Acta Scientiae Veterinariae, 2025. 53(Suppl 1): 1014.

CASE REPORT
Pub. 1014

ISSN 1679-9216

Colitis and Proctitis in a Dog Caused by *Pythium insidiosum*

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ABSTRACT

***Background*:** Gastrointestinal pythiosis, caused by *Pythium insidiosum*, is a severe and underdiagnosed disease in dogs, posing significant diagnostic and treatment challenges. Brazil ranks 2nd globally in reported pythiosis cases, with 29 cases occurring in dogs, which exhibited the highest fatality rate among reported cases in the country, with most showing gastrointestinal involvement. Understanding this condition's epidemiology and diagnostic intricacies is crucial for improving management strategies and outcomes in affected animals. We aimed to elucidate the clinical presentation, diagnostic findings, and outcomes of a gastrointestinal pythiosis case in a young dog from Mossoró, Brazil.

***Case*:** A 1-year-and-1-month-old male mixed-breed dog, weighing 20 kg, was attended with gastrointestinal symptoms (vomiting, diarrhea, hematochezia, and weight loss) following the rainy season in Mossoró, Brazil. The dog, which had access to a balcony and brick-paved yard, had no direct rain exposure but fell ill shortly after the rainy period. Initial veterinary examination revealed eosinophilia (3,432 eosinophils/mm³), suggesting bacterial or parasitic gastroenteritis. Treatment included deworming, enrofloxacin, and multivitamins, leading to initial improvement. However, symptoms recurred, and 5 months later, the dog exhibited worsened symptoms, including significant weight loss (from 20 kg to 13 kg) and increased eosinophilia (4,224 eosinophils/mm³), prompting further evaluation. Abdominal ultrasonography indicated thickened colon walls (0.99 cm), loss of wall stratification, and a suspected neoplasm (4.25 cm × 2.90 cm). Exploratory laparotomy revealed extensive intestinal adhesions and hypervascularization, leading to euthanasia due to poor prognosis. Necropsy revealed whitish necrotic areas in the colon and rectum with enlarged lymph nodes showing necrotic foci. Histopathological examination confirmed transmural pyogranulomatous inflammation with fibrous tissue proliferation and infiltrating macrophages, plasma cells, eosinophils, and neutrophils. Multinucleated giant cells surrounded caseous necrotic areas containing intralesional fungal hyphae (4–10 µm in diameter, irregular branching). Grocott-Gomori's methenamine silver (GMS) staining highlighted these hyphae, with strong immunostaining for *P. insidiosum* using immunohistochemistry.

***Discussion*:** This report describes a case of colitis, proctitis, and lymphadenitis in a young mixed-breed dog from Rio Grande do Norte's semi-arid region, caused by *P. insidiosum* infection confirmed via immunohistochemistry. *Post mortem* diagnosis, following exploratory laparotomy, revealed advanced intestinal involvement that precluded surgical resection, highlighting the critical need for early diagnosis to improve prognosis. A previous case in the same region involved anal mucocutaneous junction lesions treated with itraconazole and terbinafine. In this present case, clinical signs including vomiting, diarrhea, hematochezia, and weight loss initially suggested parasitic gastroenteritis. Histopathological analysis confirmed pyogranulomatous inflammation with eosinophilic infiltrates and necrotic areas indicative of *P. insidiosum* hyphae, visualized with GMS staining. Immunohistochemistry confirmed *P. insidiosum* involvement, which was essential for a definitive diagnosis. This case highlights the diagnostic complexities and severe outcomes of gastrointestinal pythiosis in dogs, emphasizing the need for early detection and precise management to improve treatment outcomes in affected animals.

Keywords: fungal-like infection, gastrointestinal infection, histopathology, immunohistochemistry, oomycetes, pythiosis.

DOI: 10.22456/1679-9216.141158

Received: 8 September 2024 Accepted: 20 December 2024 Published: 19 January 2025

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INTRODUCTION

Pythium insidiosum is a filamentous eukaryotic microorganism that causes pythiosis [3] by direct contact of zoospores to an individual or animal [2]. The disease has been reported in mammals, especially horses, dogs, and humans; however, other domestic and wild species may also be susceptible to infection [5,15].

