CASE REPORT

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Canine gastrointestinal pythiosis: a case report in Brazilian Pantanal from a diagnostic approach

Pitiose gastrointestinal canina: relato de caso no Pantanal brasileiro a partir de uma abordagem diagnóstica

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ABSTRACT

Pythiosis is caused by an aquatic fungus-like organism (*Pythium insidiosum*). It is considered an important public health issue as it can affect both animals and humans. This paper reports a case of gastrointestinal pythiosis in a dog. The patient was hospitalized for four days, during which the animal received supportive and symptomatic treatment. But the applied treatment was unsuccessful and the dog's clinical condition worsened, culminating in death. Complementary imaging tests such as radiography and ultrasonography, as well as hematological tests, were performed during the hospitalization period. The definitive diagnosis was reached in the postmortem as macroscopic and microscopic characteristics suggested the presence of intestinal granuloma and accentuated multifocal suppurative necrotic enteritis. Additionally, the histological evaluation revealed morphological structures compatible with *P. insidiosum*. Also, the results of nested PCR performed showed partial amplification (105 bp) of the ITS1 region of the ribosomal gene of *P. insidiosum*.

Keywords: Diarrhea. *Pythium insidiosum*. Mammals. Granulomatous disease. Necropsy.

RESUMO

A pitiose é causada por um organismo aquático semelhante a um fungo (*Pythium insidiosum*) e considerada um importante problema de saúde pública, pois pode afetar animais e humanos. Este artigo relata um caso de pitiose gastrointestinal em um cão. O paciente ficou internado por quatro dias, período em que o animal recebeu tratamento de suporte e sintomático. No entanto, o tratamento aplicado não teve sucesso e o quadro clínico do cão piorou, culminando com a morte. Exames de imagem complementares, como radiografia e ultrassonografia, bem como exames hematológicos, foram realizados durante o período de internação. O diagnóstico definitivo foi feito na autópsia, pois as características macroscópicas e microscópicas sugeriam a presença de granuloma intestinal e acentuada enterite necrótica multifocal supurativa. Além disso, a avaliação histológica revelou estruturas morfológicas compatíveis com *P. insidiosum*. Além disso, a nested PCR foi realizada e mostrou amplificação parcial (105 pb) da região ITS1 do gene ribossomal de *P. insidiosum*.

Palavras-chave: Diarreia. *Pythium insidiosum.* Mamíferos. Doença granulomatosa. Necropsia.

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Introduction

Pythiosis is an infectious disease caused by the etiologic agent *Pythium insidiosum* (*P. insidiosum*) (De Cock et al., 1987). This disease affects a wide variety of mammal species, including humans (Mendoza & Newton, 2005), but it is most frequently described in horses (Bosco et al., 2016).

The species *P. insidiosum* belongs to the phylum Oomycota, family *Pythiaceae* and genus *Pythium*. It differs from true fungi in its structural and biochemical characteristics, particularly due to the absence of chitin and ergosterol. *P. insidiosum* is a microorganism that is notably present in regions with standing water. Its life cycle involves the production of hyphae in plant or animal tissue and zoospores, the free infectious form in the aquatic environment (Bosco et al., 2016).

Pythiosis occurs mainly in tropical to subtropical regions, where higher temperatures favor the proliferation of the microorganism in the water (Gaastra et al., 2010). The disease acts indiscriminately, regardless of sex, age, or breed, and the source of infection is the environmental zoospores. It is non-contagious and direct transmission between animals and humans is of minor epidemiological concern (Gaastra et al., 2010).

The course of infection by *P. insidiosum* varies in mammals, often affecting the skin or subcutaneous tissue. This is attributed to primary infection by zoospores, but once the zoospores germinate, the proliferating hyphae cause tissue damage. Horses are particularly prone to the formation of cankers resulting from the deposition of eosinophils and mast cells on the hyphae, forming small granulomas (Bosco et al., 2016).

In dogs, albeit rarely, the disease most commonly affects the gastrointestinal tract, and its main clinical signs are anorexia, weight loss, emesis, diarrhea, and the presence of nodular intestinal masses (Fischer et al., 1994). Unfortunately, diagnosis of canine pythiosis is often reached post-mortem based on pathological, microbiological, and molecular findings (Gaastra et al., 2010).

