Case Report

Treatment of a mare for Conidiobolus coronatus infection


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Introduction

Zygomycosis refers to a group of uncommon mycoses caused by fungi in the class Zygomycetes, which includes the orders Mucorales and Entomophthorales. The order Entomophthorales includes the saprophytic fungi Conidiobolus (previously Entomophthora) spp. coronatus, incongruus and lamprauges, and Basidiobolus spp. ranarum and haptosporus (Ribes et al. 2000; Gonzalez et al. 2002; Prabhu and Patel 2004). Basidiobolus and Conidiobolus spp. may cause chronic subcutaneous infection in immunocompetent hosts, and infection caused by C. coronatus has been described in horses, mules, dogs, llamas, dolphins and man (Carvalho et al. 1976; Sweeney et al. 1976; Gugnani 1992; Moll et al. 1992; French and Ashworth 1994; Grooters 2003). The first reported occurrence of infection caused by C. coronatus in horses was by Bridges et al. (1962). Since then, Conidiobolus infection in horses has been sporadically identified in the southern United States, South America, Australia and India (Miller and Campbell 1982, 1984; French et al. 1985; Korenek et al. 1994; Zamos et al. 1996; Steiger and Williams 2000; Taintor et al. 2004).

In all species, conidiobolomycosis (also described as nasofacial zygomycosis or rhinoentomophtoramycosis) is characterised by pyogranulomatous inflammation that is generally localised to the nasopharyngeal tissue, with or without local dissemination to the face, retropharyngeal region, retrobulbar space, soft palate or trachea (Korenek et al. 1994; Zamos et al. 1996; Steiger and Williams 2000). Disseminated zygomycosis due to C. coronatus has been reported occasionally in both immunocompromised and immunocompetent human patients (Ribes et al. 2000), but has not been reported in the horse. Conidiobolus incongruus has caused rhinocerebral and nasal infection in sheep and systemic infection in man and deer (Carrigan et al. 1992; Ketterer et al. 1992; Walsh et al. 1994; Stephens and Gibson 1997). Conidiobolus lamprauges caused pharyngeal zygomycosis in an Arabian mare (Humber et al. 1989).

This case report describes the persistence progression of C. coronatus infection in a mare over 4 years, despite prolonged oral administration of potassium iodide, and the resolution of infection after aggressive resection of infected tissue. To our knowledge, this is the first report that describes removing the nasal septum and infected nasal mass for the treatment of a horse with chronic conidiobolomycosis.

Case details

History

A 4-year-old Arabian filly was presented to The Ohio State University in October 2001 because of intermittent, bilateral, mucosaemorrhagic nasal discharge of approximately one year’s duration and poor body condition, despite a normal appetite. The owner had observed the nasal discharge since the filly was purchased from a farm in West Virginia but did not know when the abnormal nasal discharge first appeared prior to purchase. Since being acquired by the owner, the filly had been housed in a pasture on a farm in Ohio. Antimicrobial drugs (unknown type and dosage) had been administered, prescribed by the referring veterinarian, for 2 weeks without improvement of clinical signs. The referring veterinarian had identified multifocal ulcerative and granulomatous lesions in the nasopharynx during endoscopic examination of the upper portion of the filly’s airways.

Clinical and diagnostic findings

When presented to our hospital in October 2001, the filly appeared bright and alert. Estimated body condition score was 4/9. The heart rate at rest was 56 beats/min, respiratory rate 30 breaths/min and rectal temperature 38°C. Slight, bilateral serosanguinous nasal discharge, harsh inspiratory sounds during auscultation of the trachea and lungs, and enlarged submandibular lymph nodes were identified. Additionally, endoscopic examination of the upper portion of the airways revealed bilateral, multifocal to coalescing granulomatous, and occasionally ulcerated, lesions on the nasal septum.

