


Rhinosinusitis in an Australian mare caused by *Flavodon flavus*, a recently recognized invasive fungal pathogen of the horse

Journal of Veterinary Diagnostic Investigation
2020, Vol. 32(1) 162–165
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DOI: 10.1177/1040638719897610
jvdi.sagepub.com

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Abstract. We describe herein the clinical, endoscopic, computed tomography (CT), pathologic, and microbiologic features of an infection caused by an under-recognized fungal pathogen, *Flavodon flavus*, in a 25-y-old Australian Quarter Horse. The horse had a unilateral obstructive nasal mass, resulting in stertor and dyspnea. On endoscopy, the mass was tan, multinodular, and completely obstructed the nasal passage. CT analysis revealed a large, soft tissue–attenuating and partially mineralized mass in the right nasal passage and dorsal-conchofrontal sinus, expanding into adjacent paranasal sinuses with associated bone lysis and rhinosinusitis. Histopathology of the mass on 2 occasions revealed suppurative inflammation initially, and pyogranulomatous inflammation subsequently. The inflammatory reaction surrounded numerous spherical fungal structures (~60–80 µm diameter) that stained positively on periodic acid–Schiff and Grocott methenamine silver stains. PCR for the fungal internal transcribed spacer 1 and 2 regions followed by Sanger sequencing on a cultured isolate identified the agent as *F. flavus*, which has only been reported previously as pathogenic in one horse in the United States, to our knowledge. Previous reports described this fungus as a nonpathogenic, environmental commensal fungus associated with insects and plants.

Key words: equine; fungal granuloma; fungal rhinitis; nasal granuloma; sinusitis.

Mycosis of the equine respiratory tract is rare, and only a small number of fungal species have been implicated as causative agents.¹⁸ The recognized upper respiratory tract fungal infections in the horse include aspergillosis caused by *Aspergillus* spp. associated with guttural pouch mycosis and nasal granuloma formation⁴; nasal phycomycosis caused by *Conidiobolus* spp.,¹ *Basidiobolus* spp.,⁵ and *Pythium* spp.¹⁷; nasal coccidioidomycosis caused by *Coccidioides immitis*⁷; sinonasal cryptococcosis caused by *Cryptococcus neoformans*^{3,18}; and sinonasal maduromycosis caused by the *Pseudallescheria boydii*/*Scedosporium apiospermum* complex.² The resultant infection often leads to the development of granulomas and/or pyogranulomas that partially or completely obstruct the upper respiratory tract.^{2–5} A 2018 North American report described a series of equine mycotic rhinitis cases caused by a variety of fungal agents previously not known to be pathogenic in animals, and one of these included the environmental fungus, *Flavodon flavus*.¹⁴ The pathogenic potential of *F. flavus* does not appear to have been reported prior to that study. In the present brief report, we present a case of obstructive rhinosinusitis in an Australian mare caused by *F. flavus* (NCBI: txid559739). We describe the clinical, radiographic, pathologic, in vitro growth, and molecular features of this fungus. With our case, we propose that *F. flavus* may be an under-recognized and emerging equine pathogen.

A 25-y-old Quarter Horse mare from coastal northern New South Wales, Australia, was presented to the Equine Specialist Hospital, The University of Queensland, in February 2017 (mid-summer), following a year described as generally wet and warm (Australian Bureau of Meteorology archive, <http://www.bom.gov.au/climate/current/annual/nsw/archive/2016.summary.shtml>). The mare had an 8-wk history of increasingly thick mucopurulent discharge from the right nostril. The owner reported discomfort in the horse during breathing, and a right facial contour deformity. A first-opinion veterinarian treated presumptively for a nasal granuloma with potassium iodide. The owner administered herbal remedies, which consisted of in-feed treatment with a mixture of garlic, turmeric, yellow sulfur, pepper, honey, colloidal

