



Aspergillus nidulans as an agent of subcutaneous mycetoma in a horse

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ABSTRACT

Mycetoma is a fungal chronic skin proliferative lesion rarely encountered in horses and often associated with fungi such as *Scedosporium* spp., *Madurella* spp., *Phialophora* spp., *Curvularia* spp., and less frequently with *Aspergillus* spp. A case of subcutaneous mycetoma in the cervical region in a 7-year-old male Quarter Horse, diagnosed through cytological, histological, cultural and molecular methods, is reported. *Aspergillus nidulans*, usually implicated in guttural pouch mycosis, was identified. This unusual case represents the second case report of mycetoma caused by *Aspergillus nidulans* in the horse and highlights the importance of including mycotic diseases in the differential diagnosis of nodular skin masses in equine patients.

1. Introduction

Mycetomas are chronic, granulomatous and proliferative infections of the skin and subcutaneous tissue caused by either actinomycetes (referred to as actinomycotic mycetoma or actinomycetoma) or filamentous fungi (known as eumycotic mycetoma or eumycetoma) (McGinnis and Fader, 1988). Eumycetoma can lead to the formation of sinuses and fistulae that exude purulent material containing fungal aggregates embedded in a matrix of granules; the size, color and shape of the granules vary depending on the causative agent (Elad et al., 2010). The disease is often associated with trauma, which introduces into the tissue the infectious agent. The mycete continues to spread in surrounding tissue over the following months and years, leading to the formation of a granulomatous lesion, which are often localized and characterized by tumefaction (McEntee, 1987; Lopez et al., 2007). Although mycetoma is more frequently documented in humans, it can also rarely affect various animal species, including horses. A case of eumycetoma in a horse is reported, from which *Aspergillus nidulans* was isolated by culture and confirmed by molecular analyses.

2. Case presentation

A 7-year-old male Quarter Horse was presented with a spherical subcutaneous mass, approximately measuring 6 × 7 cm, in the cervical region just cranial to the withers (Fig. 1), which had developed

approximately 12 months prior. The mass showed a solid consistency and seemed strongly attached to the underlying tissues. No other abnormalities were found during the examination. The mass wasn't painful and the neck movements were normal.

A fine needle aspiration (FNA) of the mass was performed, and the smears showed high cellularity with well-preserved morphology. The cellular population was composed of a mixed inflammatory element with many neutrophils, often hypersegmented, a large number of histiocytes, epithelioid cells, multinucleated giant cells, and small lymphocytes. Interspersed among the inflammatory cells is also observed the presence of small fungal hyphae of 5 to 10 μm in size, slightly-septate, rod-shaped with slightly bulbous ends (Fig. 2). The cytological diagnosis was of pyogranulomatous dermatitis with the presence of fungal elements, consistent with mycetoma.

On the basis of these findings, a second FNA sample was obtained and cultured on Difco™ Sabouraud Dextrose Agar (BD, USA) medium with 0.05 g/l chloramphenicol (Sigma, USA) (SAB-CAF) in duplicate at 26 and 37 °C. After 48 h, both macroscopic and microscopic examination of growing colonies was performed, identifying them as belonging to the genus *Aspergillus*. In particular, the colonies appearance was consistent with *Aspergillus nidulans*, macroscopically characterized by rapid growth and colonies ranging in color from green to cream to ochre yellow. The reverse of the plate exhibits a typical reddish to purplish hue. Microscopically, the conidia appear rough, the hyphae range from yellowish to cinnamon-colored, and the conidial heads are columnar

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Fig. 1. Horse, neck. External appearance of the mass in the cervical region.

