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Disseminated phaeohyphomycosis in a dog

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ABSTRACT

Phaeohyphomycosis is a rare but emerging disease caused by dematiaceous fungi. Here we describe the case of an immunosuppressed dog with disseminated phaeohyphomycosis secondary to *Bipolaris spicifera* infection. Regionally extensive infiltration of the paw pads, skin, myocardium, liver, renal interstitium and diaphragm was identified on histopathology. *Candida glabrata* and *Fusarium oxysporum* were also cultured from multiple sites post-mortem. The dog was treated with fluconazole, itraconazole, terbinafine and liposomal amphotericin B, but was euthanized due to its poor prognosis after 12 days of therapy.

1. Introduction

Phaeohyphomycosis is a term used to describe infection caused by dematiaceous (pigmented) fungi belonging to over 60 genera within the orders *Pleosporales, Ochroconiales, Chaetothyriales, Capnodiales, Dothideales, Botryosphaeriales, Microascales, Sordariales, Calosphaeriales* and *Ophiostomatales* [1]. These are typically non-pathogenic soil saprophytes found ubiquitously around the world; however, they can be responsible for life-threatening opportunistic infections in an immunocompromised host [1]. Phaeohyphomycosis has been documented in invertebrates, cold-blooded vertebrates, birds and numerous mammalian species, including ruminants, horses, dogs, cats and humans. A wide variety of clinical syndromes associated with phaeohyphomycosis has been identified, including localized or generalized cutaneous and subcutaneous infections, fungal keratitis, pneumonia or localized pulmonary infections, cerebral abscesses or encephalitis, and disseminated infections [2]. Here we report on a case of disseminated phaeohyphomycosis with concurrent candidiasis and *Fusarium* sp. infection in an immunocompromised dog. This is the third known case of phaeohyphomycosis secondary to *Bipolaris* sp. infection in the dog, and the first to report treatment outcomes.

2. Case

A 6-year old spayed female Miniature Australian Shepherd was presented for evaluation of several ulcerative and inflammatory lesions on the pads of all four paws (day 0, date of first symptom ultimately attributed to phaeohyphomycosis). On physical exam, a deep fissure was present in the left metacarpal pad, which was associated with a small amount of serosanguineous exudate. Several digital pads on the remaining three feet had small areas of purple to black discoloration on their surface. The remainder of the physical exam was unremarkable.

The dog was diagnosed with immune-mediated thrombocytopenia (IMTP) three weeks prior, and was hospitalized for supportive therapy relating to severe gastrointestinal hemorrhage secondary to her IMTP (days −25 to −15). She required multiple blood product transfusions, and vincristine (0.015 mg/kg IV) was administered once on day −24 to stimulate platelet release from the bone marrow. Current medications included prednisone (1.1 mg/kg PO in AM, 0.74 mg/kg PO in PM), cyclosporine (5.5 mg/kg PO q24hr), doxycycline (3.7 mg/kg PO q12hr), melatonin (0.22 mg/kg PO q24hr), omeprazole (0.74 mg/kg PO q12hr), and mirtazapine (0.28 mg/kg PO q24hr).

The dog was hematologically stable by day 0, with a platelet count of 252,000/uL (Reference Interval, RI: 170,000/μL-400,000/μL) and a hematocrit of 36% (RI: 36–60%). However, a chemistry profile revealed newly elevated liver enzymes: AST 76 IU/L (RI: 15–66), ALT 575 IU/L (RI: 12–118), ALP 448 IU/L (RI: 5–131) and hyperbilirubinemia (0.7 mg/dL, RI: 0.1–0.3). Cytology of an impression smear from the open left metacarpal pad yielded suppurative inflammation with predominantly degenerate neutrophils and rare macrophages in a proteinaceous eosinophilic background. No infectious organisms were appreciated, but a sterile culturette of the wound was submitted for aerobic bacterial culture (Agar Gel Amies with Charcoal, COPAN Diagnostics Inc, Murrieta, CA). The dog was presumptively started on

