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Sino-orbital aspergillosis with obstructive cervical lymphadenopathy in a cat caused by *Aspergillus viridinutans* complex

Zhe Wang[1], Joshua B Daniels[2], Michael Lappin[1], Tawfik Aboellail[2] and Sarah Shropshire[1]

Abstract

Case summary This report describes an indoor-only cat with a rare form of sino-orbital aspergillosis (SOA) with cervical lymphadenopathy causing local obstruction. Extensive work-up on initial presentation failed to identify the underlying etiology and the diagnosis was not determined until the disease progressed during a prolonged course of glucocorticoid therapy.

Relevance and novel information SOA caused by *Aspergillus viridinutans* complex is increasingly recognized as a significant cause of mortality in cats in recent years, with most cases reported in Australia, Europe and Asia. Feline SOA carries a poor prognosis owing to its invasive nature and resistance to antifungal therapy. This case demonstrates the importance of clinical awareness of SOA as a differential for cats with chronic nasal signs and exophthalmos in the USA. Moreover, it demonstrates a rare form of presentation and potential difficulty in achieving a correct diagnosis.

Keywords: Aspergillosis; lymphadenopathy; sino-orbital; fungal

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Case description

A 10-year-old female spayed domestic shorthair cat was presented for chronic sneezing, coughing and nasal congestion of 4 months duration. A treatment trial with amoxicillin trihydrate/clavulanate potassium (Clavamox; Zoetis), cefovecin (Convenia; Zoetis) and a tapering course of prednisolone did not yield clinical improvement. In the interim, the cat became lethargic and hyporexic and was presented for further evaluation to our Urgent Care service (day 1). Physical examination revealed increased upper airway sounds with mildly increased inspiratory effort, gingivostomatitis, bilateral ocular and nasal mucopurulent discharge, and exophthalmos OS with decreased retropulsion and marked pain, as well as an obese body condition. Complete blood count (CBC) revealed mature neutrophilia (23,000/µl, reference interval [RI] 2000–12,000/µl). Chemistry panel showed hyperglycemia (182 mg/dl, RI 68–140 mg/dl), hyperglobulinemia (8.0 g/dl, RI 2.7–4.2 g/dl) and hypocholesterolemia (75 mg/dl, RI 95–270 mg/dl). Urinalysis revealed a concentrated urine (urine specific gravity 1.064) with proteinuria (2+). Thoracic radiographs revealed a faint

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increased soft tissue opacity in cranial mediastinum but was otherwise normal. A point-of-care retrobulbar ultrasound was performed, showing a hyperechoic structure OS with no obvious foreign material. Fine-needle aspiration and cytology was performed, revealing suppurative inflammation with necrotic material. Culture and additional stains, such as acid fast and Grocott methenamine silver (GMS) stain, were not performed at this time. A bacterial retrobulbar abscess was suspected, so gabapentin (compounded from gabapentin; Camber), amoxicillin–clavulanate, mirtazapine (Miratraz; Dechra) and robenaxocib (Onsior; Elanco) were dispensed with the plan to follow up with internal medicine in 3 days. Amoxicillin–clavulanate was chosen because of its broad-spectrum coverage and information about previous antibiotic dosage and duration was unavailable at the emergency visit.

On day 3, a physical examination revealed resolving OS exophthalmos, stertor, decreased bilateral nasal flow (left worse than right) and mildly enlarged bilateral mandibular lymph nodes. A rapid immune-migration assay for feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) (Witness FeLV/FIV Rapid Test; Zoetis) were negative. CT of the head revealed marked soft tissue attenuating material within the nasal cavities bilaterally with no turbinate lysis or mass effect, mild lysis of the mid lateral aspect of the left frontal sinus, and marked bilateral medial retropharyngeal, mild lateral mandibular and mild right superficial cervical lymphadenopathy. Bilateral ultrasound-guided lymph node aspirates revealed marked mixed inflammation (eosinophilic, histiocytic and suppurative). Culture of lymph node fluid was submitted and showed no growth. Antegrade rhinoscopy did not reveal any fungal plagues or mass-like structures and was consistent with severe diffuse rhinitis. Retrograde rhinoscopy was not performed. Nasal cavity biopsies were submitted for histopathology and revealed severe, diffuse, chronic-active, erosive and eosinophilic rhinitis with no organism identified on Gram, GMS and acid-fast stains. An upper respiratory infectious PCR panel (feline herpesvirus/*Chlamydia*/*Mycoplasma*) on oral pharyngeal swab was performed and the results were negative. A presumptive diagnosis of idiopathic feline chronic rhinitis with a suspected secondary bacterial infection was made and amoxicillin–clavulanate (14.2 mg/kg PO q12h, [Clavamox; Zoetis]) for a total of 4 weeks was recommended, as well as an anti-inflammatory tapering dose of prednisolone (2.5 mg PO q12h for 14 days, 2.5 mg PO q24h for 7 days, 2.5 mg PO q48h for 7 days).

