

ANATOMOPATHOLOGICAL ASPECTS OF A SUBCUTANEOUS FUNGAL GRANULOMA IN A BLUE-AND-YELLOW MACAW

(Aspectos anatomo-patológicos de um granuloma fúngico em uma arara-canindé)

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RESUMO

A ordem Psittaciformes é composta por aves encontradas principalmente na América do Sul, incluindo a arara-canindé (*Ara ararauna*). Em vida livre e cativeiro, psitacídeos são altamente suscetíveis a infecções, sobretudo fúngicas. O objetivo deste artigo é descrever um granuloma fúngico em uma arara-canindé, fêmea, 22 anos, proveniente de um mantenedouro de fauna, que foi atendida devido a um aumento de volume em região inguinal direita, com tempo de evolução indefinido. Após citologia sugestiva de xantoma, a massa foi excisada e encaminhada para avaliação anatomo-patológica. Ao corte, evidenciou-se um conteúdo superficial ressecado, sendo grande parte recoberta por uma parede firme e espessa. Histologicamente, o subcutâneo estava substituído por um tecido esclerosado com aglomerados de heterófilos, macrófagos epitelioides e células gigantes multinucleadas. O conteúdo superficial era constituído de um material caseoso repleto de estruturas filamentosas levemente basofílicas, melhor evidenciadas através da reação com o ácido periódico de Schiff e da impregnação pela prata segundo Grocott, demonstrando parede celular delicada, septação evidente e ramificação dicotômica. Tais achados permitiram definir o diagnóstico como um granuloma heterofilico roto de etiologia fúngica, muito provavelmente por *Aspergillus* sp. Manejo deficiente, superpopulação e alta contaminação ambiental estão associados à imunossupressão e desencadeamento de micoes oportunistas.

Palavras-chave: Aspergilose, *Aspergillus* sp., micoe, psitacídeos, Brasil.

ABSTRACT

The order Psittaciformes comprises primarily Neotropical birds, including the blue-and-yellow macaw (*Ara ararauna*). Both in their natural habitat and under captive conditions, psittacines exhibit a high susceptibility to infectious diseases, particularly those of fungal etiology. This article aims to describe a case of fungal granuloma in a 22-year-old female blue-and-yellow macaw housed in a wildlife shelter. The bird was admitted for evaluation due to a swelling in the right inguinal region with an undetermined duration of progression. Following cytological findings suggestive of xanthoma, the mass was surgically excised and submitted for histopathological analysis. Gross examination revealed a superficially desiccated core, largely encased by a firm, thickened wall. Histopathological evaluation demonstrated subcutaneous tissue effacement by sclerotic connective tissue, interspersed with aggregates of heterophils, epithelioid macrophages, and multinucleated giant cells. The superficial material consisted of caseous necrotic debris containing numerous faintly basophilic, filamentous structures. These were most prominently visualized using periodic acid-Schiff (PAS) staining and Grocott's methenamine silver (GMS) impregnation, which highlighted a delicate cell wall, distinct septations, and dichotomous branching—morphological features consistent with fungal hyphae. These findings supported a definitive diagnosis of a ruptured heterophilic granuloma with fungal etiology, most likely caused by *Aspergillus* spp. Suboptimal husbandry conditions, overcrowding, and elevated environmental fungal load are known risk factors for immunosuppression and the subsequent development of opportunistic mycoses.

Keywords: Aspergillosis, *Aspergillus* sp., ringworm, psittacines, Brazil.

INTRODUCTION

Fungi represent significant pathogenic agents in wild birds, manifesting in three primary clinical forms: invasive mycoses (direct tissue colonization), allergic disorders

(hypersensitivity reactions to fungal antigens), and mycotoxicoses (ALBANO, 2009). Among avian fungal infections, aspergillosis and candidiasis are the most clinically relevant (FRIEND e FRANSON, 1999). The onset of these diseases is frequently associated with predisposing factors such as compromised host immunity, chronic stress, and debilitation. (ABUNDIS-SANTAMARIA, 2000).

Within this context, psittacine birds (family Psittacidae) serve as significant fungal reservoirs, with some species contributing to the epidemiology of zoonotic mycotic diseases (KURTZMAN *et al.*, 2011; WILLE *et al.*, 2013; YAMAMOTO *et al.*, 2013). Few studies in the current literature systematically document or characterize the epidemiology of mycotic infections in psittacine species. (KAPLAN *et al.*, 1975; BENGOLA *et al.*, 1994; HOPPES *et al.*, 2000; FRAGA *et al.*, 2011; DONNELLY *et al.*, 2019). Although current research has identified potentially pathogenic fungal species in psittacine excreta and food sources, comprehensive studies on infection rates and transmission dynamics in these birds remain scarce (LIMA, 2005; SIMI *et al.*, 2019).