P. insidiosum is an important public health concern in Brazil, especially in places that favor the occurrence of the agent (Bosco et al., 2016). Adding to the few reports of this infection in dogs, the most common household pet adopted in Brazil, this paper describes a case of gastrointestinal pythiosis with a focus on the diagnostic in a dog admitted and treated at the Department of Infectious Diseases of the Veterinary Hospital of the Federal University of Mato Grosso, Brazil.

In January 2018, a 21-kg, 15-month-old mixed breed male dog from a rural environment in the Pantanal region of Mato Grosso was taken to the aforementioned veterinary hospital for consultation and posterior treatment. The animal had been suffering from a gastrointestinal disease for about a month.

The dog presented with apathy, anorexia, emesis, and diarrhea, and had reportedly been in contact with a horse carcass at a lake where he lived. A physical examination revealed a good general and nutritional aspect. Although the animal appeared lethargic, its mucous membranes presented normal coloring, lymph nodes of normal size and consistency, and body temperature was within the normal level. However, careful palpation revealed hyperesthesia and the presence of a cylindrical abdominal mass.

Samples of blood in EDTA (ethylene diamine tetraacetic acid) and serum tubes were collected for hematological and biochemical, plus molecular detection of *Ehrlichia canis*, *Babesia* sp., *Anaplasma platys,* and *Leishmania* spp. (Francino et al., 2006; Melo et al., 2016) on the day of the vet consultation and the fourth day of hospitalization. Table 1 summarizes the results of the laboratory tests. In the first complete blood count (CBC), the erythrogram and leukogram levels were within the reference range adopted by the clinical pathology laboratory (Feldman et al., 2016), but serum biochemical changes showed hyperproteinemia, hypoalbuminemia, hyperglobulinemia, and, therefore, a low albumin/globulin ratio. Molecular detection of hemoparasites was negative. The second CBC revealed leukocytosis due to neutrophils with relative and absolute left shift and relative monocytosis, thrombocytopenia, and slightly jaundiced plasma.

Table 1 – Hematological and biochemical data of the canine patient on the day of admission (1st day) and during hospitalization (4th day)

Blood count and biochemical parameters	Moment		Reference	
	1st	2nd	Minimum	Maximum
Red Blood Count (10^6)	6.09	5.99	5.5	8.5
Hemoglobin (g/L)	13.9	13.8	12	18
Hematocrit (%)	42	40.5	37	55
Mean corpuscular volume (fl)	68.9	67.6	60	70
Mean cell hemoglobin (%)	33.1	34.1	32	36
Leukocyte (/mm^3)	11,700	20,600	6,000	17,000
Band neutrophils (x10^3)	0	1.6	0	0.3
Neutrophils (/mm3)	7,700	13,200	3,000	11,500
Eosinophils (/ mm3)	0,800	0,200	0,1	1,250
Lymphocytes (/ mm3)	2,900	2,700	1,000	4,800
Monocytes (x10^3/ mm3)	0,2	2,9	0,150	1,350
Platelets (x10^3/ mm3)	309	163	200,000	500,000
Alanine transaminase (UI/L)	29.2	-	21	73
Alkaline phosphatase (UI/L)	19	-	20	156
Gamma-glutamyltransferase (UI/L)	12.8	-	1.2	6.4
Total protein (g/dL)	8.8	-	5.4	7.10
Serum albumin level (g/dL)	2.4	-	2.6	3.3
Serum globulin (g/dL)	6.4	-	2.70	4.40
Albumin/Globulin ratio (g/dL)	0.37	-	0.5	1.7
Bilirubin (mg/dL)	0.3	-	0.07	0.60
Direct bilirubin (mg/dL)	0.11	-	0.06	0.12
Indirect bilirubin (mg/dL)	0.19	-	0.01	0.49

Complementary tests were performed, such as abdominal radiography and ultrasound imaging (Figures 1 and 2). The radiographic findings of the abdomen in the laterolateral position revealed normal bone density; correct alignment and space between lumbar vertebrae; the moderate presence of fecal content and minor volume of gas in the intestinal lumen, and spleen topography with increased radiodensity. There was no evidence of a radiodense foreign body. The ultrasound examination revealed marked mucosal thickening (1.54 cm) of the small intestine, with a hypoechoic and slightly heterogeneous aspect, associated with the loss of stratification in some portions of the affected segment. Other parts showed moderate dilation due to anechoic content in the lumen, with reduced motility and the presence of retrograde movement, with no evidence of thickening or changes in parietal stratification. Intestinal lesions were associated with mild mesenteric lymphadenopathy.