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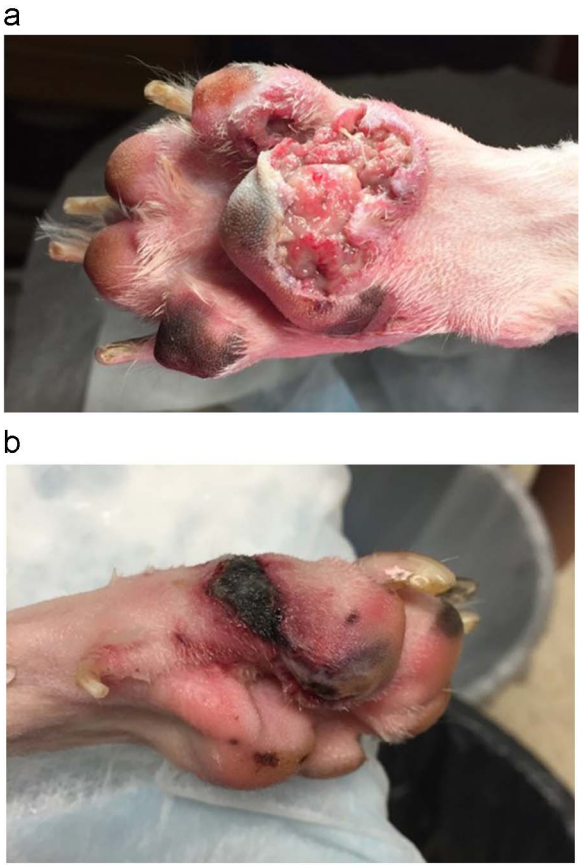

Fig. 1. Paw pad lesions observed on day +3, just prior to biopsy. A) Full-thickness ulceration and sloughing of the left metacarpal pad and fifth digital pad of the left forelimb. B) Eschar extending along the plantaromedial aspect of the left hind paw.

treatment with niacinamide (37.0 mg/kg PO q8hr) and pentoxifylline (22.2 mg/kg PO q12hr) for immune-mediated vasculitis until further diagnostic tests could be performed.

Culture of the wound swab on 5% sheep blood agar did not yield bacterial growth; however, on day +2, growth of small numbers of fungal colonies was observed. Fungal colonies were subcultured on Sabouraud's dextrose agar and identified as *Bipolaris* sp. based on morphologic characteristics. Cyclosporine was discontinued at that time, but prednisone was continued with the dose unchanged. Treatment with fluconazole (8.7 mg/kg PO q12hr) and tramadol (4.3 mg/kg PO q8-12hr) was also initiated on day +2.

Punch biopsies of the right metacarpal pad and fifth digital pad of the right rear limb were performed on day +3. By that time, all the paw pad lesions had progressed. The dermal tissue of the left metacarpal pad had sloughed, revealing the underlying adipose tissue. This sloughing extended through the caudal aspect of the fifth digital pad of the left forelimb (Fig. 1A). A similar necrotic lesion was noted on the third digital pad of the right forelimb. The right metacarpal pad was erythematous and edematous, but had not yet sloughed when biopsies were performed at this site. In addition, pitting subcutaneous edema extended proximally up the right forelimb to the level of the mid-antebrachium. A 2-cm long eschar was present along the plantaromedial aspect of the left hind paw (Fig. 1B). Lastly, erythema and edema were also appreciated on the fifth digital pad of the right hind limb, and punch biopsies were obtained from this site.

Histopathology of the right metacarpal pad revealed full-thickness epidermal ulceration and infiltration of the dermis with lymphocytes and plasma cells (Fig. 2A). The dermal vessels were frequently thrombosed with vascular necrotic debris and neutrophilic inflammation. The deep adipose tissue was focally necrotic with moderate neutrophilic and histiocytic inflammation (Fig. 2B). No infectious agents were initially identified on H & E staining, and an immune-mediated vasculitis was suspected considering the dog's history of immune-mediated disease. However, fungal culture again yielded *Bipolaris* sp. Subsequent Gomori's Methenamine Silver (GMS) staining demonstrated many fungal hyphae and yeast-like bodies within dermis, epidermis and vessel walls (Fig. 2C).

