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# Pneumonia, airsacculitis and osteomyelitis caused by *Aspergillus fumigatus* in an African grey parrot (*Psittacus erithacus*) – case report

[*Pneumonia, aerossaculite e osteomilelite por* Aspergillus fumigatus *em um papagaio-cinzento (*Psittacus erithacus*) – relato de caso*]

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## ABSTRACT

Aspergillosis is a fungal disease with high morbidity and mortality in wild and exotic bird species. The aim of this report is to describe a case of acute aspergillosis caused by *Aspergillus fumigatus* in a young African grey parrot (*Psittacus erithacus*). A three-month-old male African grey parrot from a commercial breeding site presented serous nasal discharge, inappetence, and wheezing on pulmonary auscultation. The parrot died three days after the onset of clinical signs. Postmortem evaluation demonstrated multiple smooth gray plaques in air sacs and left lung with dark red areas and adhesions to the ribs. Microscopically, there were intralesional hyphae and conidiophores in the lungs, air sacs, and ribs, which were associated with pneumonia, airsacculitis, and osteomyelitis, respectively. DNA samples were extracted from paraffinized tissues and subjected to PCR targeting the ITS-2 region, followed by sequencing, which yielded a sequence with 100% coverage and 100% identity to *Aspergillus fumigatus* sequences. Although *A. fumigatus* infection is quite common in birds, a particular aspect of interest in this case was the finding of conidiophores in the bone marrow, which may occur in birds due to air circulation through pneumatic bones.

Keywords: *Aspergillus* sp., mycoses, fungus, Psittacidae, paraffinized tissue, sequencing

## RESUMO

*Aspergilose é uma doença fúngica com alta morbidade e mortalidade em aves domésticas e exóticas. O objetivo deste relato foi descrever um caso de aspergilose aguda causada por* Aspergillus fumigatus *em um papagaio-cinzento (*Psittacus erithacus*). Um papagaio-cinzento de três meses de idade de um criatório comercial apresentou secreção nasal serosa, inapetência e estertores pulmonares. O papagaio morreu três dias após o início dos sinais clínicos. Exame* post mortem *demonstrou múltiplas placas acinzentadas nos sacos aéreos, e o pulmão esquerdo apresentava áreas vermelho-escuras aderidas às costelas. Microscopicamente, havia hifas e conidióforos intralesionais nos pulmões, nos sacos aéreos e nas costelas, associados à pneumonia, aerossaculite e osteomielite, respectivamente. Amostras de DNA extraídas de tecidos parafinizados e submetidas à PCR para amplificação da região ITS-2, seguida de sequenciamento, resultaram em sequência com 100% de cobertura e 100% de identidade com sequências de* Aspergillus fumigatus*. Embora a infecção por* A. fumigatus *seja comum em aves, um aspecto de particular interesse neste caso foi o achado de conidióforos na medula óssea, que pode ocorrer em aves devido à circulação de ar em ossos pneumáticos.*

*Palavras-chave: Aspergillus sp., micoses, fungo, Psittacidae, tecido parafinizado, seqüenciamento*

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## INTRODUCTION

*Psittacus erithacus*, commonly known as African grey parrot, is a medium-sized parrot with a white mask, yellow eye, and red tail. It is a native species from Africa, and it is currently experiencing a decrease in the population, being classified as an endangered species by the IUCN Red List of Threatened Species due to the extent habitat loss, and trapping for the wild bird trade (Birdlife International, 2021). Parrots are popular pets in many countries, being one of the most traded birds listed in the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Martin *et al.*, 2018).