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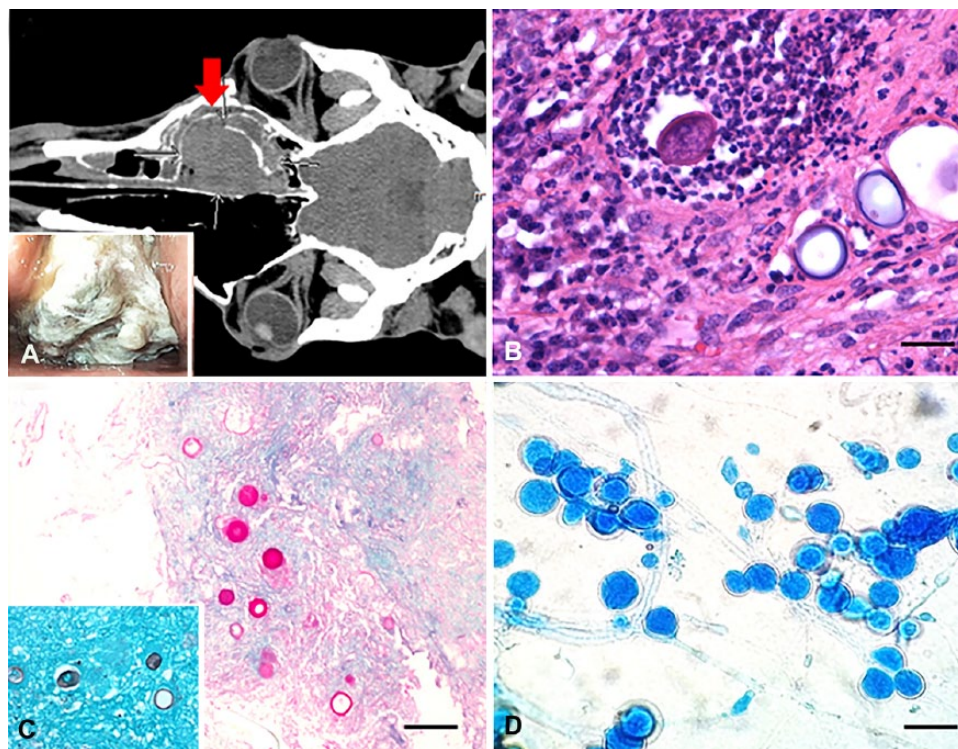


Figure 1. An obstructive nasal pyogranuloma caused by *Flavodon flavus* infection in a horse. **A.** Dorsal plane computed tomography image of the head (soft tissue algorithm) demonstrates the large, soft tissue–attenuating, partially mineralized mass (arrow) occupying the width of the right nasal passage and dorsal-conchofrontal sinus. Inset: nasal passage endoscopy shows a large obstructive irregular mass covered with necropurulent exudate. **B.** Multiple spherical fungal structures admixed with many degenerate neutrophils and other inflammatory cells. H&E. Bar = 80 μ m. **C.** The wall of the round fungal structures is periodic acid–Schiff stain positive and Grocott methenamine silver stain positive (inset). Bar = 200 μ m. **D.** Lactophenol cotton blue stain of the fungal colonies identified numerous conidia in clusters, associated with parallel-walled and branching hyphae. Bar = 150 μ m.

silver, maritime pine, and potassium iodide, and topical treatment over the dorsal aspect of the sinuses and nose with frankincense, myrrh, and cannabis oil. The horse had no history of overseas travel.

Initial physical examination identified abundant mucopurulent exudate discharging from the right nostril, with subtle facial deformity over the right nasal bone and absent airflow from the right nostril. Dyspnea and stertor were also evident. Routine blood analysis including a complete blood cell count and serum chemistry showed only a moderate increase in the acute-phase protein serum amyloid A (382 mg/L, reference interval [RI]: 0–20 mg/L). On non-contrast computed tomography of the head, the right nasal passage and dorsal-conchofrontal sinus was filled with a large (10 \times 9 \times 6 cm), soft tissue–attenuating (average Hounsfield units (HU), 37 HU; RI: 14–66 HU), partially mineralized mass. The mass extended into the surrounding right-sided paranasal sinuses with bone erosion, mild leftward displacement of the adjacent septum, effacement of the ethmoturbinates, and disruption of the infraorbital canal (Fig. 1A). Dependent fluid density was present in the right maxillary, frontal, and sphenopalatine sinuses, with some fluid and mucosal thickening noted in the right dorsal meatus; these findings were most

consistent with rhinosinusitis. On endoscopy, a large tan multinodular mass was identified at \sim 10 cm from the right nostril opening, occluding the entire lumen of the right nasal passage (Fig. 1A inset).