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Cytological smear examination of a lesion of the nasal septum revealed numerous eosinophils and neutrophils amongst clusters of epithelial cells with cytoplasmic basophilia and enlarged nuclei. Two wedge biopsies of 2 nasal septum lesions, obtained under sedation, were submitted for histopathology, and bacterial and fungal culture. Tissue submitted for culture was immediately plated onto Sabouraud’s agar with dextrose and placed into a brain heart infusion medium.

Radiographic examination of the filly’s skull revealed a moderately thickened nasal septum on a dorsoventral view, and radiography of the thorax revealed a mild, diffuse interstitial pattern, compatible with mild, chronic lower airway disease. A complete cell blood count revealed leucocytosis (19.1 x 10^9 cells/l; reference range [rr] 4.7–10.6 x 10^9 cells/l), due to a mature neutrophilia (14.5 x 10^9 cells/l; rr 2.4–6.4 x 10^9 cells/l), and serum biochemistry profile revealed hyperglobulinaemia (47 g/l; rr 36–43 g/l). Fibrinogen concentration was within the reference range (3.84 g/l; rr 1.93–4.22 g/l). No lesions or abnormalities were noted during ultrasonographic examination of the abdomen or during palpation per rectum. No abnormalities were noted on cytological examination of peritoneal fluid. At the time of examination no signs of systemic disease were recognised.

Histological examination of one of lesions on the nasal septum confirmed the presence of multifocal ulceration and hypertrophy of the nasal glandular epithelium. The submucosa was diffusely effaced by multiple eosinophilic nodules surrounded by epithelioid macrophages and occasional multinucleated giant cells. Numerous eosinophils, lymphocytes and plasma cells infiltrated the submucosa surrounding these areas of granulomatous and eosinophilic inflammation. Within the eosinophilic nodules, inclusions...
suspected to be fungal in origin were identified (Fig 1a). Histochemical periodic acid-Schiff (PAS), Gomori methenamine-silver (GMS) and Gridley’s stains performed on the tissue sections confirmed the presence of fungal hyphae. All hyphal capsules stained positively with PAS and Gridley’s stain (Figs 1b and 1d) and lightly with GMS stain (Fig 1c). The fungal hyphae were 10–30 µm in diameter, rarely branched and minimally septated.

Bacterial culture revealed numerous species of opportunistic bacteria normally resident in the nasal cavity. Fungal culture performed on a different portion of the same tissue resulted in the rapid growth of fungal colonies on the culture plates (Fig 2). Morphological characteristics of the conidia produced from these colonies, such as prominent papilla at the site of former attachment to the sporangiophore, resulted in the identification of the organism as Conidiobolus coronatus (Fig 3) (Ribes et al. 2000).

The collective results of the diagnostic tests performed to this point were consistent with an eosinophilic and granulomatous, chronic, mycotic rhinitis localised to the nasal cavities. Conidiobolus coronatus was determined to be the primary aetiological agent.

Treatment and follow-up

Following initial examination in 2001, the horse was placed on therapy for fungal rhinitis using orally administered potassium iodide starting at a dosage of 10 g/day (approximately 20 mg/kg bwt) and gradually increasing to 20 g/day over 3 weeks. Administration of potassium iodide was continued for several weeks, but follow-up information was unavailable until 2003, when the owner contacted our hospital because he observed a small mass at the right external naris of the mare. Administration of potassium iodide therapy (20 g/day, per os) was reinstated. Potassium iodide was administered for several months without side-effects. Two years later, in March 2005, the owner reported to the referring veterinarian that the mass in the right nostril had dramatically increased in size during the preceding 5–8 weeks, that another mass was growing in the left nostril, and that the mare had an increased respiratory effort. The mare was referred to the Ohio State University Veterinary Teaching Hospital.