Aspergillosis, a fungal infection associated with morbidity and mortality in companion birds, is a disease caused by *Aspergillus* spp., which are thermophilic and opportunistic fungi widely distributed in the environment. *Aspergillus fumigatus* is the species most associated with infections, although less commonly *A. flavus, A. niger, A. nidulans*, and *A. terreus* may also cause infection and disease in birds (Arné *et al.*, 2021). Aspergillosis has been reported in African grey parrots (Arné *et al.*, 2021). Immunosuppression is the most important predisposing factor for aspergillosis. Therefore, the environment, diet, and presence of other animals are aspects to be considered since those may affect immunocompetence of the bird (Crosta, 2021). The environment is critical since these pet birds may be subjected to inadequate temperature, light cycle, humidity, and cage that may be detrimental to immunity. In addition, chronic stress, as well as poor diet, particularly hypovitaminosis A may favor infection (Arné *et al.*, 2021; Crosta, 2021). Considering that airborne infection is the most common route of infection, *Aspergillus* colonizes primarily the respiratory tract, from where it may spread to other organs such as the coelomic cavity, central nervous system, and pneumatic bones (Greenacre *et al.*, 1992). Therefore, respiratory and nervous system infections are common findings of aspergillosis and are well documented in numerous bird species (Greenacre *et al.*, 1992). Bones and joints affected by *Aspergillus* spp. infection has been reported in turkeys, ducklings, and poultry (Greenacre *et al.*, 1992; Arné *et al.*, 2011, Hurley-Sanders *et al.*, 2015). In contrast, reports on bone involvement in cases of *Aspergillus* spp. infection in parrots are rare, with a reported case of infection affecting the rhinotheca of an adult yellow-naped Amazon parrot (*Amazona orchocephala auropalliata*) (Mans and Guzman, 2017).

The aim of this report was to describe a case of pneumonia, airsacculitis, and osteomyelitis in a young African grey parrot (*Psittacus erithacus*) due acute aspergillosis caused by *Aspergillus fumigatus*.

## CASE REPORT

A 3-month-old male African grey parrot (*Psittacus erithacus*) from a commercial breeder died after 3 days presenting nasal secretion, inappetence, and wheezing on respiratory auscultation. Another parrot chick died in the same period with the same signs, but with a hyperacute clinical course and was not subjected to necropsy.

Grossly, the clavicular and left anterior thoracic air sacs were thick, opaque, with deposition of smooth gray plaques on the surface. The caudal portion of the left lung was diffusely dark red, firm, with adhesion to the cavity wall adjacent to the 4th, 5th, and 6th ribs (Figure 1A). On the cut surface of the affected lung, there were multifocal white nodules with 0.1 x 0.1 mm. No other gross changes were observed. Soft tissue samples were fixed in 10% buffered formalin, paraffin embedded, cut, and stained with hematoxylin and eosin (HE), periodic acid–Schif (PAS) and Grocott-Gomori's methenamine silver (GMS). Samples of the ribs were fixed in 10% buffered formalin for 48 hours and decalcified in a solution of 10 M ethylenediaminetetraacetic acid (EDTA) for 14 days prior to further histological processing as described above.

Microscopically, the lung, bronchi and parabronchi had marked multifocal necrosis with fibrin accumulation and a heterophilic and histiocytic inflammatory infiltrate and large numbers of fungal hyphae with parallel walls ranging from 5 to 15 µm in diameter (Figure 1B), with abundantly septate, acute angle branching, and occasional formation of conidiophores filled with spores, characterizing a

fungal pneumonia. Blood vessels were filled with fibrin thrombi with hyphae (Figure 1C), with vascular wall necrosis and heterophilic infiltrate (vasculitis). There was airsacculitis with large numbers of intralesional hyphae, with morphologic features similar to those present in the lungs, and abundant formation of conidiophores (Figure 1D). In the ribs, there were multifocal areas of osteomyelitis with necrosis and loss of trabecular bone, fibrin accumulation, and heterophilic and histiocytic inflammatory infiltrate (Figure 2A). In the adjacent bone marrow, there was multifocal heterophilic and histiocytic inflammatory infiltrate, accumulation of fibrin, and large numbers of intralesional fungal hyphae with abundant formation of spore-filled conidiophores (Figure 2B, 2C and 2D). In ribs, hyphae infiltrated the bone, muscle, nerves, and ganglia. PAS and GMS stains were performed on sections of the lung, air sacs, and ribs, resulting in strong staining of hyphae, conidiophores, and spores (Figures 1C, 2C and 2D).

