



Granulomatous tracheitis and dermatitis due to *Curvularia* sp. in a horse: case report

[Traqueíte e dermatite granulomatosas associadas a *Curvularia* sp. em um equino: relato de caso]

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ABSTRACT

Tracheal fungal infections in horses are rare. This case report describes surgical and clinical management of a filly with a *Curvularia* sp. infection within the trachea and skin that caused severe intraluminal granulomas and cutaneous nodules, respectively. The patient was successfully treated with itraconazole and surgical excision.

Keywords: equine; trachea; phaeohyphomycosis; surgery; itraconazole

RESUMO

Infecções fúngicas traqueais em equinos são raras. Este relato de caso descreve condutas clínicas e cirúrgicas em uma égua com infecção por Curvularia sp. na traqueia e na pele, causando granulomas intraluminais severos e nódulos cutâneos, respectivamente. O animal foi tratado com sucesso com itraconazol e exérese cirúrgica.

Palavras-chave: equino, traqueia, feo-hifomicose, cirurgia, itraconazol

INTRODUCTION

Fungal infections can occur in every anatomical region of the respiratory tract in horses, but are most frequently observed in paranasal sinuses, guttural pouches, and lungs, where they are acquired via inhalation and from wounds (Stewart and Cuming, 2015). *Curvularia* is a genus of pigmented (dematiaceous) fungi that is widely distributed in plants, soil, and decaying matter. Although saprobic, it can behave as a phytopathogen or cause disease in humans, where it can cause keratitis, allergic or infiltrative sinusitis, brain abscesses, and lung and skin infections (Revankar and Sutton, 2010). There are few references to *Curvularia* infections in horses, but there are reports of mycetoma (Broomker *et al.*, 1977), rhinitis (Cunha, 2014), and cutaneous granuloma (More *et al.*, 2019) caused by this fungus.

Only two cases of tracheal fungal granuloma have been previously described in horses; both were caused by *Conidiobolus coronatus* (Steiger and Williams, 2000; Wallace, 2019). Here, we describe a clinical case of granulomatous tracheitis, and dermatitis associated with *Curvularia* sp. in a young horse.

MATERIAL AND METHODS

An 18-month-old female horse was brought to the Medical and Surgical Clinic for Large Animals at the Federal University of Mato Grosso do Sul for evaluation for a 30-day history of severe respiratory noise. The animal had been kept in a *Panicum maximum* cv. Mombaça pasture, which had swampy areas and sandy soil, and she was unique among a herd of 90 horses, which were raised together, in developing clinical signs. At admission, the filly was alert, cachectic, and had

marked mixed dyspnea at rest, which was exacerbated when she moved or became agitated. Breathing was thoracoabdominal with remarkable effort, and respiratory rate was 28 breaths per minute.

There was no nasal discharge or cough. Tracheal palpation revealed rings that were firm and thick, and on the ventral portion of the trachea was a circular swelling that was firm, sessile, and painless. Laryngotracheal auscultation detected stridor, and tracheobronchial and bronchobronchiolar sounds were impaired. The filly was tachycardic (96 beats per minute), but body temperature and gut sounds were normal. Circumscribed, flat, and non-ulcerated integumentary nodules were observed on the left ischial tuberosity, auricles, and dorsum (Figure 1). In the left pectoral region, there was an exophytic nodule that was different from the others mentioned.

Lateral tracheal radiographs revealed masses of various diameters, with diffuse, isolated, or clustered distributions, over the entire length of the lumen and almost completely obstructing the airway. The neoformations in radiopaque areas were adhered to tracheal rings, which in affected regions stood out as thick and irregular (Figure 2).

Hematological analysis showed anemia (erythrocytes $5.92 \times 10^6/\mu\text{L}$; hemoglobin 9 g/dL; hematocrit 27%), normocytosis (mean corpuscular volume 45.6 fL), normochromia (mean corpuscular hemoglobin concentration 33.3%), and leukocytosis ($17,600 \text{ cells}/\text{mm}^3$) due to neutrophilia (85%). Serum biochemistry was within normal limits. To provide immediate comfort for the patient, a tracheostomy was performed above the largest intraluminal mass. A 2cm \times 2cm flap was excised from the trachea and sent for histopathological and microbiological assays, along with cutaneous nodule samples (Figure 3).



