600052>>. Accessed: Nov. 16, 2020. doi: 10.1590/S0103-84782008000600052.

SONNE, L. et al. *Prototheca zopfii* genotype 2 disseminated infection in a dog with neurological signs. **Ciência Rural**, v.47, p.1-5, 2017. Available from: <<https://doi.org/10.1590/0103-8478cr20160877>>. Accessed: Nov. 10, 2020. doi: 10.1590/0103-8478cr20160877.

STENNER, V. J. et al. Protothecosis in 17 Australian dogs and a review of the canine literature. **Sabouraudia**,

v.45, n.3, p.249-266, 2007. Available from: <<https://doi.org/10.1080/13693780601187158>>. Accessed: Nov. 19, 2022.

SUDMAN, M. S. et al. Primary mucocutaneous protothecosis in a dog. **Journal of the American Animal Hospital Association**, v.163, p.1372-1374, 1973. Available from: <<https://pubmed.ncbi.nlm.nih.gov/4760084/>>. Accessed: Nov. 12, 2020.

TYLER, E. et al. Disseminated protothecosis with central nervous system involvement in a dog. **Journal of the American Animal Hospital Association**, v.176, p.987-993, 1980. Available from: <<https://europepmc.org/article/med/7380720>>. Accessed: Nov. 12, 2020.

WESSELOWSKI, S. et al. Pancarditis as the sole clinical manifestation of protothecosis in a Boxer dog. **Journal of Veterinary Cardiology**, v.41, p.128-133, 2022. Available from: <<https://doi.org/10.1016/j.jvc.2022.02.004>>. Accessed: Dec. 20, 2023.