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PATHOLOGY

Esophageal and gastric pythiosis in a dog

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ABSTRACT: Pythiosis is the disease caused by aquatic oomycetes of the genus *Pythium*. In dogs the typical course of the disease involves the gastrointestinal tract, characterized by transmural thickening of the stomach or intestines. However, infection by *Pythium* spp. has only rarely been recognized as a cause of esophagitis in dogs. Thus, the present reports a case of esophageal and gastric pythiosis in an eight-month-old female pit bull dog. The dog was attended at the hospital after two months presenting regurgitation and dyspnea. It was reported that the dog lived in an urban area and had no previous sanitary issues. At clinical examination it was noted that the dog presented crackling sounds at pulmonary auscultation. A support therapy accompanied by antibiotics has been employed; however, 22 days after hospitalization the clinical condition worsened, and the dog died. At necropsy, the wall of the distal segment of the esophagus and the cardia and part of the fundus of the stomach were expanded by a focal extensive irregular intramural annular mass. Additionally, there was a transmural esophageal fistula. At histology, the walls of the esophagus and stomach were extensively expanded by multifocal extensive areas of necrosis, associated with a pyogranulomatous infiltrate and abundant granulation tissue containing multiple negative images of hyphae that were highlighted by silver impregnation (Grocott). Furthermore, immunohistochemistry and PCR for *P. insidiosum* were both positive in samples of paraffin-embedded esophageal tissue.
Key words: disease of dogs, oomycosis, canine, esophagitis, immunohistochemistry, molecular analysis.

Pitiose esofágica e gástrica em cão

RESUMO: A Pitiose é uma doença causada por oomicetos aquáticos do gênero *Pythium*. Em cães o curso típico da doença envolve o trato gastrointestinal, caracterizado por espessamento transmural do estômago ou intestinos. No entanto, a infecção por *Pythium* spp. raramente foi reconhecida como causa de esofagite em caninos. Assim, o presente trabalho tem como objetivo relatar um caso de pitiose esofágica e gástrica em um canino Pit Bull fêmea de oito meses de idade. O cão foi recebido no hospital com histórico de regurgitação e dispneia durante dois meses. Foi relatado que o animal era de um domicílio de área urbana e não possuía histórico pregresso de enfermidades. No exame clínico contatou-se ainda crepitações à ausculta pulmonar. Foi instituído tratamento de suporte e antibioticoterapia, porém após 22 dias de internação, o cão evoluiu para o óbito. Na necropsia, as paredes do segmento distal do esôfago e o cárdia e parte do fundo do estômago estavam expandidos por uma massa anular intramural focalmente extensiva e irregular. Além disso, havia uma fístula esofágica transmural. À histologia, as paredes do esôfago e do estômago apresentavam-se difusamente expandidas por áreas multifocais extensas de necrose, associadas a infiltrado piogranulomatoso, abundante proliferação de fibroblastos imaturos, neovascularização e contendo múltiplas imagens negativas de hifas realçadas pela impregnação por prata (Grocott). Além disso, tanto a imuno-histoquímica como PCR para *P. insidiosum* foram positivas em amostras de esôfago embebidas em parafina.
Palavras-chave: doença de cães, oomicose, canino, esofagite, imuno-histoquímica, análise molecular.

Pythiosis is a disease caused by oomycetes of the genus *Pythium*. Originally considered the only mammalian pathogen in the genus, *P. insidiosum* was recently split in different clusters, including a divergent cluster now considered a novel species, named *P. periculosum* (MIRAGLIA et al., 2022; PEANO et al., 2023). The oomycetes are found as free-living zoospores roaming in aquatic environments, eventually infecting different species of mammals that come in contact with the organisms such as humans, horses, dogs, cattle, and sheep and sporadically cats, camelids, and coatis, among other species (DE COCK et al., 1987; HELMAN & OLIVER, 1999; GAASTRA et al., 2010; SOUTO et al., 2019; BEZERRA et al., 2020). The course of the disease varies between host species, being

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predominantly cutaneous and progressive in horses, cutaneous and self-limited in cattle, rhinofacial in sheep, and enteric in dogs (GAASTRA et al., 2010; MARTINS et al., 2012; UBIALI et al., 2013; FRADE et al., 2017).