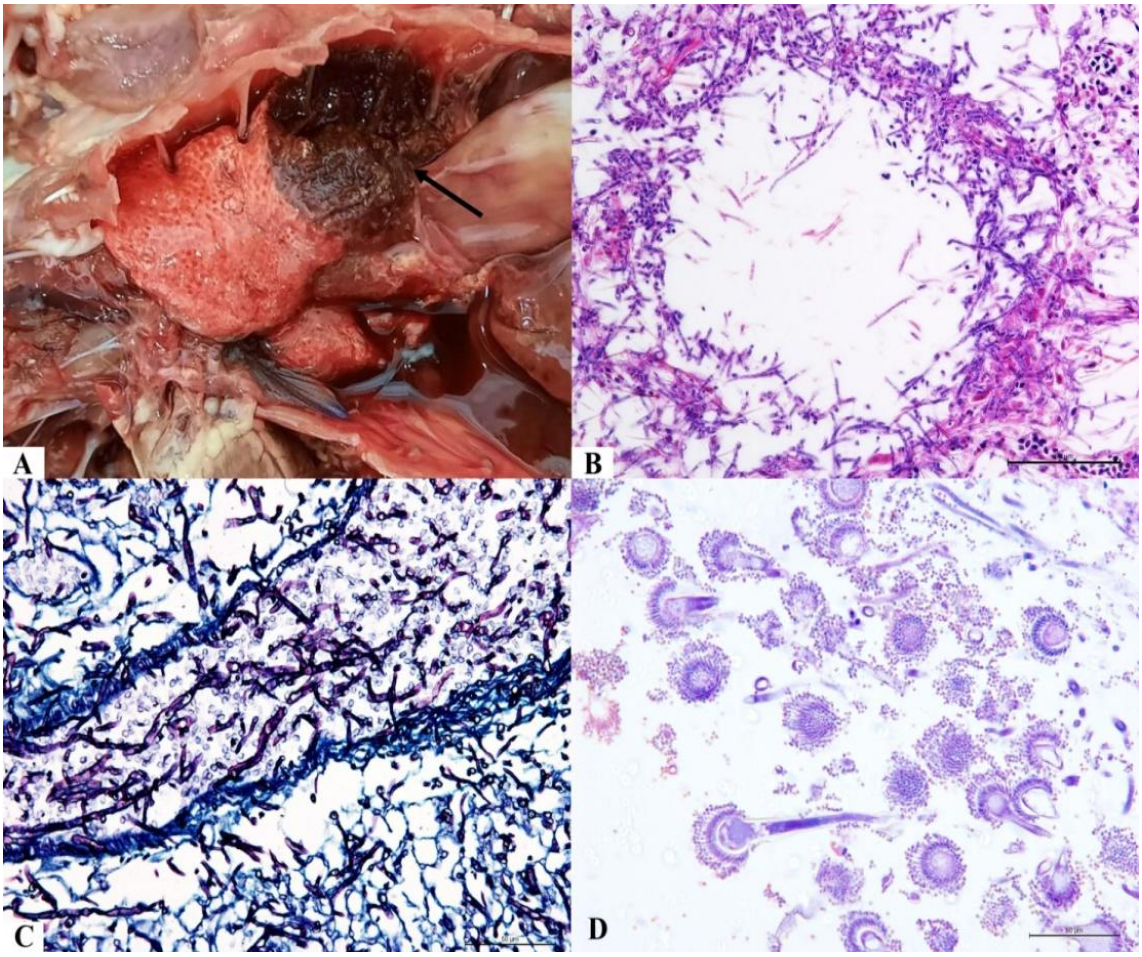


Figure 1. Lung from African grey parrot (*Psittacus erithacus*). A) Left lung, caudal portion with firm dark red focally extensive area (arrow), with adhesions to the wall of the cavity adjacent to the 4th, 5th and 6th left ribs. B) Lung, parabronchi lined by many hyphae with parallel walls, septate, branching at an acute angle (45°) and approximately 5 µm in diameter, HE, scale bar = 60 µm. C) Lung, blood vessel lumen, vascular wall and parenchyma filled with hyphae, GMS stain, scale bar = 50 µm. D) Air sac with conidiophore formation and spores, HE, scale bar = 50 µm.