Specific reports of mycoses in *Ara ararauna* remain limited. Bengoa *et al.* (1994) described a case of necrotic beak lesion involving destruction of the stratum corneum in a 10-year-old specimen, with *Penicillium cyclopium* identified as the causative agent. More recently, Donnelly *et al.* (2019) documented an infection by *Candida glabrata* in a blue-and-yellow macaw presenting with diarrhea, regurgitation, and melena. Notably, while these yeasts constitute normal avian microbiota, the authors emphasize they may become opportunistic pathogens following gastrointestinal dysbiosis. (DONNELLY *et al.*, 2019).

Most recently, Galosi *et al.* (2022) conducted a comprehensive pathological investigation of two *Ara ararauna* specimens (one male, one female). The male specimen exhibited necrotizing granulomatous proventriculitis and hepatitis, accompanied by severe splenic lymphoid depletion - pathological findings consistent with profound immunosuppression. Molecular diagnostics confirmed *Alternaria alternata* as the etiological agent. The female specimen presented with diffuse hepatocellular degeneration and chronic hepatic inflammation, along with pulmonary and air sac pyogranulomas containing fungal hyphae, subsequently identified as *Fusicladium* spp. through molecular characterization (GALOSI *et al.*, 2022).

Therefore, this study aims to document a case of subcutaneous fungal granuloma in a blue-and-yellow macaw (*Ara ararauna*), with particular emphasis on its histopathological characteristics. This report is clinically significant as it: (1) describes an unusual manifestation of fungal infection in psittacines, distinct from classical presentations; and (2) contributes to the limited literature on atypical mycotic lesions in this species. The findings may enhance clinical awareness of diverse fungal disease presentations in captive psittacines and inform diagnostic approaches.

PATIENT CARE

A 22-year-old female blue-and-yellow macaw (*Ara ararauna*) under care at a wildlife shelter was referred to an avian veterinary clinic (CRMV-RS-16641-PJ) for evaluation of a right inguinal mass with undetermined progression time. Physical examination revealed a well-demarcated, crateriform subcutaneous mass measuring $6 \times 5 \times 3$ cm, characterized with slightly

elevated edges, covered by featherless, with superficially ulcerated skin, in a typical crateriform pattern (Fig. 01A e 01B).

A fine-needle aspiration biopsy (FNA) was performed, and the collected material was submitted for cytological evaluation. Microscopic examination revealed macrophages with abundant foamy cytoplasm containing clear, well-defined vacuoles consistent with lipid accumulation, alongside scattered multinucleated giant cells and a sparse population of small lymphocytes. These cytomorphological features supported a preliminary diagnosis of xanthoma.

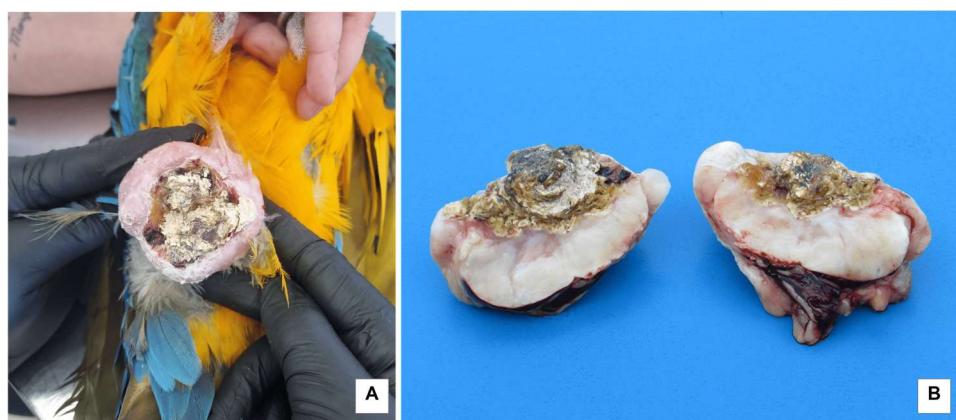


Figure 01: Subcutaneous fungal granuloma in a blue-and-yellow macaw (*Ara ararauna*).