Fresh samples from the right nasal mass were obtained on 2 separate occasions. An endoscopic incisional biopsy was obtained on admission. Subsequently, the mass was excised through a frontonasal sinusotomy. The mass was bluntly dissected free of its attachments and removed with sponge forceps. Hemorrhage was controlled by packing the sinus with gauze. An attempt was made to remove all visibly abnormal tissue. However, hemorrhage obscured the field and made inspection of the surgical margins difficult after the mass was removed. Therefore, as clean surgical margins could not be ascertained, potassium iodide therapy was continued for an additional 6 wk: 2 wk in hospital at a dose of 40 mg/kg/d; 4 wk following discharge at a dose of 20 mg/kg/d.

Both fresh samples were divided in half, one of which was submitted for bacterial and fungal culture, and the other for histopathology. The fresh sample from the latter collection was also submitted for fungal PCR, targeting the pan-fungal internal transcribed spacer (ITS) 1 and 2 regions, followed by conventional Sanger sequencing for fungal identification. On

multiple follow-up calls until 24 mo after discharge, the owner reported complete resolution of the problem; the mare was retired, in good health, and with no indication of recurrence of the sinonasal granuloma. We consider the resolution of the infection was mainly attributable to surgical resection of the granuloma, with potassium iodide administered as an ancillary therapy for treatment of any remaining unresected infected tissue.

On histopathology, both the initial endoscopic and debulked samples contained numerous circular-to-ovoid structures of ~60–80 µm diameter, with a refractile, thin, basophilic wall, and morphology consistent with either spore-like structures such as chlamydo-spores of basidio- and ascomycetes or spherules consistent with *Coccidioides* spp. (Fig. 1B). Notably, hyphae were not observed in the sections examined. The wall of these spherical structures was periodic acid–Schiff and Grocott methenamine silver stains positive, supporting a fungal origin for the spherical structures (Fig. 1C and inset). Most of these round bodies contained an empty center, but occasionally they contained irregular eosinophilic contents or infiltrating degenerate neutrophils; the latter likely represented leukocytic attempts to phagocytize or destroy these structures.

The inflammatory reaction surrounding these spherical structures consisted of an admixture of degenerate neutrophils, macrophages, and lesser numbers of lymphocytes and plasma cells in no particular arrangement in the initial biopsy. The subsequent debulked sample contained multiple pyogranulomas centered on the spherical structures. Multinucleate giant cells were frequent and often contained phagocytized fungal bodies. Within the inflammatory mass were multiple necrotic blood vessels, expanded by fibrin thrombi, consistent with mycotic vascular injury. There were also multifocal areas of necrosis, likely a result of infarction. Superficially, colonies of bacterial cocci of ~1 µm diameter were identified. Bacterial culture isolated a mixed but predominant growth of *Streptococcus equi* ssp. *zoepidemicus*, consistent with secondary superficial bacterial colonization by the most common commensal organism identified in the upper respiratory tract of horses.¹⁹

Fungal culture from the second biopsy sample performed on routine Sabouraud dextrose agar grew an initially glabrous (day 4 post-inoculation) to subsequently wrinkled (day 10 post-inoculation) white fungal colony, with light aerial mycelia, and no color change on the reverse side of the agar. Lactophenol cotton blue staining of the colonies identified numerous conidia in clusters, often associated with parallel-walled and branching hyphae (Fig. 1D). The absence of hyphal elements in the *in vivo* sample may represent a different growth pattern compared to that under *in vitro* conditions.

PCR was performed on both the fresh sample and the *in vitro* grown fungal colony, targeting the ITS1 and ITS2 regions. DNA sequence analysis of the ITS1, 5.8S, and ITS2 regions of the ribosomal DNA gene cluster using published

primers and standard sequencing methodologies was performed on the fungal culture.²⁰ The isolate's sequence was compared to those in GenBank using the BLASTn search tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and showed > 99.5% identity (597 base pairs [bp]) to *F. flavus* (FJ478126.1). Fresh tissue was referred for pan-fungal PCR testing also targeting the ITS1 and ITS2 regions of the ribosomal DNA.¹² *F. flavus* DNA was detected in both targets. The ITS1 (228 bp) and ITS2 (310 bp) sequences showed > 99% identity to GenBank sequences FJ478126.1 and MK075023.1. This confirmed that the intralesional mycotic agent was *F. flavus*.

