DERMATITIS BY *Aspergillus fumigatus* IN *Python molurus bivitattus*

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Received: 01/04/2024
Accepted: 06/06/2024

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ABSTRACT

Snakes in captivity are more prone to skin diseases, often stemming from fungal infections due to suboptimal care practices. Environmental factors such as temperature and humidity play a role in health and immune system functionality of reptiles. Typically, skin infections occur in immunocompromised animals exposed to high environmental humidity. The objective of this study is to describe a case of a *Python molurus bivitattus* in captivity exhibiting mild multifocal dermatitis on the ventral scales from the middle to the posterior third of its body. A definitive diagnosis was achieved through histopathological assessment and molecular analysis, confirming discrete multifocal fungal dermatitis caused by *Aspergillus fumigatus*. The treatment was obtained by topical ointment of ketoconazole for 21 days and total recovery was observed as the animal exhibited normal ecdysis.

Keywords: Aspergillosis. Fungal dermatitis. Histopathology. Burmese Python.

INTRODUCTION

Snakes, as members of the Class *Reptilia*, are ectothermic organisms whose metabolism relies on environmental temperature, impacting physiology, coherent, environmental factors such as temperature, humidity, and light play crucial roles in their health and immune system functionality (VON CZEKUS et al., 2021). Inadequate environmental conditions, including extremes of temperature and humidity, as well as inadequate lighting, under human care, can disrupt homeostasis, leading to increased susceptibility to diseases, tissue fragility, weight loss, and immunosuppression, rendering snakes vulnerable to pathogens and opportunistic infections (ACCO et al., 1999; FREIRE et al., 2019; JACOBSON, 1977; VON CZEKUS et al., 2021). Mycotic dermatitis primarily affects immunosuppressed snakes exposed to high environmental humidity. Effective diagnostic and epidemiological measures are essential, particularly as fungal diseases have been implicated in the decline of wild snake populations in the United States (ALLENDER et al., 2011; CLARK et al., 2011; FRANKLINOS et al., 2017; SUTHERLAND et al., 2014).

Dermatitis in snakes can be caused by various pathogens including *Fusarium* spp., *Candida* sp., *Aspergillus* sp., *Trichophyton mentagrophytes*, *Mucor* sp, *Blastomyces* sp., *Histoplasma* sp., *Coccidioides* sp., *Paracoccidioides* sp., *Microsporum* sp., *Trichophyton* sp., and *Chrysosporium ophiodiicola* (FREIRE et al., 2019; LORCH et al., 2016). Infection typically begins with the degradation of the stratum corneum and penetration through the epidermis, often facilitated by skin abrasions (LORCH et al., 2016). *Aspergillus fumigatus* is the species most frequently associated with tissue infection and typically associated with immunosuppression (VAN DE VEERDONK et al., 2017). Fungal infection affects primarily the epidermis and dermis, although it can also extend to bone and muscle tissue (ALLENDER et al., 2011). Diagnosis relies on a combination of fungal cultures, histopathological examination of the lesion, and visualization of fungal structures (JACOBSON, 1980; KOSTKA et al., 1997).

Bacterial pathogens may be isolated in reptile skin, as asymptomatic and diseased reptiles may harbor and excrete these pathogens (EBANI, 2017). Reptiles excrete *Escherichia coli*,

Klebsiella sp., *Campylobacter* spp, and *Yersinia* spp. in their feces and these pathogens are frequently isolated from clinical healthy and symptomatic individuals (EBANI, 2017).

The objective of this case report is to detail the clinical presentation, diagnostic workup, and successful treatment of mild multifocal fungal dermatitis caused by *Aspergillus fumigatus* in an adult male *Python molurus bivitattus*. The report aims to document the dermatological findings, laboratory procedures, histopathological evaluation, and therapeutic approach, providing a comprehensive overview of the case for veterinary professionals dealing with similar conditions in reptiles.

CASE REPORT

A male adult specimen of *Python molurus bivitattus* maintained under human care was presented at a veterinary hospital for evaluation. Upon physical examination, necrotic and ulcerative areas measuring between 0.5 to 1.5 centimeters were observed on the ventral scales from the middle to posterior third of its body, exhibiting a yellow to dark brown coloration (Figure 1). The animal's vital parameters were within normal ranges, with no abnormalities other than the dermatological condition. Blood samples were collected for hematology and biochemistry analyses, along with a Stuart swab sample for microbiology. Biopsies of the affected areas were taken for histopathological evaluation.

