# PRIMARY CUTANEOUS ASPERGILLOSIS CAUSED BY *ASPERGILLUS FLAVUS* IN CAT - CASE REPORT

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***Abstract***

*Aspergillosis is recognized as an opportunistic infection in human and animals often occuring in association with other chronic diseases (immunodeficiency, diabetes mellitus, long-term antibiotherapy, chemotherapy, surgery, etc.). Over 95% of human aspergillosis are produced by A.fumigatus, A.flavus and A.niger commonly found in the environment. Primary cutaneous aspergillosis with A.flavus has been rarely reported in human, mainly in immunocompromised and diabetic patients after surgery.*
*Aspergillus infections have been more less reported in cat than dog, with two clinical forms: nasal and systemic. Early detection and treatment are important factors in infection control.*
*This paper illustrated a primary cutaneous aspergillosis of the tail in a spayed mixed-breed female cat, 10 years old, with no general symptoms. The samples prelevated from tail lesions were submitted to bacteriological, mycological and cytological investigation. The results demonstrated the infection with a strain of A.flavus. The case is still under investigation and represent a real therapeutic challenge for us considering the chronic infection and the age of patient.*

***Key words:*** *aspergillosis, cat, tail.*

## INTRODUCTION

Aspergillosis is recognized as an opportunistic infection both in human and animals, often occuring subsequently to other conditions (diabetes mellitus, immunosuppression, trauma/surgery, long-term antibio- cortico- or chemotherapy, etc.). In human, the most infections are caused by *A.fumigatus, A.flavus* and *A.niger*, species with a worldwide distribution in the environment (soil, plants, air, water, food, etc.). The primary mode of fungal transmission is by inhalation of *Aspergillus* conidia.
*A.flavus* is the common cause of human synusitis and superficial dermatitis and the second responsible agent for invasive aspergillosis after *A.fumigatus,* predominantely in arid dry regions: Middle East, Africa, Southeast Asia (Krishnan, 2009). Human cutaneous aspergillosis was classified as primary (following direct inoculation at sites of skin injury) and

secondary infection (by hematogenous spread from pulmonary sites or by contiguous extension from neighbouring sinus) - Hedayati, 2007.
Aspergillosis has been rarely reported in pets (more frequent in dog than cat) developping two clinical forms: nasal-sino-orbital infection with *A.fumigatus* and systemic infection with *A.terreus* (Kano, 2008; De Lorenzi, 2006; Barachetti, 2009).
Due to a high invazivity and allergic, immunosuppressive, toxic, teratogen and carcinogen potential of *A.flavus*, early fungal detection and treatment are very important to clear *Apergillus* infection. According to the latest data, *A.flavus* seems to be more virulent and more resistant to antifungal drugs than most other *Aspergillus* species (Hedayati, 2007).
This paper reported a case of primary cutaneous aspergillosis in a cat with chronic evolution and sarcoma transformation which in our opinion has been promoted by environmental conditions and repeated trauma of the tail.

## MATERIALS AND METHODS

**Patient history and clinical findings:** A 10 years old spayed female cat from common breed was dermatologically examined at Faculty of Veterinary Medicine of Bucharest. Initially, we have received a tail fragment after surgery for microbiological evaluation, but subsequently to laboratory tests we decided the clinical examination of the cat to get a complete view of the case. So, patient history revealed repeated trauma of the tail consisting in an initial fracture resolved by surgery which was followed by a second intervention one year later due to persistent self-mutilation to the tail. Routine biochemical and hematological analysis demonstrated a hepatic insufficiency (high values of ALT and bilirubin) and a moderate polycitemia with leucopenia. Another key-element from patient history was constant exposure of the cat to an inadequate damp habitat.
Tail lesions were characterized by hair-loss, diffuse edema and induration, superficial brown crusting with the expression of petechiae and pus after crust removing (fig. 1 a,b,c ). Moreover, a fatty-sarcomatous aspect was detected on the cut-section. No general symptoms have been associated with these cutaneous lesions.
**Paraclinical evaluation** included cytological, bacteriological and fungal examination. Cytology was performed on the smears obtained from scraping and aspiration of superficial and cut-section lesions which were stained by May-Grünwald Giemsa and Gram method. For bacteriological investigation, cutaneous samples were inoculated into brain-heart infusion broth (BHI

broth) and blood agar with incubation at 37˚C. Fungal cultures were prepared in Sabouraud dextrose broth, Sabouraud dextrose agar CAF CEX (with chloramphenicol and cycloheximide) and Czapek Dox agar which were incubated at 27˚C and 37˚C. The identification of *A. flavus* was made based on gross colony morphology and microscopic features (in lactophenol cotton blue-stained wet mounts).