When presented, the mare had mild respiratory distress, a respiratory rate of 48 breaths/min, and audible upper airway noise. A large ulcerated mass, approximately 8 cm in diameter, was attached to the right alar fold, and a smaller mass, approximately 4 cm in diameter, was attached to the left alar fold (Fig 4). Endoscopical examination of the nasal cavities revealed a pitted, and mottled appearance to the right and left side of the nasal septum. Radiographic examination of the skull revealed a thickened nasal septum on a dorsoventral view; the 2 masses attached to the alar folds did not appear to involve bony and cartilaginous structures (Fig 5).

A complete cell blood count revealed a mild, microcytic anaemia (PCV 25%; rr 27–44; haemoglobin 90 g/l; rr 97–156 g/l; MCV 38 fl; rr 43–55 fl) and a leucocytosis (14.9 x 10⁹ cells/l; rr 4.7–10.6 x 10⁹/µl) due to a mature neutrophilia (12.1 x 10⁹ neutrophils/l; rr 2.4–6.4 x 10⁹/µl) and mild monocytosis (0.6 x 10⁹ monocytes/l; rr 0–0.5 x 10⁹/µl). The fibrinogen concentration was within the reference range, and a serum biochemistry profile revealed hypoalbuminaemia (19 g/l; rr 28–36 g/l), and hyperglobulinaemia (58 g/l; rr 36–43 g/l). Activated partial thromboplastin time (APTT) and one-stage prothrombin time (OSPT) were within the reference range.

Because the masses were obstructing the nasal cavities, causing difficult respiration, resection of the masses was planned for both diagnostic and therapeutic purposes. The evening prior to surgery, in the presence of a mild anaemia and with considerable risk of profuse bleeding during surgery, the horse received 4 l of fresh, whole blood from a compatible donor (based on cross-matching testing). Prior to surgery the horse received antibiotics (ampicillin 10 mg/kg bwt i.v. q.i.d., gentamicin 6.6 mg/kg bwt i.v., s.i.d.), phenylbutazone (2.2 mg/kg bwt i.v., b.i.d., followed by 4.4 mg/kg bwt, s.i.d. per os) and tetanus toxoid. The horse also received a temporary tracheotomy tube a few hours preceding the surgery because it had severe respiratory distress attributed to bilateral nasal obstruction.

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**Fig 5:** Radiographs of the skull (dorsoventral, a): Nasal septum thickening, and (lateral, b): Large pedunculated soft tissue mass in the region of the nares.
Surgery

The mare was anaesthetised and initially placed in dorsal recumbency. A small section of the right submandibular lymph node was excised and submitted for histology. The nasal septum was removed using a modification of the previously described 3 wire method (McIlwraith and Robertson 1998; Doyle and Freeman 2005). A ventral midline laryngotomy was performed and 2 flexible plastic, artificial insemination (AI), pipettes were inserted through the laryngotomy incision and directed rostrally one on each side of the nasal septum, until they were exposed at the nostrils. An end of a loop of obstetrical wire was inserted into each one of the pipettes at the laryngotomy site and the wire ends exited at the nostrils, in order to form a loop around the caudoventral nasal septum (ventral wire). The pipettes were removed, and the wire ends were secured with forceps. The mare was then placed in left lateral recumbency and transported to the surgical suite.

Each nasal mass was completely dissected from the alar fold. The head position was adjusted during dissection of the left nasal mass. Haemorrhage was controlled with haemostatic forceps. The skin of the alar folds was apposed applying traction to the septum with Vulsellum forceps (Fig 6). A 1.5 cm trephine hole was made on the dorsal midline immediately rostral to the conchofrontal sinus in order to access the right and left dorsal meatus of the nasal cavities. Two AI pipettes were inserted through the trephine hole and directed caudally, one on each side of the nasal septum, until the ends were exposed at the laryngotomy. Each end of a loop of obstetrical wire was passed through a pipette to form a loop around the caudal aspect of the ventral nasal septum in preparation for the caudal septal cut, and the pipettes removed.

