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CASE REPORT
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Systemic Phaeohyphomycosis in a Dog Caused by *Cladophialophora bantiana*

Rodrigo Cruz Alves[1], Yanca Góes dos Santos Soares[3], Gian Libânio da Silveira[1], Francisco Cézar da Silva[2], Fabrício Kleber de Lucena Carvalho[4], Almir Pereira de Souza[1], Glauco José Nogueira de Galiza[1] & Antônio Flávio Medeiros Dantas[1]

ABSTRACT

***Background*:** *Cladophialophora bantiana* is a dematiaceous fungus that causes phaeohyphomycosis, a generic term used to describe a variety of unusual mycoses caused by fungi that have melanin in their cell wall. *C. bantiana* targets the central nervous system, commonly causing localized brain infections that may result in disseminated infections. In Brazil, minimal phaeohyphomycosis data are available, and information about *C. bantiana* infections in animals, especially canines, is scarce. Thus, the aim of this study was to describe the clinical and pathological aspects of systemic phaeohyphomycosis caused by *C. bantiana* in a dog.

***Case*:** A 1-year-old female Pit Bull presented with weight loss, reduced appetite, and a history of cutaneous lesions on the right thoracic limb; however, clinical evolution was not reported. The bitch had reportedly given birth recently. Physical examination revealed thinness, pale ocular and oral mucosa, submandibular lymph nodes, and enlarged popliteal lymph nodes. The bitch died after convulsive crises during hospitalization. At necropsy, white-yellowish multifocal nodules were observed in the liver and right kidney. The brain featured left cerebral hemisphere asymmetry with blood vessel congestion in the leptomeninges and an irregular brownish focal area on the surface of the right occipital cortex. Cross-sections of the formalin-fixed brain exhibited compression of the left lateral ventricle and the presence of grayish and friable multifocal areas in the gray matter of the left parietal and right occipital cortices. Fragments of the lesions were collected for histopathological and microbiological examination. Histologically, the lesions were similar, characterized by hepatitis, nephritis, and granulomatous and necrotizing meningoencephalitis, multifocal to coalescing, accentuated, chronic, and associated with numerous pigmented fungi. Fontana-Masson–stained fungi exhibited a strong black color. In cleared and unstained histological slides, brownish pigmentation was observed in the cytoplasm and walls of the fungi. *C. bantiana* was identified via microbiological cultivation.

***Discussion*:** A diagnosis of phaeohyphomycosis caused by *C. bantiana* was made based on the characteristic morphology of the microscopic lesions and confirmed via isolation in microbiological culture. As numerous species cause phaeohyphomycosis, specific confirmation of the etiologic agent using several diagnostic techniques is necessary. In histopathological examinations, pigmented fungal organisms are easily seen among lesions. However, in some cases, the pigment is not apparent in the tissues. FM staining is necessary to demonstrate the presence of the melanin in fungi. As in most phaeohyphomycosis cases, it was not possible to determine the primary portal of entry. However, the lesion on the right thoracic limb probably favored the penetration of the agent. In addition to cerebral lesions, severe lesions in the hepatic and renal parenchyma were observed, which are characteristic of systemic mycosis. Infection and clinical diseases are usually associated with immunocompromised; here, the gestation period may have had an immunosuppressive effect, favoring the proliferation and dissemination of the agent. It was concluded that phaeohyphomycosis caused by *C. bantiana* produced severe systemic lesions in the brain and organs of the abdominal cavity. Although uncommon, phaeohyphomycosis caused by *Cladophialophora bantiana* should be included as a differential diagnosis for other canine diseases that present with similar clinical symptoms.

Keywords: canine, fungal diseases, dematiaceous fungi, *Cladosporium trichoides*, *Xylohypha bantiana*, melanin, Fontana-Masson.

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[1]Programa de Pós-Graduação em Ciência e Saúde Animal, Centro de Saúde e Tecnologia Rural (CSTR), [2]Hospital Veterinário & [3]Programa de Residência Multiprofissional em Saúde, Hospital Veterinário, Universidade Federal de Campina Grande (UFCG), Patos, PB, Brazil. [4]Centro Universitário de Patos (UNIFIP), Patos. CORRESPONDENCE: R.C. Alves [rodrigo_ cruz90@live.com]. Av. Universitária s/n. CEP 58708-110 Patos, PB, Brazil.