Recently, Yolanda and Krajaejun [19] conducted an analysis on the global distribution of pythiosis cases in both animals and humans, noting that Brazil ranked as the 2nd country in the number of reported cases of the disease (n = 843). Among the cases reported in the country, 29 occurred in dogs, with this species exhibiting the highest fatality rate and the majority of cases (86.4%) with gastrointestinal involvement.

Pythiosis is considered an under-diagnosed and difficult-to-treat infectious disease [12] and treatment of the gastrointestinal disease is frustrating, and the prognosis ranges from poor to grave [13].

Since pythiosis case reports significantly increased in the last decade [19], successful identification and treatment of the disease will require veterinary practitioners and pathologists to be familiar with characteristic clinicopathologic presentations, available confirmatory tests, and therapeutic alternatives [6]. The aim of this report was to contribute to the knowledge about gastrointestinal pythiosis in dogs in the northeastern semiarid region of Brazil by discussing clinicopathological aspects and diagnostic methods that may advance the treatment and prognosis of cases of the disease.

CASE

A 1-year-and-1-month unneutered male mixed-breed dog, weighing 20 kg, was brought for a private clinical consultation in the city of Mossoró (5°11'15''S, 37°20'39''W), Rio Grande do Norte, in January 2021. The dog resided in the Belo Horizonte neighborhood, an urban area of Mossoró.

At the residence, the dog stayed in a balcony with access to a partially brick-paved yard, without any ponds, puddles, mud, garden, plants, or pots. The owner reported that the dog fell ill shortly after the rainy season but was not exposed to the rain and only had access to the yard the following day. In the area where the dog stayed, there was a water dispenser and a feeder, with mineral water provided and changed approximately three times a day. The dog was fed only bulk-purchased puppy food and did not usually receive other types of food. It had no access to other animals or the street and rarely went for walks.

During the consultation, the owner reported that the animal had been experiencing vomiting, diarrhea, hematochezia, and progressive weight loss. Based on the clinical suspicion of bacterial and/or parasitic gastroenteritis (helminthiasis), a blood sample was collected for a complete blood count, which revealed only eosinophilia (absolute values: 3,432 eosinophils/mm³; Reference: 120-1,800/mm³). Treatment was initiated with a broad-spectrum anthelmintic[1] [Vetmax Plus®, Vetnil], along with enrofloxacin[1] [150 mg - orally, twice daily for 7 days] and a multivitamin serum. Following the completion of the treatment, the animal showed clinical improvement; however, the owner reported sporadic recurrence of the clinical signs.

In May of the same year, 5 months later, the animal underwent another home clinical consultation, during which the owner reported the same symptoms with a worsening clinical condition and significant weight loss, with the dog's body weight now being 13 kg. New laboratory and imaging tests were conducted, and the new complete blood count revealed an intensification of eosinophilia (absolute values: 4,224 eosinophils/mm³; Ref.: 120-1,800/mm³). The other hematological and biochemical parameters (urea and creatinine) were within the reference values for the species.

Ultrasonography of the abdominal cavity revealed the most significant alterations in the large intestine (Figure 1A & B). The colon showed a small amount of liquid and gaseous content with a diffusely thickened wall (0.99 cm), a diffuse loss of differentiation in the colonic wall stratification, and an apparent neoplasm more evident in the transverse/ascending colon region, measuring approximately 4.25 cm x 2.90 cm. The mesentery displayed increased echogenicity in the

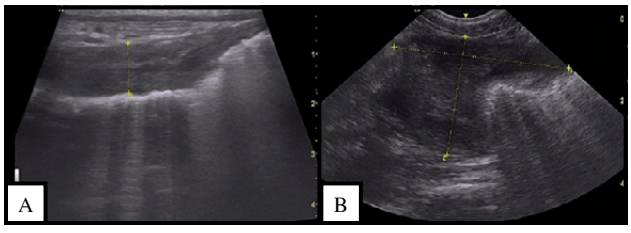

Figure 1. Ultrasonographic findings in a dog with intestinal pythiosis. A- In sagittal plane (longitudinal), the colonic wall appears diffusely thickened (0.99 cm) with loss of stratification differentiation {+ yellow]. B- In transverse plane, a hyperechoic area suggestive of a neoformation is observed (4.25 cm x 2.90 cm) [+ yellow].

area adjacent to the neoplasm. The colonic and splenic lymph nodes were enlarged. Additionally, a small amount of free fluid was observed in the abdominal cavity, located between the hepatic lobes and the area adjacent to the colonic neoplasm. Based on these ultrasonographic findings, the diagnostic conclusion was thickening and diffuse loss of stratification in the colonic wall, indicative of an infiltrative neoplasm of undetermined origin. Differential diagnoses proposed included neoplastic infiltration, infectious granuloma, and intussusception.