Prior to 2018, *F. flavus* was only known as an environmental basidiomycete fungus with a coastal distribution associated with decaying vegetation.¹⁵ There were also reports of a symbiotic relationship with plants and insects,¹⁰ antibacterial and antifungal properties,¹⁰ and potential biotechnologic application.¹⁵ The pathogenic potential of *F. flavus* in a vertebrate host has been reported in a 12-y-old Fjord gelding, with a nasal granuloma similar to the one described in our report.¹⁴ The horse resided in an area of Florida with access to wetland and decaying wood (S. More and W. Castleman, pers. comm., 2019). This was comparable to the year of occurrence (2017), the coastal inhabitation, and climatic conditions at the time of presentation of our case. These similarities suggest that *F. flavus* is an under-recognized mycotic pathogen of the horse, and the relatively recent occurrence of these cases implies a recent emergence of *F. flavus* as a vertebrate-infecting fungus. Whole genome sequencing is required to identify potential newly acquired virulence determinants conducive for this host switching. Alternatively, infrequent molecular characterization of equine mycotic granulomas may have led to under-diagnosis and -reporting of *F. flavus* as a pathogenic fungus. Further studies into the epidemiology, genomic characterization, and *in vivo* pathogenesis of this fungus are required.

Despite some of the similarities mentioned above, there are a few differences between our case and the prior reported case.¹⁴ Most notable is the absence of hyphal elements associated with the spherical fungal structures in our biopsy samples. *F. flavus* belongs in the phylum of Basidiomycota.¹⁵ Basidiomycetes have been reported to produce chlamydo-spores, which are enlarged vegetative cells formed within hyphae or at hyphal tips and are hypothesized to be rest or survival structures.¹³ Although the prior report¹⁴ described the intralesional round structures as chlamydo-spores, we are cautious in identifying our structures as chlamydo-spores because hyphae were not observed in our samples. Alternative fungal structures such as yeast cells (e.g. *Cryptococcus neoformans*, a basidiomycete that can exist as yeast cells) or arthroconidia should be considered.^{8,11}

The character of the inflammation is also slightly different in our case compared to that described in the Florida case.¹⁴ The presence of more neutrophils in our samples is consistent with chronic-active inflammation. The ongoing inflammatory

stimuli could be a combination of factors within the wall of the fungal spherical structures and the presence of secondary bacterial infection with *Streptococcus equi* ssp. *zooepidemicus*. The latter was not present in the lesion of the Florida case,¹⁴ and thus may account for the difference in the inflammatory reaction. Alternatively, the variation in the inflammatory profile may reflect a difference in the chronicity of the cases or could, at least in part, have been triggered by the various topical treatments being applied by the owner.

In our investigation, differential diagnoses for pathogenic fungal or fungal-like agents with spherical morphology within lesions included *Coccidioides* spp.,⁹ *Rhinosporidium seeberi*,⁶ and *Emmonsia* spp.¹⁶ The 2 former differentials are exotic to Australia, and *Coccidioides* spp. was especially concerning given its zoonotic potential.⁹ Our horse was placed in quarantine during the interim phase of the diagnostic process. Other common differentials for mycotic rhinosinusitis in the horse such as *Conidiobolus* spp. and *Basidiobolus* spp. were also considered. The possibility of nonpathogenic artefacts such as micelles originating from the herbal remedies applied by the owner, however unlikely, was also considered. Sanger sequencing results confirmed that the causative agent was *F. flavus*. The possibility of environmental contamination of *F. flavus* in our samples was excluded based on: 1) the localization of numerous fungal structures within the suppurative and pyogranulomatous lesions; 2) ITS PCR of biopsy detected the presence of a fungus that was identified as *F. flavus* upon DNA sequencing; and 3) fungal culture of the biopsy isolated a fungus that was also identified as *F. flavus* by ITS PCR and sequencing. These findings confirmed that the etiologic agent causing the sinonasal pyogranuloma in this mare was *F. flavus*.

Acknowledgments

We thank Sunil More and William Castleman from the University of Florida for providing details of their case for comparison with our case.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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