INTRODUCTION

Cladophialophora bantiana is a dematiaceous fungus belonging to the group of agents that cause phaeohyphomycosis [2], a generic term used to describe a variety of unusual mycoses caused by fungi that have melanin in their cell wall [9,12]. The fungi in this group are saprophytes, which are widely distributed and well adapted to diverse environments. These fungi can cause serious infections in animals and humans, especially in immunocompromised individuals [3,6].

Cladophialophora bantiana has a predilection for the central nervous system, causing localized brain infections [9] that can lead to disseminated infections [2]. In Brazil, phaeohyphomycosis data and information about infections in animals, especially in dogs, are limited. Thus, the objective of this study was to report the clinical and pathological features of systemic phaeohyphomycosis in a dog caused by *C. bantiana* in northeastern Brazil.

CASE

A 1-year-old female Pit Bull presented with a chief complaint of weight loss, in addition to reduced appetite and a history of cutaneous lesions on the right thoracic limb. Clinical evolution was not reported. According to the owner, the bitch had recently given birth. Thinness, pale ocular and oral mucosa, enlarged submandibular lymph nodes, and enlarged popliteal lymph nodes were observed during the physical examination. Death occurred after convulsive crises during hospitalization.

Macroscopically, the liver shoved to have multiple nodules, some of which coalesced. The nodules were yellowish, delimited by a reddish halo, and slightly elevated on the capsular surface, measuring approximately 0.2 to 0.8 cm in diameter (Figure 1A). The nodules extended into the parenchyma with depressed and friable centers forming small cavities surrounded by a discrete whitish capsule (Figure 1B). In the right kidney, there was a whitish nodule on the subcapsular surface. In the brain, asymmetry of the left cerebral hemisphere with blood vessel congestion in the leptomeninges and an irregular brownish focal area measuring 0.5 cm in diameter on the surface of the right occipital cortex (Figure 1C) were observed. Cross-sections of the formalin-fixed brain revealed compression of the left lateral ventricle and grayish and friable multifocal areas in the gray matter of the left parietal (Figure 1D) and right occipital cortices.

Tissue samples from all organs were collected and fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax. The sections were stained with haematoxylin[1] and eosin[2] (HE). Histochemical techniques, including Grocott methenamine silver (GMS)[3] staining, periodic acid-Schiff (PAS)[3] staining, and Fontana-Masson (FM) staining, were used to determine the morphological characteristics of the infectious agent. Additionally, some clarified and unstained histological slides were used to visualize the pigmentation of the agent. Samples of tissue containing lesions were plated onto blood agar (5% defibrinated sheep blood) and MacConkey agar[4] and incubated at 37°C. Also, tissues were cultured onto 4% Sabouraud dextrose agar with chloramphenicol[5] and incubated at 25°C for 5 days.

Histologically, the lesions were similar, characterized by hepatitis, nephritis, and granulomatous and necrotizing meningoencephalitis, multifocal to coalescing, accentuated, chronic, and associated with numerous pigmented fungi. In the liver, there were multiple granulomas characterized by central areas of necrosis containing fungal structures, which were surrounded by a marked inflammatory infiltrate consisting mainly of macrophages, epithelioid cells, multinucleated giant cells, lymphocytes, plasma cells, and rare neutrophils, surrounded by a discrete capsule of fibrous connective tissue.

In the brain, the granulomas caused distension of the leptomeninges and often infiltrated adjacent gray and white matter associated with multifocal areas of malacia that were surrounded by numerous macrophages with foamy cytoplasm (gitter cells), vasculitis, and fungi. In the kidney, there were multiple granulomas with discrete areas of central necrosis associated with fungi in the cortical region. Fungal structures were also observed in the cytoplasm of giant cells and in blood vessel lumen, where they were associated with thrombi.

The fungi presented as tubuliform structures in the longitudinal and transverse HE-stained sections, with brownish pigmentation (Figure 2A). Fungi stained with FM and GMS exhibited a strong black color (Figure 2B & C). PAS-stained fungal structures were faintly pink, and light brown pigmentation sometimes remained. In clarified and unstained histological slides, brownish pigmentation was observed in the cytoplasm and walls of the fungi (Figure 2D). Morphologically, hyphae exhibited thin walls with bulbous dilatations. They were rarely septate and were sparsely branched,

measuring 2-5 µm in diameter. Conidia, characterized by oval structures arranged in chains measuring 2-10 µm in diameter, were also observed.