Figure 1. 18-month-old filly referred for severe dyspnea. Nodules were scattered throughout the body. Circular, elevated, and alopecic nodules are shown on the pinna (A) and near the left ischial tuberosity (B).

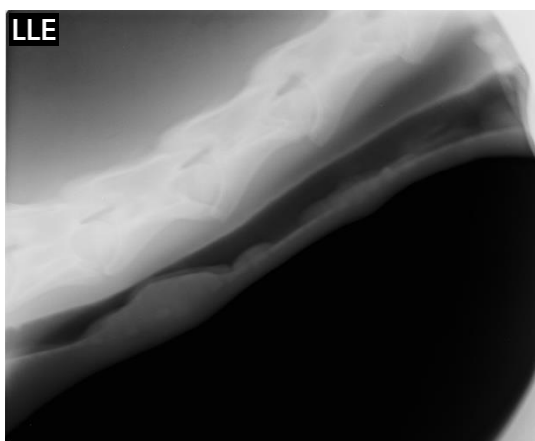


Figure 2. Latero-lateral radiographic projection of the cervical region of the filly, whose trachea was obstructed along its entire length by masses of various diameters.



Figure 3. Appearance of section of cutaneous nodule collected from the left ischial tuberosity and sent for histopathological and microbiological analysis.

Histopathology showed tangles of fibrous connective tissue from the thick and expanded tracheal submucosa, infiltrating the cartilaginous rings. Coalescent foci of giant multinucleated Langhans cells, epithelioid macrophages, neutrophils, and interspersed eosinophils were observed. In the macrophage cytoplasm, there were giant cells and numerous loose, rounded yeast organisms, measuring 10 to 20 μ m in diameter, containing a round basophilic nucleus of 8 to 10 μ m and a double, refringent, and thick cell wall, strongly impregnated by Grocott's silver methenamine stain. The diagnosis was consistent with fungal granulomatous tracheitis. Similarly, skin flaps from the dorsum and ischial tuberosity were suggestive of fungal granulomatous dermatitis, while the sample from the pectoral region was diagnosed as an equine sarcoid.

For microbiological analysis, samples were sown on blood agar, brain heart infusion agar (BHI, Kasvi, Brazil), and Sabouraud's dextrose agar (SDA, Kasvi, Brazil) with antibiotics. No significant bacterial growth was observed. Fungus cultivation resulted in the growth of gray to black colonies. Microculture was performed on SDA, and microscopy revealed curved conidia, which

are structurally similar to *Curvularia* sp. (Figure 4). Etiological confirmation was based on polymerase chain reaction (PCR) as described by Bialek *et al.* (2001), starting from fungal culture, and DNA sequencing of amplicons obtained by PCR. DNA sequencing (427 bp, SSU rRNA gene) showed an identity greater than 97% with organisms of the genus *Curvularia* found at Genbank-NCBI. After analysis, the DNA sequence was deposited at GenBank NCBI under accession number MN814437.

Based on the observed lesions and diagnostic conclusions, itraconazole (Drogavet, Brazil) (5mg/kg, PO, SID) administration was started immediately. As adjuvants, Catosal B12 (cyanocobalamin, Bayer S.A., Brazil) (25mL, IV) was given for six days, and Ripercol L solution (levamisole 5%, Zoetis, Brazil) (7.7mg/kg, PO) was given every 48 h for six doses. The tracheostomy site was cleaned daily. In the first two months of hospitalization, the animal's general condition improved, and she recovered from the anemia. The rostral granulomas decreased in size, and there was a subtle decrease in the size of the largest granuloma (Figure 5).