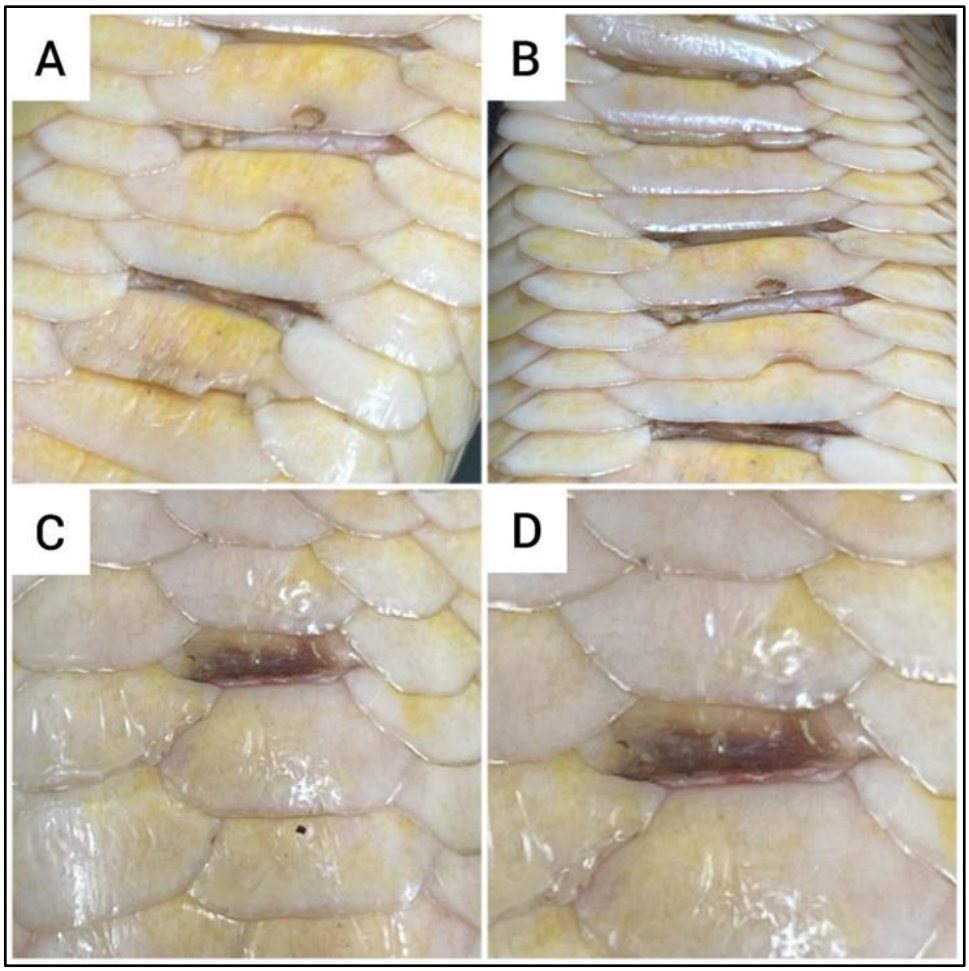

Figure 1 - Clinical evaluation of *Python molurus bivitattus* presenting yellow to dark brown lesions on its ventral scales. A, B - Ventral scales presenting multifocal areas (1.5 cm), yellow to dark brown, humid. C, D - area (0.5 cm), red to brown, humid caudal to spur.

A swab sample was enriched using Brain Heart Infusion (BHI) (Sigma-Aldrich, Brasil) broth. For initial plating, BHI and MacConkey (MC) (Sigma-Aldrich, Brasil) agar were utilized and incubated for 24 hours at 37 ºC. After conducting Gram staining and isolating colonies, biochemical tests were conducted to identify the species and determine genus. For Gram-negative bacteria, plating was performed using the Rugai method, Citrate, Catalase and Oxidase mediums. Following biochemical identification, colonies isolated on MC agar were submitted for identification via MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry). Upon species confirmation, an antibiogram was conducted on Mueller-Hinton (MH) (Sigma-Aldrich, Brasil) agar and incubated at 37 ºC for 18-24 hours.

Fungal samples were enriched in BHI broth, and DNA was extracted for Polymerase Chain Reaction (PCR).

RESULTS AND DISCUSSION

The antibiogram results for *Citrobacter amalonaticus* indicated resistance to Amoxicillin and Clavulanic acid, Ampicillin; Doxycycline; Gentamicin, Nitrofurantoin and Tobramycin. The fungal structure was identified by DNA extraction and PCR with a positive result for *Aspergillus fumigatus* in agarose agar 1.5% (Figure 2).