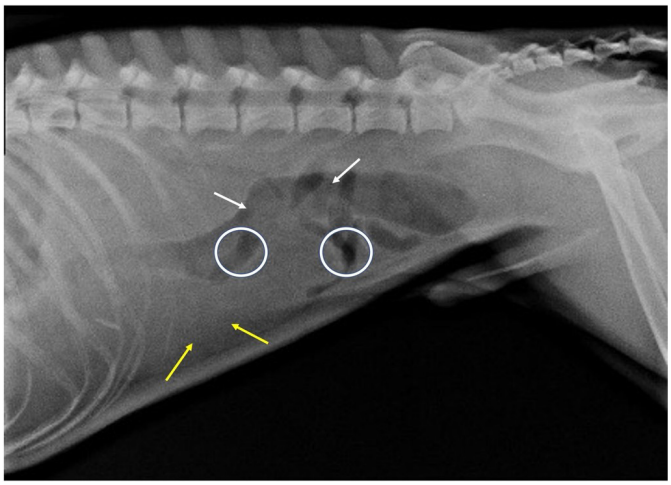

Figure 1 – Right-laterolateral radiograph of the canine abdomen showing the moderate presence of fecal content (white arrows), slight gas volume in the intestinal lumen (circles), and spleen topography with increased radiodensity (yellow arrows).

The patient was hospitalized for four days and treated with electrolyte replacement with lactated Ringer's solution containing 5% glucose, omeprazole (1.5 mg/kg/SID), maropitant citrate (0.1 mg/kg/SID), vitamin C (5 ml/SID), B complex (2 ml/SID), gentamicin (8 mg/kg/SID), ampicillin (40 mg/kg/QID) and tramadol hydrochloride (4 mg/kg/TID), but without therapeutic success. During hospitalization, the dog's clinical condition gradually worsened, with fluctuations in body temperature ranging between 36.9 and 39.9º C, emesis, abdominal distension, and dyspnea, culminating in death at the end of the fourth day.

A necropsy revealed that the main macroscopic changes were subcutaneous edema of the abdominal region, and the presence of 200 mL of cloudy, bloody liquid with yellow exudates in the cavity. The omentum showed diffuse redness and thickening, adhering to multifocal areas of the intestines. The intestines presented marked serous