Antifungal susceptibility testing performed on fungal isolates cultured from the biopsy sample (University of Texas Health Science Center, San Antonio, TX) yielded the following minimum inhibitory concentrations: fluconazole 16 mcg/mL, itraconazole 0.5 mcg/mL and posaconazole 0.25 mcg/mL (≤1 mcg/mL suggestive of potential susceptibility). A 24-hr trough, whole blood cyclosporine concentration was significantly above the therapeutic range (1731.4 ng/mL; RI 400–600 ng/mL; Clinical Pharmacology Laboratory, Auburn University, Auburn, AL).

The dog was initially treated as an outpatient. Soft padded bandages were placed on all four paws after the biopsies were performed, and the bandages were changed on days +4 and +5. Necrotic tissue was debrided when indicated, and miconazole cream (2%) was applied to the contact layer of the bandages. The dog became progressively more anorexic, lethargic and lame in the days subsequent to the biopsy. Her dose of prednisone was decreased to 0.87 mg/kg PO q12hr on day +4. Due to persistent anorexia and unwillingness to take oral medications, the dog was hospitalized on day +7 for ongoing wound care, pain management and supportive therapy. Bandages were changed every other day. Enrofloxacin (9.3 mg/kg PO q24hr) was initiated on day +7 given the growth of a susceptible *E. coli* infection on aerobic wound culture. The dose of prednisone was further reduced to 0.87 mg/kg PO q24hr on day +7. On day +8, fluconazole was discontinued and replaced by itraconazole (9.1 mg/kg PO q24hr) and terbinafine (28.4 mg/kg PO q12hr). Low numbers of hyphal elements surrounded by inflammatory cells were identified on impression smears obtained from the paw pad lesions on the left and right forelimbs on the day +9 bandage change. The hyphae were described as lightly basophilic staining, branching, and septate. They occasionally had oval to large, round conidia-like terminal elements.

An irregularly irregular arrhythmia was ausculted on day +9, and continuous ECG monitoring confirmed frequent ventricular ectopic beats occurring in singles and couplets, with occasional runs of a slow, non-sustained idioventricular rhythm. Cardiac troponin I (cTnI) was markedly increased (5.36 ng/mL, RI: < 0.1 ng/mL). An echocardiogram revealed suspected pseudohypertrophy of the left and right ventricles secondary to hypovolemia, with no evidence of vegetative valvular lesions. The myocardium appeared normal in echotexture, and contractility was normal with a fractional shortening of 35%. The dog's arrhythmia progressed on day +11 to include short runs of ventricular tachycardia with occasional R-on-T morphology. Lidocaine (2 mg/kg IV once followed by a CRI at 60 mcg/kg/min) was initiated and provided adequate control of the arrhythmia.

Liposomal amphotericin B (0.7 mg/kg IV over 2hr) was administered on day +11, with plans to continue treatment every 48 hr. However, the owners elected humane euthanasia on day +12 due to the dog's continued clinical deterioration and poor long term prognosis.

On gross necropsy, deep, regionally extensive ulceration of multiple paw pads was observed. Two circumferential, irregularly circular, 4 cm diameter ringed areas, brown in color with central clearing, were noted in the thoracic skin. Focal bruising was noted within the left mid-abdomen and right caudal thigh. Foci of necrosis were appreciated on the surfaces of the left and right atria, along with several nearly transmural foci of necrosis in the right ventricle, interventricular septum and left ventricle near a site of superficial coronary artery branching (Fig. 3). Both kidneys were swollen on cut section. The cortex of the left kidney contained a circumscribed focus of necrosis. The liver had a few small, irregular foci of discoloration (measuring up to 3 × 5 mm).

On microscopic examination, pyogranulomatous inflammation was