Indian J. Vet. Pathol., 49(2) : 175-178, 2025: DOI: https://doi.org/10.56093/ch9s7p24

SHORT COMMUNICATION

Pathomorphological findings of gastrointestinal pythiosis in a dog

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Received: 19.2.2025; Accepted: 8.3.2025

ABSTRACT

Intestinal pythiosis is a life threatening disease in dogs caused by fungus like organism called *Pythium insidiosum*. Gross examination revealed thickened intestinal wall with firm irregular masses on the serosal surface. Histopathological analysis revealed that the mass primarily consisted of fibrous tissue with multiple necrotic areas which were surrounded by macrophages, epithelioid cells, giant cells, lymphocytes and few plasma cells. In haematoxylin and eosin, both the longitudinal and transverse sections of tubuliform structures were observed within the necrotic areas. Periodic Acid-Schiff staining revealed pink fungal elements scattered in necrotic areas, while Grocott methenamine silver nitrate staining highlighted the fungal hyphae in the periphery of the necrotic regions with dark staining. Based on the histopathological and histochemical findings, it was concluded as gastrointestinal pythiosis in a dog.

Keywords: Dog, fungal hyphae, intestine, *Pythium insidiosum*

Pythiosis is a granulomatous disease caused mainly by *Pythium insidiosum*, an oomycete, belonging to the kingdom Stramenopila, affects several species of humans and animals[1]. Equines were mostly affected, followed by the dogs[2]. In dogs, the disease involves the skin[3] and gastro intestinal tract[4]. Initial studies on the life cycle of this oomycete have shown that *P. insidiosum* is present in stagnant waters and may possibly require the Australian water lily or other plants to complete its life cycle[5]. It was also found that its zoospores may play an important role in the propagation of infections amidst plants and animals. Infection picked up through minor wounds which came in contact with water or by drinking the water contaminated with motile zoospores[2].

Intestinal pythiosis commonly cause the granulomatous nodules on the serosal surface with involvement of mesentery which affect the normal function of intestine due to pressure and thickening of intestinal wall[6]. The affected dogs show the common non specific clinical signs like vomition, diarrhoea, weight loss and chronic anorexia[4]. Histologically, three different types of lesions were described. First type showed large irregular areas of necrosis surrounded by giant cells, epitheliod cells, macrophages and abundant neutrophils, eosinophils, and few plasma cells in the necrotic areas. Second type showed small, well-differentiated granuloma with small necrotic centers containing numerous neutrophils and few eosinophils. The third type contained mixture of focal granulomas and areas of necrosis followed by macrophages, with excessive connective tissue in the areas of inflammation[7].

The diagnosis of intestinal pythiosis is always challenging due its non specific clinical signs. Histological lesions associated with pythiosis in dogs were similar with other pyogranulomatous lesions so it is necessary to confirm the diagnosis other than the routine haematoxylin and eosin. The present study describes pathomorphological findings of gastrointestinal pythiosis in a dog.

A five year old dog was brought for treatment with the history of anorexia, chronic vomition and weight loss. On clinical examination, there was palpable mass in the abdominal cavity and the mass was removed surgically.

How to cite this article : Sowmya, K., Gopal, K., Sivaraj, S., Kokila, S., Balachandran, P. and Srinivasan, P. 2025. Pathomorphological findings of gastrointestinal pythiosis in a dog. Indian J. Vet. Pathol., 49(2) : 175-178.

The mass was fixed in 10 per cent neutral buffered formalin. Paraffin embedded tissue sections of 4 micron thickness was cut and stained with Haematoxylin and Eosin (H&E). Additionally, histochemistry techniques like Grocott Methenamine Silver Nitrate (GMS) and Periodic Acid-Schiff (PAS) were also performed for visualization of fungal hyphae.

Grossly, the irregular thickening of intestine with appreciable firm multiple nodules on the serosal surface were observed. On incision, the nodules were resisting to cut and grey to yellowish in colour. Each nodule was surrounded by thick fibrous connective tissue indicating multiple areas of necrosis and fibrosis (Fig. 1).

