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Diagnosis and successful management of a rare case of phaeohyphomycosis in a Doberman pinscher dog

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Summary

Skin affections in canines are common and their treatment depends upon the pathogens involved. Delayed or no response to traditional treatment indicates the involvement of unusual etiology or the multidrug resistance. This report describes the diagnosis and therapeutic management of a rare case of phaeohyphomycosis caused by *Alternaria alternata* complicated with *Pseudomonas aeruginosa* associated pyoderma in a Doberman pinscher dog. Identification of the etiological agents was based on microscopy and culture of the skin lesions and further confirmation by polymerase chain reaction (PCR) amplification. Treatment consisted of a combination of systemic antifungal and antibacterial drugs selected on the basis of *in vitro* antimicrobial susceptibility tests along with the tropical therapy. The report highlights the need of studies on opportunistic fungi to better understand the associated risks, pathogenesis and optimal treatment of these uncommon infections.

Key words: Alternaria, Concurrent, Dog, Phaeohyphomycosis, Pseudomonas

Introduction

Skin diseases in canines due to bacterial, fungal or parasitic infections, alone or in association with other conditions are very common (Kumar *et al.*, 2011). Among all, bacteria are the major infectious cause; however, fungi also contribute in a substantial proportion of cases, either alone or in association with bacterial infections. The fungal etiology in cutaneous infections can be true pathogenic fungi or opportunistic fungal infections. In recent times, many of the fungi previously considered nonpathogenic have emerged as opportunistic pathogens causing more number of infections and tends to complicate other problems (Paixao *et al.*, 2001). *Alternaria*, a dematiaceous ascomycete ubiquitous in air, soil and decaying vegetable matter, is one of such emerging pathogens. The clinical affections of *Alternaria* spp. have been reported in humans (Mayser *et al.*, 2002; Pastor and Guarro, 2008) and cats (Dye *et al.*, 2009). In dogs, the involvement of *Alternaria* spp. is comparatively less common but may cause cutaneous, subcutaneous, cerebral, corneal or disseminated phaeohyphomycosis (Grooters and Foil, 2006). This report describes a rare cutaneous infection involving two infrequent etiological agents: *Alternaria alternata* and *Pseudomonas aeruginosa*, in a Doberman pinscher dog and the resolution of the ailment with promptly selected antibacterial and antifungal drugs.

Case description

A 12-month old Doberman pinscher male dog was presented to Teaching Veterinary Clinic Complex, Veterinary University (DUVASU), Mathura, India with the problem of severe hair loss from lower jaw region extending up to the forelegs (Fig. 1). Anamnesis revealed that the problem started around two months back and since then the dog was treated with antihistaminic and steroid along with antibiotics. Other than this, there was no history of any other previous disease-treatment or underlying disease. On the day of presentation, the dog was receiving 2.6 mg/kg levocitrizine and 1.3 mg/kg prednisolone, each administered orally twice daily. The dog was also receiving 12.5 mg/kg of clavulanic acid-potentiated amoxicillin, administered orally twice daily to manage a number of non-pruritic, non-painful, crusting, erosive to ulcerative, purulent skin lesions that had developed 2 weeks earlier. The owner also revealed that many times, the dog would rush into the grass and bushes in its play area.

On general physical and clinical examination, the dog weighed 30.5 kg, had a normal rectal temperature and was alert and responsive. There was no tick or flea observed and the hair coat was rough and dull with the presence of dandruff. There was complete alopecia of lower jaw and neck region, while on the chest and legs the hair loss was in the moth-eaten pattern (Fig. 1). Clinical examination revealed erythematic and purulent

skin lesions at and near the skin folds of the lower jaw. The hematological values were within normal range except a slight neutrophilia. Erosive to ulcerative crusts and plaques dark in appearance and dry epidermal collarettes were also present over the affected skin (Fig. 1). Swabs from the moist erythemic lesions at neck skin fold and hair plucks and skin scraps from different sites of affected skin were collected aseptically for bacterial and fungal isolation. A part of the scraped skin samples was processed for potassium hydroxide (KOH) mount microscopic examination and the rest was inoculated on Sabouraud dextrose agar (SDA) media plates. For bacterial isolation, the samples were cultivated on blood agar and on MacConkey agar.



