Medical Mycology Case Reports 2 (2013) 32–36

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

Cytological, microbiological and therapeutic aspects of systemic infection in a dog caused by the fungus *Phialosimplex caninus*

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ARTICLE INFO

Article history:
Received 10 December 2012
Accepted 14 December 2012

Keywords:
Phialosimplex caninus
Canine
ITS sequencing
Lymphadenopathy
Mycosis
Splenomegaly

ABSTRACT

A seven-year-old immunocompetent dog presenting with lymphadenopathy, mesenteric masses and splenic nodules was diagnosed with *Phialosimplex caninus* infection. Cytology of a mesenteric mass aspirate demonstrated few intact cells but numerous variably sized fungal cells and rare hyphal fragments. The identity of the cultured fungus was confirmed by DNA sequencing. Itraconazole therapy improved clinical signs, but the fungus was reisolated at follow-up. *P. caninus* systemic infection should be suspected in dogs presenting with lymphadenopathy and splenomegaly.

1. Introduction

Phialosimplex was described recently for two species, *P. caninus* and *P. chlamydosporus*, that are associated with systemic infections in canines [1,2]. The genus is related to *Aspergillus*, *Geosmithia*, *Penicillium*, *Paecilomyces*, *Sagenomella* and their sexual relatives, which include species that have been implicated in opportunistic canine infection. Infections caused by *P. caninus* have been confirmed in a small number of dogs and are mostly associated with disseminated infection involving the lymph nodes or vertebrae [1]. Factors contributing to *P. caninus* infection are not clear since a detailed case history is known for a single animal in which immunosuppression appeared to contribute to onset of bone marrow infection [3]. In that case, the animal was treated with itraconazole and prednisone but died 18 months later of unknown causes. This case reports systemic infection occurring in an immunocompetent dog, demonstrates that sporulation from phialidic conidiogenous cells occurs in tissue and confirms that itraconazole controls but does not eliminate the infection.

2. Case

A seven-year-old, female spayed, Miniature Poodle cross dog presented to the Mississauga Oakville Veterinary Emergency Hospital for further evaluation of acute onset vomiting, decreased appetite, depression and abdominal pain. The vomiting had started approximately four days prior, and had continued despite treatment with intravenous fluids and supportive care at the referring clinic. A single episode of vomiting had occurred one month prior and resolved with offering a gastrointestinal diet. There was no history of travel outside of Niagara Falls, Ontario.

Abdominal palpation revealed a mid-to-caudal abdominal mass and moderate pain in the cranial abdomen as well as moderate enlargement of the left prescapular lymph node. Other findings included a depressed mentation, mild dehydration, bradycardia and mild hypertension. Abdominal ultrasound revealed a large, hypoechoic mass (7×3 cm) that appeared adherent to the serosal surface of the lesser curvature of the stomach in the proximal pylorus. The stomach wall adjacent to this mass was mildly thickened. Mild splenomegaly was present with multifocal, hypoechoic nodules up to 1.2 cm throughout the parenchyma. Several large masses were identified in the portal, hypogastric, mesenteric and sublumbar region of the abdomen with the largest measuring 3.7×1.6 cm. Thoracic radiographs demonstrated a large, radiopaque mass (7×4 cm) in the cranial mediastinum. Biochemical profile revealed moderate hyperproteinemia, hyperglobulinemia,

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2211-7539
http://dx.doi.org/10.1016/j.mmcr.2012.12.007

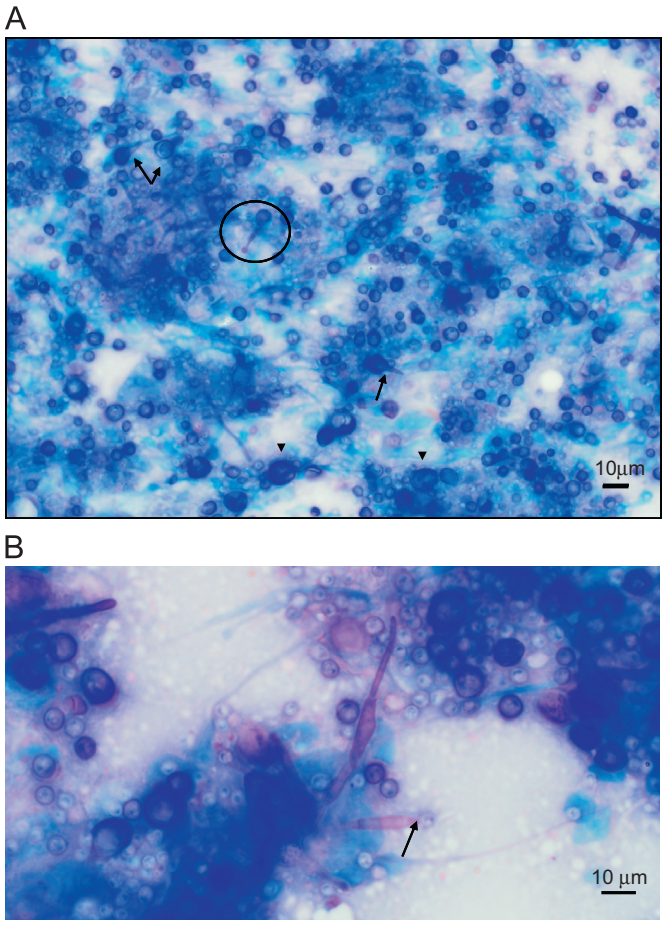

Fig. 1. Direct Wright's stained smears from aspirates of a mesenteric mass from a canine show numerous round to ovoid, variably sized fungal cells. (A) Thicker-walled cells showing secondary proliferation (arrowheads), occasional conidiogenous cells bearing conidia (circle) and cells germinating to form short hyphae (arrows) are present. (B) Fragments of branched hyphae and a conidiogenous cell producing a conidium (arrow) are present. Bars = 10 µm.

mildly elevated urea and mild hypochloremia. A complete blood count (CBC) identified a mild leukocytosis due to a primary neutrophilia and monocytosis.

