CLINICAL REPORT

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Disseminated histoplasmosis in an urban canine with access to the rural environment

Histoplasmose disseminada em canino urbano com acesso ao ambiente rural

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ABSTRACT: Histoplasmosis is a systemic disease caused by the fungus *Histoplasma capsulatum*. The microorganism causes localized granulomatous disease, with the respiratory tract being most affected, and can spread through the lymphatic and blood stream. Severe infection in dogs is associated with immunosuppressive therapies. The reported case occurred in a canine, brought to care with a serious clinical condition, which did not respond to treatment and was euthanized. The necropsy examination showed white nodules, of firm consistency and distributed throughout the omentum, liver, splenic capsule, pancreas, stomach serosa, intestines, bladder, mesenteric and inguinal lymph nodes, diaphragm, parietal pleura, aorta and aortic lunate. Microscopically, all nodular lesions consisted of areas of necrosis, with intense histiolymphoplasmacytic infiltrate, epithelioid macrophages and Langhans-type giant cells with yeast-like structures, with morphology and arrangement consistent with *Histoplasma* spp., confirmed by special Periodic Acid-Schiff (PAS) stains and Grocott. Therefore, the present study aims to describe a case of multisystemic histoplasmosis in a canine, diagnosed through anatomopathology and histochemistry, and to alert to this potential infection in urban canines with access to rural areas and/or environments with the fungal agent.

KEYWORDS: *Histoplasma* spp.; dog; fungus; systemic mycosis.

RESUMO: Histoplamose é uma doença sistêmica causada pelo fungo *Histoplasma capsulatum*. O microrganismo causa doença granulomatosa localizada sendo o trato respiratório mais acometido, podendo se disseminar pela corrente linfática e sanguínea. A infecção severa em cães está associada a terapias imunossupressoras. O caso relatado ocorreu em um canino, conduzido à atendimento com quadro clínico grave, o qual não apresentou resposta ao tratamento e foi submetido a eutanásia. No exame de necropsia apresentou nódulos brancacentos, de consistência firme e distribuídos pelo omento, fígado, cápsula esplênica, pâncreas, serosa do estômago, intestinos, bexiga, linfonodos mesentéricos e inguinais, diafragma, pleura parietal, aorta e semilunar aórtica. Microscopicamente, todas as lesões nodulares consistiam de áreas de necrose, com intenso infiltrado histiolinfoplasmocitário, macrófagos epitelioides e células gigantes do tipo Langhans com estruturas leveduriformes, com morfologia e arranjo consistentes com *Histoplasma* spp., confirmado pelas colorações especiais Ácido Periódico de Schiff (PAS) e Grocott. Assim, o presente estudo tem por objetivo descrever um caso de histoplasmose multissistêmica em um canino, diagnosticado por meio da anatomopatologia e histoquímica, e alertar para essa potencial infeção em caninos urbanos com acesso ao meio rural e/ou ambientes com o agente fúngico.

PALAVRAS-CHAVE: *Histoplasma* spp.; cão; fungo; micose sistêmica.

INTRODUCTION

Histoplasmosis is a systemic disease with worldwide distribution caused by the dimorphic fungus *Histoplasma capsulatum*. Infection occurs through inhalation or ingestion of infectious micro- or macroconidia present in soils or environments with bird and bat feces, constituting an organic matter for developing this fungus (Brömel; Sykes, 2005). The agent presents itself in mycelial form in the environment, becoming yeast-like in the host. The disease can affect humans, canines and felines and has already been reported in horses, rodents, marsupials, gazelles and birds (Teles *et al.*, 2014; Guerra *et al.*, 2020). It can remain in the environment for years if the soil

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pH is acidic, the temperature is low, and the relative humidity is high (Agostinho *et al.*, 2021).