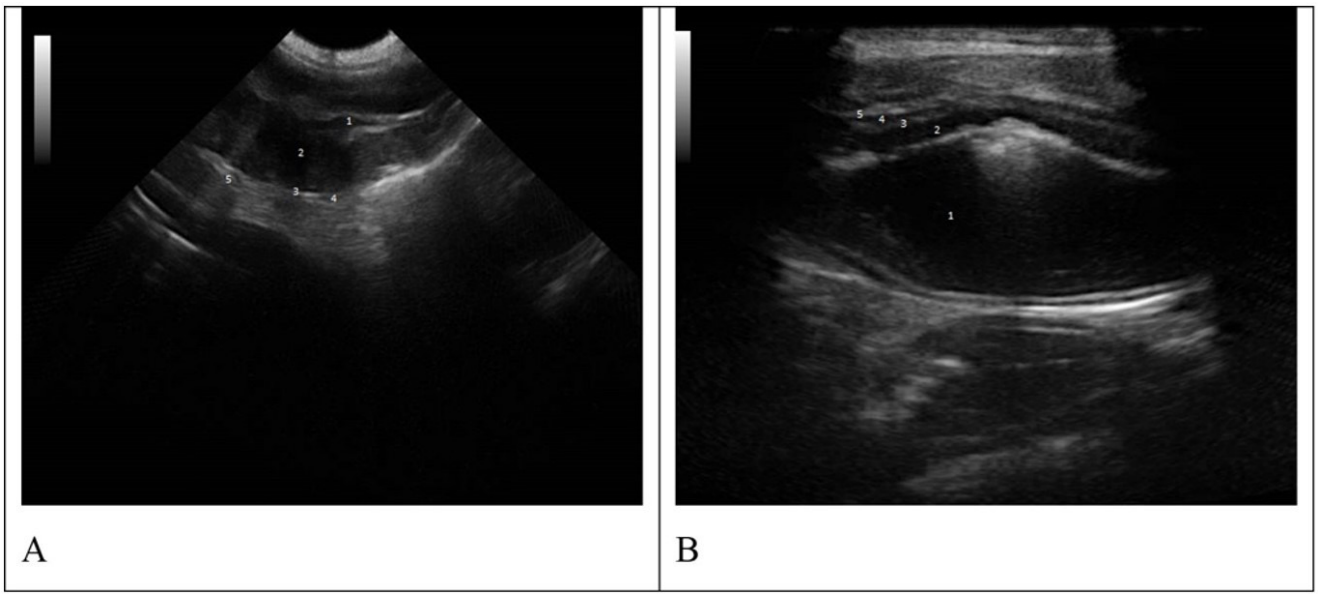

Figure 2 – Ultrasound images of canine small intestine segments. (A) Marked mucosal thickening of the small intestine, with a hypoechoic and slightly heterogeneous appearance; (B) Moderate dilation due to anechoic luminal content, with no evidence of thickening or changes in parietal stratification. (1) lumen; (2) mucosa; (3) submucosa; (4) muscle; (5) serum. Normal intestinal mucosa thickening value: 1-2mm (Nyland et al., 2015).

hemorrhage, transmural bowel wall thickening with multifocal perforation areas, omentum adhesion, and fibrin plaques. The ileum and rectum showed marked thickening of the wall with the formation of a firm whitish mass involving the intestinal loops, approximately 9 cm in diameter, with multifocal yellowish and hemorrhagic areas. Thickening of mesenteric arteries and lymph nodes and lumen filled with reddish lumpy content were identified (Figures 3 and 4).

A microscopic examination was performed using hematoxylin-eosin (H&E) and Gomori's methenamine silver (GMS) staining techniques. The marked proliferation of fibrous connective stroma was observed, involving the intestinal loops from the submucosa to the serous muscle, containing multifocal areas of conspicuous coagulative necrosis. Negative images showed hypha-like structures, accompanied by hemorrhage, neutrophilic inflammatory infiltrate, multinucleated giant cells, and epithelioid macrophages. Multifocal areas containing numerous bacterial coccoid to bacillary and basophilic forms were also present.

The macroscopic and microscopic characteristics of the intestines and the mass suggested the presence of intestinal granuloma and accentuated multifocal suppurative necrotic enteritis, associated with morphological structures compatible with *P. insidiosum* (Figure 5). Grocott-Gomori staining of the intestines and mass revealed silver permeated hyphal walls, showing segmented and sometimes branched structures morphologically similar to *P. insidiosum* (Figure 6).

The diagnosis of pythiosis was confirmed by serological identification based on immunohistochemical analysis, using