Histologically, the granulomatus reactions were extending

from the serosa to mucosa. The luminal surface was free of lesions. Diffuse fibrous connective tissue with multiple areas of liquefactive necrosis surrounded by varying degrees of inflammatory cells was observed on the serosal surface of intestine (Fig. 2). The granulomas were characterized by necrosis in the centre infiltrated by neutrophils (Fig. 3), surrounded by epithelioid cells, giant cells, discrete lymphocytes, plasma cells and moderate fibrosis (Fig. 4). The sections stained with sirius red revealed collagen fibres appearing bright red while the necrotic areas showing yellow colour (Fig. 5). Accompanied with these necrotic areas, unstained tubuliform structures were observed with haematoxylin and eosin (Fig. 6). Both longitudinal and transverse

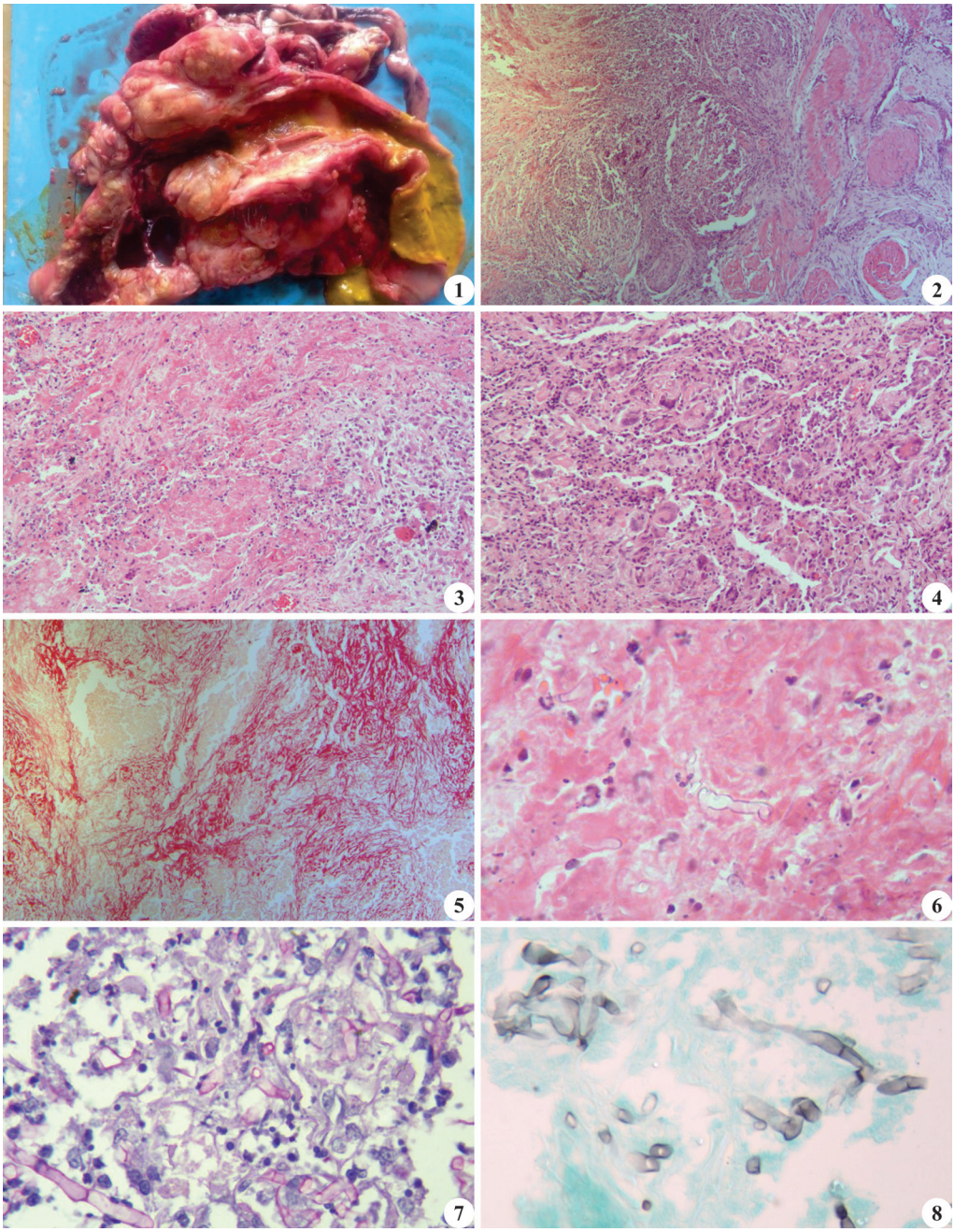

Fig. 1. Thickened intestinal wall with firm irregular masses and areas of grey to yellowish nodules on the serosal surface; **Fig. 2.** Multiple necrotic foci with fibrous tissue on the serosal surface of intestine (H&E x40); **Fig. 3.** Infiltration of neutrophils, necrotic debris in the necrotic area with fibrosis (H&E x100); **Fig. 4.** Fibrous tissue around the necrotic foci with infiltration of giant cells, macrophages and plasma cells (H&E x100); **Fig. 5.** The area of fibrosis and collagen deposition are stained red and necrotic area yellow in picro-sirius red x100; **Fig. 6.** Longitudinal and transverse sections of tubuliform structures were observed within the necrotic areas (H&E x400); **Fig. 7.** Both longitudinal and transverse sections of pink stained fungal hyphae were observed (PAS x400); **Fig. 8.** Fungal hyphae were stained black (GMS x400).

sections of pink coloured fungal hyphae structures and weakly stained hyphal walls were observed in the histological sections stained with PAS (Fig. 7). The GMS-stained sections showed that the hyphal walls were weakly stained black (Fig. 8). The weak staining could be due to the specific characteristics of the fungal species, as variations in the composition and thickness of cell wall can affect the intensity of stain.

Canine infections were first reported in dogs with cutaneous and gastrointestinal lesions from the Gulf of Mexico in the USA[5]. Subcutaneous pythiosis lesions have been recorded also on the legs, face and tail in dogs[8]. Disseminated pythiosis with involvement of internal organs like lung and liver has also been reported in dogs[8,9]. In the present study, lesions were confined to small intestinal serosal surface with involvement of mesentery. There were clinical cases caused by an unidentified species in the genus *Lagenidium* which were comparable to those observed in canine pythiosis[10] must be differentiated from canine cases.