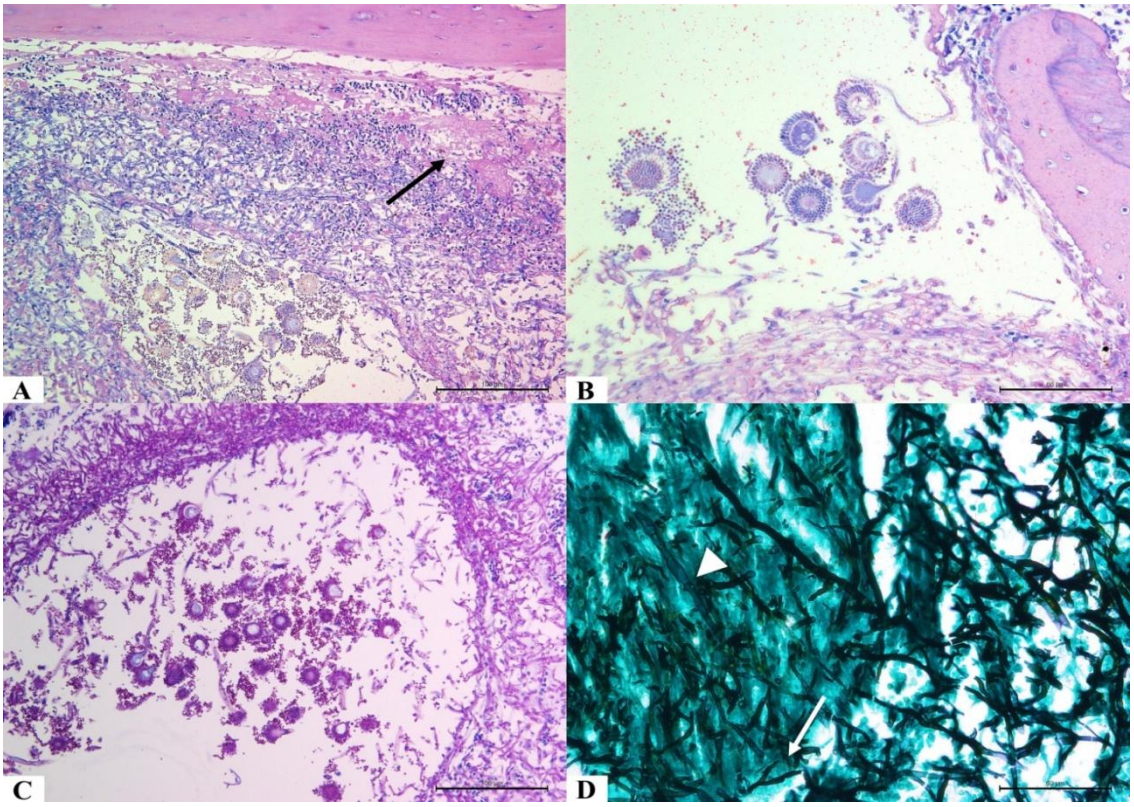


Figure 2. Rib from an African grey parrot (*Psittacus erithacus*). A) Bone and bone marrow with large number of hyphae and abundant formation of conidiophores, and areas of necrosis and fibrin (arrow), HE, scale bar = 100µm. B) Bone with hyphae and conidiophores with spores, HE, scale bar =100µm. C) Bone marrow with hyphae and PAS-positive conidiophores, PAS, scale bar = 500µm. D) Rib, arrow shows hyphae with branching area at an acute angle of approximately 45° (arrow) with septated hyphae (arrowhead), GMS, scale bar = 60µm.

DNA samples were extracted from paraffin-embedded samples of the lung and ribs using a commercial kit (ReliaPrep FFPE gDNA Miniprep System extraction kit; Promega) according to manufacturer's instructions. Amplification of internal transcribed spacer-2 (ITS-2) was performed by polymerase chain reaction (PCR) using a pair of panfungal primers (Meason-Smith *et al.*, 2017). PCR reaction included 2.5µL of 10x Taq-Buffer (Invitrogen), 0.5µL of dNTPs (10µM), 0.5µL of each primer (10µM), 0.75µL $MgCl_2$ (50µM), 0.25µL Taq-DNA polymerase (Invitrogen), 18.0µL of DEPC-treated water, and 2.0µL of template DNA. Amplification parameters were previously described by Meason-Smith *et al.* (2017). The amplicon was purified using the QIAEXII kit (Quiagen) selecting 350 to 400 pb DNA fragments from agarose gels. Amplicons were subjected to capillary sequencing. The sequence obtained was subjected to basic local alignment search tool (BLAST) analysis (Altschul *et al.*, 1990). DNA amplified with panfungal primers from lung samples containing intralesional fungus yielded a sequence with 100% coverage and 100% identity with *Aspergillus fumigatus*. There was no amplification of the material extracted from the rib lesions.