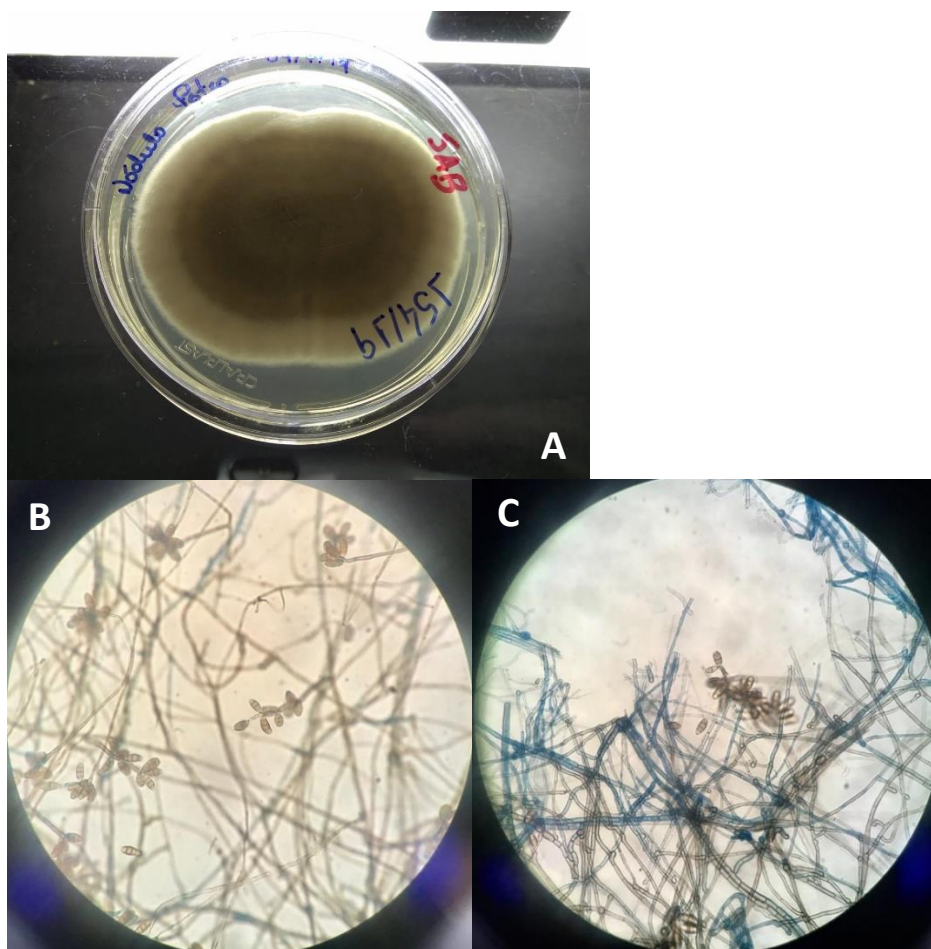


Figure 4. Gray to black colonies obtained from fungal culture, from samples of skin and trachea, indicating that infection was caused by dematiaceous (pigmented) fungi, phaeohyphomycosis (A). Microscopy of this material showed curved conidia, structurally similar *Curvularia* sp. (B and C).

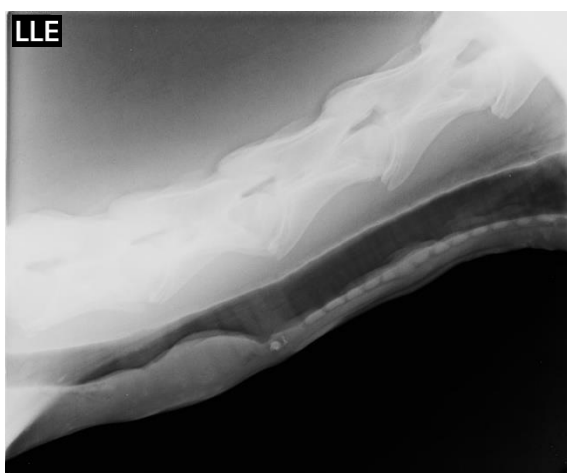


Figure 5. Latero-lateral radiographic projection of the cervical region of the filly after two months of itraconazole therapy. Note regression of the rostral granulomas and improvement in the cartilaginous tracheal rings. The diameter of the largest granuloma is also decreased.

After two months of itraconazole therapy, the granulomas had not completely vanished, so they were surgically excised. Under sedation with Equisedan (xylazine, J. A. Animal Health, Brazil) (0.5mg/kg, IV), induction with Cetamin (ketamine, Syntec, Brazil) (2mg/kg, IV) and Dormire (midazolam, Cristalia, Brazil) (0.5mg/kg, IV), and maintenance with inhaled anesthesia (isoflurane, Cristalia, Brazil), the filly was given a tracheotomy. A 20-cm incision was made in the midline neck as described in Santamaria-Martínez *et al.* (2014). Identifying and incising the trachea was difficult due to the presence of fibrotic tissue.