On day 39, the cat re-presented for a recheck. The owner reported resolution of exophthalmos but rapidly growing cervical masses causing obstructive breathing and dysphagia. Markedly enlarged retropharyngeal lymph nodes were noted bilaterally (Figure 1), as well as resolved exophthalmos OS, stertorous breathing and weight loss.

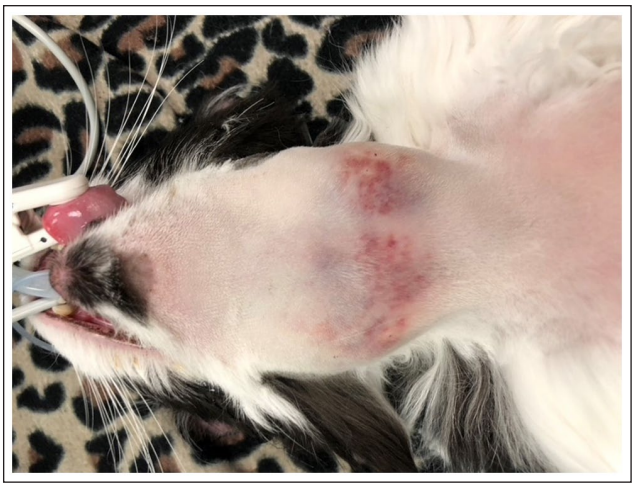

Figure 1 Marked bilateral medial retropharyngeal lymphadenopathy with bruising owing to fine-needle aspiration

Fine-needle aspiration of the retropharyngeal lymph node and cytology revealed pyogranulomatous inflammation with intra- and extracellular fungal hyphae (Figure 2). Owing to the significant negative impact on the ability to eat and breathe, bilateral medial retropharyngeal extirpation (Figure 3) was performed on day 41. Histopathology of the retropharyngeal lymph nodes showed necrotizing, granulomatous and eosinophilic cellulitis. The lymph node was effaced by inflammation and necrosis with numerous fungal hyphae. Fungal culture grew *Aspergillus* species. Bacterial culture (aerobic and anaerobic) was negative. Clinical improvement occurred over the next 3 days with ampicillin and sulbactam (Unasyn; Mylan) before culture results, tapering dose of dexamethasone-SP (0.1 mg/kg IV q24h for 2 doses, 0.05 mg/kg IV q24h for 1 dose), intravenous fluids, maropitant (Cerenia; Zoetis), mirtazapine, gabapentin (compounded; Ascend) and itraconazole (compounded from Amneal Pharm, 100 mg capsules, 8 mg/kg, 50 mg PO q24h). The cat was discharged with mirtazapine, maropitant and itraconazole.

On day 62, serum (6 h after administration) was submitted for itraconazole levels (MiraVista) and the level was 3.1 µg/ml, which is within the reported therapeutic range (2–7 µg/ml). On day 83, bilateral decreased retropulsion was noted and OS had mild third eyelid thickening. Progressive bilateral exophthalmos and hyporexia developed in the following 2 weeks. Humane euthanasia was elected on day 103 and marked bilateral exophthalmos (left worse than right) with bilateral mucopurulent ocular discharge with protruding nictitating membrane was noted (Figure 4).

On necropsy, a fungal granuloma was found in the right pterygopalatine fossa contralateral to the more affected eye (left) in the oral cavity. The tracheobronchial lymph node was enlarged 4–5 times its normal size and