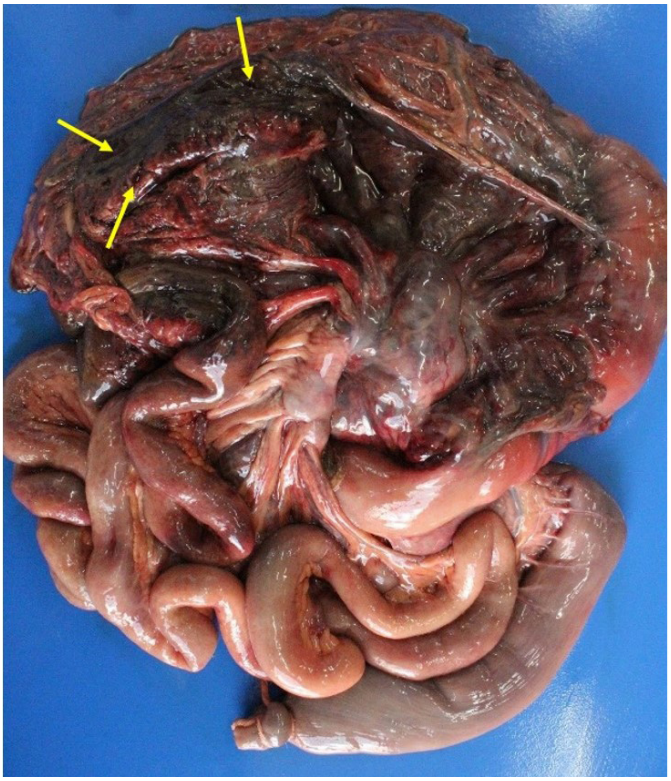

Figure 3 – Canine small intestine loops showing serous hemorrhage (yellow arrows), transmural wall thickening with areas of perforation, fibrin plaques, and thickening of mesenteric arteries.

primary anti-*P. insidiosum* antibodies (1:500 dilution) and biotinylated secondary antibodies and peroxidase-conjugated streptavidin solution (Figure 7) (Ubiali et al., 2013) and partial amplification (105 bp) of the ITS1 region of the ribosomal gene of *P. insidiosum* by nested PCR (Grooters

& Gee, 2002; Ubiali et al., 2013) from fragments collected from the mass.

This paper describes a case of gastrointestinal pythiosis in a dog from the Pantanal region of Brazil. *P. insidiosum* infection is commonly acquired through small wounds in animals that have been in contact with zoospore-contaminated water (Gaastra et al., 2010). It is noteworthy that the patient lived in a rural environment and had frequent access to a pond on the farm. A chronic condition of canine gastrointestinal pythiosis takes a long time to develop after exposure. Canine death two months after the onset of clinical signs of *P. insidiosum* infection has been reported (Fischer et al., 1994), thus reinforcing the clinical case diagnosed in this study.

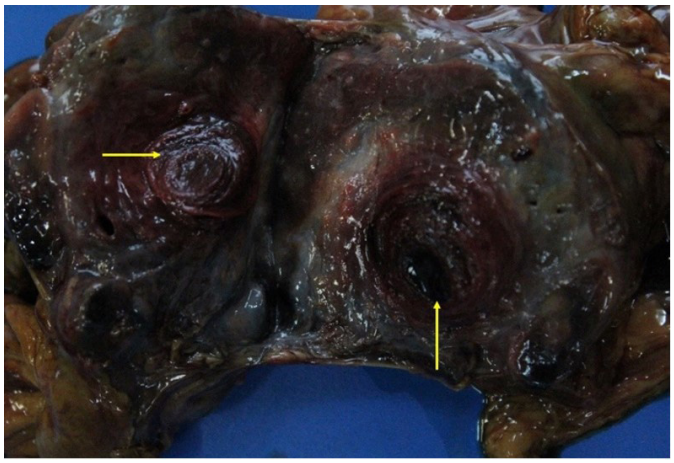

Figure 4 – Cross-section of granuloma Hemorrhagic and necrotic mass (yellow arrows) involving portions of the ileum and rectum.

The CBC revealed intense leukocytosis with a left shift associated with monocytosis and thrombocytopenia (Table 1). There is not much information about hematology tests on animals affected by pythiosis, although reports in the literature are compatible with the patient's hematological findings (Gaastra et al., 2010).

During hospitalization, the dog received treatment for gastroenteritis based on hydroelectrolytic replacement, vitamins, and an association of antimicrobials such as gentamicin and ampicillin. Most *P. insidiosum* infections respond poorly to treatment. Yet treatment should be initiated as soon as possible and include extensive surgical excision, the association of antifungals such as terbinafine and itraconazole, and immunotherapy (Gaastra et al., 2010). However, the success associated with these antifungals is small because of the lack of ergosterol that is targeted by these drugs. Since it was too late to send samples for microbiological culture or to subject the patient to exploratory laparotomy, antifungal treatment was not applied.

