Systemic canine histoplasmosis: A case report from Ecuador

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ABSTRACT

Histoplasmosis is a zoonotic systemic mycosis caused by Histoplasma capsulatum. We report a case of a female canine, 4 years old, presenting multifocal lymphadenitis and skin and gingival lesions, in Ecuador. Based on cytological, histopathological, histochemical analyses, fungal culture and DNA sequencing of the ITS region of the fungus, the diagnosis confirmed the presence of H. capsulatum as the agent of infection. The treatment plan included ketoconazole with a satisfactory outcome.

1. Introduction

Ajellomyces capsulatus is the holomorphic name for Histoplasma capsulatum, the etiological agent of histoplasmosis, a systemic mycosis. This thermo-dependent dimorphic fungus is soil-borne [1] and in organic nitrogen rich soils fungal growth and sporulation are accelerated [2,3]. Therefore, places where bird and bat droppings enrich the soil with organic nitrogen, represent a potential environment for proliferation of the fungus and increases the risk of infection [4,5]. In this environment, the fungus exists in mycelial form, whereas in the host it exists as a yeast-like form [6,7].

Commonly, exposure occurs when histoplasma spores become airborne and are inhaled, less frequently, ingestion and infection via open wounds can occur [4,8–10]. In its most severe form (disseminated) histoplasmosis can affect almost any part of the body; which depends strongly on the genetic predisposition of the subject, according to recent experimental evidence [7,11]. Severe clinical disease can result from high doses of infectious spores or if the infected host is immunocompromised (10).

H. capsulatum is distributed worldwide [3] with certain evidence of its origin in Latin America [12]. Despite this knowledge, there are still few reports published about histoplasmosis in domestic animals in this region [9,13–15], considering that it is common in Latin America for domestic animals to live in backyards with poultry where the potential risk of infection is higher. Here, we report a case of histoplasmosis with multifocal lymphadenopathy, skin and gingival lesions in a dog that lived in close proximity to bird guano, used as fertilizer.

2. Case

A 4-year-old female sterilized dog (Schnauzer) presented initially mild gastrointestinal problems (mucous diarrhea) and respiratory affections (sneezing), which were treated with oral hydration (day 0) after veterinary evaluation. In day +20, the patient returned to the veterinary hospital because several dermatological problems appeared. These consisted of multiple crusted papules located over lips, back of head, neck, thorax and lumbar region (Fig. 1a). Furthermore, the dog presented swelling of submandibular and popliteal lymph nodes and left maxillary gingiva (Fig. 1b). Hematologic findings showed thrombocytopenia (44 K/\text{\mu}L). No imagenological abnormalities were identified.

Cytological examination using Diff-Quick® staining method of a fine needle aspiration of papules and submandibular and popliteal lymph nodes, showed mixed cellularity represented by lymphocytes, plasma cells, neutrophils, eosinophils and epithelioid histiocytes in the background inflammatory component. The latter

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contained numerous rounded to oval yeast-like structures in their cytoplasm, 2–4 μm in diameter, surrounded by a thin clear halo and an eccentric purple core of crescent shape, compatible with *H. capsulatum* (Fig. 2a).

Excisional biopsy of a skin nodule located in the dorsal neck was submitted for histopathology. Macroscopic examination revealed that the sample consisted of crusted papules composed of a well-defined, solid, yellow and firm granuloma (Fig. 2b). Microscopy showed a partially delimited non-encapsulated pyogranuloma inside the epidermis, dermis and adipose tissues (Fig. 2c). The inflammatory cells were mainly comprised of neutrophils (many degenerated), epithelioid macrophages (some binucleated) and a few reactive lymphocytes. Inside the macrophages’ cytoplasm, numerous yeast-like structures (2–4 μm in diameter) were present, with shapes from round to oval, and surrounded by a clear thin halo, and an eccentric nucleus, compatible with yeast-like structures of *H. capsulatum*.

Fungal samples obtained by fine needle aspiration of submandibular and popliteal lymph nodes were cultured in Potato Dextrose Agar (PDA) and Brain Heart Agar (BHA) + 5% human blood; both media were supplemented with antibacterial agents, amoxicillin/clavulanate 5 μg/mL, tobramycin 2 μg/mL and chloramphenicol 5 μg/mL. In order to obtain the filamentous form of *submandibular and popliteal lymph nodes* were cultured in Potato Dextrose Agar (PDA) and Brain Heart Agar (BHA) + 5% human blood; both media were supplemented with antibacterial agents, amoxicillin/clavulanate 5 μg/mL, tobramycin 2 μg/mL and chloramphenicol 5 μg/mL. In order to obtain the filamentous form of

![Fig. 1. Macroscopic view of the lesions (day +20). (a) Dorsal neck skin presented multiple crusted papules and (b) gingiva of left maxilla is erythematous and swollen.](image1)