Two AI pipettes were inserted from the trephine hole rostrally, one on each side of the nasal septum until the ends were exposed at the nostrils. A small stab incision was made dorsally at the juncture of the nasal bones and septum, approximately 3 cm caudal to the rostral limit of the septum. Obstetrical wire was passed through the pipette in the left nasal cavity. Using a haemostat, the wire was pulled through the stab incision from the left nasal cavity to the right and passed caudally up the pipette in the right nasal cavity and out of the trephine opening. The pipettes were pulled from the trephine opening.

The cranial portion of the septum was incised in a slightly semicircular fashion. The wire ends for the ventral cut were both exited at the right nostril to prevent accidental cutting of the cranial strut of the septum, which must be left intact to support the nostrils. The ventral, dorsal and caudal septal cuts were then performed using the preplaced loops of obstetrical wire, and the resected portion of the nasal septum was exteriorised en bloc through the right nostril by grasping and applying traction to the septum with Vulsellum forceps (Fig 6). A small section of the nasal septum was submitted for histological examination. To control haemorrhage, a stockinette filled with absorbent packing material was inserted through the trephine hole and passed into the nasal cavity. An absorbent cloth was inserted through the right nostril into the nasal chamber and secured with 2–0 polypropylene suture. The trephine hole and the laryngotomy were left unsutured to heal by secondary intention.

Histological examination of the submandibular lymph node biopsy revealed reactive lymph node tissue. Histological examination of the biopsy of the nasal septum and nasal masses revealed a pyogranulomatous eosinophilic dermatitis, and fungal organisms were seen at the centre of some of the granulomatous foci. Conidiobolus coronatus was cultured from one of the lesions.

Antibiotics were discontinued the day after surgery. The nasal packing was removed 72 h after surgery. Administration of phenylbutazone was discontinued at that time. While the horse was hospitalised, a persistent, mild mucopurulent exudate drained from both nostrils, and the laryngotomy site. The tracheotomy tube was removed 4 days after surgery, and the mare was discharged 6 days after surgery. Care of the laryngotomy, tracheotomy and the trephine hole was continued at home. At the time of discharge the owners were instructed to orally administer sodium iodide daily (35 mg/kg bwt).

Outcome

Forty-four days after surgery, the mare was readmitted to the hospital because of facial cellulitis, periorbital abscesses, and a profuse, mucopurulent malodorous nasal discharge (Fig 7). The owner reported that care of the surgical incisions had been difficult due to the mare’s fractious nature. A serum biochemistry profile revealed a polyclonal hyperglobulinaemia, compatible with inflammation, and a mild hyperfibrinogenaemia (5.31 g/l). Endoscopic examination of the nasal cavities revealed the presence of necrotic tissue and distortion of what remained of the caudal aspect of the nasal septum. A large amount of mucopurulent discharge was also observed at the drainage angle in the middle meatus of the left naso-maxillary opening. Radiographic examination of the skull revealed the presence of small osseous fragments adjacent to the trephine hole and opacities of soft tissue within the left and right maxillary sinuses. Two small periorbital abscesses were lanced routinely with the horse sedated, and the nasal chamber was flushed daily for 10 days through the trephine hole with a dilute solution of povidine iodine. On the tenth day, the nasal cavity and the caudal left maxillary sinus were copiously flushed with sterile physiological saline solution with the horse anaesthetised. During the procedure, fragments of necrotic bone and tissue from the nasal septum were extracted and submitted for bacterial and fungal culture and histological examination. Streptococcus zooepidemicus was isolated during culture, and histology revealed a severe, suppurative osteitis with osteonecrosis, osteolysis and numerous coccoid bacteria. Fungal organisms were not identified.

The mare was discharged and continued to be treated with orally administered sodium iodide (35 mg/kg bwt) at home.
Facial cellulitis resolved completely, and the nasal discharge ceased within 2 weeks. The trephine hole healed by secondary intention. A follow-up endoscopic examination of the nasal passages 2 months after surgery revealed healing of the caudal stump of the nasal septum. The nasal chamber was devoid of discharge, necrotic tissue and debris (Fig 8). Eight months after surgery, the owner reported that the mare had no nasal discharge, facial swelling or visible masses, so administration of sodium iodide was discontinued. One year after surgery the referring veterinarian and the owner reported that the mare showed no signs of recurrence of mycotic infection.