The remaining granulomas were excised (Figure 6) and the trachea was sutured with 3-0 polyglycolic acid suture material in a simple interrupted pattern. The resected tissue was white, yellowish, and firm, and it was submitted for bacteriology and histopathology, which reaffirmed the presence of *Curvularia* sp. Postoperative medications consisted of Gentopen (potassium penicillin, J.A. Animal Health, Brazil) (22.000 UI/kg, IV, QID) for 10 days and Flumax (flunixin meglumine, J.A. Animal Health, Brazil) (1.1mg/kg, IV, SID) for 3 days.

The patient was discharged after five months of hospitalization, with antifungal medications prescribed for an additional two months, for a

total of seven months of systemic antifungal therapy. The filly fully recovered from the dyspnea, showed improvement on radiographs (Figure 7), and showed no signs of recurrence. The skin nodules became flat and smaller but did not fully disappear. Furthermore, she gained approximately 100kg. Six months after discharge, the owner reported that the filly remained eupneic, showing no nostril dilatation or thoracoabdominal effort.

DISCUSSION

Cases of *Curvularia* sp. infection in horses are rare. Broomker *et al.* (1977) described a deeply ingrained mycetoma in the ventral thoracic muscle region in a racehorse, and verrucous growths in the skin of the neck, shoulder, chest, and front legs of a pony. Cutaneous fungal granulomas due to *Curvularia* sp., characterized by firm, single or multiple, non-progressive nodules, have also been reported. It is thought that infection occurs by penetrating plant material or in traumatized skin, and lesion severity depends on the host's immune system (Antoniassi *et al.*, 2010). In Florida, a 12-year-old mare was diagnosed with granulomatous rhinitis caused by *C. lunata*, presenting with a large obstructive pink mass in one nasal cavity and purulent nasal discharge (More *et al.*, 2019).

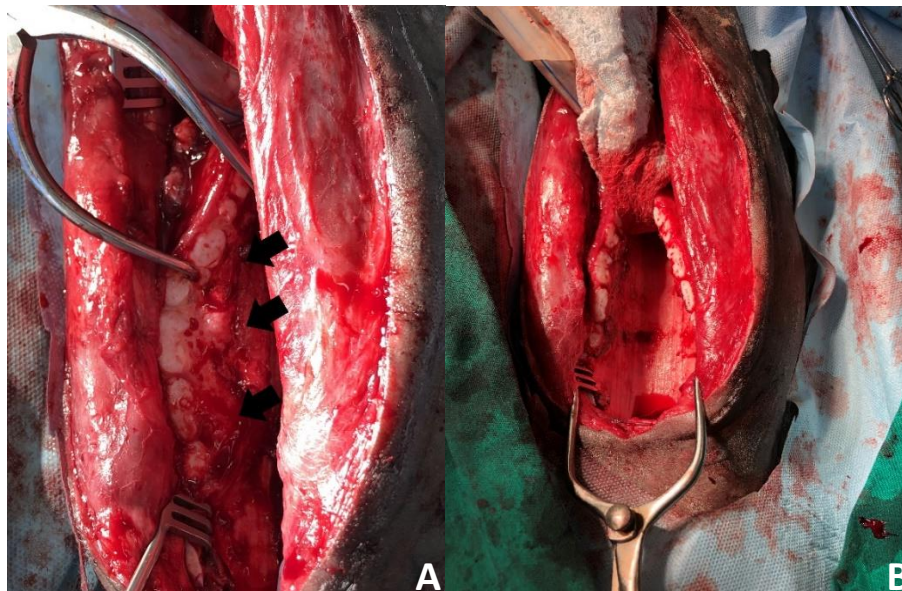


Figure 6. Appearance of the largest intraluminal granuloma in the trachea, closely adhered to the cartilages, which were thick and deformed, during the tracheotomy procedure for excision (arrows, figure A). Tracheal mucosa after extirpation of the remaining granulomatous tissue, before tracheal suture (B).

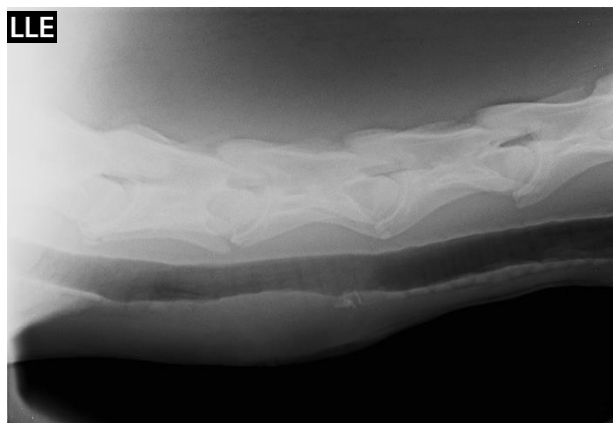


Figure 7. Latero-lateral radiographic projection of the cervical region of the filly three months post-surgery. After five months of systemic itraconazole therapy, the tracheal rings remained thick and irregular, but air passage was not compromised.