Due to the suspicion for neoplasia on diagnostic imaging, fine needle aspirates of the mesenteric mass adjacent to the stomach, caudal abdominal mass and the spleen were obtained under sedation. No complications were noted and recovery was uneventful. While awaiting results, the patient was hospitalized and treated with maropitant, famotidine, sucralfate and fentanyl for pain control. No vomiting occurred overnight, and a slow improvement in appetite was noted with symptomatic therapy.

Direct Wright's stained smears were prepared from the aspirates submitted for cytology. The mesenteric mass aspirate contained rare intact neutrophils and macrophages; however no lymphocytes were identified. Numerous, extracellular, round to ovoid, variably sized pleomorphic fungal organisms ranging from 3–12 µm in diameter were present in dense aggregates (Fig. 1A and B). Their cell walls exhibited variable staining characteristics and ranged from a thin to thick appearance. Occasional cells appeared to proliferate to form secondary cells or produced short germ tubes. Conidiogenous cells bearing conidia and fragments of septate, irregular, occasionally branched hyphae were also present (Fig. 1A and B). There was abundant, amorphous basophilic material on the smears consistent with cellular debris. Smears from the spleen revealed fewer numbers of similar pleomorphic organisms, but no hyphae were present. Fungal culture had not been requested at the time of initial aspiration so a fine needle aspirate of the enlarged left prescapular lymph node was taken the day following presentation.

Therpay with itraconazole (30 mg orally q 24 h) was begun and the patient was monitored in hospital over 48 h with a gradual improvement in appetite, mentation and energy level. A single dose of intravenous dexamethasone was administered as a precaution to limit an excessive inflammatory response to antifungal therapy. At discharge, the owner was instructed to administer itraconazole, tramadol as needed for pain control, maropitant as needed for vomiting, prednisone (daily for 3 days, then every other day for 3 treatments, then discontinue) and famotidine for 14 days.

The lymph node aspirate submitted for fungal culture to the Animal Health Laboratory, University of Guelph revealed 1+ fungal elements on wet mount preparation. Due to concern for zoonotic potential, the sample was forwarded for culture to the Public Health Ontario Laboratory, Etobicoke. Culture yielded numerous colonies of a yellowish-white fungus on Sabouraud dextrose agar (BD Diagnostic Systems, Mississauga, ON) and no growth on the same medium amended with cycloheximide. Preliminary identification as *Phialosimplex caninus* was determined by microscopic observations of round to ovoid conidia borne in chains from solitary phialides and by sequencing of the internal transcribed spacer 2 (ITS2) region of the ribosomal RNA gene using primers ITS3 and ITS4. Comparison with nucleotide sequences in the GenBank (National Center for Biotechnology Information, Washington, DC) yielded several *P. caninus* sequences having 99–100% similarity. Both the culture and the sequence were forwarded to the University of Alberta Microfungus Collection, Edmonton, AB and deposited as accession number UAMH 11502. Subsequently the complete ITS region was sequenced using primers ITS1 and ITS4 following protocols previously described [1]. The sequence was deposited as GenBank accession number JX218036.

The owner reported a significant clinical improvement in the dog with a normal appetite, good energy level and no vomiting over the first few weeks of itraconazole therapy. Physical examination performed at three months showed resolution of the enlarged left prescapular lymph node. Thoracic radiographs and abdominal ultrasound revealed minimal change in size of the thoracic, mesenteric and splenic masses. The cranial abdominal mass adjacent to the serosal surface of the stomach was slightly smaller and had developed a large central cavitation with a thick, irregular, hyperechoic capsule. The stomach wall adjacent to the mass appeared normal. The owner was advised to increase the itraconazole dose to 10 mg/kg orally q 24 h (80 mg). At the 6th month examination, clinical signs had stabilized and repeat CBC and biochemical profile were unremarkable. Radiography and ultrasound again revealed only marginal reduction in size of the masses and splenic nodules. Therefore, fine needle aspirates of two mesenteric masses and the splenic nodules were obtained under sedation. Cytology from the masses revealed very few small lymphocytes and macrophages, a large amount of cellular debris and many organisms of varying size along with hyphal fragments, consistent with the previous *P. caninus* observations. The spleen cytology had predominantly blood with a few organisms.

Fungus cultures performed on specimens from the mesenteric masses submitted to the University of Alberta Microfungus Collection yielded heavy growth of *P. caninus* on phytone yeast extract agar (BD) after incubation for 5 days at 30 °C (Fig. 2A). The isolate from this sampling was retained as accession number UAMH 11532 and demonstrated characteristics similar to the first isolate (UAMH 11502). Colonies grown on PDA (BD) were yellowish-white, flat and felty and attained diameters of 60 cm at 30 °C and 47 cm at 35 °C after 14 days incubation (Fig. 2B). Microscopy