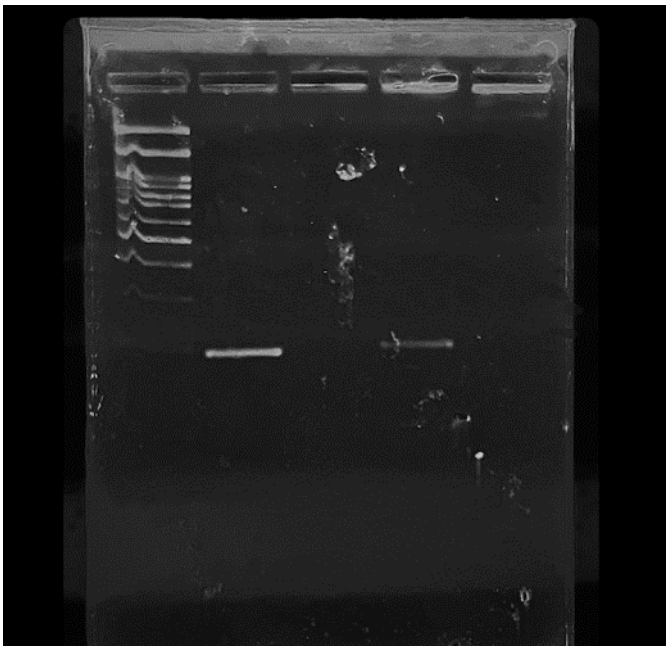

Figure 2 - Gel electrophoresis showing *Aspergillus fumigatus* positive reaction on 1.5% agar and 2.5% ethidium bromide. Lane 1: molecular weight ladder; Lane 2: positive control; Lane 3: negative control (all reagents, except DNA template); Lane 4: sample isolated from *Python molurus bivitattus.*

Histopathological examination revealed a focal area of epidermal erosion characterized by discontinuity of the epithelium. Additionally, multifocal areas contained a discrete quantity of pigmented cells, with focal moderate deposition of amorphous, homogeneous, and discretely basophilic material (myxoid matrix) in the superficial dermis associated with a discrete amount of hyphae. The diagnosis was determined as mild multifocal fungal dermatitis. Based on the histopathological characteristics and the clinical condition of the animal, the indicated

treatment was topical ointment of ketoconazole, applied twice a day for 21 days, was recommended. The specimen recovered well and exhibited normal ecdysis.

Fungal dermatopathies in reptiles, particularly within the suborder *Ophidia*, are increasingly concerning (LICITRA et al., 2019). Main causes linked to these infections include low temperatures, nutritional deficiencies, immunological disorders, and high relative humidity (SCHUMACHER, 2003). While environmental challenges are inherent in the wild, under human care, these factors may indicate inadequate husbandry practices, leading to disruptions in an individual's homeostasis and subsequent infection development (FREIRE et al., 2019). *Aspergillus fumigatus,* a saprotrophic and environmental fungus, is the species most frequently associated with tissue infection (VAN DE VEERDONK et al., 2017). Differential diagnosis for bacterial dermatitis is crucial for tailored therapy, and interpretation is essential to distinguish between pathogenic and commensal microorganisms of the skin microbiota (HARKEWICZ, 2002).

Gram-negative bacteria are frequently isolated in reptile dermatitis, often opportunistically (BRANCH et al., 1998). Some mentioned genera include *Proteus* sp., *Pseudomonas* sp., *Citrobacter* sp., *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp., and *Morganella* sp. (BRANCH et al., 1998; FERREIRA et al., 2012; FERREIRA et al., 2014; MARTINS et al., 2017). The prevalence of multidrug-resistant strains is rising due to environmental contamination and the indiscriminate use of antimicrobials, facilitating the transfer of resistance genes. This phenomenon is associated with mutations and the expression of resistance genes (DOLEJSKA; LITERAK, 2019; FUKUDA et al., 2016).

In this study, *Citrobacter amalonaticus* was isolated from the biopsy material taken from the ventral scale of *Python molurus bivitattus*. The antibiogram results showed resistance to 6 out of 13 antimicrobials (Amoxicillin and Clavulanic acid, Ampicillin, Doxycycline, Gentamicin, Nitrofurantoin, and Tobramycin). However, bacteria were not identified in the histopathological evaluation, suggesting that it might be an opportunistic agent unrelated to the dermatitis.

The genus *Aspergillus* comprises emerging and pathogenic agents that affect both free-ranging reptiles and those in human care (VAN DE VEERDONK et al., 2017). Diagnosis typically involves histopathology and fungal culture, essential for assessing pathogenicity. Environmental conditions play a crucial role in altering the microbiota, potentially influencing susceptibility to opportunistic agents and snake fungal disease (SFD) (ALLENDER et al., 2018). The mortality and morbidity associated with SFD underscore the importance of diagnosis and epidemiological control measures to understand their ability to cause disease and confer resistance.

