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CUTANEOUS PHAEOHYPHOMYCOSIS IN A DOG WITH COLOR DILUTION ALOPECIA - CASE REPORT

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Abstract

Phaeohyphomycoses are recognized as opportunistic fungal infections caused by several genera of melanin-pigmented moulds (dematiaceous fungi) which are ubiquitous saprophytic agents found in soil, water and decaying vegetable matter. These infections are usually acquired by direct traumatic implantation of fungal elements into tissues or by contamination of open wounds, being invariably associated with an immunosuppressive or debilitated status.
Phaeohyphomycoses are rarely reported in dogs, most appearing as focal or multifocal subcutaneous intact or ulcerated/fistulized nodules or plaques usually found in the facial area, the distal part of extremities or the tail, without any systemic signs. According to the literature data, Alternaria spp. were identified on the coat from 20-80% of healthy dogs and cats without any skin lesions.
In this paper, we have reported a case of cutaneous phaeohyphomycosis with Alternaria spp. in a 3-year-old unspayed male Cane corso dog with chronic skin lesions, not responding to antibiotherapy. The diagnosis of fungal infection was based on cytology, fungal culture and clinical response to long term oral administration of itraconazole.
In our opinion, the infection likely occurred by direct implantation into defective hairs as well as by contamination of ruptured follicular cysts with Alternaria spp.originated from skin colonization and the outdoor habitat. We also considered the inherited follicular dysplasia (color dilution alopecia) to be a promoting factor in acquisition of this opportunistic fungal infection. Finally, complete resolution of lesions under itraconazole therapy and lack of reccurence for 14 months were decisive features for diagnosis.

Key words: *alopecia, dog, hyperpigmentation, itraconazole, phaeohyphomycosis.*

INTRODUCTION

Phaeohyphomycosis (chromomycosis, eumycotic mycetoma) is an opportunistic fungal infection caused by the ubiquitous saprophytic pigmented moulds (dematiaceous fungi), often associated with immunesupperssive therapy or immunodeficient diseases.
The agents of phaeohyphomycosis in human and animals have been classified in 60 genera including more than 100 species. In dog and cat, the recognized pathogens are *Alternaria*, *Bipolaris*, *Cladophialophora* and *Curvularia* (Lloret et al., 2013; Taboada, 2016). Some of these organisms have been isolated from skin and mucosal areas of dogs with and without clinical lesions. In several studies of fungal carriage, *Alternaria spp.* were isolated on the coat from 20-80% of healthy dogs and cats without skin lesions (Dedola et al., 2010).
Most fungal infections are acquired by traumatic implantation or wound contamination, but not trough direct transmission between hosts (Lloret et al., 2013; Herráez et al., 2001). Nodules, tracts or masses in the skin or nasal mucosa are common clinical forms of phaeohyphomycosis which is more less reported in dogs than cats. Skin chronic infections are usually localized in the face, the limbs or the tail (Lloret et al., 2013; Medleau and Hnilica, 2006).
Diagnosis and treatment of most infections are quite difficult because of indistinguishable lesions from other skin disorders (bacterial pyoderma, demodicosis, dermatophytosis, foreign body/sterile granuloma, acral lick dermatitis, tumors, etc.), resistance to most antifungal medications and common recurrence after drug or surgical therapy. Itraconazole is currently the drug of choice with good results

in treatment of multiple lesions and post-surgical ablation (Medleau and Hnilica, 2006; Lloret et al., 2013).

MATERIALS AND METHODS

The case was submitted to the Department of Dermatology from Faculty of Veterinary Medicine-Bucharest, in october 2015, after previous treatments in a private clinic.

Case presentation: The patient was a 3-year-old unspayed male *Cane corso* dog of outdoor with chronic symmetrical skin lesions localized in the face and the digits showing no resolution after more series of antibiotherapy. Facial lesions appeared as focal alopecia with diffuse hyperpigmentation and fistulous tract constantly expressing small hair clumps (figure 1).

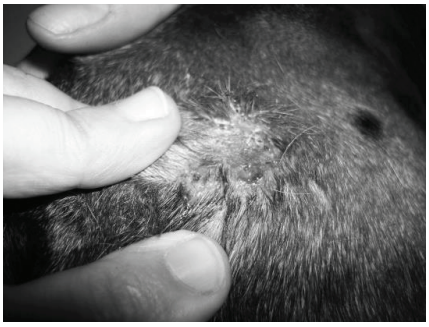

Figure 1. Facial lesion

Digital lesions were quite similar to the facial ones consisting in well-circumscribed alopecia with hyperpigmentation and hyperkeratosis (figure 2).

