

Case report

Invasive hyphomycotic rhinitis in a cat due to *Metarhizium anisopliae*

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Invasive mycotic rhinitis was diagnosed in a cat with a 4-month history of stertor, nasal discharge and subcutaneous swelling of the nasal bridge. Histology demonstrated fungal hyphae in representative biopsies of the nasal cavity and subcutaneous mass. Mycological culture demonstrated a pure growth of *Metarhizium anisopliae* var. *anisopliae*. The infection was treated with orally administered itraconazole. This is the first well documented case of infection by *M. anisopliae* in a mammalian host.

Keywords cat, hyalohyphomycosis, itraconazole, *Metarhizium anisopliae*, rhinitis

Introduction

In the dog, hyphomycotic rhinitis due to infection caused by *Aspergillus* or *Penicillium* spp. is a well described form of nasal cavity disease [1]. Over the years various treatment regimens have evolved and, currently, topical treatment with enilconazole [2] or clotrimazole [3] is considered the treatment of choice, except in cases of invasive disease in which adjunctive systemic therapy with itraconazole is indicated [2].

In the cat, *Cryptococcus neoformans* [4] is encountered much more frequently as a cause of nasal cavity disease than are filamentous fungi [5]. In fact, so few cases of hyphomycotic and phaeohyphomycotic rhinitis have been documented in the literature [6–11] that treatment regimens for the cat have not really been evaluated. Of the reported cases, only three cats [8,10,11] were treated successfully, although this may have reflected the limited number of antifungal agents available when these cases were seen. Widespread access to new effective broad-spectrum antifungals such as itraconazole [12,13] and new strategies for delivering older drugs such as amphotericin B [14] are likely to improve the prognosis for these infections in feline patients.

The diagnosis and treatment of a cat with hyphomycotic rhinitis are presented primarily because the causal organism *Metarhizium anisopliae* var. *anisopliae* has not been reported previously as a cause of mammalian disease.

Case report

History

A 7-year-old castrated domestic short-haired cat (7.5 kg) was presented to another veterinarian with stertorous breathing and sneezing of 1 months duration. A firm subcutaneous swelling was present over the nasal bridge, and palpation of this swelling resulted in discharge from the left nostril. The purulent material was submitted for laboratory examination. Cytology demonstrated neutrophilic (mainly) and eosinophilic inflammation, and a heavy growth of bacteria was reported. Neoplastic cells, capsulate yeasts or other fungal elements were not observed. Skull radiographs were unremarkable. The cat was given amoxycillin/clavulanic acid (125 mg combined orally every 12 h for 3 weeks) and clindamycin (75 mg orally every 12 h for 1 week), but stertor continued and the cat began sneezing with increased frequency. One month later bilateral mucoid nasal discharge was evident and sneezing persisted, although the lump on the nasal bridge was less prominent. A trial of prednisolone (10 mg

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orally every other day) combined with amoxycillin/clavulanic acid was then implemented, but failed to produce any discernible improvement.

Physical findings

Three months after initial presentation, the cat was seen at East Chatswood Cat Clinic for a second opinion. Physical findings included stertorous breathing, absence of air movement through the right nostril and a hard, prominent swelling of the nasal bridge. A slight bilateral nasal discharge was evident, the mandibular lymph nodes were enlarged, particularly on the right, and the cat frequently resorted to open-mouth breathing. It was considered that the cat had invasive nasal cavity disease, with the differential diagnosis consisting of infectious rhinitis, sinonasal neoplasia or a migrating foreign body.

Investigation

Diagnostic procedures were performed at Sydney University Veterinary Teaching Hospital under general anaesthesia. Multiple 'pinch' biopsies were obtained from the right side of the nasal cavity via the right dorsal nasal meatus using cup-tipped alligator forceps. In addition, an incision was made on the midline directly over the swollen nasal bridge and abnormal tissues debrided down to bone using a No. 15 scalpel blade. The abnormal subcutaneous swelling consisted of 'veins' of brown marbled tissue interspersed with off-white fibrous tissue. The incision on the nasal bridge was closed using single interrupted sutures. The various tissue specimens were submitted for laboratory investigations, although only excised material obtained aseptically from the nasal bridge was used for culture.

Cytopathology, mycology and histopathology

Diff-Quik stained squash preparations of the excised brown material demonstrated necrotic tissue containing masses of fungal hyphae (Fig. 1). Eosinophils featured in the cellular reaction, in addition to numerous neutrophils and scattered macrophages, mast cells, plasma cells and spindle cells. Tissue specimens were diced and implanted onto duplicate Sabouraud glucose agar containing chloramphenicol and gentamicin and incubated at 28 °C and 37 °C. Biopsy material was also ground using a sterile mortar and pestle and inoculated onto duplicate plates at 28 °C and 37 °C. A filamentous fungus was isolated after 3–4 days of incubation at 28 °C, with growth occurring, but more slowly, at 37 °C. Moderate to heavy pure growth occurred on all plates. Cycloheximide-containing medium was not inoculated.