Transmission of *H. capsulatum* from canines to humans has not been reported. In humans and dogs, the disease is mainly related to immunosuppressive therapies or some condition that leads to immunosuppression (Brömel; Sykes, 2005). As for breeds, sporting groups, working groups, Terriers and breed groups such as Brittany, English Pointer and Weimaraner are more susceptible (Wilson *et al.*, 2018). In felines, its occurrence is associated with chronic corticosteroid therapy and/or the occurrence of lymphoma (Brömel; Sykes, 2005). It can occur at any age, considering dogs and cats, but is more commonly reported in young animals. (Teles *et al.*, 2014).

In Brazil, cases have already been reported in canines and felines (Teles *et al.*, 2014; Castro *et al.*, 2017). The microorganism causes local granulomatous disease, mainly affecting the respiratory tract and can spread through the blood and lymphatic stream to other organs. Canines with the infection generally have non-specific chronic signs such as hyperthermia, lethargy, anorexia, weight loss, cough, increased respiratory effort, diarrhea and lymphadenopathy (Wilson *et al.*, 2018).

Diagnostic methods involve the association between clinical signs and complementary exams such as radiography, endoscopy to analyze gastrointestinal abnormalities, cytopathology, fungal culture, histopathology, histochemical stains, immunohistochemistry, serology and molecular techniques such as polymerase chain reaction (PCR) (Teles *et al.*, 2014). However, the gold standard for diagnosis is cytological or histological confirmation of *H. capsulatum* (Wilson *et al.*, 2018). Depending on the degree of exposure and prolonged time for diagnosis, mycosis can present a severe evolution and lead to the animal's death. Regarding treatment, the recommended therapeutic protocol is based on the use of itraconazole, at a dose of 10 mg/kg orally, for long periods of 3 to 6 months; ketoconazole and amphotericin B can also be used (Teles *et al.*, 2014; Silva *et al.*, 2013).

The present report aims to describe a case of multisystemic histoplasmosis in a canine, diagnosed through anatomy pathology and histochemistry, and to alert to this potential infection in urban canines with access to rural areas and/or environments with the fungal agent.

CASE REPORT

The case occurred in a three-year-old male Yorkshire dog. The animal was taken for veterinary medical care and had been vomiting after eating, diarrhea and emaciation for around 30 days. During the anamnesis, it was found that the animal lived in an urban environment but frequented rural areas frequently. The patient was hospitalized for symptomatic treatment and additional tests. The blood count revealed monocytosis and mild regenerative anemia, while the biochemical examination revealed increased alkaline phosphatase and hypoalbuminemia. Supportive treatment was established, but the canine did not show clinical improvement, and due to the patient's severe impairment, euthanasia was suggested, which was authorized by the owner. The canine was sent for a necropsy examination.

At necropsy, white nodules of firm consistency were observed distributed throughout the omentum (Figure 1A), liver (Figure 1B), splenic capsule, pancreas, stomach serosa, intestines, bladder, mesenteric and inguinal lymph nodes, diaphragm (Figure 1C), parietal pleura, aorta and aortic lunate (Figure 1D). The lungs showed severe congestion, edema and areas of consolidation. Samples from all organs were collected, fixed in 10% buffered formalin, processed according to conventional histochemical methods and stained with Hematoxylin and Eosin (HE).

Microscopically, the nodular lesions consisted of areas of necrosis, with histiolymphoplasmacytic infiltrate, epithelioid macrophages and Langhans-type giant cells with foamy cytoplasm (Figure 2A-B). Mild multifocal pyogranulomatous bronchopneumonia was also observed in the lungs. In the areas of necrosis of all organs and the cytoplasm of macrophages and giant cells, rounded, yeast-like structures were found, suggestive of fungi. Therefore, samples from the affected organs were subjected to special Periodic Acid-Schiff (PAS) staining and Grocott staining to confirm fungal structures. In both special stains, numerous fungal structures were observed with morphology and arrangement consistent with *Histoplasma* spp. (Figure 2C-D).

DISCUSSION

The canine lived in an urban environment with frequent access to rural areas and interaction with domestic birds, an epidemiological condition extremely favorable to infection. After all, *H. capsulatum* is frequently isolated from bird farms and caves, where bats are abundant. The presence of the fungus is also reported in open spaces, such as parks, where bird excrement is frequently found (Guerra *et al.*, 2020), proving that the presence of waste alone is sufficient for transmission of the pathogen (Figueiredo *et al.*, 2016). Studies show that *H. capsulatum* was detected through environmental soil isolation in seven Brazilian states (Almeida *et al.*, 2019), which shows that contamination involves a unique health issue, as it encompasses animals, humans and the environment.