and short, as described by De Hoog et al. (2020) (Fig. 3). Considering the presence of fungal elements in the microscopic examination of the FNA and the isolation of an *Aspergillus* species in culture, a eumycotic mycetoma was suspected, and surgical removal was performed. With the horse standing and sedated (detomidine 10 mg/kg, bwt followed by morphine 0.1 mg/kg bwt detomidine i.v.) a local anaesthesia was performed with subcutaneous infiltration of lidocaine 2 % (10 ml). The surgical site was surgically prepared and draped, and an elliptical skin incision was made encircling the mass about 1 cm apart. The subcutaneous tissue was carefully dissected around the neoplasm, and the mass easily separated from it and removed. The surgical site was left open with topical application of 10 % povidone-iodine cream for four days. Additionally, 2 % clotrimazole cream was administered topically for a duration of 10 days. Given the localized presentation of the mycetoma, systemic antifungal therapy was deemed unnecessary. A part of tissue removed was stored in 10 % neutral buffered formalin for histological analysis, and another portion used for culture examinations on SAB-CAF at 26 and 37 °C to confirm the etiology. A fragment of the grown colonies was processed for DNA extraction, using Pure Link® Genomic DNA Mini kit (Invitrogen by Thermo Fisher) as reported by the manufacturer's protocol. PCR for the β -tubulin gene was performed, according to Burrough et al. (2012), using the primers described by Romanelli et al. (2014). For sequencing, the amplicons were excised and

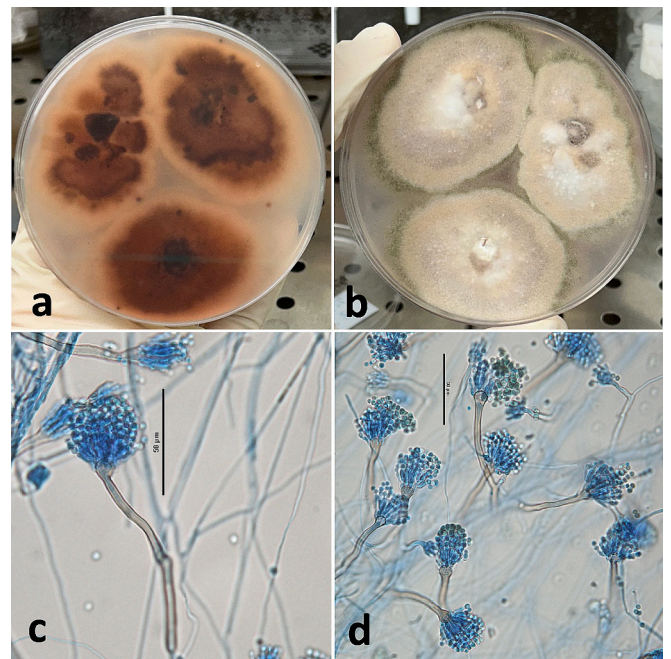


Fig. 3. a-b: Macroscopic appearance of the colonies of *Aspergillus nidulans* subcultured on Czapek agar 1 week, 26 °C (a: reverse; b: obverse). c-d: Lactophenol blue staining of conical heads of *A. nidulans* 400× magnification.

purified by Nucleo-Spin Gel and PCR Cleanup (Mackerey-Nagel, Düren, Germany), and sequenced with an ABI 3730 DNA analyzer (StarSEQ, Mainz, Germany). The β -tubulin sequence obtained were assembled with Contig Express (VectorNTI Advance 11 software, Invitrogen, Carlsbad, CA, USA), and the consensus sequences were compared with published data by BLAST tools (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), showing a 99,6 % similarity to *A. nidulans* (MN594520).

A multifocal, severe, pyogranulomatous dermatitis was recognized at histology. The whole mass appeared composed of variably sized aggregates of many neutrophils, epithelioid macrophages, multinucleate giant cells, lymphocytes, plasma cells, with frequent central intralesional clusters of fungal hyphae (mycetomas). Hyphae were 5–15 μ m wide, non-parallel, non-pigmented, poorly septate, with bulbous morphology and irregular branching highlighted most using Periodic Acid-Schiff (PAS) and Grocott histochemical special staining (Fig. 4).

The skin closure took 3 months to be completed because of the position where the movements, especially the dorso-ventral ones, create tension on the margins.