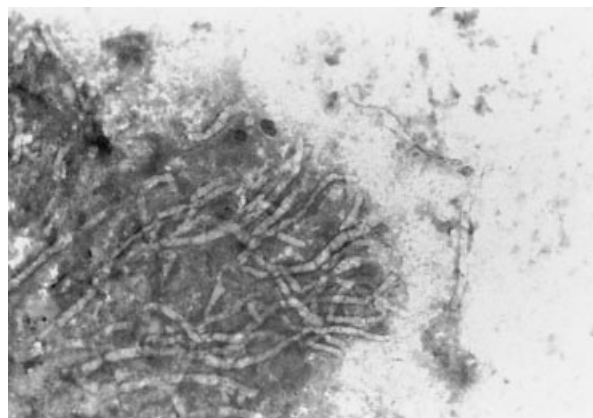


Fig. 1 Cytopathology of a squash preparation from excised subcutaneous tissue from the nasal bridge. Masses of hyphae are evident within necrotic tissue. Diff Quik; $\times 132$.

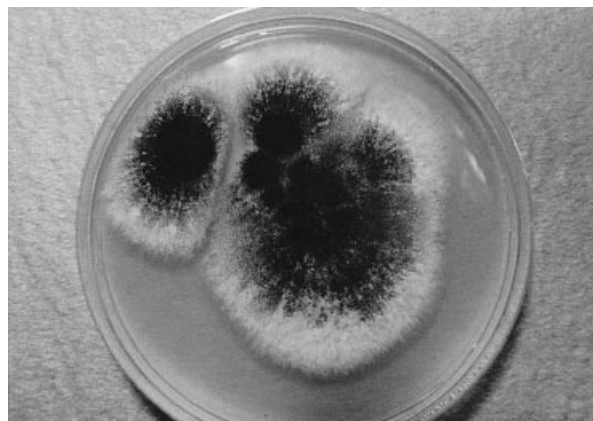


Fig. 2 Colonies of *M. anisopliae* on potato glucose agar.

The isolate was identified as the hyphomycete *Metarhizium anisopliae* [15,16] by a reference laboratory. On subculture, the isolate grew well at 25 °C (35 mm after 7 days) on potato glucose agar (BBL), producing a dark olive-green colony with a colourless reverse (Fig. 2). It did not grow at either 5 °C or 35 °C. This would represent another strain of the type 14 designated by Yip *et al.* [17]. The fungus was identified as *Metarhizium anisopliae* var. *anisopliae* on the basis of conidial dimensions and descriptions reported by Tulloch [15]. It produced long, dry, adherent chains of dark olive green cylindrical conidia (average $6.4 \times 2.5 \mu\text{m}$) with a rounded apex and slightly tapering truncate base. The side walls were straight. The conidia were produced from phialides in aggregated conidiophores (Fig. 3). The isolate has been retained in the culture collection of the Australian National Reference Laboratory in Medical Mycology as AMMRL 154.01.

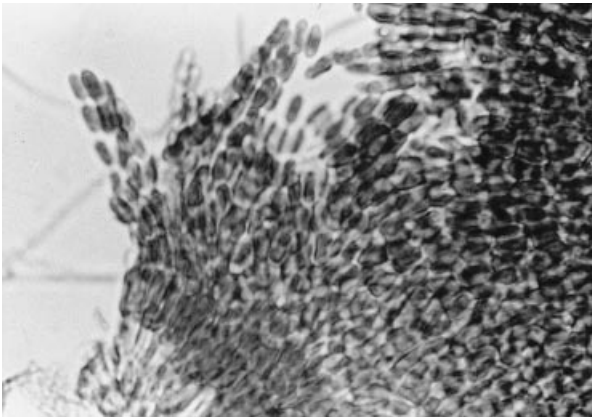


Fig. 3 Photomicrograph showing conidial morphology of the *M. anisopliae* var. *anisopliae* strain isolated from a cat with invasive hyphomycotic rhinitis, grown on cornmeal Tween 80 agar. Lactophenol cotton blue; $\times 309$.

Histologically, the subcutaneous swelling over the nasal bridge was composed of masses of inflammatory cells, featuring plasma cells, eosinophils, macrophages and abundant necrotic material in which there were many fungal hyphae. The nasal biopsies showed chronic inflammation, some epithelial and glandular hyperplasia and a small number of fungal elements. Fungal hyphae were much more discernible in periodic acid-Schiff stained sections, but could also be visualized in haematoxylin and eosin-stained sections.