In this case, *Aspergillus fumigatus* is reported to cause ulcerative dermatitis and necrosis in the ventral scales, consistent with findings in snakes (LORCH et al., 2016). Alterations in skin barriers or imbalances in the metabolic equilibrium of the microbial flora enable colonizing fungal organisms to proliferate, with infections typically associated with immunosuppression (LANGFELDT et al., 2022). Although the isolation of *Aspergillus* spp. from the skin and hair of mammals often represents contamination, the agent can produce opportunistic infections by invading mucosal or cutaneous surfaces in compromised individuals, as observed in this case through histopathological analysis (BARRS; DEAR, 2021).

Treatment of *Aspergillus* nasal infections with topical intranasal administration of antifungals has been reported to be more effective than oral medication (BARRS; DEAR, 2021). In the present case, topical treatment with ketoconazole was effective, eliminating the need for systemic administration of antifungals. This case describes dermatitis caused by *Aspergillus fumigatus*, likely due to inadequate environmental conditions. This agent was probably opportunistic and not the initial or primary cause of the dermatitis. Histopathological visualization of filamentous fungal organisms confirms the involvement of the pathogen but cannot determine the initial cause of the pathology.

CONCLUSION

This case report details the presentation, diagnosis, and treatment of a male adult specimen of *Python molurus bivittatus* with ulcerative dermatitis and necrosis on its ventral scales, caused by *Aspergillus fumigatus.* The lesions were likely exacerbated by inadequate

environmental conditions, leading to the proliferation of opportunistic fungal organisms. Histopathological examination confirmed the presence of fungal hyphae, supporting the diagnosis of mild multifocal fungal dermatitis. Effective treatment with topical ketoconazole without the need for systemic antifungals highlights the potential for localized therapy in similar cases. Additionally, the isolation of multidrug-resistant *Citrobacter amalonaticus* emphasizes the importance of identifying and addressing opportunistic bacterial infections in reptile dermatopathies.

This report underscores the necessity of proper husbandry practices to prevent environmental stressors that can predispose reptiles to opportunistic infections. It also demonstrates the importance of comprehensive diagnostic workups, including histopathology and microbiology, to accurately identify and treat the underlying causes of dermatological conditions in reptiles.

DERMATITE FÚNGICA POR *Aspergillus fumigatus EM Python molurus bivitattus*

RESUMO

Serpentes mantidas em cativeiro são mais predispostas ao desenvolvimento de doenças de pele, comumente associadas a infecções fúngicas devido ao manejo ambiental inadequado. Fatores ambientais como temperatura e umidade são fundamentais para a manutenção da saúde e do sistema imune dos répteis. Infecções cutâneas tendem a ocorrer em animais expostos a condições de alta umidade ambiental. Este relato de caso tem o objetivo de relatar uma infecção fúngica por *Aspergillus fumigatus* em *Python molurus bivitattus,* exibindo leve a moderada dermatite multifocal nas escamas ventrais do terço médio a posterior do corpo. O diagnóstico definitivo se deu a partir de avaliação histopatológica e análise molecular, confirmando dermatite micótica. O tratamento foi realizado através de aplicação tópica de creme antifúngico à base de cetoconazol, atingindo os resultados esperados e ecdise adequada.

Palavras-chave: Aspergilose. Dermatite fúngica. Histopatologia. Píton birmanesa.

DERMATITIS FÚNGICA POR *Aspergillus fumigatus* EN *Python molurus bivittatus*

RESUMEN

Las serpientes mantenidas en cautiverio son más propensas a desarrollar enfermedades de la piel, comúnmente asociadas a infecciones fúngicas debido a un manejo ambiental inadecuado. Factores ambientales como la temperatura y la humedad son de gran importancia para la salud y el sistema inmunológico de los reptiles. Las infecciones cutáneas tienden a ocurrir en animales expuestos a condiciones de alta humedad ambiental. Este reporte de caso tiene como objetivo describir una infección fúngica por *Aspergillus fumigatus* en *Python molurus bivittatus*, exhibiendo una dermatitis multifocal leve a moderada en las escamas ventrales del tercio medio a posterior del cuerpo. El diagnóstico definitivo se realizó mediante evaluación histopatológica y análisis molecular, confirmando la dermatitis micótica. El tratamiento se llevó a cabo mediante la aplicación tópica de una crema antifúngica a base de ketoconazol, logrando los resultados esperados y una muda adecuada.

Palabras clave: Aspergilosis. Dermatitis por hongos. Histopatología. Pitón birmana.

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