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# Paracoccidioidomycosis in a Dog: Case Report of Generalized Lymphadenomegaly

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**Abstract** Paracoccidioidomycosis (PCM) is a severe systemic mycosis, endemic in Latin America and highly prevalent in Brazil, where it ranks eighth as a mortality cause among infectious and parasitic diseases in humans. The disease in animals has been little explored. It is observed that armadillos can harbor the fungus at high frequencies, although the active disease has not been well documented in this wild mammal. Dogs are susceptible to experimental infection, and the naturally acquired PCM-disease was reported only recently in a dog from Brazil. The present work reports the second case of naturally acquired PCM in a 6-year-old female dog that presented emaciation, lymphadenomegaly, and hepatosplenomegaly. Biochemical and pulmonary radiographic evaluation did not reveal any abnormalities. PCM was diagnosed by clinical findings, culturing, immunohistochemistry, and histopathology of popliteal lymph node. The fungus was recovered from popliteal lymph node, and the molecular analysis showed respective sequencing similarities of 99 and 100% for 803 nucleotides of the *Gp*43 gene and 592 nucleotides from the *ITS-5.8S* region of *Paracoccidioides brasiliensis*. Immunohistochemistry revealed severe lymphadenitis and presented numerous yeasts, which reacted against the gp43 antibody. Histopathology revealed a severe

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granulomatous lymphadenitis associated with numerous single or multiple budding yeasts. After diagnosis, the dog was successfully treated with itraconazol for 2 years. Veterinarians should be aware of the importance of considering PCM for differential diagnosis, especially in dogs from PCM-endemic areas, whose monophagocytic system involvement is evident.

**Keywords** *Paracoccidioides brasiliensis* · Dog · Lymphadenomegaly · Granulomatous disease

## Introduction

*Paracoccidioides brasiliensis*, the etiological agent of paracoccidioidomycosis (PCM), is a thermodimorphic fungus and was recently classified into the Ajellomycetaceae family [1]. PCM is endemic in Latin America, especially in Brazil, which has reported the majority of human cases [2]. In Brazil, PCM is the eighth most frequent cause of death among the infectious and parasitic diseases, with a mean annual mortality rate of 1.45 per million inhabitants [3]. The infection is acquired through airborne inhalation of the infective conidia present in the environment [2].

Human PCM is observed mainly in two distinct clinical manifestations: (1) acute or subacute form, also called the juvenile form and (2) chronic or adult form [4]. The chronic form is the most frequently observed and affects mainly men from rural areas. This clinical form of PCM is characterized by granulomatous lesions in lungs, skin, and mucocutaneous junctions. The acute or subacute form occurs less frequently, and it is observed in children and young adults with severe involvement of the phagocytic mononuclear system [4, 5].

Although human PCM has been the subject of a large number of clinical, epidemiological, and pathological studies, the infection in animals has not yet been elucidated. Several species of wild and domestic animals have demonstrated the infection by serology and skin test, but no clinical manifestations were observed [6–9]. It was observed that among the wild animals, the armadillos, especially *Dasypus novemcinctus*, can harbor the fungus at high frequencies [10], although the active disease seems to be uncommon. It was demonstrated recently that dogs are susceptible to naturally acquired PCM-disease when the first case of canine PCM was reported in a dog from Brazil presenting lymphadenomegaly [11].

The present report aimed to describe the clinical and pathological features of the second case of canine PCM and to address for the importance of the differential diagnosis in clinical cases that involve the monophagocytic system.

## Case Report

A 6-year-old non-neutered female dog, Doberman breed, was admitted to the veterinary hospital at the Pontifícia Universidade Católica do Paraná, Curitiba, Parana state, Brazil, after having presented progressive emaciation and generalized lymphadenomegaly for 2 weeks (Fig. 1). The dog was born and lived in Argentina until 2 years of age, and then it moved to Brazil. This animal was a breeding dog and travelled to dog shows in the Brazilian states of Rio de Janeiro, Mato Grosso do Sul, São Paulo and Parana, with the latter being the location of its permanent residence and manifestation of clinical signs. It had a history of cohabitation with other dogs of the same breed, including young animals, but all were asymptomatic.

Generalized lymphadenomegaly and hepatosplenomegaly were observed in the clinical examination. The pulmonary radiography showed a mild bronchial lung pattern and an increase in mediastinal radiopacity. The hemogram revealed neutrophilia (11,508/$mm^3$), reactive lymphocytes, and macrocytic platelets. Serum biochemistry tests for renal and hepatic functions revealed increase in alkaline phosphatase (654 UI/l).