## RESULTS AND DISCUSSIONS

Clinical lesions were reproduced in figure 1 a,b,c.

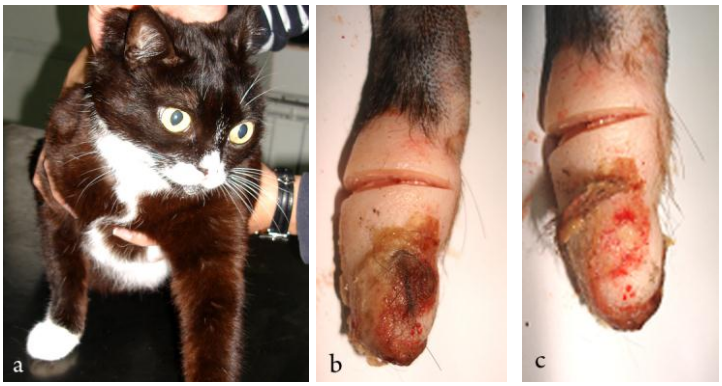


Figure 1 a, b, c. Tail lesions

Routine cytology supplied the first clue for diagnosis. In Gram stained smears, few septate hyphae intricated by numerous cellular remnants were observed (figure 2).

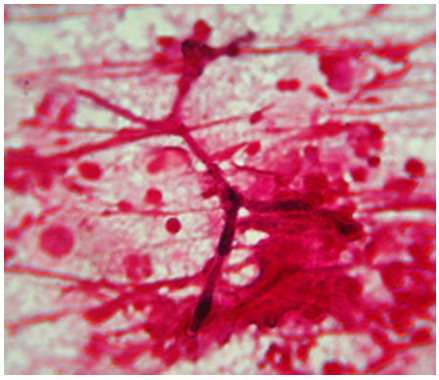

Figure 2. Branched septate hyphae in Gram stained smear (X100)

In May-Grünwald Giemsa staining, smears obtained from superficial scrapings revealed an inflammatory infiltrate with predominant degenerate neutrophils (nuclear pyknosis and karyorrhexis), red blood cells and fibrin filaments. Surprisingly, the aspirates from fatty cut-section lesions evidenced a moderate relatively uniform population of hystiocyte-like cells entrapped into an oxyphil matrix lacking other inflammatory cells (figure 3 a,b).

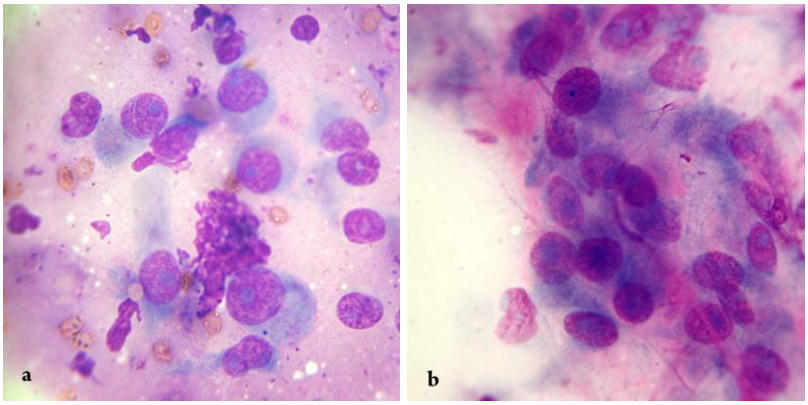


Figure 3 a,b. Hystiocyte-like cells entrapped into an oxyphil matrix from the aspirate of tail lesion (MGG stain, X100)

No granulomatous reaction typically found in fungal infection has been detected. Sarcomatous transformation in the deep tissue of the tail could be the result of combined action of repeated trauma and slow releasing of mycotoxins by *Aspergillus* isolated from these lesions.

Routine bacteriology was negative since no bacterial colony was identified in inoculated broth and agar. Instead of bacterial growth, fungal colonies have developped both in BHI broth (at 10 days of incubation) and blood agar (at 3 days of incubation) under 37˚C (figure 4 a,b).