Obs.: A= Gross appearance of the exophytic mass featuring irregular surface contours and a central ulceration containing caseous, dehydrated material; B = Cross-sectional view demonstrating laminated, desiccated content with heterogeneous pigmentation and well-demarcated margins.

To establish a definitive diagnosis, the mass was surgically excised and submitted for histopathological evaluation.

RESULTS AND DISCUSSION

Gross examination of the sectioned specimen revealed a dry, laminated superficial core exhibiting heterogeneous pigmentation (Fig. 01B). The lesion was circumscribed by a firm, thickened fibrous wall, except at the ulcerated surface where this demarcation was disrupted.

Histologically, the normal subcutaneous architecture was entirely effaced by dense sclerotic connective tissue containing numerous inflammatory foci. These foci consisted of aggregates of heterophils interspersed with epithelioid macrophages and multinucleated giant cells, consistent with a heterophilic granuloma pattern. The granulomatous inflammation showed a distinct zonal arrangement, with central clusters of heterophils surrounded by concentric layers of macrophages and fibrous tissue. Scattered lymphoid aggregates were frequently observed throughout the lesion (Figs. 02A, 02B). The superficial regions contained abundant caseous necrotic material harboring numerous faintly basophilic, filamentous fungal hyphae measuring approximately 5 μ m in diameter (Fig. 02C). The fungal hyphae were more clearly visualized using periodic acid-Schiff (PAS) and Grocott's methenamine silver (GMS) stains (Figs. 02D and 02E), which highlighted their delicate cell wall, distinct septations, and dichotomous branching patterns—morphological features consistent with invasive fungal hyphae.

These findings established the diagnosis of a ruptured heterophilic granuloma of fungal etiology. Differential diagnoses included infections by *Aspergillus* spp., *Penicillium* spp., zygomycetes (particularly mucorales such as *Mucor* spp., *Rhizopus* spp., and *Absidia* spp., or entomophthorales including *Conidiobolus* spp. and *Basidiobolus* spp.) and *Pythium insidiosum* (BENGOA *et al.*, 1994; DAHLHAUSEN, 2005; PESAVENTO *et al.*, 2008; GALOSI *et al.*, 2022). Based solely on hyphal morphology (as fungal culture was unavailable in this case), the most probable etiological agent was *Aspergillus* spp. Histopathological examination revealed characteristic fungal hyphae measuring approximately 5 μm in diameter with parallel walls, regular septation, and acute-angle (45°) dichotomous branching - features quite common for *Aspergillus* species. (CASWELL e WILLIAMS, 2016) (Figs. 02D, 02E).

The etiological diagnosis was further supported by the well-documented predominance of aspergillosis as the most frequently reported fungal infection in captive avian species (FRIEND e FRANSON, 1999). This epidemiological prevalence, combined with the characteristic histomorphological findings, strongly reinforced the diagnosis of aspergillosis in this case. Respiratory aspergillosis, which primarily involves the lungs and air sacs, represents the most clinically significant manifestation in avian species (ANDREATTI FILHO, 2006). However, the infection can disseminate to affect virtually any organ system, demonstrating the systemic potential of this mycosis.

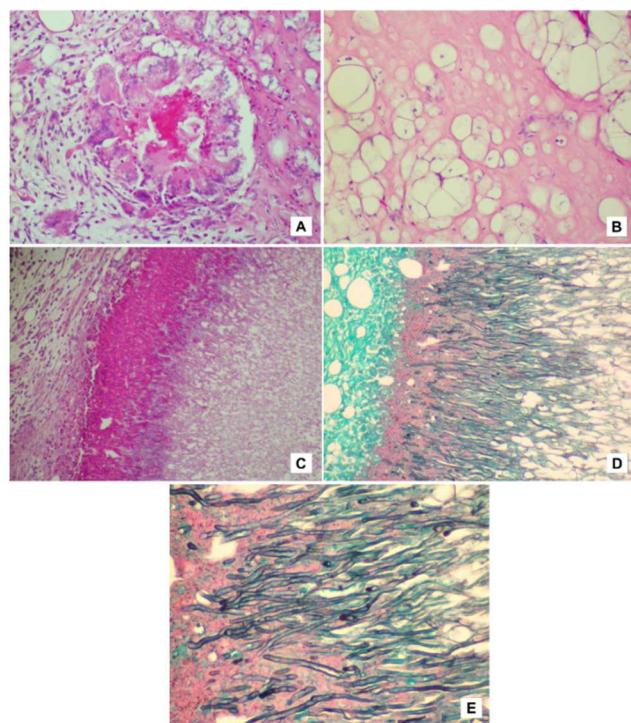


Figure 02: Subcutaneous fungal granuloma in a blue-and-yellow macaw (*Ara ararauna*).