In addition, *Curvularia* sp. has been isolated and identified from the microbiota of the ocular conjunctiva of horses in Alagoas, Brazil, from animals with no ocular disease or any systemic changes that could cause an ocular modification (Sousa *et al.*, 2011). The patient presented in this case study lived in a hot climate for an extended time, an environmental factor propitious to the existence of dematiaceous fungi, which are cosmopolitan. It is believed that rhinitis occurs due to contact with pathogens residing in decomposing plants on which horses feed, aerosols, or even insects, and which may spread to other areas (More *et al.*, 2019). However, it is difficult to predict how a primary infection may occur if previous injuries related to other agents exist, or to answer why only this horse had severe simultaneous respiratory and tegumentary disease.

Systemic presentation of phaeohyphomycosis is uncommon. In small animals, infections by *Curvularia* sp. are reported to attack several organs, and are correlated with neoplasia, intercurrent disease, or immunosuppressive therapy (Ferreiro *et al.*, 2007). Although it is believed that this animal was immunocompromised, she had a serum globulin concentration within the reference range (4.99 g/dL) and was negative for Equine Infectious Anemia in immunodiffusion on agar gel (IDAG). To date, there have been no reports of granulomatous tracheitis related to *Curvularia* sp. in any other species. Two cases of tracheitis caused by fungal infections in horses have been reported; one was confirmed as *Conidiobolus*

coronatus through culture and serology (Steiger and Williams, 2000), and the second was related to *Conidiobolus* spp., based on microscopic characteristics (Wallace, 2019). Unlike those cases, the present patient did not demonstrate nasal discharge, blood in the nostrils, or cough. However, her dyspnea was extremely severe, and her body condition score was critically low at 1 out of 5. Tracheal thickening was identified by external palpation of the trachea, as described in previous reports.

Unfortunately, it was not possible to perform endoscopy on this animal. The author considers this to be a flaw of the work.

Equids with fungal infections are challenging to diagnose and treat. Definitive diagnosis was established through clinical findings of upper respiratory disease, followed by histopathological observations, culture, PCR, and DNA sequencing. In this case, oral itraconazole was elected because *Curvularia* sp. are sensitive to this active principle *in vitro* and in clinical experience (Cunha, 2014). There are no previous descriptions of horses treated for infections by *Curvularia* sp. with itraconazole but it is known that it is well absorbed orally at a dose of 5mg/kg every 24 hours, and maintains concentrations higher than the minimum inhibitory concentration without side effects (Stewart and Cuming, 2015). In one report of a 19-year-old immunocompetent human with chronic polypoid rhinosinusitis caused by *C. spicifera*, therapy consisted of endoscopic surgery, oral itraconazole, and topical corticosteroids, with good results (Buzina *et al.*, 2003).

Levamisole was employed as a synthetic immunostimulant because it promotes cell-mediated immunity and neutrophilic mobility (Rush and Mair, 2004). The surgical approach was elected after two months of itraconazole because the larger granuloma did not adequately decrease in size. Surgical excision with a wide margin is recommended to be used alongside systemic antifungals, mainly azole derivatives, in order to decrease the frequency of recurrence (Ferreiro *et al.*, 2007). The only post-surgical complication was subcutaneous emphysema for the first five days. The prognosis for complete resolution is generally guarded (Stewart and Cuming, 2015). Some of the relevant difficulties include the lack of defined treatment schedules, long and costly treatment periods, and recurrence (Ferreiro *et al.*, 2007), as fungal infections are insidious.

CONCLUSIONS

This case represents, to the authors' knowledge, the first report of granulomatous tracheitis and dermatitis associated with *Curvularia* sp. in a horse. Although these lesions are clinical peculiarities, fungal infections in the respiratory tract must be considered as differential diagnoses in horses. Excision by classical surgical intervention seems to be a good adjunct option for prolonged use of itraconazole.

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