The fine needle aspiration cytology of the popliteal lymph node, stained with Wright, showed an intense pyogranulomatous inflammation. Histopathological evaluation of the same lymph node revealed numerous oval and eosinophilic structures, presenting thick walls not well stained, which resembled yeast cells. Around these structures was also observed an intense pyogranulomatous infiltrate containing giant cells, macrophages, and neutrophils (Fig. 2). Immunohistochemical analysis was also carried out using polyclonal anti-gp43 antibody diluted at 1:2000 in 1% of bovine serum albumin solution (provided by Dr. Maria Irma Seixas Duarte and Dr. Elaine Raniero Fernandes,

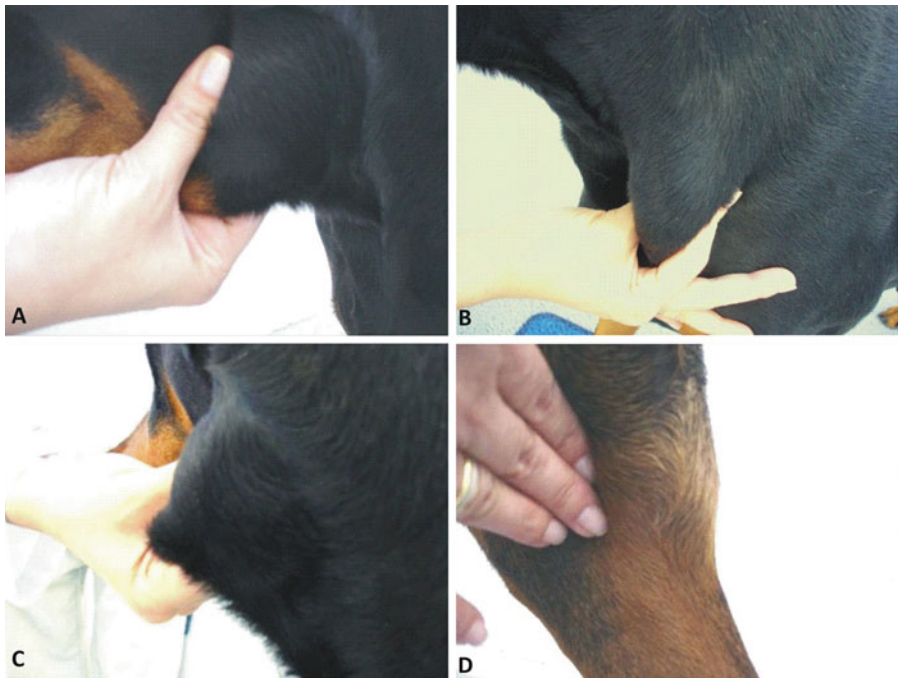


**Fig. 1** Generalized lymphadenomegaly in a six-year-old female Doberman. **a** Submandibular lymph node; **b** prescapular lymph node; **c** inguinal lymph node; **d** popliteal lymph node

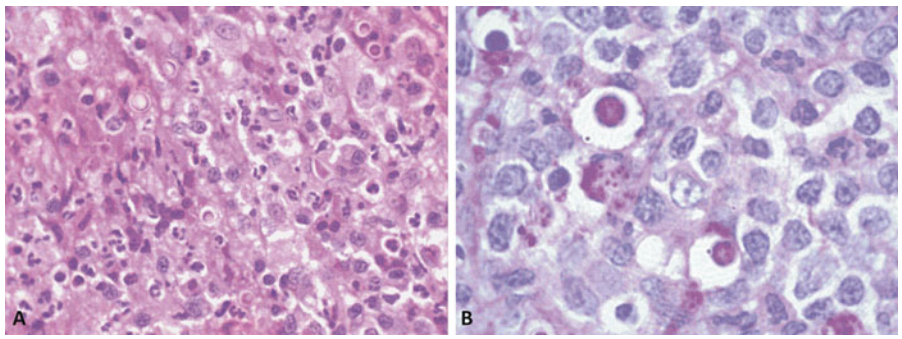


**Fig. 2** **a** Histological fragment of popliteal lymph node stained with hematoxylin and eosin showing *oval structures* (40×). **b** Histological fragment of popliteal lymph node stained with periodic Acid-Schiff showing the same poorly stained *oval structure* surround by neutrophils, macrophages and giant cells (100×)

produced at Department of Pathology-School of Medicine/USP) and showed structures similar to fungal organisms observed in the histopathological sections of the popliteal lymph node (Fig. 3).