Discussion

Fungi of the genus *Conidiobolus* are ubiquitous in the soil and are typically found in decaying vegetable matter in tropical and subtropical climates (Ribes et al. 2000; Gonzalez et al. 2002). Infection in horses is uncommon. The principal mode of transmission is through inhalation of spores from environmental sources, though cutaneous or percutaneous inoculation is possible. Although most Zygomycetes are opportunistic pathogens that may cause infection in immunocompromised individuals, members of the genera *Conidiobolus* and *Basidiobolus* are able to cause infection in immunocompetent hosts (Gonzalez et al. 2002). Prolonged housing on damp bedding rich in organic matter and fungal spores may overwhelm a competent host immune system through the persistent inhalation of the infecting spores (Gugnani 1992; Gonzalez et al. 2002).

Clinical signs of *Conidiobolus* infection are similar among species. *Conidiobolomycosis* develops as a slowly progressive pyogranulomatous disease of the external nares, nasal cavities, paranasal sinuses, pharynx, larynx and trachea. Infection is typically restricted to the submucosa and is characterised by polyps or palpable subcutaneous masses. Clinical signs may include serosanguinous or mucopurulent bilateral nasal discharge, multiple granulomatous masses, facial swelling and occlusion of the nasal cavities resulting in dyspnoea (Bridges et al. 1962; Korenek et al. 1994; Zamos et al. 1996; Ribes et al. 2000; Steiger and Williams 2000; Taintor et al. 2004). The condition in man is typically described as being nonpainful and, as in this horse, the most severe consequence of infection tends to be related to the occlusion of air flow by masses occupying the nasal cavities (Ribes et al. 2000; Prabhu and Patel 2004).

Fungi implicated in upper respiratory mycotic infections in horses include *Aspergillus*, *Blastomyces*, *Cryptococcus*, *Rhinosporidium*, *Coccidioides immitis*, *Pythium insidiosum*, *Coniodobolus coronatus*, *C. lamprauges*, *Basidiobolus ranarum*, *B. haptosporus*, fungi of the order *Mucorales*, and *Pseudoallescheria boydii* (Greet et al. 1981; Nickels 1993;

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**Fig 6:** Resected nasal septum, lateral view (a) and dorsal view (b). The nasal septum appeared thickened and mildly distorted.

**Fig 7:** Post operative facial cellulitis and periorbital abscesses surrounding the trephine hole that occurred 44 days post operatively.

**Fig 8:** Nostrils appearance approximately 2 months post operatively.
Davis et al. 2000). Neoplasia, amyloidosis and glanders (Burkholderia mallei) are other causes of nasal occlusion.

Diagnosis of conidiobolomycosis is based on identification of the causative organism during histological examination and culture of the infective organism. Histologically, pyogranulomatous inflammation with diffuse eosinophilic dermatitis is typical of Zygomycosis, but recognition of this alone does not permit differentiation between Conidiobolus, Basidiobolus and Pythium infections. An amorphous to granular eosinophilic material that surrounds fungal hyphae (eosinophilic sleeves or Splendore-Hoepli phenomenon) is present in tissues infected with either Conidiobolus or Basidiobolus (Ribes et al. 2000). Conidiobolus hyphae are broad, thin-walled and occasionally septate. Conidiobolus hyphae may not be identified with H&E stain. They are generally recognised when stains intended for the identification of fungal hyphae are used (e.g. PAS and GMS). However, in some cases, the paucity of organisms, the presence of a diffuse, and often obscuring, infiltration of inflammatory cells (i.e. eosinophils), or the failure of hyphal walls to uptake the stain, can render the identification of Conidiobolus hyphae difficult.