Figure 2. Digital lesion

Both lesions were nonpainful and nonpruritic. Under a more careful examination we could detected some areas of mild hair color dilution around the facial and digital lesions.

Diagnostic protocol included the following standard procedures: trichogram, cytology, bacterial and fungal cultures. Additionally, therapy monitoring represented a diagnostic-key by the resolution of lesions after long treatment with itraconazole.

Trichogram was used to visualize the hairs collected from perilesional areas on wet mounts prepared with mineral oil and lactophenol cotton blue (LPCB).

Cytology was performed on the smears made from lesional aspirates and stained by May-Grunwald Giemsa method, proving to be a useful tool in the detection of inflammatory origin of lesions, excluding neoplasia. Bacterial cultures were obtained by the inoculation of skin aspirates from facial lesions onto sheep blood agar 5% (SBA) and incubation at 37°C for 72h with the growth of non-haemolytic colonies of *Streptococcus spp.*.

For fungal cultures, skin aspirates and hairs from the edge of digital alopecic lesions were plated directly onto Sabouraud dextrose agar (SDA) containing chloramfenicol and incubated at 27°C for 21days when some woolly grey-green-coloured colonies could be seen growing over the inoculated hairs. Microscopic examination of cultures on native preps with lactophenol cotton blue (LPCB) revealed typical dematiaceous septate hyphae and brown multicellular conidia with both transverse and longitudinal septa of *Alternaria spp.*. In the direct preps from inoculated hairs we have also found a cluster of hyphae growing over an intact hair shaft.

RESULTS AND DISCUSSIONS

Skin lesions were quite irrelevant so that differential diagnosis should be made from other alopecic conditions like the bacterial pyoderma, demodicosis, dermatophytosis, nocardiosis, foreign body granuloma, acral lick dermatitis, squamous cell carcinoma.

Trichogram was the first step helping us to exclude demodicosis and dermatophytosis from the mentioned list of suspicions, but also supplied more information showing small melanin clumps distributed predominantly in the cortex beside some irregularities of hair

cuticle enabling the insertion of a brown multiseptate fungal conidia (figure 3). This pigmented conidia has been most likely implanted by trauma from the environment.

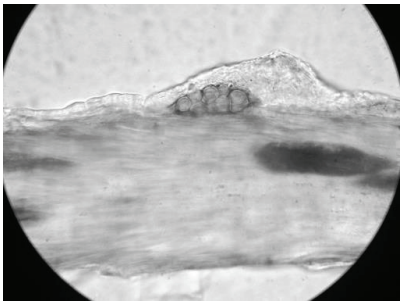

Figure 3. A brown multiseptate fungal conidia under hair cuticle; see also a melanin clump on the right (wet prep, 1000 X)

Moreover, we have considered melanin clumping and cuticle defects together with macroscopical discoloration of the coat around the lesions to be very suggestive for a follicular dysplasia known as color dilution alopecia. In this way, *Cane corso* is a known breed to have genetic predisposition to this follicular disorder (Guaguère et al., 2008). In our opinion, the lower resistance of hair shafts associated to color dilution alopecia might promote fungal inoculation especially into more exposed skin areas like the face and the limbs.

Cytology was useful to preclude skin neoplasia, demonstrating a chronic inflammatory response in both lesions. Thus, the aspirates from digital lesions evidenced a pyogranulomatous reaction comprising a mixed population of neutrophils and epithelioid macrophages satellite to hair fragments (figure 4).

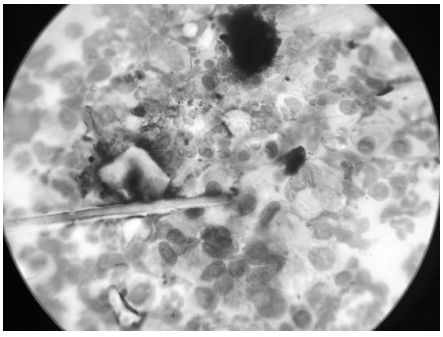

Figure 4. Pyogranulomatous reaction in digital lesions (MGG stain, 1000X)

Under a careful examination we could hardly found a fragment of melanized hyphae surrounded by degenerated neutrophils showing cariolysis (figure 5).

