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Case report

Systemic canine protothecosis

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Abstract

Protothecosis is a rare disease caused by achlorophyllic algae of the genus *Prototheca*. This case involved a 5 year-old intact female Cocker Spaniel, with a history of mild and persistent diarrhea that was not responsive to treatment. Clinical signs progressed to watery and bloody diarrhea followed by development of ocular signs, including complete and bilateral retinal displacement, glaucoma, synechia, and blindness. At necropsy, lesions observed consisted of necro-hemorrhagic enteritis, and multiple white nodular lesions in the heart, pancreas, kidneys, and thyroid gland. Microscopically, the nodular lesions were characterized by a chronic histiocytic inflammatory infiltrate associated with a severe accumulation of organisms with morphology and histochemical features compatible with *Prototheca* sp.

Key Words: dog, *Prototheca* spp., chronic diarrhea, colitis

Introduction

Protothecosis is a rare disease of human and animals caused by achlorophyllic algae of the genus *Prototheca* (11), which is closely related to members of the genus *Chlorella* (6). The organism frequently infects cats, dogs, and cattle. In cattle, *Prototheca* spp. is known to cause a severe mastitis that is difficult to treat, resulting in considerable economic losses (12).

Prototheca species have worldwide distribution and are considered to be ubiquitious in nature (6). The algae is found in three slime flux, sewage systems, soil, lakes, ponds, and feces (8). The genus *Prototheca* was established by Krüger in 1894, and five species have been recognized, namely *P. moriformis*, *P.stagnora*, *P. ulemera*, *P. wickerhammi* and *P. zopffi* (13). However, only *P. wickerhamii* and *P.zopfii* have demonstrated pathogenic potential for humans and animals (5, 6, 13).

Clinical signs often include weight loss associated with chronic episodes of diarrhea and intestinal hemorrhage, usually about four months prior to the development of the disseminated phase of the disease. (5, 11). Generalized manifestations include lymphadenopathy, fever, lameness, dehydration, deafness and head tilt due to involvement of ocular and nervous systems (6). Nodular skin lesions might be present in a minority of cases (6, 12).

Gross lesions in canine protothecosis consist of white to gray, 1 to 3 mm nodules, or occasionally streaks, diffusely scattered throughout multiple tissues (5, 6, 12). Organs usually affected include the large bowl, eyes, kidney, liver, skeletal muscle, myocardium, lymph nodes, thyroid gland, pancreas, peritoneum, and diaphragm (6). Gross ocular examination of patients with protothecosis may reveal retinal detachment and uveitis, synechiae and development of iris bombe (2, 6, 12). Histologically, the nodules noted on gross examination represent granulomas, composed of areas of necrosis, surrounded by an

inflammatory infiltrate with lymphocytes, plasma cells, macrophages, and a few neutrophils, with large number of intralesional organisms with morphology consistent with *Prototheca* sp. (5, 6, 10). Within the host tissues, the algae are round to ovoid, with 2 to 20 μm in diameter, and a characteristically thick hyaline cell wall that is partially birefringent. Different stages of their life cycle can be observed, and organisms containing daughter cells are usually apparent (6). The organism stains moderately well with hematoxylin and eosin (HE) and strongly with periodic acid-Schiff (PAS), Gomori's methenamine silver (GMS), Gridley's, and Giemsa stains (4, 6). Here we describe a case of systemic protothecosis in a Cocker Spaniel bitch.

Case Report

This case involved a 5 year-old intact female Cocker Spaniel. The dog was first presented in July 2003 when it was 2 years old with a history of mild but persistent episodes of diarrhea. Initially the animal was treated with antihelminthic drugs (febantel and pyrantel pamoate) followed by association of sulfadiazin, probiotics and activated coal. Therapeutic protocols based on corticosteroids, metronidazole, disophenol and several formulations of hypoallergenic diet were also attempted with no success. During the course of the disease several laboratory exams were performed: parasitological, hematology, serum chemistry, fecal microbiology, and colonoscopy; all laboratory evaluations were inconclusive. In spite of the progression of the intestinal signs to watery and bloody diarrhea, the dog remained active and without any other relevant clinical changes for two years. In November 2005, the animal started to present ocular signs, including complete and bilateral retinal displacement followed by glaucoma, synechia and sudden blindness. In January 2006, when the ocular condition worsened, an enucleation of the left ocular globe was performed, and followed by the insertion of a polimetilmetacrilate implant (Figure 1A). When the dog reached five years of age, the progression of the intestinal symptoms to severe effusive and bloody diarrhea resulted in death. The dog was then sent to the Veterinary Pathology service at the Universidade Federal da Bahia (UFBA) for *post mortem* examination.