In the microbiological examination, after the incubation period, the presence of grayish colonies with a cottony appearance was verified in samples incubated in Sabouraud dextrose agar with chloramphenicol (Figure 2D, inset). Microscopic analysis revealed undifferentiated conidiophores forming long chains of slightly brownish ellipsoidal conidia. The species was identified as *Cladophialophora bantiana*, using the microculture method.

DISCUSSION

Phaeohyphomycosis caused by *Cladophialophora bantiana* was diagnosed based on the presence of microscopic lesions with the morphological characteristics of the agent. This diagnosis was confirmed by isolation in microbiological culture. Due to the wide variety of species that cause phaeohyphomycosis, it is essential to use several diagnostic techniques to specifically confirm the etiologic agent causing an infection, including culture and isolation, molecular methods, and immunohistochemistry [9]. In histopathological examinations, pigmented fungal organisms are easily seen among lesions. Melanin is the pigment responsible for the coloration of hyphae [6] and can act as a virulence factor in the development of the infection. However, in some cases, the pigment is not apparent in the tissues. FM staining is necessary to demonstrate the presence of the melanin in fungi [7].

Infections can occur via cutaneous injuries resulting from trauma, or via contamination from previous lesions [1,13]. The limbs and trunk are the most commonly affected anatomical sites [5]. The inhalation of spores is also common, as fungi are saprophytes, which are abundant in environmental sources [13]. However, the primary portal of entry for infection is not identified in most cases of phaeohyphomycosis