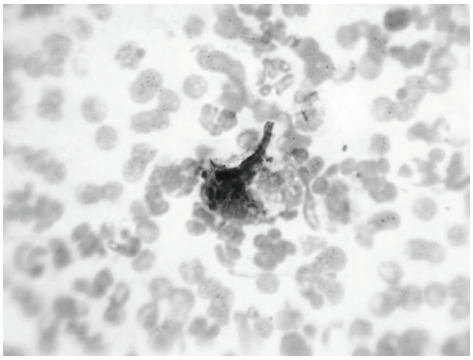

Figure 5. Melanized hyphae with satellite degenerated neutrophils (MGG stain, 1000X)

In a Gram-stained smear from digital aspirates we have also caught an ovoid dark-coloured poroconidia with lateral growing hyphae (figure 6).

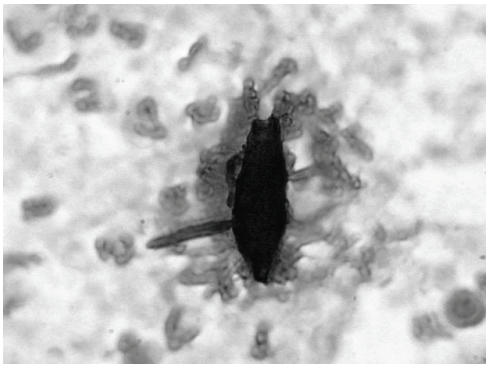

Figure 6. Dark-coloured poroconidia with lateral growing hyphae (Gram stain, 1000X)

Cytology from facial lesions were relevant for follicular cysts by the presence of abundant basophilic keratinaceous debris, numerous corneocytes and nucleated superficial keratinocytes combined with a secondary pyogranulomatous reaction just like the digital lesions.

In some fields, numerous extracellular bacterial cocci scattered in the background along with few phagocytized cocci by neutrophils could be observed indicating a pyoderma subsequently

confirmed by the isolation of *Streptococcus spp.* in the bacterial cultures. Taken together, cytology more sustained the diagnosis of primary color dilution alopecia responsible for defective hair formation and pigmentation, hair breaking, cystic accumulation of hairs and keratin into follicles and foreign body reaction with fistulization resulted from cyst rupture (Medleau and Hnilica, 2006).

Additionally, the exposure of the dog to outdoor habitat most likely promoted the secondary contamination of ruptured follicular cysts by environmental fungi.

Indeed, the isolation of *Alternaria spp.* from fungal cultures (figures 7, 8 and 9) was definitive for the diagnosis, but only in correlation with the history, clinical features and therapy response since the isolated agent is known as a resident of normal cutaneous mycobiota in dog as well as an ubiquitous contaminant.

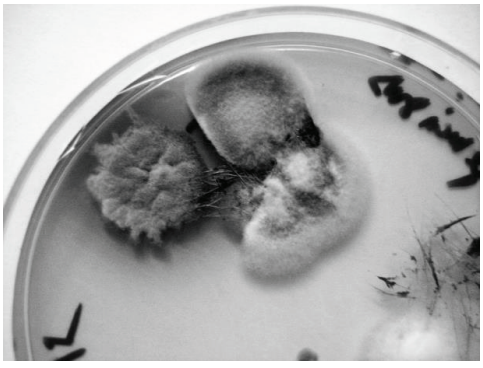

Figure 7. Fungal culture on SDA (7days of incubation)

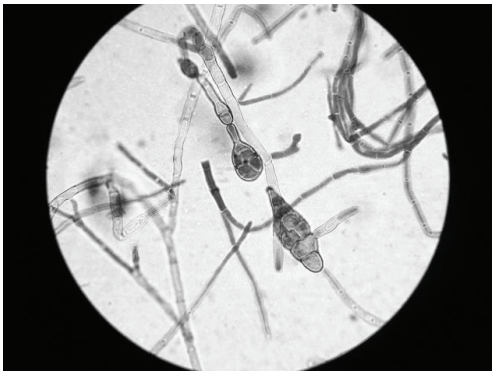

Figure 8. Culture of *Alternaria spp.*- microscopical appearance (wet prep in LPCB, 1000X)