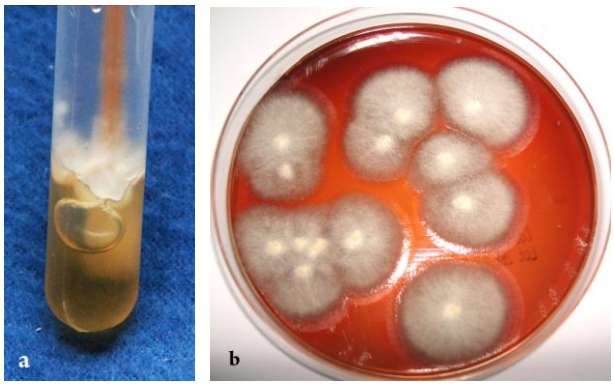


Figure 4 a,b. Cultures in BHI broth (10 days, 37˚C) and blood agar (3 days, 37˚C)

Fungal cultures proved the most relevant for diagnosis by isolation of *Aspergillus flavus* in pure culture. Typical flat powdery colonies ranging in colour from yellow-greenish to olive green-brown on averse side and cream to gold on reverse with radial grooves were observed in Sabouraud dextrose agar CAF CEX and Czapek Dox agar both at 27˚C and 37˚C at 10 days of incubation (figure 5 a,b).

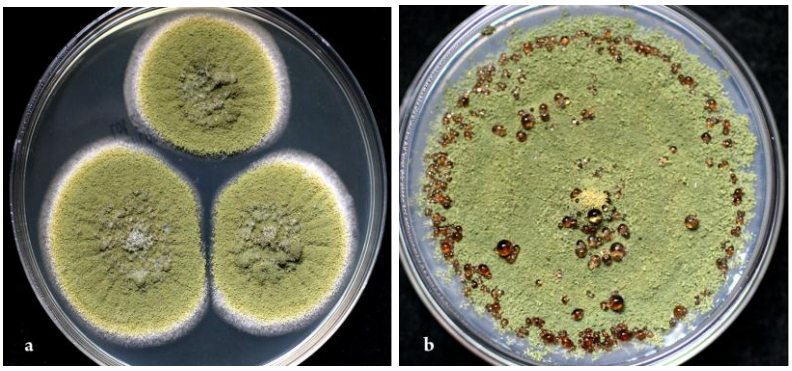


Figure 5 a,b. Colonies of *A.flavus* on SDA CAF CEX and Czapek Dox agar (10 days, 27˚C)

Sclerotia production was also observed in fungal colonies on Czapek Dox agar, at 27˚C and 37˚C at 10 days of incubation (figure 6 a,b).

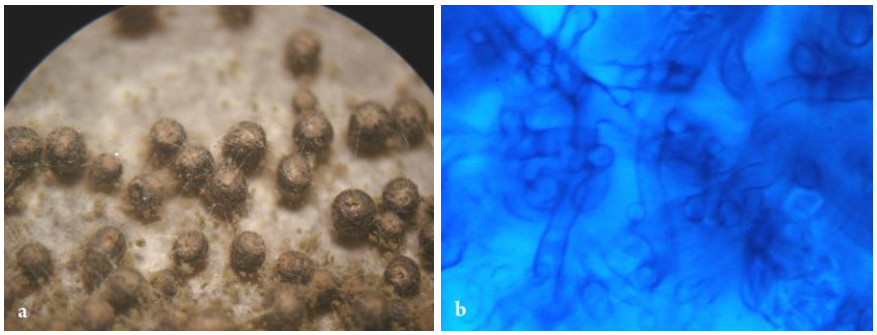


Figure 6 a,b. Macroscopic and microscopic features of sclerotia on Czapek Dox agar (10 days, 37˚C)

These gross findings of fungal cultures has been correlated with the typical microscopic features of *A.flavus* (figure 7 a,b).