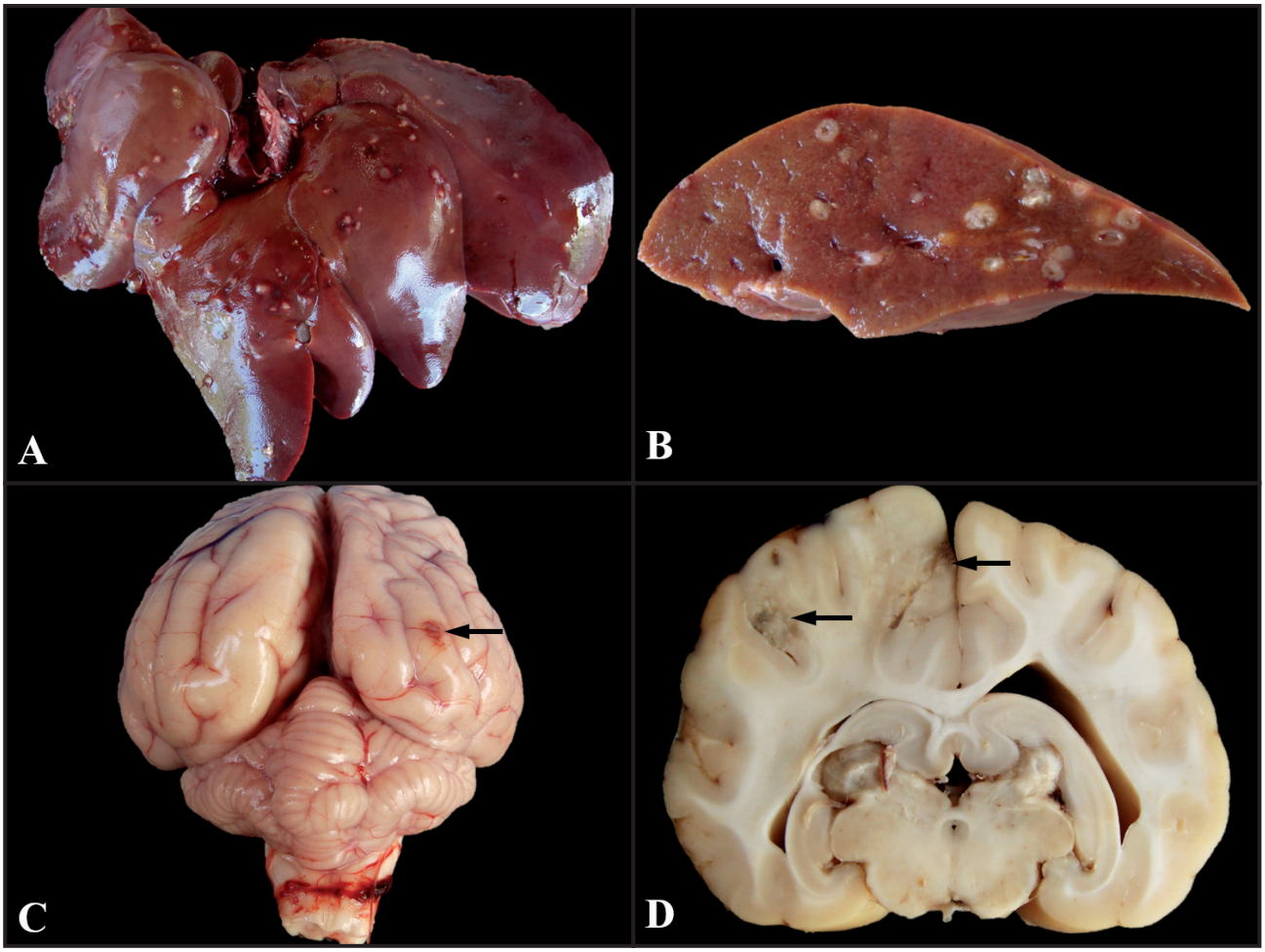

Figure 1. Systemic phaeohyphomycosis in a dog caused by *Cladophialophora bantiana*. A- Liver: Capsular surface with slightly elevated, multifocal to coalescing yellowish nodules bordered by a reddish halo. B- Liver: Cut surface with multifocal areas delimited by discrete whitish capsules with depressed and friable centers forming small cavities. C- Brain: Left cerebral hemisphere asymmetry with blood vessel congestion in the leptomeninges and a brownish irregular focal area on the surface of the right occipital cortex (arrow). D- Brain: Cross section of the brain with asymmetry of the left cerebral hemisphere, compression of the left lateral ventricle, and light grayish friable multifocal areas in the parietal cortex (arrows).