Grossly, there were petechial and ecchymotic hemorrhages in the intestinal serosa and pleura. The wall of the colon was diffusely thickened and the mucosa was diffusely hemorrhagic with abundant hemorrhagic content in the large intestine (Figure 1B). In the heart, there was several multifocal small white to grayish nodules ranging from 3 to 5 mm in diameter, particularly in the myocardium of the left ventricle, projecting to the endocardial and epicardial surfaces (Figure 1C). Similar nodular lesions were observed in the pancreas (Figure 1D), kidneys, and thyroid gland. There were multiple gastro-duodenal ulcers, with hemorrhagic contents in the stomach and small intestine. In addition, there were a moderate pulmonary edema and an aortic pseudo-aneurism. Samples of the heart, thyroid gland, pancreas, small and large intestines, lymph nodes, kidneys, and lung were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin.

Microscopically, the colon presented diffuse superficial necrosis of the mucosa with loss of luminal and glandular epithelium, moderate diffuse histiocytic infiltrate, severe accumulations of intralesional organisms extending to the submucosa and multifocally into the muscular layer, and diffuse fibroplasia and fibrosis in the mucosa (Figure 2A). The organisms were round or oval ranging from approximately 4 to 20 μm, with a thick cell wall. Some of the larger parent cells (sporangia) contained multiple progeny cells. The organisms stained strongly with GMS and PAS. In the heart there was multifocal to coalescing lympho-plasma-histiocytic inflammatory infiltrate in the myocardium, associated with moderate degeneration, necrosis, and loss of cardiomyocytes (Figure 2B). In the heart there was a severe accumulation of intralesional organisms with morphological features identical to those observed in the colon. Similar lesions to those described in the colon and heart, associated with the presence of the organisms, were observed in other tissues such as pancreas, liver, thyroid gland (Figure 2C), kidney and lymph nodes.

The morphological features of the organism associated with the positive GMS (Figure 2D) and PAS staining allowed the diagnosis of a disseminated infection caused by algae of the genus *Prototheca* in the present case. A definitive etiologic diagnosis to the species level was attempted using the PCR technique described by Tsuji et al. (2006). However, we failed to amplify rDNA from paraffin embedded samples presumably due to DNA degradation. Fresh tissue samples were not available for DNA extraction.

Discussion

To the best of our knowledge, with the exception of a communication in a scientific meeting reviewed by Siqueira et al., 2008 (12), the present case is the first published report of systemic canine protothecosis diagnosed in Brazil. The above mentioned communication in a scientific meeting also described a case describing hemorrhagic colitis caused by *Prototheca zopfii* in a 8 year-old male crossbreed dog from a rural area in the Southern part of the country (12).

The enteric clinical signs observed in this case are similar to those described in the literature, which reports occurrence of bloody diarrhea months prior to development of the systemic signs of the disease (6, 11). The ocular lesions observed characterized by retinal displacement, glaucoma, synechia and blindness are considered to be a major complication of protothecosis in dogs (6). Changes in the central nervous system (CNS) were not observed in this case, although the CNS is