The recent studies indicating that from 1980 to 2021, 4203 cases of pythiosis in humans (n = 771; 18.3%) and animals (n = 3432; 81.7%), with an average of 103 cases/year, were reported worldwide[11]. The number of pythiosis cases was significantly increased in the recent past and more importantly, 94.3% of human cases were recorded in India and Thailand, while 79.2% animal cases were reported in the USA and Brazil[11]. The canine pythiosis in India was not reported up to the year 2022[11] and the first case of canine intestinal pythiosis reported in the year 2023 in India[12].

There are several potential mechanisms by which environmental changes related to water may contribute to an increased incidence of pythiosis[1]. In the present study, the history of dog played in the stagnated water or any other water based activities were not available to trace the possible route of infection. In dogs, digestive lesions are more frequently observed in the stomach and small intestine. These lesions can sometimes spread via the serosa to neighbouring structures such as pancreas, uterus, mesenteric lymph nodes, lymphatics and frequently causing the omentum to adhere to the inflamed peritoneum[6].

Both granulomatous/pyogranulomatous and necrotizing eosinophilic inflammatory reactions were recorded in affected dogs[13] but only granulomatous pattern was observed in the present case. Extensive Hoeppli-Splendore-like phenomenon from degranulated eosinophils scattered over the hyphal elements in the sections stained with H&E were recorded in canine cases[9], where as extensive necrosis with unstained tubuliform structure without any eosinophilic infiltration around the fungal hyphae were recorded in the current case.

Some authors have suggested that the histological lesions of pythiosis in dogs were very comparable to those seen in the infections caused by other oomycetes and zygomycetes[14]. Therefore, other diagnostic methods should be considered, including culture, immunohistochemistry, serological tests and DNA amplification using PCR[2]. Ultrasound and X-ray examination were used to evaluate the intestinal lesions and the wall thicknesses exceeding 7 mm in the stomach and 5 mm in the small intestine are considered pathological[15,16]. In immunohistochemistry, hyphae give strong immune reactivity to a polyclonal anti-*Pythium insidiosum* antibody in the cutaneous and intestinal lesions[14]. Another differentiating feature is the diameter of fungal hyphae in the tissue sections. The hyphae diameter of *Pythyium insidiosum* is ranging from 5-7 micron[7] whereas zygomycete fungi diameter ranging from 7-25 micron[10]. In the present case the diameter of fungal hyphae was 4-6 micron. Using antifungal drugs and surgical removal of masses have shown good results in some cases[17].

Based on the histological, histochemical and morphological analysis this case was confirmed as gastro intestinal pythiosis in a dog.

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