The diagnosis was invasive hyphomycotic rhinitis due to *M. anisopliae* var. *anisopliae* with spread of the infection through the nasal bones to the subcutaneous tissues overlying the nasal cavity. Blood was subsequently collected for determination of feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) status: the cat tested negative for FeLV antigen and FIV antibodies.

Treatment

The infection was treated using itraconazole (initially one capsule [100 mg] orally with food once a day [13]). After 2 weeks the cat's breathing had improved, with better air movement through the right nostril, reduction of sneezing and nasal discharge and healing of the biopsy incision over the nasal bridge. Because anorexia developed after 23 days of therapy, itraconazole was suspended for 10 days, then re-implemented at a reduced dose of 50 mg once a day for 80 days. The dose was then increased to 75 mg per day for a further 32 days, when therapy was altered to ketoconazole (50 mg once a day for 80 days) for financial reasons. Clinical signs of mycotic rhinitis, including involvement of the subcutaneous tissues of the nasal bridge, recurred 3 months after discontinuing treatment,

at which time the cat's owners were unprepared to pursue further therapy.

Discussion

This case represents infection of an apparently immunocompetent host with a soil organism thought to be of inherently low virulence. Infection may have arisen in a variety of ways. A large inoculum of hyphae or phialoconidia may have been introduced traumatically, resulting in a slowly progressive infection of the nasal cavity with later extension through the nasal bones to the subcutaneous tissues over the nasal bridge. Alternatively, the inhalation of conidia with subsequent germination and growth due to unknown factors, combined with some breach in the integrity of the nasal mucosa, may have permitted invasion of the fungus. Penetration of bone with spread of infection to adjacent soft tissues has been a feature of nasal aspergillosis, *Penicillium* hyalohyphomycosis and pythiosis in cats, whereas invasion occurs in less than 10% of dogs with comparable infections. The long time course of the infection and failure to disseminate, despite inappropriate dosing with corticosteroids, suggest that the cat was immunocompetent; presumably the initial infection resulted from a very transient disruption of the host-parasite relationship which permitted implantation of the fungal elements into susceptible host tissues from which they could not be eliminated readily. The prompt improvement following appropriate antifungal therapy supports this notion.

Although *M. anisopliae* has been isolated from the sputum of a human patient [18] and a corneal lesion in a 19-year-old Columbian man [19–22], this report is the first detailed account of infection in a mammalian host. We are also aware of a case of widely disseminated disease due to *M. anisopliae* involving skin, lung and brain in a 9-year-old boy receiving chemotherapy for acute lymphoblastic leukaemia (D. Muir, unpublished observations [23]).

Interestingly, *M. anisopliae* is known as the cause of 'green muscardine' disease in a variety of insect hosts [20]. Attempts have been made to use the fungus for the biological control of insect pests in pastures in New Zealand [20], and its potential use for the biological control of termite infestations has been reported recently [21]. Given the potential for this organism to cause disease in immunocompromised hosts, precautions in dispersing high concentrations of this agent would be prudent if its use in the biological control of termites and other insect pests were to become more commonplace.

The present case highlights the need to obtain representative samples of tissue when investigating the aetiology of

nasal cavity disease. Superficial samples, such as discharges, are unlikely to provide a definitive diagnosis, because they are not necessarily representative of the primary disease process. Deeper samples from within the nasal cavity are obtained readily using small cupped alligator forceps which pinch off small tissue specimens. Furthermore, in cases such as this when the infection has penetrated through overlying bone, representative tissue is even more readily obtained by biopsy of affected nearby subcutaneous tissues. Indeed, debulking infected subcutaneous tissues is of therapeutic as well as diagnostic value, and has the additional benefit of providing the laboratory with a superior sample for mycological studies. Although not performed in the present case, rhinoscopy may provide useful additional information [22].

Itraconazole provided effective antifungal therapy in the present case, producing a prompt improvement in clinical signs, despite long-standing invasive disease and prior dosing with corticosteroids. Itraconazole is a broad-spectrum triazole antifungal that is generally more effective than the imidazole agent ketoconazole, although it is substantially more expensive [12]. Therapeutic antifungal doses of itraconazole are generally better tolerated by cats than doses of ketoconazole of similar clinical efficacy. At high doses side effects such as decreased appetite and vomiting referable to hepatotoxicity may appear but are reversible following appropriate dose reductions [12,13]. In hyphomycotic infections refractory to monotherapy with itraconazole, treatment with amphotericin B, either alone, or in concert with itraconazole or flucytosine, may provide other options for treatment. A safe, practical and effective means for administering amphotericin B to cats with minimal nephrotoxicity has been described recently [14]. Intranasal instillation of antifungal agents [2,3] may be considered as another therapeutic option. Recurrence of infection in the present case was most probably due to an insufficiently long period of treatment, as the cat had become completely asymptomatic during administration of itraconazole. A longer period of treatment would probably have given more time for residual foci of infection to have been permanently eliminated by normal host mechanisms.

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