![Fig. 2. Cytological and histopathological examination. (a) Diff-Quick® stain of fine needle aspiration of submandibular and popliteal lymph nodes, samples show *H. capsulatum* structures within macrophages (1000 × ); (b) skin biopsy; (c) focal pyogranulomatous dermatitis, hematoxylin-eosin stain (1 × ); (d) Yeast-like structure of *H. capsulatum* inside macrophages, Grocott’s methenamine silver stain (1000 × ) and (e) Yeast-like structure of *H. capsulatum* inside macrophages, Periodic acid-Schiff stain (1000 × ).](image2)

![Fig. 3. Histoplasma capsulatum cultures. Fungal samples were obtained by fine needle aspiration of submandibular and popliteal lymph nodes. (a) *H. capsulatum* filamentous form in Potato Dextrose Agar (PDA) cultured for 28 days at 25°C; (b) *H. capsulatum* yeast-like form in Brain Heart Agar (BHA) cultured for 28 days at 35°C and (c) microscopic structure of filamentous form of *H. capsulatum* stained with a drop of methylene blue (400 × ).](image3)
the fungus and yeast-like structures, PDA and BHA plates were 28 days incubated at 25 °C (Fig. 3a) and 35 °C (Fig. 3b), respectively. Fungal growth was visible after 18 days of incubation. PDA plates showed the filamentous form of the fungus and BHA plates the yeast-like form, as expected. Microscopic structures of filamentous growth were examined with an optical microscope, the slide was prepared by placing a drop of methylene blue over the culture sample (Fig. 3c). The strain was named MZ&ZUDLA1.

In order to confirm the diagnosis of *H. capsulatum*, molecular testing was conducted. Genomic DNA of filamentous and yeast-like forms was extracted using the High Pure PCR Template Preparation Kit (Roche). The amplification and sequencing of the internal spacer (ITS) region was carried out with universal primers ITS1 (5′-TCCGTAAGGTGAACCTGCGG-3′) and ITS4 (5′-TCTCCGCTATTGATATGC-3′). The amplification protocol consisted of 1 cycle of initial denaturation for 5 min at 95 °C, 30 cycles of denaturation for 1 min at 95 °C, annealing for 30 s at 52 °C, and extension for 1 min at 72 °C, and a final extension for 5 min at 72 °C. Positive PCR products were visualized in 1% agarose electrophoresis gels. Sequencing was performed at Macrogen Inc. (South Korea). The MZ&ZUDLA1 ITS sequence was analyzed using BLAST, then compared with previously described sequences via an UPGMA analysis (Geneious 5.6.4. software) (Fig. 4). The ITS sequence of the MZ&ZUDLA1 strain was deposited in GenBank, accession no. [KM279010].

After histochemical confirmation of initial diagnosis (day +33), the treatment plan included cephalexin: 25 mg/Kg taken orally every 12 h for 21 days, and ketoconazole: 10 mg/Kg taken orally every 12 h for 60 days. During examination at day +140 the dog exhibited a 95% skin lesion recovery (Fig. 5). Additionally, there was a size reduction of the lymph nodes. It was unnecessary to proceed with further examinations due to the dog’s full recovery and discharge.

3. Discussion

During the first veterinary evaluation (day 0), the patient showed mild digestive and respiratory problems. As these symptoms are not specific to histoplasmosis [2,3], the first diagnosis was missed. In the second medical evaluation (day +20), the patient presented lymphadenopathy (submandibular and popliteal), cutaneous and gingival lesions and thrombocytopenia. It is known that disseminated histoplasmosis usually presents dermatological problems [16,17] and thrombocytopenia may be observed in up to half of dogs with histoplasmosis (10); thus, at the second examination there were signs indicating the disease. Furthermore, the data provided by the dog’s owner revealed that they lived in the valley around Quito where the weather is warm and humid, suitable conditions for *H. capsulatum* [2]. The owner also revealed that bird guano is used to fertilize her garden and, as reported previously, this material enhances the growth and sporulation of this fungus [2,3], suggesting that infection was via inhalation or ingestion of *H. capsulatum* spores.

Results of cytological, histopathological and histochemical tests were compatible with disseminated histoplasmosis, due to the presence of intracellular yeast-like structures in lymph nodes and skin [2,6,7]. In addition to biochemical analyses, molecular tools provided important information about the pathogen genotype. The ITS region has been used successfully in the identification of pathogenic and non-pathogenic fungal species [18,19]. In our study, we combined several diagnostic methods to confirm the presence of *H. capsulatum*.

Although itraconazole is the recommended treatment for histoplasmosis due to its effectiveness and lesser secondary effects [6], Ketoconazole was used having the advantage of being less expensive, not nephrotoxic and excreted in low amounts by the kidneys [20] and proved just as effective in treatment.

In conclusion, the current case report describes a case of histoplasmosis in a female canine diagnosed via cytology and culture of *H. capsulatum* from the lesions. It is widely accepted that the use of itraconazole is the treatment of choice for histoplasmosis in dogs; however, the use of ketoconazole, in this case, proved to be an adequate treatment option. This sreep mainly helps veterinarians, but also doctors and researchers, to better understand the epidemiology, symptoms and laboratory diagnosis of this disease. Finally, we suggest that histoplasmosis should be considered for diagnosis in cases with mild symptoms, especially in areas with identified environmental risk factors.

Conflict of interest

No conflicts of interest have been declared.
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