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Canine rhinitis caused by an uncommonly-diagnosed fungus, Scedosporium apiospermum

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ABSTRACT

A Golden Retriever cross was presented with a four week history of violent sneezing and licking at the nasal planum. Nasal mycosis was diagnosed and Aspergillus sp. presumed the causative agent, until culture, PCR and DNA sequencing showed that Scedosporium apiospermum, an uncommonly diagnosed, yet emerging, fungal pathogen, was the agent responsible. Debridement of the fungal plaques and systemic itraconazole therapy resulted in complete resolution of clinical disease. We discuss the current literature on S. apiospermum, review its clinical significance and question the validity of its 'complex' taxonomy.

1. Introduction

Scedosporium apiospermum is a saprophytic fungus with a worldwide distribution that causes a range of disease presentations in human and animal hosts [1]. There has been a steady increase in infection rates of this species in both immunocompromised and immunocompetent individuals [2,3]. Taxonomy of *S. apiospermum* and related species has undergone considerable change over the last 15 years [2,4]. Currently, *S. apiospermum* is part of a 'species complex', comprising six pathogenic species [5], which collectively can be referred to as the *Scedosporium/Pseudallescheria* complex fungi (SPCF) [2].

Histopathologic and clinical features of *S. apiospermum* infection are very similar to those of *Aspergillus* and *Fusarium* spp., making diagnosis difficult [6]. SPCF infections, including mycotic rhinitis, have been misdiagnosed as *Aspergillus* in human and veterinary cases [2]. A delayed or erroneous diagnosis is correlated with a poor clinical outcome [7,8]. Optimal treatment of SPCF infections is currently unknown [9]. Most cases in veterinary medicine are systemic and fatal [2]. Cases where the infected tissue can be surgically excised or debrided have an improved prognosis [2,10].

It is hypothesised that some cases of fungal rhinitis in dogs are presumptively misdiagnosed as *Aspergillus* spp., based on histopathologic morphology which may, unless definitive confirmation of the diagnosis is made, lead to the unnecessary administration of invasive or expensive therapy, or even euthanasia.

2. Case

An eight-year-old male neutered Golden Retriever cross presented to the Companion Animal Health Centre at the University of Adelaide with a four-week history of violent sneezing and licking at the nasal planum. Rhinoscopic examination of the nasal passages, under sedation using an otoscope, revealed inflamed nasal mucosa and some blood in the right nasal passage only. The remainder of the physical examination was unremarkable.

Rhinoscopy under general anaesthesia was performed, which revealed a large rotting grass seed in the right middle meatus along with an extensive mucopurulent build-up and destruction of the local turbinates. The grass seed was removed and a nasal flush performed. Cytology of this flush showed clusters of degenerate and non-degenerate neutrophils with squamous epithelial cells.

While the traditional diagnostic approach to such a case would usually involve computed tomography (CT) scans prior to rhinoscopy, the discovery of the grass seed during the preliminary nasal exam led the owner to permit a nasal biopsy procedure in place of a CT scan.

Biopsy revealed expansion of the submucosa by degenerate and non-degenerate neutrophils, macrophages, lymphocytes and plasma cells, with erosion and ulceration of the epithelium. The inflamed mucosa was frequently covered by large plaques formed by septate hyaline fungal hyphae with parallel walls exhibiting dichotomous branching (Fig. 1). Rarely, simple branching conidiophores bearing single-celled ovoid conidia were observed. Based on hyphal morphology only, an interim diagnosis of fungal rhinitis due to *Aspergillus* spp. was made, with definitive diagnosis of the agent pending culture

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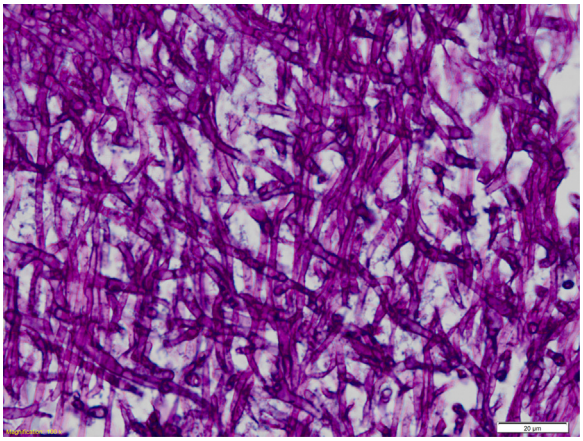

Fig. 1. Fungal plaques identified by histology (Periodic acid Schiff stain) on the inflamed nasal mucosa. Fungi are septate and hyaline with parallel walls and exhibit dichotomous branching. Simple branching conidiophores bearing single-celled ovoid conidia are also present.

results.