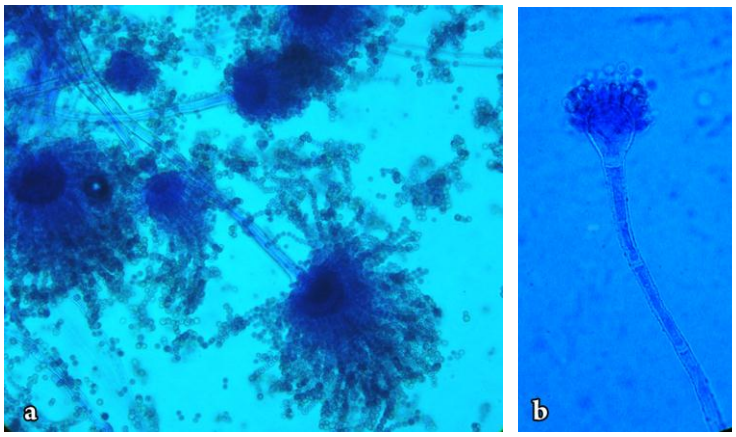


Figure 7 a,b. Conidiophores of *A.flavus*

*A.flavus* is known as a relative fast growing thermotolerant fungus able to grow at temperatures from 12 to 48˚C (Hedayati, 2007). Moreover, the growth of the fungus in the presence of cycloheximide indicated the isolation of a pathogenic strain of *A.flavus* from the tail lesions.
Sclerotia production is considered of key importance for identification of an *A.flavus* strain (Krishnan, 2009) and may be also a reliable marker of aflatoxins production (Leema, 2010; Hedayati, 2007). According to Leema's opinion (2010) the production of aflatoxins by a *A.flavus* may contribute to the severity of clinical lesions in human keratitis being

necessary the suppression or neutralization of deleterious effects of aflatoxins for a good response to usual antifungal therapy.The same author has found that aflatoxin production occured more frequently in isolates of *A.flavus* from patients with keratitis compared to *A.flavus* isolated from environment (Leema, 2010). Surprisingly, other studies on human aspergillosis demonstrated a reduced genetic diversity amongst isolates of *A.flavus* in comparison with *A.fumigatus* though the strains of *A.flavus* group (with 9 species and 2 varieties) are highly polymorphic in nature (Hedayati, 2007).

In our case, a strain of *A.flavus* was incriminated in a primary cutaneous infection in cat. The chronic infection (about 1 year) most likely was due to the inoculation of fungal spores into deep tissues of the tail secondarily to repeated trauma (surgery and persistent self-mutilation) and exposure to dampness. The isolated strain of *A.flavus* may be also toxigen inducing a local sarcomatous reaction, but not clasical granulomatous response (in cytology) with no general symptoms excepting a subclinical hepatic inssuficiency (in routine biochemistry).

The case is still under investigation and represent a real therapeutic challenge considering the chronic infection possibly combined with chronic toxicity beside the advanced age of patient.

## CONCLUSIONS

In this paper, we reported a case of primary cutaneous aspergillosis in cat with an atypical localisation to the tail.

Detailed history, clinical and paraclinical investigations helped us in diagnosis. Repeated trauma of the tail by fracture, surgery and self-mutilation beside a persistent exposure of the animal to dampness were the most significant data from patient history. Routine bacteriology was negative, while the mycological examination (morphological and cultural evaluation) was definitive for diagnosis, confirming the infection with an *A.flavus* strain.

Moreover, sclerotia production on Czapek Dox agar beside a particular tissue response consisting in sarcomatous, but no granulomatous reaction are indicative for the toxic potential of the isolated strain of *A.flavus*. A role of aspergillar toxins from cutaneous site in subclinical hepatic insufficiency detected in this case cannot be excluded.

## REFERENCES

Barachetti et al., 2009. Bilateral orbital and nasal aspergillosis in a cat (case report). Veterinary Ophtalmology, 12(3), 176-182.

De Lorenzi D., Bonfanti U., Masserdotti C., Caldin M., Furlanello T., 2006. Diagnosis of canine nasal aspergillosis by cytological examination: a comparison of four different collection techniques. Journal of Small Animal Practice, 47, 316-319.

Hedayati M.T., Pasqualotto A.C., Warn P.A., Bowyer P., Denning D.W., 2007. Aspergillus flavus: human pathogen, allergen and mycotoxin producer. Microbiology, 153, 1677-1692.

Kano R., Itamoto K., Okuda M., Inokuma H., Hasegawa A., Balajee S.A ., 2008. Isolation of *Aspergillus udagawae* from a fatal case of feline orbital aspergillosis. Mycoses, 51, 360-361.

Krishnan S., Manavathu E.K., Chandrasekar P.H., 2009. Aspergillus flavus: an emerging non-fumigatus Aspergillus species of significance (Review article). Blackwell Verlag GmbH, 1-17.

Leema G., Kaliamurthy J., Geraldine P., Thomas P.A., 2010. Keratitis due to *Aspergillus flavus*: Clinical profile, molecular identification of fungal strains and detection of aflatoxin production, 16, 843-854.