Biopsy fragments of the popliteal lymph node were submitted to microbiological culture in Sabouraud dextrose agar, Mycosel$^{TM}$ agar (Becton–Dickinson and Company, Cokeysville, MD, USA) and Brain–Heart-Infusion agar (Difco Laboratories, Detroit, MI, USA) at 37°C. A white cream cerebriform colony was observed in BHI agar after 20 days of incubation. Globous structures, each with a thick wall and multiple budding forms attached, resembling a "pilot wheel," were found via lactophenol cotton blue–stained smear (Fig. 4). The conclusive diagnosis was established after molecular sequencing of the *ITS1-5.8S-ITS2* rDNA and *Gp43* regions, which showed respective similarities of 100 and 99% to *P. brasiliensis* [12]. Both sequences were deposited at NCBI databases ( www.ncbi.nlm.nih.gov), and the accession numbers are JF289270 and HQ878437, respectively. In Table 1 are presented the polymorphic sites, in relation to the

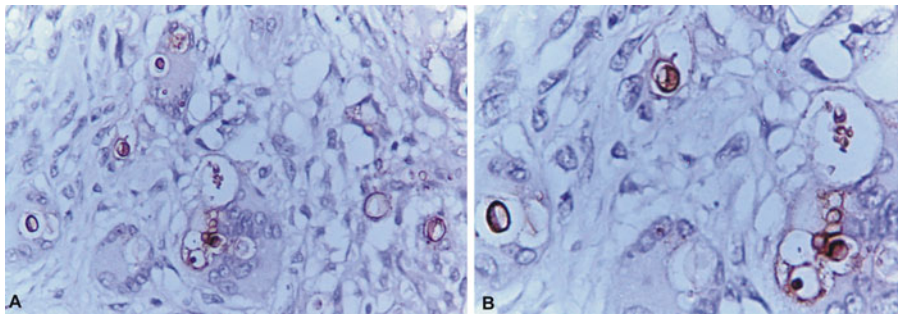


**Fig. 3** Yeasts of *P. brasiliensis* stained through an antibody directed against the protein *Gp43* in immunohistochemical section of popliteal lymph node (40×—**a**, 100×—**b**)

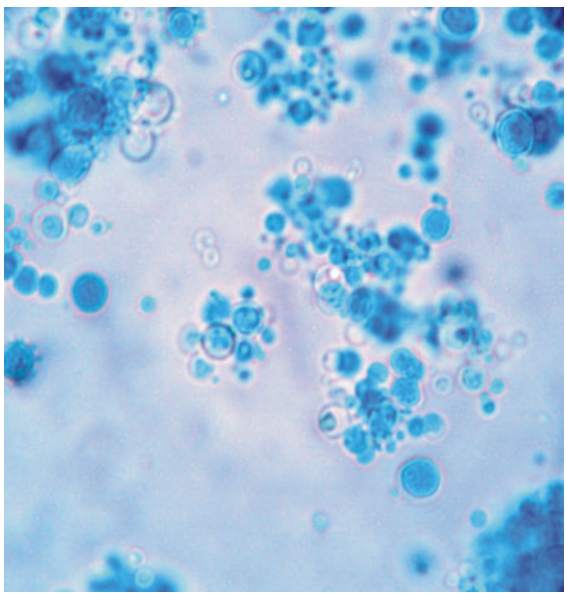

**Fig. 4** Microscopy showing many globous yeast cells stained by lactophenol cotton blue smear (40×)

reference Pb-18 strain, whose structural genome is deposited at Broad Institute (http://www.broadinstitute.org). The analyses were carried out by MEGA 4.0 software.

This dog was treated with itraconazol (10 mg/kg/day/PO, for 24 months) and showed remission of clinical signs.

## Discussion

This report describes the second known case of canine PCM. Similarly, to the first case [11], the animal presented lymphadenomegaly, apathy, and hepatosplenomegaly, characterizing non-specific clinical signs. Both cases were reported in adult Doberman pinscher females that had the same history of contact with apparently asymptomatic other animals.

The infection, in both humans and other animals, is transmitted in an airborne manner by inhalation of conidia present in the environment. The habit of sniffing and digging in the soil could expose dogs to *P. brasiliensis* infection [13]. Despite the knowledge of the airborne mode by which the infection is acquired, there are some ecological features of the pathogen that remain unknown such as the exact place where the fungus occurs in nature. Few reports in the literature indicate the successful isolation of the fungus from the environment [14]. The knowledge of the whereabouts of *P. brasiliensis* in the environment should permit the adoption of preventive measures to avoid infections, as observed in histoplasmosis, using adequate masks when visiting caves or other places with bats [15].