## DISCUSSION

The morphologic features of the intralesional fungal agent and PCR amplification of ITS-2 followed by sequencing allowed us to identify the pathogen at the species level as *Aspergillus fumigatus*. Clinical signs associated with histopathological lesions such as necrosis, fibrin exudate, a predominantly heterophilic and histiocytic inflammatory infiltrate, and vasculitis prompted us to classify this case as acute aspergillosis, which is usually associated with inhalation of large amounts of spores, more often

affecting young birds with a poorly developed immune system (Arné *et al*., 2021).

Fungal pneumonia and airsacculitis are important causes of mortality in captive birds (Crosta, 2021). As in this case, *A. fumigatus* is the most common *Aspergillus* spp. that causes respiratory disease in a variety of bird species (Paixão *et al.*, 2004; Talbot *et al.*, 2018; Sabino *et al.*, 2019). As it is an environmental opportunistic fungus, pre-existing diseases, poor sanitary conditions, and low immunity are predisposing factors for infection. (Kang *et al.*, 2017; Arné *et al.*, 2021). In this case, separation of the chick from the mother for artificial feeding, when the immune system was not fully developed may have contributed to the occurrence of the disease.

The protocol employed for decalcification likely resulted in degradation of fungal DNA, preventing PCR amplification, but due to the histopathological features and proximity of the lesions, we can safely assume that the pathogen was the same in all affected tissues. Fungal osteospondylitis has been described in two bufflehead duckling (*Bucephala albeola*) with morphology of the agents involved compatible with *Aspergillus* spp. and *Mucor* spp. (Hurley-Sanders *et al.*, 2015). There are also reports of infection by *Aspergillus flavus* and *Aspergillus niger* causing rib osteomyelitis in a farmed ostriches (*Struthio camelus*) and sternum osteomyelitis in chickens due by *Aspergillus* spp. (Arné *et al.*, 2011). In this case, the bone involvement may have occurred due to the extension of the lung lesions and air sacs since these organs were adhered. The hematogenous route is unlikely, given the absence of lesions in other distant organs. Abundant conidiophores are usually observed in lung and air sac infections, due to the presence of oxygen, which is required for the formation of conidiophores. Conidiophores have been rarely reported in bones. The large number of conidiophores in the bone marrow in this case may be due to the communication of the air sacs with the osseous tissue, which form pneumatic bones, and by extension of the pulmonary lesion (Greenacre *et al.*, 1992; Arné *et al.*, 2011; Arné *et al.*, 2021).

*Aspergillus* spp. are known to have intense angiotropism so vascular lesions such as aneurysms and thromboembolism are often observed in these cases (Barathidasan *et al.*, 2013; Veiga *et al.*, 2021). Intravascular hyphae in the lung and rib with vasculitis demonstrate this tropism, and if this bird had survived, disseminated aspergillosis could have developed later in course of infection. In addition to the tissue damage caused by vascular lesions, fungi of the genus *Aspergillus* can produce mycotoxins, such as gliotoxin, which is associated with necrotic lung lesions in birds, including an African gray parrot (Oca *et al.*, 2022).

Mycotic infections in African grey parrots including pulmonary aspergillosis by *Aspergillus fumigatus* (Oca *et al.*, 2022), cryptococcosis (Schunk *et al.*, 2017), mucormycosis (Desmidt *et al.*, 1998), and penicillosis (Lanteri *et al.*, 2011) have been reported, so the gray parrot is thought to be highly susceptible to mycotic infections (Arné *et al.*, 2021). Clinical signs of avian aspergillosis may be nonspecific, with hyperthermia, weight loss, anorexia, and polydipsia, and may be directly related to the affected systems, such as coryza, unproductive cough, wheezing on auscultation, and dyspnea in cases of respiratory infections (Arné *et al.*, 2021). In this case, the parrot presented dyspnea and coryza. Importantly, aspergillosis should always be considered in the differential diagnosis of birds with respiratory signs or acute death, and the differential diagnosis should also include mycobacteriosis, trichomoniasis, chlamydiosis, poxvirus and avian influenza (Crosta, 2021).

As seen in this case, aspergillosis in parrots can be an acute lethal disease. An accurate diagnosis of the disease based on necropsy, histopathology, PCR, sequencing, among other techniques, is essential for adopting control actions (Arné *et al.*, 2021). Although the air sacs and lungs are the main sites affected, the infection can also occur in other sites, such as the bones, and these must always be evaluated in cases of aspergillosis.

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