A head CT, performed one week after the interim diagnosis, revealed focal turbinate destruction of the right dorsal nasal cavity with no evidence of soft tissue mass or free fluid (Fig. 2).

Culture on Sheep's Blood agar (SBA) yielded growth of a white cottony colony (Fig. 3), inconsistent with that expected of *Aspergillus* spp. cultured on the same medium [11]. The isolate was sent to the National Mycology Reference Centre (NMRC) in Adelaide, South Australia for further morphological identification. Microscopic examination showed predominantly slender and elongate conidiophores some of which were simple and some branched. Several shorter conidiophores were also present. Conidiophores bore ovoid-shaped to clavate conidia with truncate bases, some of which were borne directly off hyphae. Based on this, the isolate was identified as *Scedosporium apiospermum* and showed susceptibility to both clotrimazole and ketoconazole.

DNA was extracted from the cultured sample using the Ultraclean® Microbial DNA Isolation Kit from MO BIO Laboratories Inc. according to manufacturer's instructions. A conventional PCR was performed, targeting two gene regions- ITS1, 5.8S and ITS2 genes (using primers ITS-1 and ITS-4) and partial β-tubulin gene (using primers bt2a and bt2b). DNA purification and sequencing was performed at Macrogen labs (Seoul, Republic of Korea). Resulting sequences were edited with BioEdit Sequence Alignment Editor © version 7.2.5 [12] and species identified using the Basic Local Alignment Sequence Tool (BLAST ®) online software provided by the National Centre for Biotechnology Information [13]. The isolate's internal transcribed spacer (ITS) and β-tubulin (TUB) sequences were consistent with *Scedosporium apiospermum* (with an identification percentage match of 100% and 98% respectively based on GenBank BLAST search analysis).

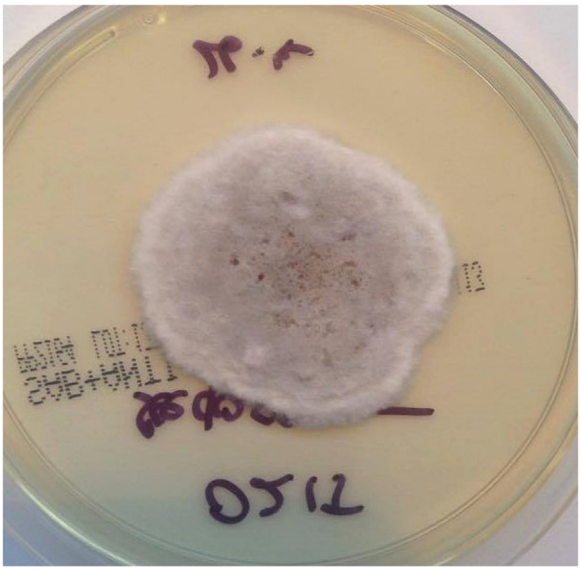

Fig. 3. Grey–white cottony colony cultured from nasal swabs of the patient presented in this report, grown on Sabouraud Dextrose Agar (SDA).

Bacterial cultures of nasal swabs and flush yielded a mixture of organisms, primarily *Staphylococcus pseudintermedius* and *Fusobacterium varium*; both of which were deemed to be opportunistic pathogens, secondary to the fungal infection.

Based on preliminary fungal identification and MIC data provided, the dog was treated with oral itraconazole at a dose of 2.5 mg/kg twice daily for 3 months. Anti-fungal treatment began approximately one month after the first consultation.

At the three month re-check, the dog was no longer sneezing or licking at the nasal planum and treatment with itraconazole was discontinued. The remainder of this exam was unremarkable, aside from a resolving hotspot (superficial bacterial skin infection) on the right dorsal carpus.

At the final re-examination, which occurred 5 months later and 8 months after initial presentation, nasal swabs were negative for fungal growth, and a follow-up CT scan showed no progression of turbinate destruction. The clinical conclusion was that mycotic rhinitis had resolved.