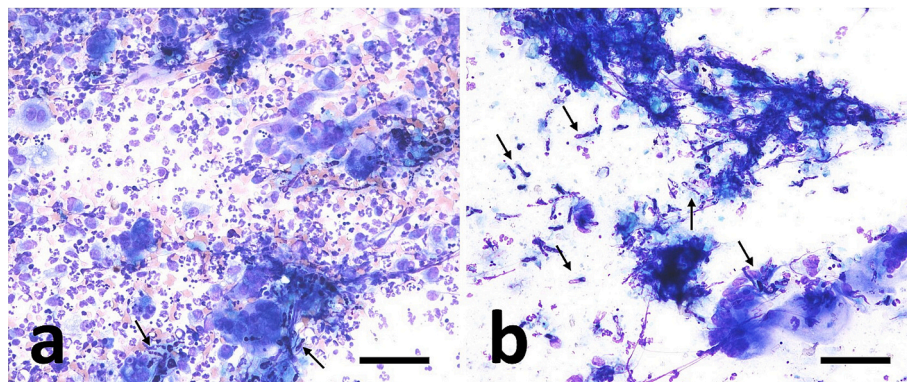


Fig. 2. Fine-needle aspiration cytology of the mass. (a) Smears are composed of a mixed population of inflammatory cells interspersed with some fungal elements (arrows). MGG stain, Bar: 100 μ . (b) Numerous fungal hyphae, slightly septate and parallel sided, present at the bottom of the smears (arrows). MGG stain, Bar: 100 μ .

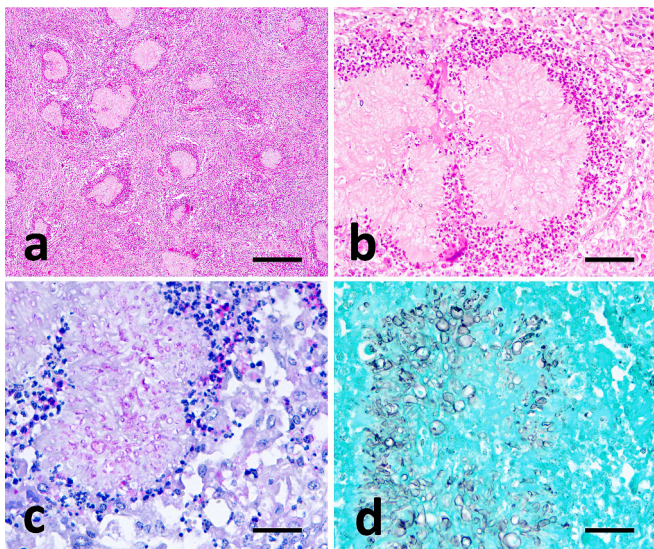


Fig. 4. Histological features of equine subcutaneous mycetoma. (a) Multifocal to confluent fungal granulomas surrounded by a chronic inflammatory infiltrate. HE. Bar: 500 μ . (b) At higher magnification, a mycetoma characterized by central clusters of fungal hyphae surrounded by large numbers of neutrophils, epithelioid macrophages, lymphocytes, and fibroplasia is observed. HE. Bar: 100 μ . Fungal hyphae are displayed using Periodic acid–Schiff (PAS) stain (c) and Grocott's methenamine silver (GMS) stain (d) and appear slightly septate with bulbous ends and irregularly branched. Bar: 100 μ .