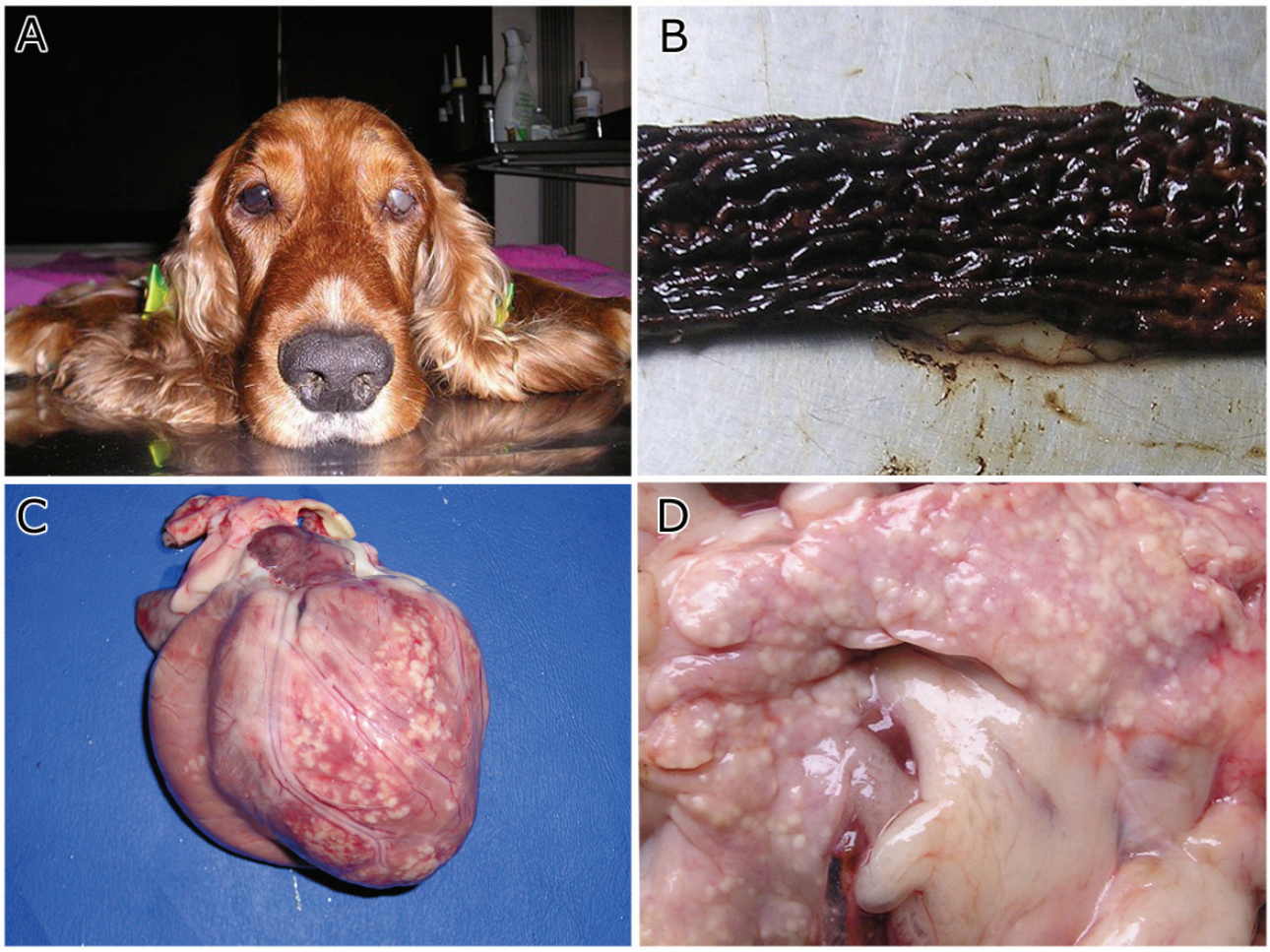

Figure 1.- Dog, Cocker Spaniel, female. Systemic protothecosis. (A) Mild opacification of the left cornea and prosthesis in the right orbit. (B) Heart with multiple white to grayish nodules with multifocal distribution in myocardial surface. (C) Colon with diffusely thickened and edematous wall with diffuse and severe hemorrhage in the mucosa. (D) multiple small whitish nodular lesions in the pancreas.

recognized as a major site of infection in the disseminated phase of the disease (6, 11, 12). The macroscopic nodular lesions with necrohemorrhagic colitis are also compatible to the previous reports (10, 11). Microscopic findings demonstrated presence of a marked histiocytic infiltrate in affected organs. However, the lack of epitheloid macrophages and multinuclear giant cells in the inflammatory infiltrate in the present case prevented us from characterizing this process as granulomatous. However, a granulomatous reaction has been previously described as the most common histological presentation of the disease (6, 11).