Cases of disseminated histoplasmosis in dogs have already been reported inside and outside Brazil (Mitchell; Stark, 1980; Meadows *et al.*, 1992; Džaja *et al.*, 2004; Gilor; Ridgway; Singh, 2011; Ortiz-Yépez *et al.*, 2015). According to the literature, in cases of systemic histoplasmosis, there is a wide variation in the presentation and duration of clinical signs. Between the treatment of the canine in the report and the death, 31 days they were passed. The disseminated form commonly presents signs that include weight loss, inappetence, high blood pressure and lethargy, as shown by the dog in this case. However, these clinical signs are non-specific and occur

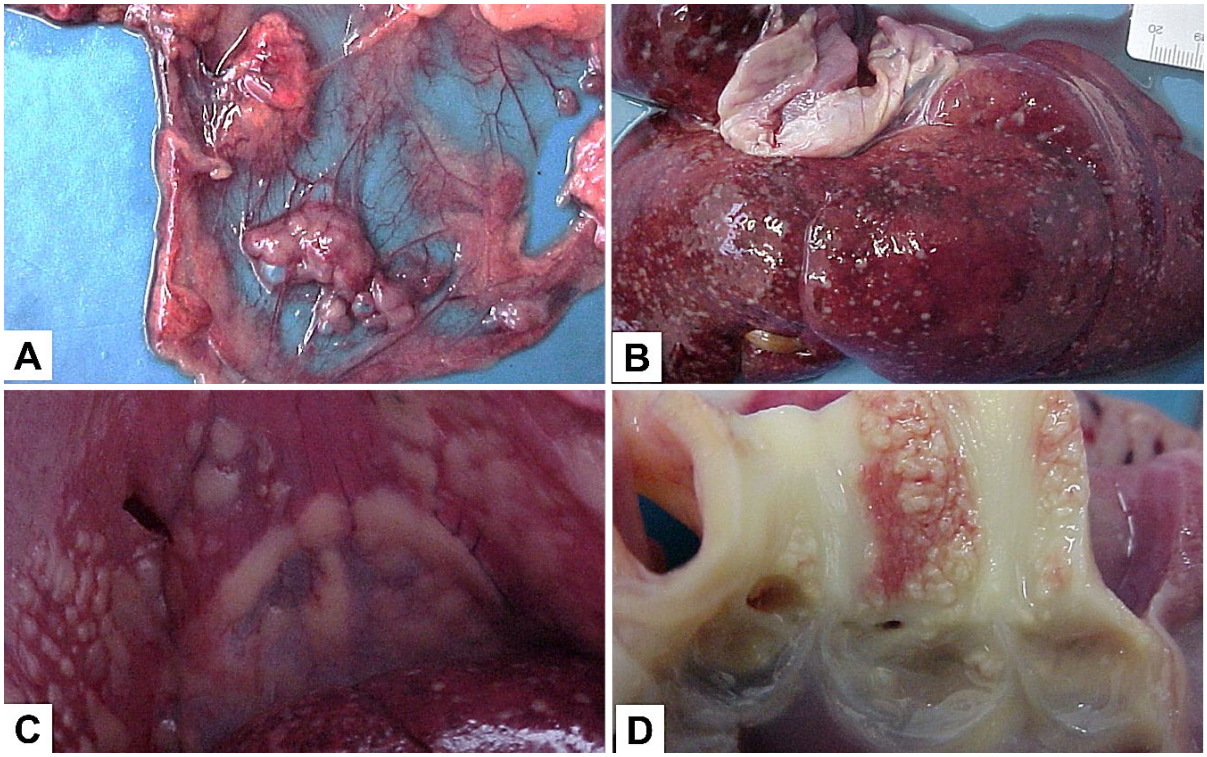

Figure 1. Disseminated histoplasmosis in a canine. **A)** Omentum showing white nodules of firm consistency. **B)** Liver showing a capsular surface with numerous small white nodules. **C)** Diaphragm with white nodules of firm consistency. **D)** Heart with aorta and aortic lunate showing white nodules of firm consistency.