Based on these results, no therapeutic medication was administered, and the animal was referred to a private clinic for an exploratory laparotomy. During the laparotomy, several segments of the intestines were identified as bright red, with adhesions of the hypervascularized omentum, which encased the intestinal loops, forming indistinct intestinal masses and preventing the physical separation of the jejunum, ileum, cecum, and colon. The mesentery also exhibited adhesions and hypervascularization. Given the severity of the findings and the poor prognosis, the animal was euthanized. This was performed while the animal was under inhalation anesthesia, maintained under an anesthetic plane with isoflurane. Shortly after the surgical incision was sutured, the anesthesia was deepened, and potassium chloride was administered intravenously. Subsequently, with the owner's permission, the animal was sent for a necropsy examination.

At necropsy, the intestinal loops were intensely adhered, and the intestinal wall of the colon (Figure 2A) and rectum (Figure 2B) appeared whitish with multifocal yellowish areas of necrosis and severe transmural thickening, leading to luminal stenosis. The colonic and mesenteric lymph nodes were enlarged, and on sectioning, whitish areas with multiple yellowish millimetric necrotic foci were observed.

For histopathological analysis, fragments of the affected intestinal portions and lymph nodes were collected and fixed in 10% buffered formaldehyde for 48 h, then cleaved, dehydrated in increasing concentrations of alcohol, cleared in xylene, and embedded in paraffin. Sections of 4 µm thickness were prepared and stained using Hematoxylin-Eosin (HE) and Grocott-Gomori's methenamine silver (GMS) histochemical techniques and examined under a light microscope. Immunohistochemistry (IHC) was performed for the identification of the agent. The IHC protocol, Martins *et al.* [10] modified method, was performed using a rabbit polyclonal anti-*P. insidiosum* antibody (non-commercial). Briefly, sections were dewaxed and rehydrated, and endogenous peroxidase activity