Obs.: A = Granulomatous and heterophilic cellulitis (H&E, 20x); B = Nodular sclerosing cellulitis (H&E, 10x); C = Central caseous necrosis containing faintly basophilic, filamentous fungal hyphae (H&E, 20x); D = Fungal hyphae embedded in necrotic heterophilic debris (H&E, 20x); E = High-power view demonstrating characteristic hyphal morphology: delicate cell wall, sparse septations, and acute-angle dichotomous branching (H&E, 40x).

Aspergillosis frequently affects immunocompromised birds or those exposed to high environmental fungal loads (BAUCK, 1991). The disease has also been documented secondary

to traumatic injury (JENKINS, 1991), nutritional deficiencies (McMILLIN e PETRAK, 1989), and prolonged antibiotic therapy (BAUCK, 1991).

This study documents a case of subcutaneous fungal granuloma in a blue-and-yellow macaw (*Ara ararauna*), with histomorphological features consistent with *Aspergillus* spp. infection. Fungal culture remains an essential diagnostic tool for confirming mycotic infections, enabling both species identification and guidance of targeted antifungal therapy (MUELLER *et al.*, 2007; ROMANI *et al.*, 2020). However, fungal culture was not pursued in this case. Instead, histopathological examination was performed directly following cytological findings suggestive of xanthoma—a benign tumor commonly reported in psittacines and gallinaceous birds. This lesion type is particularly associated with self-trauma and secondary hemorrhage in avian species. (KOSKI, 2002).

While mycotic dermatitis and cellulitis are uncommon clinical presentations in avian species, fungal organisms have been isolated from the plumage of clinically healthy free-ranging birds (GUNGNANI *et al.*, 2012). Notably, cutaneous infections associated with *Aspergillus* spp. have been documented in both columbiformes (particularly pigeons) and psittacines, typically manifesting as ulcerative dermatitis or feather follicle involvement. (TUDOR, 1983). *Aspergillus* species have also been implicated in several cases of epidermal cysts and keratitis in hens and chicks (BECKMANN *et al.*, 1994; SUEDMEYER *et al.*, 2022). HOPPES *et al.* (2000) reported keratitis due to *Aspergillus fumigatus* in the left eye of a blue-fronted parrot (*Amazona aestiva*), suggesting direct environmental contamination, due to the absence of respiratory lesions. Further supporting the dermatotropic potential of aspergillosis, Abrams *et al.* (2021) documented right periocular blepharitis and dermatitis in a 3-year-old female peregrine falcon-gyrfalcon hybrid (*Falco peregrinus* x *Falco rusticolus*). Fungal culture of lesional tissue yielded *Aspergillus* spp., confirming mycotic etiology. This case reinforces the species' capacity for primary cutaneous invasion, independent of respiratory involvement.

The histopathological findings in most reported cases, consistent with our observations, revealed characteristic heterophilic granulomas featuring a central necrotic zone with radially organized, degenerate heterophils. Fungal elements were typically enmeshed within this necrotic core and surrounded by a rim of viable heterophils. The periphery consistently showed a granulomatous response comprising epithelioid macrophages, multinucleated giant cells, and scattered lymphocytes or plasma cells, forming the classic trilaminar structure of avian mycotic granulomas (ARNÉ *et al.*, 2021).

While mycotic dermatitis and cellulitis represent uncommon clinical entities in avian medicine, they should remain important differential diagnoses for cutaneous and subcutaneous pathologies, particularly when lesions prove refractory to conventional therapy. This diagnostic consideration becomes especially relevant in cases with a history of prolonged antibiotic administration, which may predispose to fungal overgrowth and opportunistic infections. (HOPPES *et al.*, 2000).

CONCLUSIONS

This study documents a case of subcutaneous fungal granuloma in a blue-and-yellow macaw (*Ara ararauna*), with histopathological features consistent with *Aspergillus* spp. infection. The findings underscore the critical importance of including mycotic etiologies in the

differential diagnosis of cutaneous and subcutaneous lesions in avian patients, particularly for captive wildlife where environmental and management factors may predispose to fungal infections. This case serves to alert avian practitioners to consider fungal pathogens when evaluating non-responsive dermatological conditions, even when such presentations are uncommon.

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