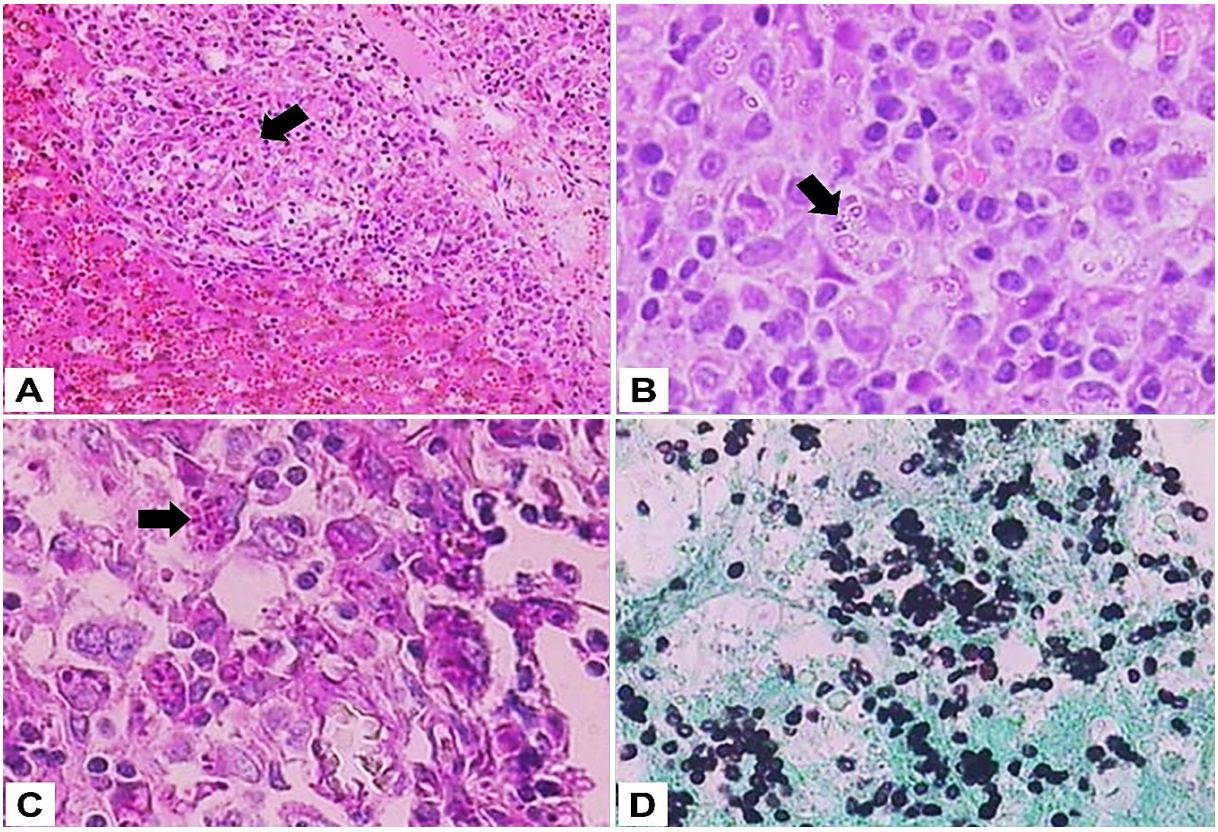

Figure 2. Disseminated histoplasmosis in a canine. **A)** Liver showing granulomatous hepatitis (arrow). HE, 100X. **B)** Pancreas showing yeast-like structures in the macrophage cytoplasm (arrow). HE, 400X. **C)** Omentum with yeast-like structures in the macrophage cytoplasm (arrow). PAS, 400X. **D)** Omentum with yeast-like structures dispersed in the tissue. Grocott, 400X.

in several illnesses, undermining the clinician's presumptive diagnosis and leading him to consider several possibilities and request several tests to elucidate the case (Teles *et al.*, 2014). It is known that disseminated histoplasmosis is uncommon and generally affects young dogs between two and four years of age (Meadows *et al.*, 1992), corroborating the animal's age in the present report.