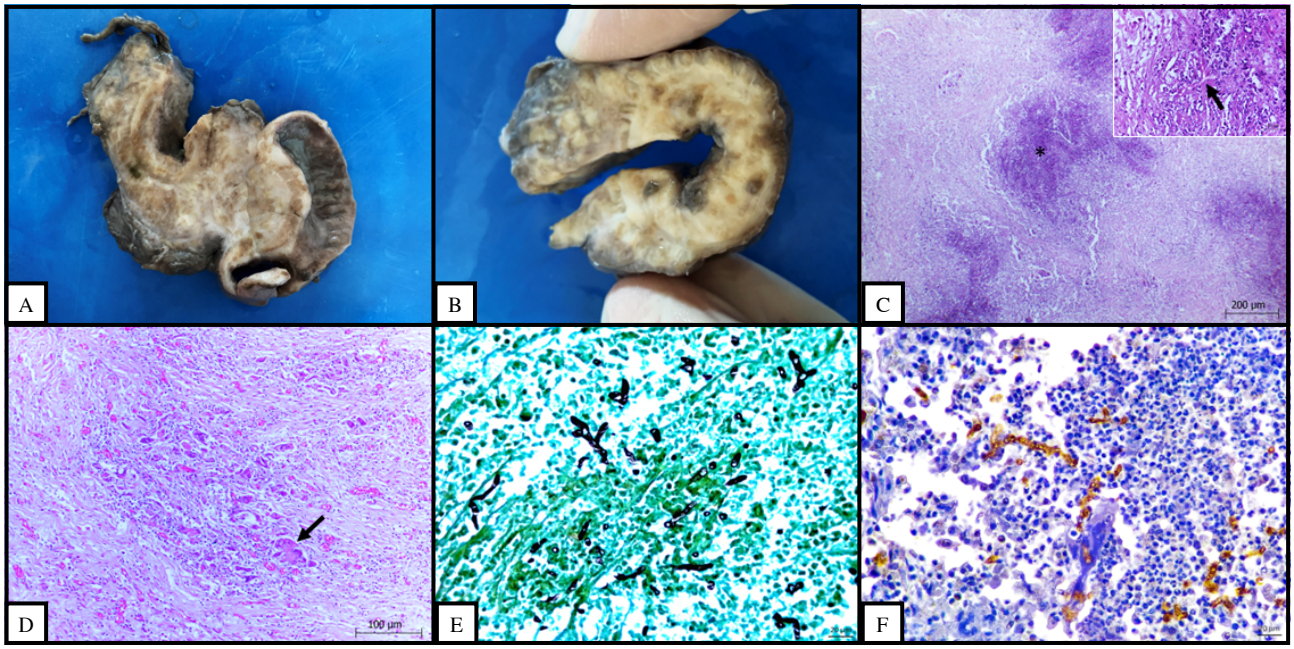

Figure 2. Macroscopic and histopathological lesions in a dog with intestinal pythiosis. A- Thickened and whitish colonic intestinal walls. B- Rectum with intense and diffuse thickening and yellowish multifocal areas of necrosis leading to luminal stenosis. Fragments after fixation in 10% buffered formaldehyde. C- Colonic submucosa showing intense fibrosis and multifocal to coalescent areas of necrosis (*) surrounded by multinucleated giant cells associated with intralesional hyphae structures (inset, →), in addition to D- Infiltration of multinucleated giant cells (→), macrophages, plasma cells, eosinophils, and neutrophils [HE]. E- Hyphae strongly stained black [GMS]. F- Strong immunolabelling of the hyphae (in brown) for anti-*P. insidiosum* antibody [IHC, DAB; Bar = 20 µm].

was blocked with 3% hydrogen peroxide in distilled water. Antigen retrieval was done by microwaving (10 min at full power) in TRIS-EDTA (pH 9.0). Sections were incubated at 37°C for 60 min with the primary antibody diluted at 1:1000. The secondary antibody was a polymer-HRP[2] [EasyLink One; EasyPath] followed by chromogen 3.3-diaminobenzidine[3] [DAB; DakoCytomation]. Sections were counterstained with Harris hematoxylin and coverslipped. As a positive control, histologic sections from a confirmed case of equine pythiosis were used. Sections from the same animal were used as negative controls, with the primary antibody replaced by phosphate buffered saline containing 0.5% polysorbate 20.

The histopathological analysis of the colon and rectum revealed transmural pyogranulomatous inflammation with marked thickening of the organ walls, particularly affecting the mucosa and submucosa. These lesions were characterized by intense proliferation of fibrous connective tissue with diffuse infiltration of macrophages, plasma cells, eosinophils, and neutrophils, along with multifocal to coalescent areas of caseous necrosis surrounded by multinucleated giant cells associated with intralesional fungal hyphae observed within the cytoplasm of multinucleated giant cells or free (Figure 2C & D). The hyphae, which were negatively stained with hematoxylin and eosin, exhibited diameters ranging from 4 to 10 µm, irregular branching, and rare septations, highlighted in black by GMS staining (Figure 2E). Immunohistochemistry demonstrated strong positive immunostaining for anti-*P. insidiosum* antibody (Figure 2F), confirming the involvement of the agent in the case.

DISCUSSION

This report describes a case of colitis, proctitis, and lymphadenitis associated with *P. insidiosum* infection in a dog from the semi-arid region of Rio Grande do Norte, confirmed by immunohistochemical examination. In this case, confirmatory diagnosis was made post-mortem, as during exploratory laparotomy advanced intestinal involvement was observed, precluding surgical resection and highlighting the need for early disease diagnosis to improve prognosis.

A previous case of pythiosis in a dog was reported in in the same city (Mossoró), Rio Grande do Norte [9]. Unlike the case presented here, the previously affected dog had a lesion at the anal mucocutaneous junction. After confirmatory incisional biopsy for pythiosis, the animal received treatment with itraconazole and terbinafine hydrochloride, achieving complete resolution of the lesion in approximately three months.