Diagnostic imaging exams, such as ultrasonography, showed thickening of the intestinal wall and an increase in regional lymph nodes. Ultrasound examinations are often performed to assess the gastrointestinal tract of patients and suggest that lesions caused by gastrointestinal pythiosis are quite extensive at the time of clinical presentation, similar to descriptions of neoplasms (Graham et al., 2000), which coincides with what was observed in the present case. Even in cases of an ante-mortem diagnosis and the introduction of treatment consisting mainly of surgical resection, treatment is rarely successful, and animals usually die within three months after diagnosis.

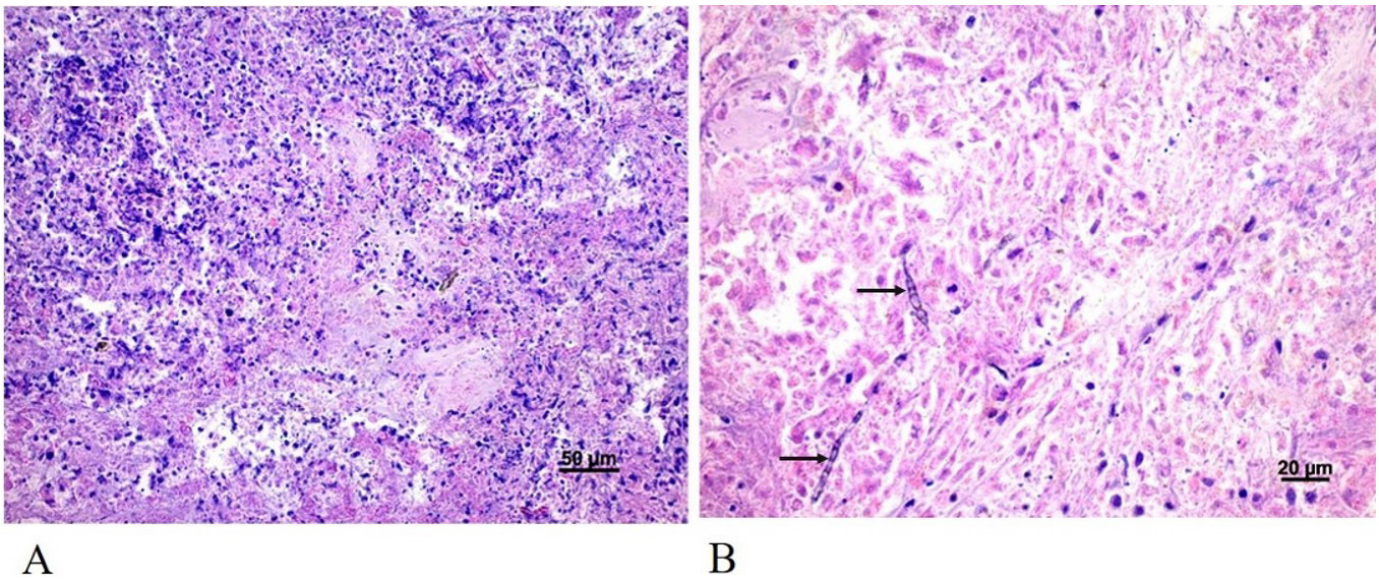

Figure 5 – Micrograph of intestine infected with *Pythium insidiosum*. (A) The proliferation of fibrous conjunctive stroma, areas of coagulative necrosis, negative images, neutrophilic inflammatory infiltrate, giant and epithelioid cells; (B) Between areas of coagulative necrosis are septate hyphae (black arrows) stained with H&E from a fragment of the intestine.