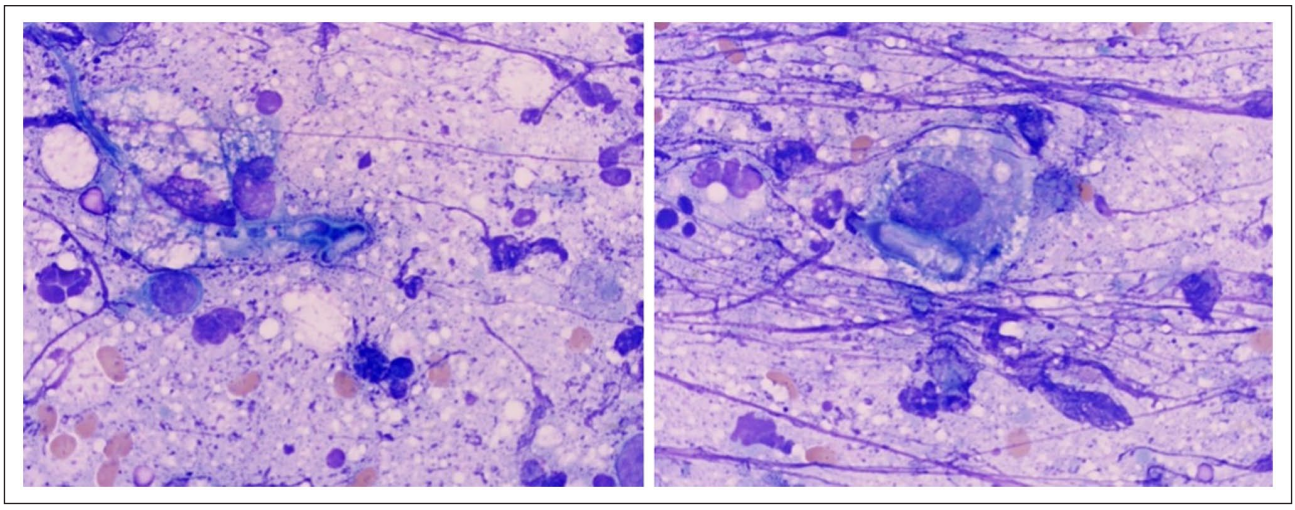

Figure 2 Hematoxylin and eosin stain. Cytology of retropharyngeal lymph nodes showing marked necrosis and extra- and intracelluar branching, septate fungal hyphae

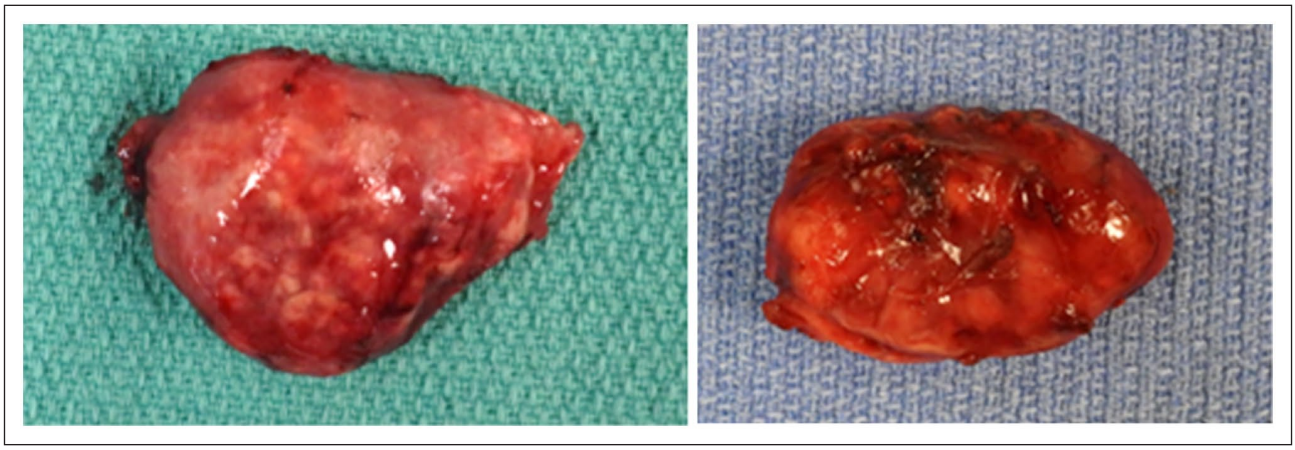

Figure 3 Surgically excised bilateral retropharynheal lymph nodes

had coalescing areas of purulent to caseous necrosis containing mats of fungal hyphae consistent with *Aspergillus* species. Different sections of decalcified skull at the level of nasal sinuses, periorbital and hard palate showed extensive granulomatous to eosinophilic inflammation. The brain and lung parenchyma showed no evidence of fungal invasion. Direct Sanger sequencing of the PCR product of the ITS1-5.8s-ITS2 region (Primers ITS4 and ITS5)[1] was performed on the isolate sourced from post-mortem tissue. The product had a 99.68% nucleotide identity to *Aspergillus udagawae* strain CBS 114217 (GenBank OL711845). The species *A. udagawae* resides in the *Aspergillus viridinutans* complex clade.[2] Antifungal susceptibility via Clinical and Laboratory Standards Institute reference methods was performed by Cornell University Animal Health Diagnostic Center and the results are shown in Table 1.