Fig. 1: Skin lesions on the day of presentation (A: Hair loss and skin lesions on lower jaw and neck, and B: Hair loss and skin lesions on chest and fore limbs)

Skin scraping's KOH mount smear from all skin scrapings were found negative for mites and revealed thick walled septate fungal hyphae and multicellular obclavate shape macroconidia (Fig. 2). On SDA plates, culture was isolated as a pure growth of olivaceous greyish colonies with aerial mycelia (Fig. 3A). The growth was in the form of distinct concentric rings with a light border and the reverse was brown (Fig. 3B). Microscopic examination of culture stained with lactophenol cotton blue (LPCB) revealed septate hyphae with acropetal chains of large, brown, obclavate to obpyriform shaped conidia with short conical beaks (Fig. 4). Further, the conidia had both vertical and horizontal septae (Fig. 5). The conidial chains were long and profusely branched. Hence the species identified in the present case is *A. alternata*. On the basis of cultural, morphological and biochemical tests, the bacteria isolated from purulent skin lesions were identified as *P. aeruginosa*. The identity of the isolate was also confirmed by the sequencing of 618 bp of the 16S rDNA polymerase chain reaction (PCR) amplicon (GenBank Accession No. KY930658). The *P. aeruginosa* isolate was subjected to antimicrobial drug sensitivity test against 18 antibacterial drugs using disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines for selecting the most effective therapy.

While tests are being performed for the identification of species of *Alternaria* and *Pseudomonas*, the dog was put to gradual withdrawal of prednisolone with 0.7 mg/kg and advised ciprofloxacin once daily. For the treatment of cutaneous alternariosis, oral itraconazole was prescribed once daily. Along with the systemic therapy, topical application of skin soothing spray (Exmarid Skin Soother; Bob Martin Vetcare Ltd., Bristol, UK) thrice daily and bathing twice weekly using shampoo (Ketochlor Shampoo; Virbac AH, Cambridge, Ontario) was recommended. The skin lesions showed gradual improvement after a week with the absence of exudates and reduction in the size of erythemic lesions. After two weeks, the prednisolone dosage was reduced from 0.7 mg/kg twice daily to once daily for a week. The lesions were drying and contracting with subsidence of the pustules. Further re-inspections were performed after

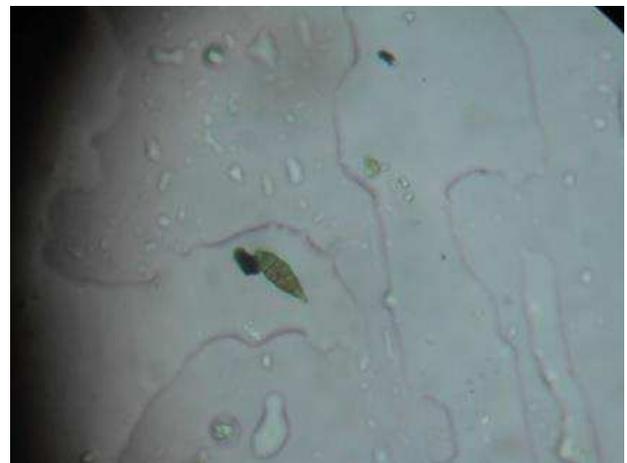


Fig. 2: KOH mount of skin scraping showing pigmented macroconidia with transverse and longitudinal septa

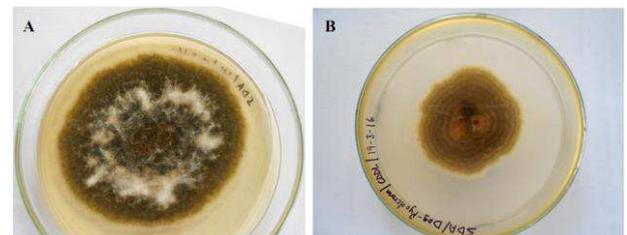


Fig. 3: Fungal growth obtained from skin samples on Sabouraud dextrose agar (SDA) media (A: Observe, and B: Reverse)