Most of the cases of pythiosis described in dogs comprise males of large breeds, up to three years of age, with access to water from ponds, lakes, lagoons, or streams (FISCHER et al., 1994). Lesions reported in gastrointestinal pythiosis of dogs are characterized by transmural thickening in any part of the stomach, small intestines or colon, that can give rise to expansive masses, which can compress adjacent organs and, obstruct or perforate bowels leading to subsequent peritonitis (JAEGER et al., 2002; GROOTERS & GEE, 2002; UZAL et al., 2016). However, infection by *P. insidiosum* has rarely been recognized as a cause of esophagitis in dogs (PATTON et al., 1996; BERRYESSA et al., 2008; SOUTO et al., 2022). This report described the clinical and pathological findings in a dog with pythiosis involving the esophagus, stomach and the adjacent respiratory tract.

An eight-month-old female pit bull dog from the urban area of Cuiaba City, Central West, Brazil was evaluated at the Veterinary Hospital of the University of Cuiaba after a two-month history of regurgitation and emaciation. At clinical evaluation, the dog was in poor body condition, with frequent episodes of regurgitation, dehydration, dyspnea, and crackling sounds at pulmonary auscultation. The owner; however, did not allow radiographic studies to be performed. Hematologic results revealed neutrophilia (15,604x10³/mm³ [reference: 4,140 – 10,200x10³/mm³]) and marked lymphopenia (996x10³/mm³ [reference: 2,700-6,750x10³/mm³]). Biochemical profiles demonstrated hyperglobulinemia (6.8g/dL [reference: 2.7 – 4.4g/dL]). The dog received supporting therapy with 250 mg amoxicillin, dipyrone, ondansetron, tramadol, and ranitidine. Despite efforts to withstand the advance of the disease the clinical condition worsened, and the animal died after 22 days of hospitalization.

At necropsy the thoracic cavity contained abundant purulent exudate (pyothorax). The wall of the distal segment of the esophagus and of the cardia and fundus of the stomach were thickened (5cm thick along the 10cm distal esophagus and 5cm of the proximal stomach) by a focal extensive firm, pink and white, irregular intramural annular mass, which contained multifocal petechial to suffusive hemorrhages, with a transmural esophageal fistula measuring 2 cm in diameter. The lumen of the distal portion of the esophagus was severely distended and contained large amounts of undigested food (Figure 1a). The lungs had extensive atelectasis as well as a white-yellowish firm mass involving the caudal portion of the cranial lobe of the right lung, measuring approximately 4cm in diameter with extensive adhesion to the esophageal wall. In addition, the mediastinal and gastric lymph nodes were markedly enlarged and firm, sometimes containing whitish irregular masses on cut surface.

At histological evaluation, the walls of the distal esophagus and stomach were diffusely expanded by extensive multifocal areas of necrosis characterized by deposition of abundant amorphous eosinophilic material and cellular debris. Surrounding those areas there was a severe inflammatory infiltrate of neutrophils, epithelioid macrophages, and Langhans-type multinucleated giant cells, as well as lymphocytes, plasma cells and occasional eosinophils. At the periphery there was abundant proliferation of immature fibroblasts and neovascularization in perpendicular disposition (compatible with granulation tissue) and moderate multifocal deposition of fibrous connective tissue matrix. Fibrinoid necrosis of the arteriolar wall with moderate multifocal infiltration of neutrophils into the media was seen in multifocal distribution, as well as multifocal arterial thrombi, sometimes with secondary reorganization and recanalization. Intermingled into the necrotic foci were numerous negative images of 5-8µm cylindrical structures with non-parallel walls, occasionally septate, irregularly branching structures (Figure 1b), which stained positive with silver impregnation (Grocott's methenamine silver stain - GMS) (Figure 1c). The structures compatible with oomycete hyphae were also found in necrotic arteriolar walls. The lungs had diffused atelectasis while the right cranial lung lobe sections had similar lesions to those described in the esophagus and stomach, associated with occasional intralesional hyphae. The adjacent pleura was covered with infiltrate of neutrophils and eosinophilic fibrillar material (fibrin). The mediastinal and gastric lymph nodes were expanded by severe intra sinusoidal infiltration of macrophages and occasional multinucleated giant cells, associated with areas of necrosis and occasional hyphae.