Little is known about the pathogenesis of *Prototheca* infections. It is thought that the cutaneous manifestation of the disease in humans and cats is caused by traumatic inoculation of the organisms from contaminated environmental sources (6). In dogs, it has been suggested that the primary site of infection is the colon and that colonization of the mucosa of the organ occurs after ingestion of a large number of infectious propagules from an environmental source (11). It is also believed that disruption of the normal epithelial barrier of the bowel wall by a concurrent colonic inflammation, foreign body or parasitism is required to allow access of *Prototheca* to the submucosa, where they can establish infection (11). Systemic dissemination of the algae occurs through either by lymphatic or hematogenous routes. Once in the blood stream, *Prototheca* sp. can establish a chronic infection in multiple organs, mainly in the heart, central nervous system, eyes, and kidneys (10, 12).

Gender predisposition has been suggested in the literature as one of the features of the disease, with epidemiological studies demonstrating a higher incidence in female dogs (6, 11, 12). Immune suppressive factors and immunological hereditary dysfunctions have also been considered as predisposing factors to canine protothecosis since these factors apparently play an important role in cases of systemic dissemination of the disease from

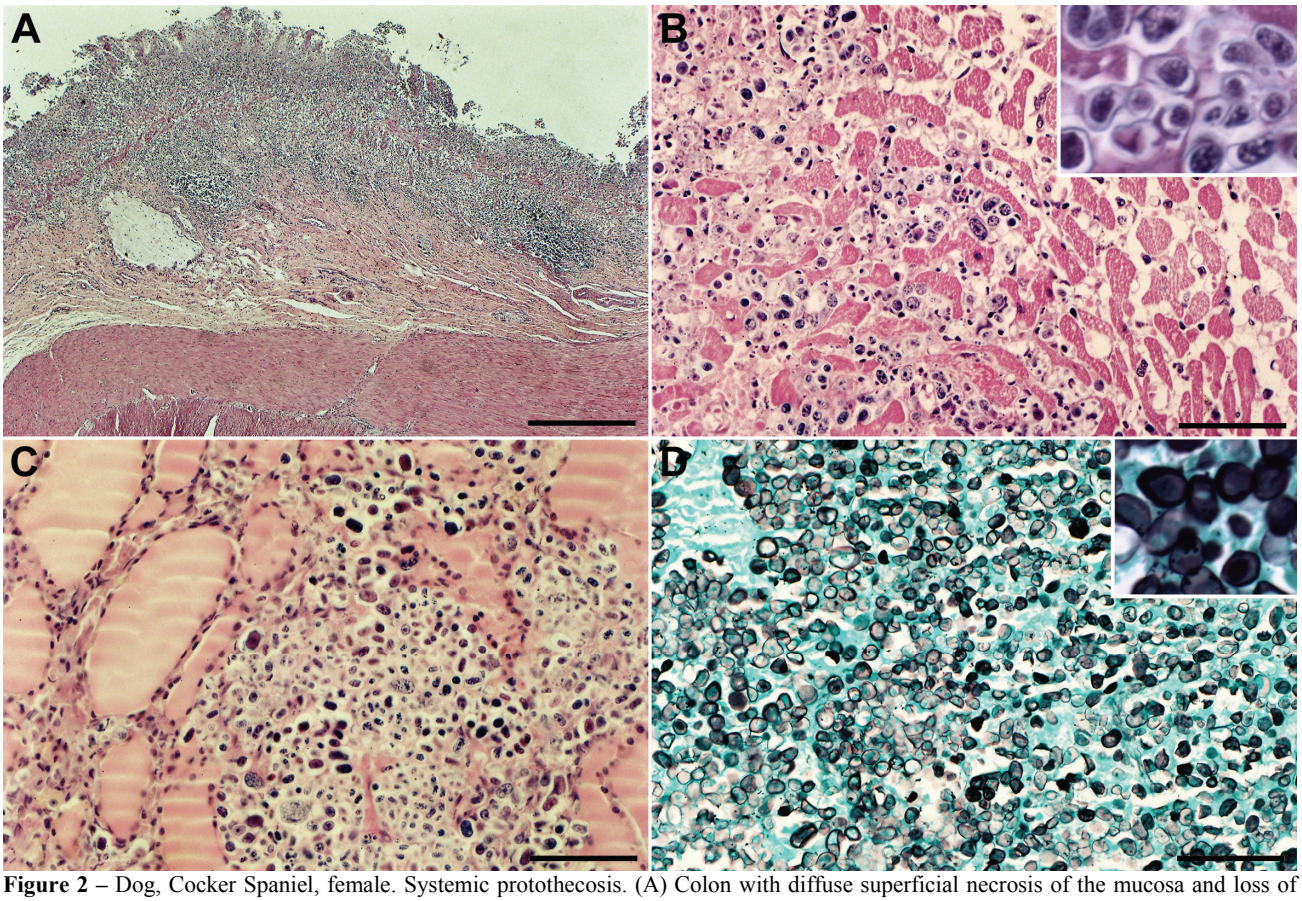

Figure 2 – Dog, Cocker Spaniel, female. Systemic protothecosis. (A) Colon with diffuse superficial necrosis of the mucosa and loss of luminal and glandular epithelium, moderate diffuse histiocytic infiltrate and a severe accumulation of intralesional *Prototheca* sp. extending to the submucosa. HE. Bar = 25 µM. (B) Heart with multifocal to coalescing lympho-plasmo-histiocytic inflammatory infiltrate in the myocardium, associated with moderate degeneration, necrosis, and loss of cardiomyocytes, and a severe accumulation of intralesional *Prototheca* sp. (inset) HE. Bar = 100 µM. (C) Thyroid with multifocal to coalescing lympho-plasmo-histiocytic inflammatory infiltrate associated with a myriad of intralesional *Prototheca* sp. organisms. HE. Bar = 100 µM. (D) Intestine with *Prototheca* sp. cellular wall strongly stained with a silver based special staining (inset). GMS. Bar = 100 µM.