Discussion

This case report describes an unusual presentation of obstructive cervical lymphadenopathy in progressive SOA in a cat. Initial presentation with nasal signs and unilateral exophthalmos is not unusual for this disease syndrome; however, the extensive work-up did not reveal evidence of aspergillosis. This is likely due to the presence of a large amount of necrotic tissue and secondary bacterial infection, as well as the sample size limitation of fine-needle aspiration and nasal biopsy. The diagnosis was achieved after a course of anti-inflammatory prednisolone and after obstructive cervical lymphadenopathy occurred. Due to its rarity, SOA was not highly considered on initial presentation and a fungal culture was not submitted. Serological testing for aspergillosis could also have been considered. However, agar gel double immunodiffusion (AGID) to

Figure 4 Day 103, on presentation for humane euthanasia. Picture showing marked bilateral exophthalmos (left worse than right) with bilateral mucopurulent ocular discharge and protruding nictitating membranes

detect aspergillosis-specific antibodies widely used for canine sinonasal aspergillosis (SNA) does not appear to be a sensitive test for cats (sensitivity 43%) although it was reported to have specificity of 100%.[3] Two cats with SNA and SOA were reported to test positive in one retrospective study.[4] A negative AGID result would not rule out aspergillosis, but a positive result would highly support SOA. In addition, the detection of *Aspergillus*-specific antibodies by IgG ELISA was reported to have a high sensitivity (95%) and specificity (93%),[3] but such an assay is not yet commercially available.

In this case, we suspect that prednisolone likely exacerbated disease progression. This highlights the importance of clinical awareness of feline SNA and SOA since the initial presentation can mimic signs of chronic rhinosinusitis where steroids are commonly prescribed for treatment. Obstructive cervical lymphadenopathy is a rare presentation for SOA and SNA in cats. To the author's knowledge, this is the second case report for this presentation. The first case was described in a cat with disseminated aspergillosis in Sydney, Australia, where a unilateral medial retropharyngeal lymph node enlargement was noted in a Ragdoll cat.[5]

In the current case, itraconazole monotherapy and bilateral medial retropharyngeal extirpation were performed but failed to stop the progression. Antifungal susceptibility was performed post mortem in this case.

Table 1 Results of antifungal susceptibility testing on the *Aspergillus viridinutans* isolate

Drug	Minimum inhibitory concentration* (ug/mL)
5-Flucytosine	No interpretation (>64)
Amphotericin B	No interpretation (8)
Anidulafungin	No interpretation (>8)
Caspofungin	No interpretation (>8)
Fluconazole	No interpretation (>256)
Itraconazole	No interpretation (0.12)
Micafungin	No interpretation (>8)
Posaconazole	No interpretation (0.06)
Voriconazole	No interpretation (2)

*There have been no interpretations of these minimum inhibitory concentration values established for the specific *Aspergillus viridinutans* species cultured

The result showed low minimum inhibitory concentration (MICs) of itraconazole. In human antifungal treatment, the area under the curve (AUC) to MIC ratio (AUC/MIC) is closely linked with efficacy, and the clinical target for AUC/MIC for *Aspergillus* species pulmonary infection is 25,[6] which was achieved in our case if the trough level of the itraconazole is at least 3.1 mg/µl. In addition, itraconazole blood concentrations above 3 µg/ml by bioassay are considered therapeutic.[7,8] However, some studies have shown that compounded itraconazole in cats may have poor absorption and bioavailability and should be avoided.[9,10] A 100 mg oral dose of itraconazole every other day was proposed as an alternative dosing regimen for the treatment of cats with systemic fungal disease, but its efficacy has not been validated in clinical cases.[11] In addition, adequate serum itraconazole concentration does not equal adequate antifungal concentration at the site of infection, considering the difficulty of tissue penetration into the necrotic tissue or chronic abscesses. A study evaluating the antifungal susceptibility of *A viridinutans* complex using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method showed high MICs of itraconazole and voriconazole and low MICs of posaconazole and echinocandins, indicating posaconazole may be a better first-line treatment.[12] A case of feline orbital aspergillosis that failed treatment of amphotericin B and itraconazole but was successfully treated with posaconazole is also reported.[13] The combination of posaconazole, casposungin and terbinafine for the successful treatment of SOA is also reported.[4,14] Bilateral medial retropharyngeal extirpation was recommended in this case due to its obstructive nature, causing clinical signs of partial airway obstruction and dysphagia. The benefits of orbital exenteration have not been demonstrated; medical treatment alone can achieve a clinical cure in the reported

cases that were successfully managed.[4,14] Cryptic aspergillosis species in *A viridinutans* complex including *Aspergillus felis*, *A udagaawae* are the most common cause of feline SOA.[15] Molecular identification of aspergillosis species is clinically relevant because inherent resistance to azole drug resistance is common.[16,17] Susceptibility testing is therefore highly important to ensure the appropriate selection of antifungal therapy for individual patients.

Conclusions

Feline SOA should be considered in cats with exophthalmos and chronic nasal signs, even in areas where aspergillosis is rarely observed in this species. Molecular identification of aspergillosis and susceptibility testing is critical for guiding antifungal therapy with the caveat that no interpretive breakpoint is available. Itraconazole may not be the most effective therapeutic choice for this fungal disease.

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Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognized high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS Open Reports*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). For any animals or people individually identifiable within this publication, informed consent (verbal or written) for their use in the publication was obtained from the people involved.

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