3. Discussion and conclusions

In horses, the most common causative agents of mycetoma are the genera *Scedosporium*, *Pseudallescheria*, and *Madurella* (McEntee, 1987; Berzina et al., 2011; Elad et al., 2010), although *Curvularia verruculosa* (Russo et al., 1994) and *Phialophora oxyspora* (Lopez et al., 2007) have occasionally been detected. In the literature, only a few documented cases of mycetoma in horses caused by *Aspergillus* species have been reported, involving *A. terreus* (Randleff-Rasmussen et al., 2017) and *A. versicolor* (Keegan et al., 1995). In addition, a very recent case of dermatitis, cellulitis, and osteomyelitis caused by *A. nidulans*, histologically compatible with a mycetoma, was described (Waitt Wolker et al., 2024). *Aspergillus* species are ubiquitous moulds, commonly found in the environment, such as in soil, water, and decaying materials with occasional potential to infect living hosts including plants, insects, birds, and mammals (Seyedmousavi et al., 2015). Usually, they don't cause mycetomas in humans or animals. In particular, in horses, aspergilloses are more commonly reported in immunocompromised subject, or in those with a history of prolonged administration of antibiotics and corticosteroids (Cafarchia et al., 2013). The most characteristic *Aspergillus* infection in horses is guttural pouch mycosis, with *A. fumigatus* and *A. nidulans* being the primary fungi involved, although other *Aspergillus* species have been reported in the literature (Ludwig et al., 2005; Freeman, 2015; Elad and Segal, 2018). In horse, *Aspergillus* species are also associated with respiratory infection (Carrasco et al., 1996; Sweeney and Habecker, 1999), corneal ulcers or keratomycoses (Samuelson et al., 1984; Sansom et al., 2005) and placentitis or endometritis in mares (Dietz and Wiesner, 1984; Orellana-Guerrero et al., 2019). In contrast to other types of infections, dermal lesions are rarely reported as a site of *Aspergillus* spp. infection in horses (Waitt Wolker et al., 2024), with only one case of superficial dermatitis caused by *A. sydowii* reported by Sang et al. (2012). To the best of our knowledge, the present is the second described case of a cutaneous mycetoma caused by *Aspergillus nidulans* in a horse. This species is included in the *Nidulantes* section, along with *A. versicolor*, which was previously reported as an agent of equine mycetoma (Keegan et al., 1995).

This case highlights the importance of including fungal infections in

the differential diagnosis of cutaneous or subcutaneous nodular proliferative lesions in horses. In our case, fine needle aspiration cytology from the lump allowed a fast and easy recognition of the inflammatory pyogranulomatous nature of the lesion, as well as the presence of fungal hyphae in the smears, directing toward in-depth mycological and biomolecular determinations. At the same time, it allowed quick as well as more appropriate surgical treatment.

From a clinical point of view, these atypical cases could be relevant depending on the location and the dimension and the potential dysfunction created by the proximity with vital structure. In our case the location of the mass and the tension created during the dorso-ventral movements hindered and delayed the healing.

This case also points to the necessity for additional research on the epidemiological and diagnostic aspects of mycetoma in equine patients, given the limited number of cases reported in the literature. It remains unclear whether such conditions are underestimated, as is often the case with other fungal diseases which are frequently unrecognized or incorrectly diagnosed. Moreover, it is crucial to employ multiple diagnostic techniques to reach a definitive diagnosis. Due to the widespread presence of *Aspergillus* spp. in the environment, it is important to isolate the pathogen directly from the affected tissue, combining cytological and histological examinations to confirm tissue colonization. With the increase in recent years of cryptic *Aspergillus* species, molecular investigations are necessary to confirm the identity of the species involved. For example, in the case of *A. nidulans*, there are very similar species belonging to the same section, such as *Emericella quadrilineata* (Verweij et al., 2008). In fact, given the high similarity between *A. nidulans* and other species belonging to the same section, it is essential to use molecular techniques as the only reliable method for making a definitive identification. A morphological diagnosis alone is not sufficient and molecular results must also be carefully evaluated considering the minimal nucleotide differences between the species in the same section. A comprehensive approach, combining morphological, molecular, and clinical findings, is essential to ensure an accurate diagnosis.

CRedit authorship contribution statement

Talita Bordoni: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Filippo Maria Dini:** Writing – review & editing, Methodology, Formal analysis. **Maria Morini:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Riccardo Rinnovati:** Writing – review & editing, Methodology, Formal analysis. **Alessandro Spadari:** Writing – review & editing, Methodology, Formal analysis. **Roberta Galuppi:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

None.

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