Since the 1st consultation, the owner reported that the animal had been experiencing vomiting, diarrhea, hematochezia, and progressive weight loss, which corroborates clinical signs associated with gastrointestinal pythiosis [6]. However, due to the non-specific nature of these signs and the eosinophilia revealed by the blood count, the clinical suspicion at that time was parasitic gastroenteritis. After treatment with an anthelmintic, the owner reported improvement in the animal's clinical condition.

Indeed, the possibility of gastrointestinal pythiosis as the cause of the disease presented by the dog at the time of the 1st consultation cannot be ruled out. However, it is interesting to note that since zoospores have an affinity for damaged skin, animals with lesions in the gastrointestinal mucosa induced by parasites or cutaneous wounds may have a greater tendency to acquire the infection, although epidemiological evidence supporting this relationship is limited [6,7]. In the case reported here, in addition to lesions in the cecum, colon, rectum, and satellite lymph nodes, involvement of other structures was not observed, such as the esophagus, stomach [14], pancreas, bile ducts, limbs, face, or tail, which can also occur in affected dogs [16].

In this case, histopathological analysis of the lesions was essential for directing the etiologic diagnosis, as it revealed pyogranulomatous and eosinophilic inflammation with areas of necrosis associated with structures poorly stained by HE, suggestive of hyphae, consistent with previous descriptions of the disease [8]. Hyphae of *P. insidiosum* are difficult to observe with HE staining alone, so suspicious tissues should also undergo staining with PAS (Periodic Acid-Schiff) or Gomori Methenamine Silver (GMS) staining [11]. In this study, GMS staining confirmed the presence of hyphae with irregular branching, rare septations, and a diameter of 4-10 µm, similar to observations in other cases of gastrointestinal pythiosis in dogs [4,18]. Immunohistochemistry (IHC) for *P. insidiosum*, however, was the method used to confirm the etiological diagnosis in the case reported here. This technique, initially employed in confirming the diagnosis of equine pythiosis [1], has been widely used for pythiosis in various animal species [10,15].

It is worth emphasizing that histological and immunohistochemical analyses can be performed on biopsy specimens, increasing the chances of diagnosing the animal during its lifetime and enabling the implementation of appropriate therapy, thereby improving prognosis, as previously reported [9]. However, in the case reported here, during exploratory laparotomy, the dog exhibited extensive involvement of intestinal loops with adhesions, indicating an advanced stage of the disease. This led the surgical team to euthanize the animal, with consent from the owner. This underscores the importance of early diagnosis of gastrointestinal pythiosis in dogs and the necessity of confirmatory tests that facilitate specific treatment. Success of treatment depends on factors such as the extent and duration of the lesion, the age, and the nutritional status of the animal [17].

The affected animal was a male just over 1-year-old, fitting the epidemiological profile commonly seen in dogs with pythiosis. In this species, pythiosis is most frequently diagnosed in young (1 to 3-years-old), immunocompetent male dogs of large breeds, particularly those engaged in outdoor work [7,15]. Despite the owner reporting that the dog became ill shortly after the rainy season, the dog did not have access to standing water sources. It is noteworthy that although dogs with pythiosis often have a history of recurrent exposure to warm freshwater habitats, a significant number of infections in dogs occur without a history of exposure to such environments. It is not uncommon to diagnose pythiosis in dogs that have not visited lakes, swamps, or ponds [3,7].

Although the northeastern region has fewer publications on pythiosis in dogs, the recurrence of cases of this disease in Mossoró, as well as in other locations in the northeastern semi-arid region, underscores the need to raise awareness among veterinarians about its occurrence in the area. In these locations, young dogs presenting with chronic signs of gastrointestinal disease, alongside stenosis or obstruction, palpable abdominal mass, or histological evidence of pyogranulomatous or eosinophilic gastroenteritis, should have pythiosis included in the differential diagnosis. This inclusion is crucial for initiating specific therapy as early as possible to improve the prognosis for affected dogs [4,6,9].

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***Declaration of interest*.** The authors declare no conflicts of interest. The authors are solely responsible for the content and writing of the article.

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