Vomiting, diarrhea, reluctance to move and dyspnea may also be present (Wilson *et al.*, 2018). Lung changes are frequent and culminate in dyspnea, coughing and wheezing (Silva *et al.*, 2013). A characterization of 24 cases of the disease demonstrated diarrhea unresponsive to medication and progressive weight loss with a clinical course of 14 weeks in most of them (Wilson *et al.*, 2018), corroborating the picture presented in the present report. Even though it is not common, dermatological diseases, neurological signs, and bone and eye involvement can also occur (Agostinho *et al.*, 2021).

Histoplasmosis can cause hematological changes, with non-regenerative normochromic normocytic anemia, neutropenia and monocytosis already described (Wilson *et al.*, 2018). In the present case, the presence of monocytosis stands out, possibly justified by the chronic inflammatory nature of the disease or in response to the increased demand of these cells for the tissues (Weiser, 2015). Regarding the mild anemia presented by the patient, it is known that in histoplasmosis, the anemia is generally non-regenerative, especially in the disseminated form of the disease when infiltration of the bone marrow occurs. Therefore, as in the present case, the anemia was characterized as regenerative. Despite being discreet, an antibody-mediated hemolytic cause, that is, immune-mediated, may be possible (Schaefer; Rizzi; Royal, 2019).

The increase in alkaline phosphatase, an induction enzyme, is related to cholestatic processes in the liver (Allison, 2015), suggesting involvement of the organ, which was later confirmed. Hypoalbuminemia may have occurred due to production failure, but in the present case, the leading causes are supposed to be diarrhea with protein loss and anorexia with low protein intake (Allison, 2015).

The lesions observed at necropsy were nodular and white, with a firm consistency and located in the omentum, splenic capsule, pancreas, diaphragm, parietal pleura, aorta and aortic lunate. According to the literature, histoplasmosis lesions have a granular to nodular appearance and are seen on the surfaces of organs, as well as granulomatous nodules within the viscera, on intestinal walls and in mesenteric lymph nodes (Wilson *et al.*, 2018). All the proliferative lesions the patient presented histologically consisted of necrosis and chronic inflammatory infiltrate, with rounded structures suggestive of fungi. Despite the absence of clinical respiratory signs in the lungs, multifocal pyogranulomatous bronchopneumonia was evident, with rounded structures suggestive of fungi. For canines with histoplasmosis, there are reports of chronic/granulomatous changes occurring in various locations, causing meningitis, encephalitis, bilateral chorioretinitis, pneumonia, hepatitis, jejunitis and lymphadenitis (Meadows *et al.*, 1992), similar to those observed in this case.

According to the majority of reported cases, and as found in the present report, upon histopathological examination, the lesions present with granulomatous inflammation and the presence of numerous rounded intra and extracellular microorganisms. These appear yeast-like structures surrounded by a clear halo and are reactive to Grocott and PAS staining (Wilson *et al.*, 2018). It is known that in humans, biopsies have positive results in identifying the fungus (Almeida *et al.*, 2019). In the case in question, identification was carried out through anatomopathological examination at necropsy, where HE staining and special stains showed rounded and yeast-like shapes. Thus, through anatomopathological examination, ruling out the possibility of neoplasia, paying attention to the morphology of the agent and using histochemistry, the diagnosis of multisystemic histoplasmosis was obtained.

CONCLUSION

The importance of carrying out a histopathological examination and other available laboratory resources to elucidate infectious disease cases, such as histoplasmosis, is highlighted. Furthermore, the diagnosis of *Histoplasma spp*. in urban companion animals with access to rural environments and/or urban contact with bird and bat feces contributes to alerting owners and the animal health service to the circulation of the agent and the potential risk of infection and severe illness in these animals.

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