Sections of paraffin-wax embedded esophagus were dewaxed and rehydrated, and antigen retrieval was performed. The technique has been done with the primary polyclonal antibody against *P. insidiosum* (dilution of 1:500), raised by immunizing rabbits after subcutaneous inoculation of zoospores,

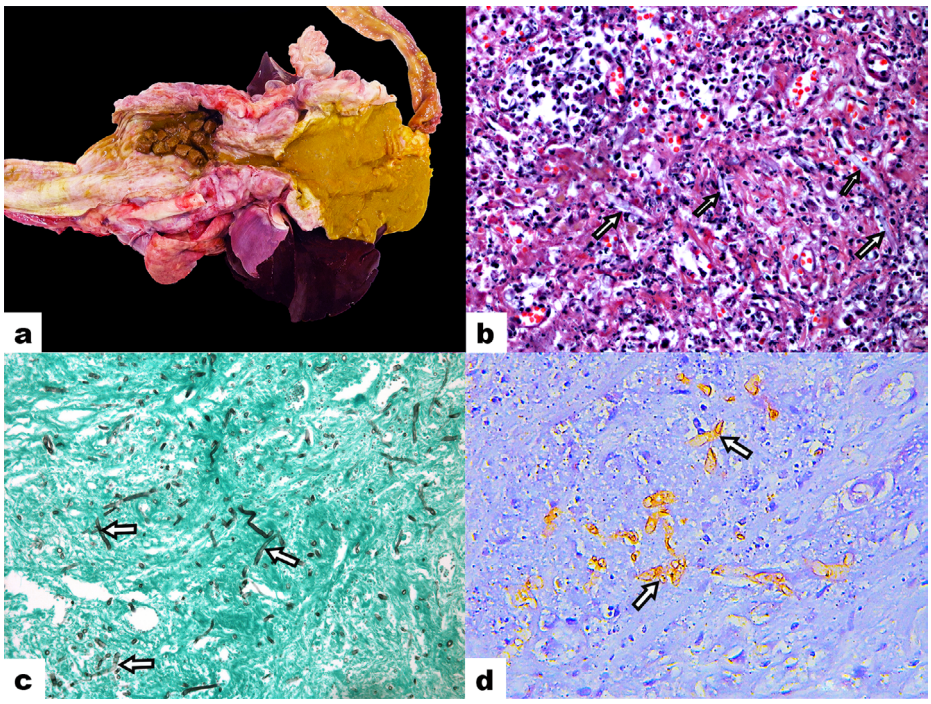

Figure 1 - Gross, histological, and immunohistochemical (IHC) aspects of a case of esophageal and gastric pythiosis in a dog. (1a) The wall of the distal segment of the esophagus and the stomach are expanded by an intramural firm pink and yellowish mass. The segment of esophagus affected was distended by large amounts of undigested food. (1b) There is extensive necrosis associated with severe infiltrate of neutrophils, macrophages and lymphocytes. Intermixed among the necrosis there are multiple negative images of 5-8 µm cylindrical structures (arrows) (HE, 40X). (1c) Note the abundant cylindrical irregularly branching hyphae highlighted by GMS (arrows) (20X). (1d) Intralesional hyphae were immunolabeled for *Pythium insidiosum* (arrows) (IHC hematoxylin counterstain, 40X).