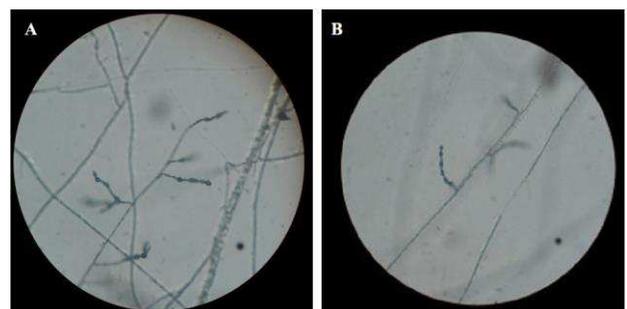


Fig. 4: Arrangement of pigmented macroconidia in acropetal chains

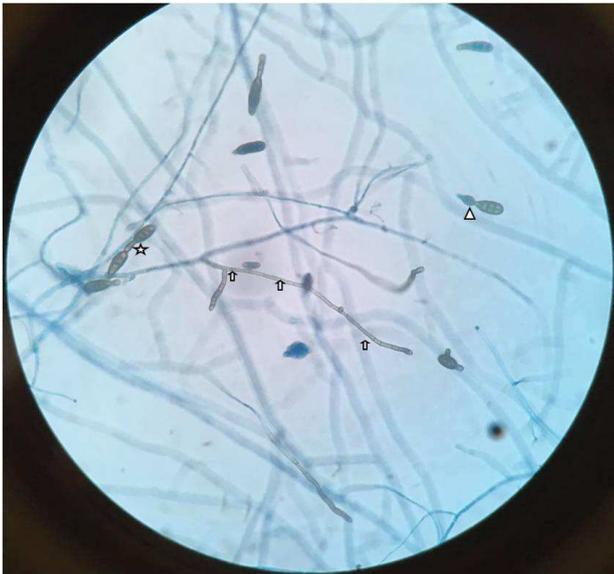


Fig. 5: Hyphae and conidia after lactophenol cotton blue (LPCB) staining showing septate hyphae (arrows) with acropetal chains of large, brown, obclavate to obpyriform shaped conidia with short conical beaks (star). The conidia had both vertical and horizontal septae (arrow head)



Fig. 6: Dog after recovery with healed cutaneous lesions and re-growth of hairs (A: Hair regrowth on lower jaw and neck region, and B: Hair regrowth on chest and forelegs region)

7 days and subsequently every 2 weeks for the following 4 months. The dog's physical condition and the hair coat continued to improve progressively. The antibiotic was stopped after 1 month and the prednisolone was gradually reduced by small decrements and was stopped after 2 months of initial presentation. At 75 days, all cutaneous lesions had healed with the re-growth of hairs on the affected parts (Fig. 6). Two consecutive skin scrapings performed 2 weeks apart were negative. The dog remains well thereafter during the period of 12 months follow-up, no hair loss occurred and there has been no recurrence of the cutaneous lesions.

Discussion

Pseudomonas aeruginosa is usually not associated with skin infections in canines but has been reported from skin of dogs with pyoderma typically associated with other pathogens, as in the present case of

phaeohyphomycosis due to *A. alternata*. Dermatitis in canines due to *Alternaria* spp. has been reported earlier (Dedola *et al.*, 2010; Gangil *et al.*, 2012; Subapriya *et al.*, 2015) due to long-term exposure to soil and garbage, a penetrating trauma, or subsequent to a course of steroid therapy. Canines are more frequently affected by the dermatophytes during the first year of life whereas non dermatophytes cutaneous infections are mostly chronic in character and recorded in dogs of 6 months to 3 years age (Prado *et al.*, 2008). Also, male dog has been reported with a higher incidence of mycotic dermatitis (Kumar *et al.*, 2011) which corroborates the findings of the present report. However, another report recorded no sex differentiation in the incidence of mycotic dermatitis in dogs (Brilhante *et al.*, 2003). Other than age, sex and breed, climatic conditions and season also play an important role in fungal dermatitis (Salo *et al.*, 2005) and such incidences increase significantly in warm and humid climate (Jand and Gupta, 1989). The presence of these predisposing factors during rainy to winter season in the locality might have contributed to the occurrence of *Alternaria* infection as also observed by Salo *et al.* (2005). The clinical signs of *A. alternata* reported in the literature were similar to the ones observed in this case, especially with regard to pigmentation of affected skin and severe hair loss.

The anatomic distribution of the lesions varies depending on the site of direct inoculation of the fungus into damaged skin following contact with a contaminated fomite or plant. The presence of the more severe lesions on the lateroventral neck, chest, and forelimbs also correlate to the inoculation from the plant sources. *Alternaria alternata* is a common saprophyte prevalent in the environment inhabiting primarily leaves and kernels of grasses and cereals (Simmons, 2007) and the exposure of dogs to such areas during playing and running into grasses and bushes might be the source of either direct or indirect inoculation. The use of corticosteroid in the initial therapy further aggravated the conditions as the prolonged use of the immunosuppressive drugs in pets also facilitates the occurrence of the cutaneous infection (Dedola *et al.*, 2010) and this might be the major contributing factor in the present case in the progress of the disease and complications arising due to *Pseudomonas* infection.

Conflict of interest

The authors declare that they have no conflict of interests.

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