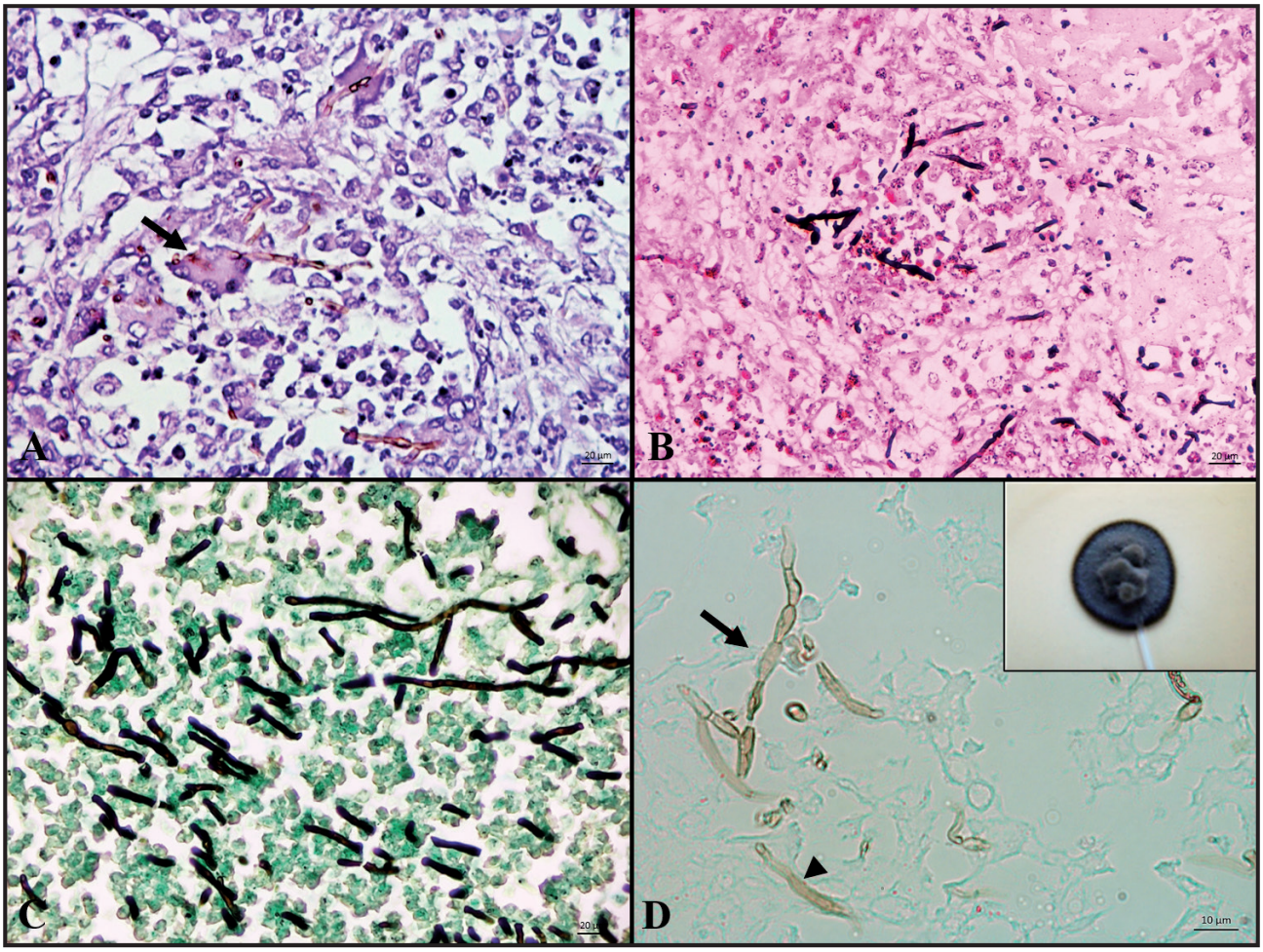

Figure 2. Systemic phaeohyphomycosis in a dog caused by *Cladophialophora bantiana*. A- Liver: Multiple granulomas associated with brown pigmented fungal structures in the cytoplasm of multinucleated giant cells (arrow) [HE; bar= 20 µm]. B & C- Brain: Fungal structures heavily stained black [FM and GMS; bar= 20 µm]. D- Liver: Hyphae with brownish pigmentation (arrowhead) and conidia (arrow) on a clarified and unstained histological slide [bar= 10 µm]. Inset: Grayish colony with a cottony appearance in Sabouraud dextrose agar with chloramphenicol.

[4,10]. In this case, although it was not possible to determine the portal of entry, it was believed that the skin lesion on the right thoracic limb probably favored the penetration of the agent.

The clinical features of these mycosis include subcutaneous, cerebral, or systemic infections [7]. *C. bantiana* has been isolated in cases of cutaneous infections [1,4]; however, due to its neurotropic characteristics, infection by this species tends to cause cerebral changes and is rarely systemic [6,13]. Commonly seen brain lesions include single abscesses, multiple abscesses, and pyogranulomatous inflammation [8,12]. In this case, in addition to brain involvement, severe lesions in the hepatic and renal parenchyma were observed, which are characteristic of the systemic form of mycosis.

Infection and the development of clinical disease are usually associated with immunocompromised and debilitating diseases [8,11]. In this case, it was believed that the gestation period may have contributed to a state of immunosuppression, favoring the proliferation and dissemination of the agent in various organs.

The clinical signs of infections vary, depending on the distribution of lesions and the affected systems. However, hepatic and cerebral involvement usually occur [13], with rapid evolution and high mortality rates [5], and infections are often diagnosed only in the post-mortem examination [4]. Thus, the wide variety of clinical manifestations and the relatively low frequency of the disease makes clinical diagnosis difficult, consequently impeding the establishment of an effective treatment.

We found that phaeohyphomycosis caused by *Cladophialophora bantiana* produced severe systemic lesions in the organs of the abdominal cavity and brain of the bitch. Although uncommon, it is important to include this disease as a differential diagnosis for canine diseases that present with similar clinical symptoms and lesions.

MANUFACTURERS

[1]Erviegas Química Fina e Plásticos Ltda. Indaiatuba, SP, Brazil.

[2]Imbralab Química e Farmacêutica Ltda. Ribeirão Preto, SP, Brazil.

[3]EasyPath - Diagnósticos. Indaiatuba, SP, Brazil.

[4]Cral - Artigos para Laboratório Ltda. Cotia, SP, Brazil.

[5]Kasvi - Produtos e Equipamentos para Laboratórios. São José dos Pinhais, PR, Brazil.

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