primary cutaneous infections in humans (7, 9). In this case there were no indications of concomitant predisposing factors such as trauma or parasitism (11) that may have contributed to the establishment of infection. Although corticoid therapy in this case may have resulted in some degree of immune suppression, contributing to progression of the infection, it may not be considered as a predisposing factor since the therapy was initiated after systemic signs of infection were already present.

In dogs, cases of protothecosis, particularly cutaneous, must be differentiated from mycotic infections such as *Blastomyces* sp., *Cryptococcus* sp., *Candida* sp., *Histoplasma capsulatum, Coccidioides immits, Geotrichum candidum* and *Pneumocystis* sp., in addition to protozoan-induced infections due to infection with *Carysospora* sp. (13). In spite of this long list of differentials, these agents have morphologic features, including size, form of cellular division, and special staining properties (3, 13), that allow their differentiation from *Prototheca* spp. In cases of ocular manifestation, the differentials include non specitic chorioretinitis, uveitis and retinal detachment caused by tumors and hypertension (13). The differentials for necrohemorrhagic colitis include parasites (particularly *Acylostoma caninum*), parvovirosis or coronavirosis (6, 11). The diagnosis of protothecosis can be achieved by culture of the organism, histopathology associated with special staining like GMS, PAS and Giemsa or by indirect immunofluorescence, (5, 6, 11) and most recently by molecular techniques such as PCR (13). In the present case, PAS coloration contributed to a differential diagnosis with organisms of the gender *Chlorella* by the absence of PAS-positive starch. The poor quality of DNA samples extracted from paraffin embedded tissues prevented us from specifically identifying the organism as previously described (13). Most of the cases of canine systemic protothecosis and bovine mastitis have been attributed to *P. zopfii* (11). Conversely, *P. wickerhamii* has been suggested to be the main causative agent of cutaneous protothecosis in humans, cats, and dogs (1, 6, 11). Therefore, the knowledge about the species

involved in different clinical manifestations may result in a better understanding of the epidemiology and pathogenesis of the disease.

In conclusion, here we describe a case of systemic protothecosis in a dog. The scarcity of information about this disease in Brazil motivated this report.

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