

# A CASE REPORT ON DIAGNOSIS AND ISOLATION OF *APOPHYSOMYCES* FROM A NONRESPONSIVE CANINE DERMATITIS

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A 3 year old female Labrador weighing 38 kgs with a nonresponsive dermatitis was presented at the Khalsa College Veterinary Hospital, Amritsar. The physical examination revealed that the patient suffering with intense pruritus and patches of alopecia on the skin; but active and presented a general demeanor. Treatment history indicated ineffective cycle of combination of oral antifungal and lotion. *Apophysomyces* species was isolated on Sabouraud dextrose agar and Brain Heart Infusion Agar. The fungal pathogen was found to be sensitive to Terbinafine and Posaconazole.

**Keywords:** Nonresponsive dermatitis, *Apophysomyces*, Antifungal susceptibility test, Emerging mycoses.

*Apophysomyces elegans* species complex is classified under the class *Saksenaaceae* and belongs to the order *Mucorales*. Approximately, 60% of global mucormycosis cases in humans have been reported from India (Pamidimukkala *et al.*, 2020; Prakash and Chakrabarti, 2019). Among the *Mucorales*, *Rhizopus*, *Mucor*, *Absidia*, *Rhizomucor*, *Mortierella*, *Saksenaea* species are commonly isolated as invasive pathogens in both man and animal.

The clinical spectrum of infections includes cutaneous and subcutaneous infections, rhino-orbital infection, osteomyelitis, renal mucormycosis and disseminated; Petrikkos *et al.*, 2021; Gnat *et al.*, 2021). However, the diagnosis is difficult as the species has significant geographic variations and the clinical manifestations and subsequent mortality depend on the mode of acquisition and the host immune status (Ingram *et al.* 2014). In Veterinary science, diagnosis is rare due mainly to lack of awareness among the practitioners. Reports of mucormycosis in animals such as ruminants, However, cases of mucormycosis are rarely described in dogs and cats with the only report being of enteritis and or systemic infections caused by the species *Mucor* and

*Saksenaea vasiformis* (Mosbah *et al.*, 2015). The purpose of this case report is to provide information on the isolation of *Apophysomyces* from a clinical sample in a dog and its subsequent treatment regimen.

## Case history and Observations

A 3-year-old female Labrador retriever was presented with dermatitis with intense pruritus and patches of alopecia on the back and sides of the body. On physical examination, rectal temperature was 104.7°C, mucous membrane was congested with swollen lymph nodes, and on the back and sides of the body. dehydration. The dog has been previously treated with antifungal lotion with no response.

## Laboratory Diagnosis

Skin scraping sample was received in the mycology laboratory. Direct microscopic examination with potassium hydroxide (KOH) and Lactophenol cotton blue (LCB) stain was performed. The skin scraping sample was cultured in duplicate on brain heart infusion agar and Sabouraud dextrose agar (SDA) with chloramphenicol, and incubated at 25°C and 40°C.

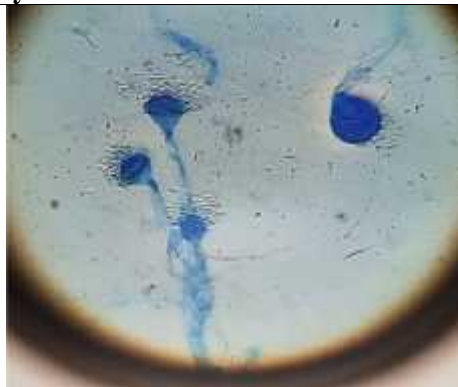
## Antifungal susceptibility test

Fungal isolate was subjected to Antifungal susceptibility test (AFST) using gradient diffusion strip technique according to CLSI standard guideline criteria for filamentous fungus (CLSI, 2010) against

common antifungals such as Amphotericin B (AMB), Itraconazole (ITC), Posaconazole (PSC), Terbinafine (TRB), Ketoconazole (KT).



**Fig. 1 Colony of fungus rapidly growing to white and fluffy (arrow). On subculture to SDA, the fungus showed erect aerial mycelia growth pushing the upperlid of the petri plates within 23 days o incubation**



**Fig. 2 The apophyses are dark, with typical champagne glass shaped (arrow). Pyriform sporangia were borne terminally at the tips of the sporangiophores, distinctly apophyseal and columellar. Spores shows mostly oblong, sub-globose, and subhyaline, light brown, smooth walled (400X).**

## Results and Discussion

Fungal isolate was provisionally identified based on gross colony morphology, sporulation patterns, and microscopic examination observed on lactophenol cotton blue mount. The colonies of *A. elegans* on Sabouraud dextrose agar were fast growing, covering the entire surface of the petri plates in 7 days at 25°C. Colony was floccose, white and became creamy white to yellow upon longer incubation. The fungus showed erect aerial mycelia growth pushing the upper lid of the petri plates within 2-3 days of

incubation (Fig.-1). The isolate was found to sporulate on SDA comparable to the species isolated and reported by Samaddar and coworkers (2019). Sporangiohores generally developed singly, unbranched, and slightly tapered towards the apex, arising at the ends of stolon-like hyphae but not always opposite to groups of underlying rhizoids. Pyriform-sporangia are located terminally at the tips of the sporangiophores, distinctly apophyseal and columellar covered by a thin-walled, transparent, and smooth Zygosporangia were not observed. When compared with two authentic

isolates of *Apophysomyces* the isolate in this report was found to be identical.

Treatment of mucormycosis in humans involves a combination of surgical debridement of involved tissues and antifungal therapy (Spellberg, *et al.*, 2009). In humans, intravenous (IV) amphotericin B (lipid formulation) is the drug of choice for initial therapy (Cornely *et al.*, 2019) followed by Posaconazole or Isavuconazole is used as step-down therapy for responsive patients to amphotericin B and also as salvage therapy for non-responsive amphotericin B treatment depending on the medical condition and treatment history of the patient with the antifungal Amphotericin B (Chander *et al.*, 2021). The patient was already under treatment with antifungal ketoconazole @ 200 mg once daily in combination with Vetalexin @ 600mg once daily for 5 days each with slow progress. AFST in the laboratory indicated high MIC value (>12) to Terbinafine and Posaconazole, MIC value of 8 to ketoconazole and 4 for amphotericin B, in contrast to human isolates with high amphotericin B susceptibility. The antifungal drug was changed to Terbitotal (Terbinafine) @ 500mg, once daily after meal for 7 days. Follow up after one week reported no untowards condition, and the patient ~~was also~~ showed improvement.

Our findings report *Apophysomyces* cutaneous infection in a dog for the first time in India. Our finding indicated that even pet animals such as dogs are also prone to *Apophysomyces* infection, under chronic stress condition, skin trauma, direct contact with the pathogen, and/or immunosuppressive drug therapy. Due to its wide geographical distribution, it was recently reported that 4.5% of all Mucorales isolated are from soil spanning Pan-India, and that 29.2% cases are healthcare related cutaneous mucormycosis with majority of cutaneous involvement directly related to inoculation from contaminated wound dressings, bandages, medical devices, tongue depressors, catheters of procedures such as intramuscular injection, wound packing or operation theatre room

contamination (Pamidimukkula *et al.*, 2020). Limited availability of contemporary treatments, lack of knowledge and vague clinical presentation delay its diagnosis in context to cutaneous mycoses. Hence, our study recommended mucormycoses to be included as one of the differential diagnosis in nonresponsive cutaneous infection/dermatitis.

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