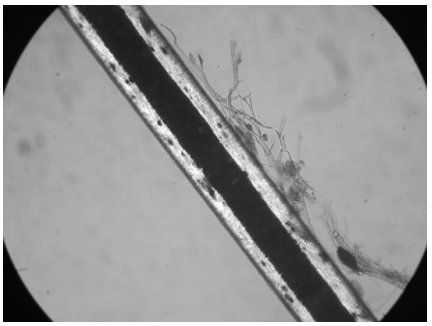

Figure 9. Fungal hyphae growing onto inoculated hairs on SDA (wet prep in LPCB, 200X)

Alternaria spp. are dematiaceous fungi with a worldwide distribution in the environment (usually in the soil and vegetal matter), most frequently isolated from mammals. They are also recognized as usual laboratory and indoors contaminants as well as the most common respiratory allergens in human (Dedola et al., 2010; Dye et al., 2009; Meason-Smith et al., 2015). A recent study about the skin fungal microbiota in dogs using molecular techniques (next-generation sequencing) demonstrated the prevalent colonization with *Alternaria* and *Cladosporium* of canine skin across all body sites and health statuses (Meason-Smith et al.,2015). The study also revealed that cutaneous mycobiota could be influenced by environmental exposure, cohabitation with other pets and skin health status. Similar fungal skin colonization were also identified in cat (Meason-Smith et al.,2017).

The isolation of a common fungal inhabitant represent a real diagnostic challenge. Dogs seem to be less susceptible to infections caused by *Alternaria spp.* than cats and human.

Clinical forms of phaeohyphomycoses range from superficial colonization to subcutaneous and systemic dissemination with encephalitis, osteomyelitis or nephritis, being not absolutely necessary a concurent immunosuppressive or debilitating disease like in human (Seyedmousavi et al., 2013; Herráez et al., 2001; Lloret et al., 2013; Taboada, 2016).

Dedola et al. (2010) isolated *Alternaria infectoria* from multiple, purulent, crusting and ulcerative skin lesions, but also from onychorrhexis and plaque-like lesions on the tongue in a dog under immunosuppressive therapy.

In human, superficial and cutaneous forms of phaeohyphomycosis are believed to result from colonization of the epidermis and hair follicles, while subcutaneous localization follows the traumatic implantation of fungus or results from the progression of superficial infections into the dermis/subcutis with induction of pyogranulomatous inflammation (Herráez et al., 2001).

In this context, the diagnosis of cutaneous phaeohyphomycosis was quite difficult due to the opportunistic nature of isolated agent. The clues for definitive diagnosis were represented by: clinical features (focal alopecia, diffuse hyperpigmentation, slow progression), the identification of pigmented fungal elements in trichoscopy and cytology, fungal cultures, lack of response to antibiotherapy and resolution of skin lesions after oral administration of itraconazole (10mg/kg) for 2 months (figure 10). It is also important to mention that no reccurence has been recorded 14 months after completion of therapy.

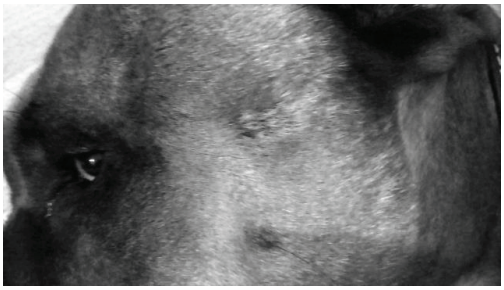

Figure 10. Clinical resolution after 2 months of itraconazole therapy

CONCLUSIONS

Phaeohyphomycoses are uncommon opportunistic infections in dog, usually occurring in pre-existing skin lesions, not always associated with an immunocompromised status.

Diagnosis of infections caused by *Alternaria spp.* represent a serious challenge since these fungi are recognized as normal residents of skin mycobiota in dog and common environmental contaminants as well.

In our case, color dilution alopecia reported in *Cane corso* breed was considered as a promoting factor in fungal infection which most likely arouse from traumatic implantation into defective hairs as well as from contamination of ruptured follicular cysts.

The complete resolution of lesions under itraconazole therapy and lack of reccurences were decisive for diagnosis.

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