Definitive diagnosis of conidiobolomycosis is based on fungal culture and morphological characteristics of the conidia. The primary conidia of Conidiobolus spp. are spherical, 12–40 µm in diameter, and have a prominent papilla at the site were they were attached to the sporangiophore. Villous conidia, with hair-like projections, may be obtained from old cultures (Grooters 2003). Serological and polymerase chain reaction tests are available (Steiger and Williams 2000; Ribes et al. 2000; Taintor et al. 2004).

Although C. coronatus infection in the horse is generally localised, treatment of affected horses can be challenging. Prompt diagnosis, aggressive excision, and prolonged medical therapy may lead to resolution. Complete excision is often limited by the location and extent of a lesion, and therefore, medical therapy is commonly prescribed. No single drug is universally effective for the treatment of human Conidiobolus infections. Medical treatment of infected horses has included parenteral administration of iodine (Zamos et al. 1996; Steiger and Williams 2000; Ribes et al. 2000; Taintor et al. 2004).

The oral dosage of sodium or potassium iodide used to treat the horse in the present case was about 35–40 mg/kg bwt. Toxicity following excessive consumption of iodine has been reported in mares and their foals (Plumb 1999; Wehr et al. 2002; Eroksuz et al. 2004). Excessive iodine supplementation to mares has been associated with greater than normal incidence of abortion and enlarged thyroid glands (goitre) and limb deformities in foals (Eroksuz et al. 2004). Lacrimation, nasal discharge, coughing, dry scaly hair coat, alopecia, inappetence and diarrhoea may develop in horses chronically exposed to large amounts of iodine. Potassium and sodium iodide were each administered to the horse in this report for several months without any of the reported signs of toxicity, and although the efficacy of these agents in the treatment of this horse could not be established, we speculate that these drugs slowed the progression of the infection.

Surgical approaches for removal of the nasal septum have been described in horses (Nickels 1993; McIlwraith and Roberston 1998; Doyle and Freeman 2005). These techniques were modified in this case to include a laryngotomy approach, because we had determined that the most caudal portion of the diseased nasal septum had to be removed. The laryngotomy approach improved access to the caudal portion of the nasal septum and probably decreased the surgical time. Post operatively, the laryngotomy site healed without complication by secondary intention. Facial cellulitis, associated with the trephine hole, was initially thought to be due to an ascending C. coronatus infection from the nasal chamber, but fungal organisms were not cultured from tissue surrounding the trephine hole despite repeated fungal culture. Therefore, this complication probably occurred due to the severe inflammation of the remaining portion of the nasal...
septum and the poor post operative care allowed by the fraxious nature of the mare. This was supported by the resolution of the cellulitis and sinusitis after lavage of the nasal chamber and caudal left maxillary sinus.

Local post operative recurrence at the site of resection is common and continuation of the antifungal medical treatment post operatively is highly recommended in other species (Grooters et al. 2003). Recurrence of infection has been reported up to 17 months post operatively (Zamos et al. 1996). Therefore, it is important to perform short- and long-term examinations to determine if there are any clinical signs to suggest a recurrence and to determine when medical therapy may be discontinued. In this case, no evidence of recurrence had been observed 12 months after surgery. Consequently, the owner elected to discontinue iodine therapy and intends to breed the mare.

In the case reported here, aggressive surgical excision of infected tissue and prolonged post operative medical treatment with potassium and sodium iodide resulted in the resolution of conidiobolomycosis. The surgical technique used is, to our knowledge, the first to incorporate a laryngotomy approach to aid excision of a caudal portion of the nasal septum. Though the contribution of prolonged potassium or sodium iodide therapy to the resolution of infection in this horse was not established, both were well tolerated at the dosages administered (35–40 mg/kg bwt/day). Although delayed resection of the infected tissue in this horse did not appear to negatively affect the outcome, prompt diagnosis of the disease and resection of infected tissue accompanied by pre- and post operative medical therapy is recommended for the treatment of horses with conidiobolomycosis.

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**References**