as previously described (UBIALI et al., 2013), incubated at 37 ˚C for 2 hours. The streptavidin-biotin-peroxidase (LSAB + System HRP, Agilent Technologies, Santa Clara, California, USA) kit was employed as the IHC detection system. The chromogen employed was 3,30-diaminobenzidine (DAB; Dako). The sections were counterstained with hematoxylin. The negative images of hyphae contained in the necrotic areas were immunolabeled for *P. insidiosum* (Figure 1d). For differential diagnosis of other oomycetes, one paraffin-embedded section of the esophagus measuring 10µm was sent for molecular characterization. The DNA was extracted from the paraffin sample according to SHI et al. (2004). The polymerase chain reaction (PCR) was performed using the oligonucleotide pair PI1 (5´-TTCGTCGAAGCGGACTGCT-3´) and PI2 (5´-GCCGTACAACCCGAGAGTCATA-3´), which amplies 105bp. This section encodes the sequence of the ITS1 rDNA gene of *P. insidiosum* (AZEVEDO et al., 2012). The amplified product was analyzed through 2% agarose gel electrophoresis, using the GelRed™ (Biotium Inc, Freemaont, CA, USA), and was observed on the ChemiDoc™ XRS documentation system using ImageLab™ software. The amplicon was purified using a Kit Illustra GFX PCR DNA and GelBand purification kit (GE Healthcare Life Sciences, Pittsburgh, PA, USA) and sequenced in the ABI 3500 Genetic Analyzer (Applied Biosystems®), using specific oligonucleotides. The sequence was analyzed using the BLAST (NCBI) software and was compatible with *P. insidiosum*.

The invasion of animal tissues by *P. insidiosum* gives rise to a wide range of manifestations, including chronic necrotizing and eosinophilic, or granulomatous reactions, either affecting the skin, the alimentary tract or the respiratory system of different animals species (DE COCK et al., 1987; GAASTRA et al., 2010; MARTINS et al., 2012; PATTON et al., 1996). The disease frequently affect horses, dogs,

humans, cattle, and sheep and sporadically camelids, cats andcoatis, among other species (BEZERRA et al., 2020; GAASTRA et al., 2010; GALIZA et al., 2014; HECK et al., 2018; MARTINS et al., 2012; SOARES et al., 2019). Although esophagitis due to *P. insidiosum* is rarely reported in dogs, its clinical and pathological presentation remains largely unknown (PATTON et al., 1996; BERRYESSA et al., 2008). However, the preferential sites of infection and the course of the disease varies among host species, being predominantly cutaneous and progressive in horses, enteric in dogs, cutaneous and auto limited in cattle, and rhinofacial in sheep (GAASTRA et al., 2010; GALIZA et al., 2014). In camels and dromedaries, *P. insidiosum* appears to affect the third compartment of the stomach more often than the stomach of monogastric species (HECK et al., 2018). Furthermore, a case of pythiosis affected the esophagus of an ostrich (*Struthio camelus*) in Brazil (SOUTO et al., 2019).

Dogs that have access to flooded areas contaminated with *P. insidiosum* zoospores are especially predisposed to developing the condition, which mainly affects the gastrointestinal tract in this species (FISCHER et al., 1994; FRADE et al., 2017). Most of the cases of pythiosis described in dogs comprise males of large breeds, such as Labrador Retrievers and German Shepherds, up to three years in age with access to water from ponds, lakes, lagoons, or streams (BERRYESSA et al., 2008; FISCHER et al., 1994). However, there are few reports of the disease affecting females or dogs from urban areas, with limited access to natural water bodies FRADE et al., (2017), like in our study In our case it was not possible to establish the epidemiology of the infection, since the dog lived in an urban environment and it was not possible to determine if it has had access to bodies of water (FRADE et al., 2017).

Although, the mechanism of action of *P. insidiosum* into the gastrointestinal tract of dogs remains unclear, it is believed that the zoospores ingested with the water can reach the organism through either intact or damaged mucosa (GAASTRA et al., 2010; SOARES et al., 2019). Additionally, some studies state that the organism is chemotactically attracted to the intestinal mucosa (GAASTRA et al., 2010). Nevertheless, the wide variation in the distribution of inflammation sites along the gastrointestinal tract is still poorly understood (GAASTRA et al., 2010; GROOTERS & FOIL, 2015).