Both the present case and the first one reported by Ricci et al. [11] showed the involvement of the monophagocytic system, the major manifestation of the acute form of PCM observed in humans, in which lymphadenomegaly is present in 100% of cases. In contrast, the chronic form of the disease in humans is characterized by pulmonary and tegumentary granulomatous lesions [4, 5]. In the present case, no sign of respiratory disease was observed. These findings suggest an acute canine form of the disease similar to acute human PCM. The pathogenicity of human PCM involves progression to the monophagocytic system, leading to granulomatous lesions in lymph nodes, lungs, liver, spleen, kidney, and adrenal glands [4, 5]. In the present case, granulomatous lesions were also observed in lymph nodes.

**Table 1** Accession numbers of *ITS1-5.8S-ITS2* rDNA and *GP43* gene sequences of *Paracoccidioides brasiliensis* from dog and polymorphic sites according to alignment with standard strain Pb-18

| Genomic region | Isolate | Distribution of polymorphic and number of position according to alignment with Pb-18 strain | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Position number | | | | | | | | | | | |
| | | 513 | | | | | | | | | | | |
| *ITS1-5.8S-ITS2* rDNA | Pb-dog accession number JF289270 | C | | | | | | | | | | | |
| | Pb-18 (Broad Institute) | – | | | | | | | | | | | |
| | | 2315 | 2599 | 2625 | 2636 | 2664 | 2675 | 2798 | 2810 | 2846 | 2877 | 2903 | 2919 |
| GP43 gene | Pb-dog accession number HQ878437 | A | C | G | A | A | G | A | G | G | T | G | T |
| | Pb-18 (Broad Institute) | G | T | A | G | G | A | G | C | T | C | A | A |

In other human and animal systemic mycoses, hematological changes can include normocytic-normochromic anemia, neutrophilia, hypoalbuminemia, hyperglobulinemia, hypercalcemia, and eosinophilia, but none of these abnormalities can be correlated with disease severity [5, 16]. Increased serum levels of hepatic enzymes due to liver involvement can occur during the spread of the disease. Azotemia and hyperphosphatemia are seen in renal involvement [5]. The present case did not present erythrogram changes, but immunological alterations showed neutrophil and lymphocyte reactivity as well as hyperproteinemia, features that clearly indicate an immunological reaction against the fungus.

The fungus was successfully recovered from popliteal lymph node and proved to be dimorphic, with a white wrinkled cottony surface in the mycelial phase and a creamy and slightly cerebriform aspect in the yeast phase. The microscopic features of the yeast phase were characteristic of *P. brasiliensis*. This present *P. brasiliensis* case provided the first isolate obtained from a dog and was subsequently characterized according to its morphological and molecular aspects. Concerning the molecular aspects, the sequencing of the *Gp43* gene, the major antigen of this fungus, and *ITS-5.8S* rDNA exhibited respective similarities of 99 and 100% with the *P. brasiliensis* sequences deposited at the NCBI database (http://www.ncbi.nlm.nih.gov/blast) [12]. As expected, the alignment with Pb-18 strain showed a high polymorphism in *Gp43* gene (12 substitution nucleotides), since this gene is under positive selection to increase its variability [17, 18].

Great advances in medical mycology therapy, including PCM treatment, have been obtained by the use of azolic drugs. This group of drugs presents few side effects and the possibility of oral administration and is thus considered a viable choice for long periods of treatment. Itraconazole is a third-generation azole that acts on the cytochrome P450 and depends on 1,4-α-dimethylase to prevent the conversion of lanosterol to ergosterol on the fungus membrane. Currently, this treatment is considered more effective than other azoles, although relapses and resistance were found in some discontinued treatments in humans [19, 20]. Due to the establishment of rapid diagnosis and effective treatment with itraconazol, no sequelae were evidenced in the present case. Another factor that probably favored the clinical recovery of the dog was the absence of coinfections, an important aspect that must be considered during the treatment [19].

There is evidence that dogs present strong resistance against PCM [13, 21]. Although natural PCM-disease seems to occur rarely in dogs, the canine PCM infection is well known in some endemic regions of Brazil [9, 22, 23]. Specifically, in the geographical region where the dog of the present case has lived (Parana state), a seroepidemiological survey using ELISA revealed 89.5 and 48.8% positivity among dogs from rural and urban areas, respectively [9].

Dogs play an important role in the epidemiology of North American Blastomycosis, caused by the related dimorphic fungus *Blastomyces dermatitidis.* In fact, dogs are considered a harbinger of human blastomycosis in the USA [24]. However, the role of dogs in PCM epidemiology needs to be further investigated.

Veterinarians should be aware of the importance of including PCM in differential diagnosis among the diseases that involve the monophagocytic system, especially in cases originating from regions endemic for this important systemic mycosis.

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