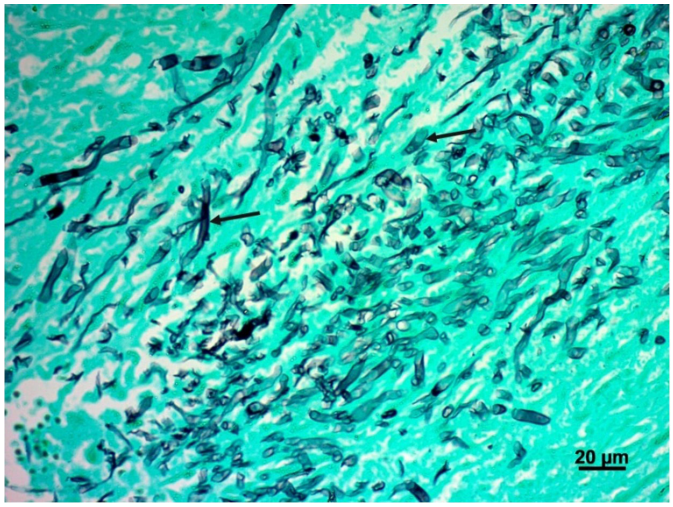

Figure 6 – Micrograph of intestine infected with *Pythium insidiosum*: Septate branching hyphae (black arrows) stained by the GMS technique.

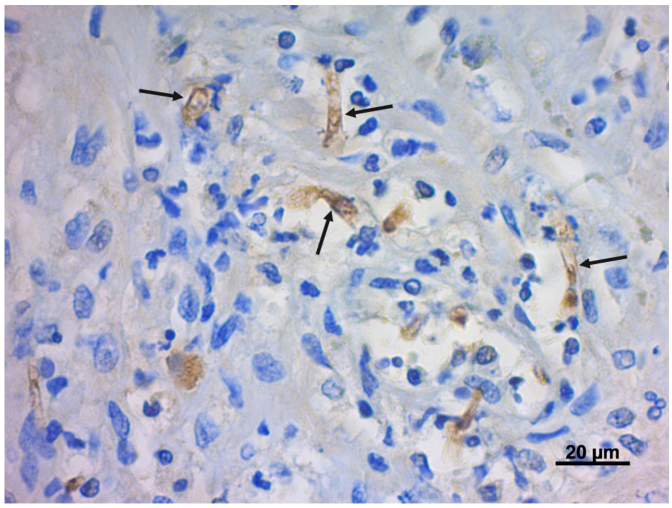

Figure 7 – Micrograph of intestine infected with *Pythium insidiosum*: Septate branching hyphae marked by immunohistochemical staining (black arrows).

A necropsy of the dog revealed peritonitis, indicated by yellowish exudates, and a whitish mass surrounding intestinal loops with points of perforation. These changes are indicative of leukocytosis, as discussed in the previous paragraph. The intestinal lesions observed in the present report are consistent with previously reported injuries (Trost et al., 2009), characterized by granulomatous inflammation. The etiological diagnosis of this infection was confirmed by the presence of hyphae characterized by the GMS, immunohistochemical, and PCR techniques.

The detection of the agent by molecular diagnosis (PCR), which is known as a useful tool for the diagnosis of *P. insidiosum* (Grooters & Gee, 2002), was adopted in the present study. A diagnosis based on cultured pythiosis is rarely definitive because it is difficult to produce and identify its reproductive structures, which resemble those produced by other diseases, and can therefore not be used alone for definitive identification. Also, professionals often do not send fecal samples for fungal culture because the lesions caused by pythiosis can be mistaken for neoplastic disease or bacterial infection. The histological characteristics of *P. insidiosum* are not exclusive to this pathogen and the histological appearance is also typical of infections caused by *Conidiobolus* sp. and *Basidiobolus* sp. (zygomycetes) and the histological appearance of the pathogen may be similar to the genus *Lagenidium* (Grooters & Gee, 2002).

It is important to differentiate pythiosis from other pathogens to adopt the appropriate treatment, predict results and better understand the epidemiological situation of the disease. The use of this molecular tool for the diagnosis of pythiosis has the potential to offer greater specificity than other diagnostic tools.

This study concluded that it is important to associate anatomical pathology and molecular biology techniques for a definitive diagnosis of canine pythiosis. Nevertheless, proper medical history taken during the clinical evaluation can guide medical decisions so that a diagnosis can be reached before the disease culminates in death.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

I declare that in the present case report there was no animal experimentation, having its final diagnosis confirmed after histopathological examination and by post-mortem molecular biology. All hospital procedures for the clinical management of the case were performed according to the veterinary medical routine of HOVET-FAVET-UFMT. In view of the above, this case was not submitted to the CEUA-UFMT

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