The poor body score of the dog in this case reflects the chronicity and debilitating nature of the condition. Regurgitation and inanition probably resulted from impaired deglutition due to stenosis, resulting from inflammation and fibrosis of the esophagus and stomach, with secondary dilatation of the esophagus. In many cases, severe consequences can follow, such as aspiration pneumonia or esophageal rupture which are immediate life threatening (GALIZA et al., 2014; HELMAN & OLIVER, 1999; REAGAN et al., 2019). In this case the esophageal fistula caused extravazation of the alimentary content, leading to pyothorax, which aggravated the clinical condition and finally caused the animal's death.

The pulmonary lesions were characterized by a mixed inflammatory infiltrate, similar to what was seen in the esophagus, containing oomycete hyphae, reinforcing the invasive nature of the pneumonia, following continuity from the lesion in the esophagus. Secondary invasion of the mediastinal lymph nodes followed the thoracic lymphatic drainage. Primary pulmonary infection by *P. insidiosum* is rarely reported in veterinary medicine, but is already described in a dog and a horse, as well as in a captive jaguar (*Panthera onca*) and two coatis (*Nasua nasua*) from a zoo in Brazil (GOAD, 1984; CAMUS et al., 2004; KEPLER et al., 2017; BEZERRA et al., 2020). In all cases with primary pulmonary infection by *P. insidiosum*, the main lesions are found in lungs and airways, differing from our case, which presents its main lesions in the upper alimentary tract lesion.

In canine pythiosis, gross lesions generally do not exhibit the typical gray-white coral-like concretions known as "kunkers" found in equine pythiosis, instead, it presents expansive masses as observed in this case (GALIZA et al., 2014; GROOTERS, FOIL, 2015). Macroscopically, esophageal granulomas in dogs should be differentiated from tumors like squamous-cell carcinomas, adenocarcinomas, osteosarcomas, fibrosarcomas, leiomyosarcomas, and sarcomas induced by *Spirocerca lupi* (COOPER & VALENTINE, 2017; UZAL et al., 2016).

There are two recognized inflammatory patterns of canine pythiosis that can be seen solely or in combination. The first is called necro-eosinophilic pattern while the second is a granulomatous reaction (MARTINS et al., 2012; PATTON et al., 1996). In our case there was predominance of the necro-eosinophilic pattern with multifocal areas containing granulomatous reaction surrounding areas of necrosis, and extensive granulation tissue reaction and fibrosis separating the inflammatory foci and causing widespread expansion of the wall, which clearly contributed to the narrowing of the distal portion of the organ and consequent dilation of the lumen.

The definitive diagnosis can be attained through the macroscopic and histologic aspects of the disease associated with the microbiological identification of the organism through the culture, molecular detection, immunohistochemistry or immunoblot. Differential diagnosis of canine pythiosis should be made considering other oomycetes, such as *Lagenidium* spp., or even *Entomophthorales* fungi, which can have similar morphological characteristics, as well as similar clinical and pathological presentation (OKADA et al., 2015). An early accurate etiological diagnosis may elicit the establishment of a specific treatment resulting in better chances of recovery (MARTINS et al., 2012; SHI et al., 2004).

CONCLUSION

As in our report, regurgitation and emaciation in dogs should be considered possible indicators of upper alimentary tract infection by *P. insidiosum*. Moreover, dogs with little or no access to flooded environments can also be affected by pythiosis. The clinical and pathological findings are associated with the immunohistochemical, and molecular detection permitted to establish the diagnosis of esophagitis and gastritis, with concurrent pneumonia and lymphadenitis due to *P. insidiosum* infection in a dog.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization, investigation, methodology and writing: EMSS, KPFM, AHBP, AHG and FHM. Review, editing and supervision: LN, EMC, LGSO and FMB. All authors critically revised the manuscript and approved of the final version.

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