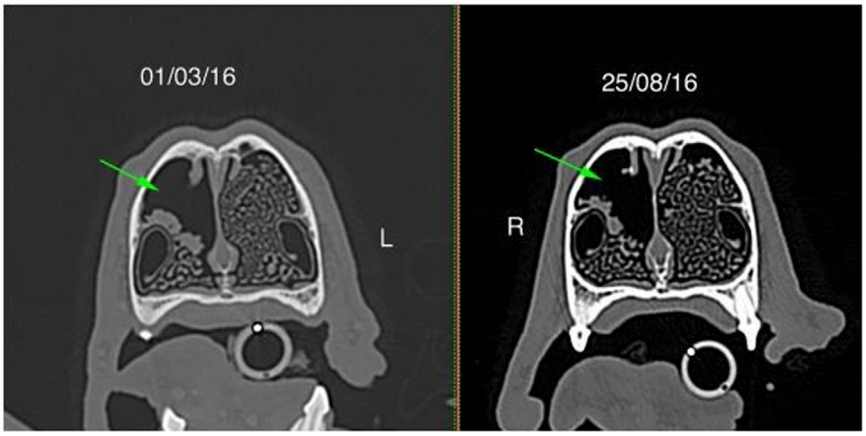

Fig. 2. Transverse multiplanar reconstruction (MPR) CT images at the level of the second pre-molar. On the left is the first CT taken one week after presentation and the right is at the five month-recheck. The green arrows indicate turbinate loss caused by the fungal rhinitis, which got no worse during or after the treatment period.

3. Discussion

Scedosporium apiospermum is commonly reported as an important emerging fungal pathogen for humans and animals [8]. Disease presentations reported in dogs include disseminated infection, keratomycosis and mycetoma [2]. Reports of *S. apiospermum* as the aetiological agent in cases of dogs with rhinitis remain relatively few. This is only the fifth reported case, and the second in Australia [1,10,14,15]. There is no specific protocol for treatment of nasal scedosporiosis documented in the literature; hence, treatment (and, importantly, the response to treatment) has generally been varied and unsystematic in its approach. All cases of nasal scedosporiosis reported resolved and became clinically normal following treatment.

Scedosporium apiospermum and related species have undergone considerable taxonomic and nomenclatural change over the last decade [2,4]. The species is currently classified in a complex with five other species- *S. boydii*, *P. angusta*, *S. minutispora*, *S. dehoogi*, *S. aurantiacum*-collectively known as the *Scedosporium/Pseudallescheria* Complex Fungi (SPCF) [5]. *S. apiospermum* is not the only species in the complex that has been shown to cause rhinitis in dogs, with *S.boydii* also reported [8]. The term 'species complex' in general is poorly defined and used taxonomically for groups of fungal entities that have uncertain taxonomic status, are closely-related and hard to identify and/or are not individually significant [16]. Genetic variation among the SPCF has been well-reported [2,4,17]. Due to difficulty in species-level identification, most diagnostic laboratories will report an SPCF as simply part of the 'complex', despite differences in pathogenicity and anti-fungal susceptibility, as well as genetics [2,3]. There is not currently sufficient molecular data, nor routine species-level identification, to consistently correlate these differences with particular species of the complex [3], hence the persistence of the 'complex' nomenclature. Whether the clinical variation is species-specific, or is more generalised across the complex, cannot be elucidated from current molecular data [2,17]. Indeed, aside from the case presented here, only one other report [8] of nasal scedosporiosis in dogs has utilised molecular methods for diagnosis.

Previous cases vary in their clinical presentation. Only two cases [1,15] report major osteolysis (to the vomer and maxillary bone, respectively). Other cases report, at most, turbinate lysis. The strains of SPCF in these cases may not have possessed the virulence to cause osteolysis, however the influence of host and environmental factors cannot be discounted. Our case showed turbinate lysis, however no evidence of destruction of other bony structures. Sneezing and licking at the nasal planum were the only clinical signs recorded. The case described by Paul et al. [15] is noteworthy for its relatively unique presentation among the cases. Novel signs included halitosis (without evidence of oral or periodontal disease) and a mild enlargement of the right submandibular lymph node. As already mentioned, genetic variation within *S. apiospermum* isolates has been documented and could account for the differing clinical presentations.

The diagnosis of *S. apiospermum* is complicated by its numerous morphologic and clinical similarities to *Aspergillus* spp. and other hyaline hyphomycetes such as *Fusarium* spp. [6], as well as clinicians' and pathologists' relative unfamiliarity with this species [7]. In fact, *S. apiospermum* can be impossible to distinguish morphologically from *Aspergillus* spp. [7] In the case presented here, histopathological findings led to an interim diagnosis of fungal rhinitis caused by *Aspergillus*. This is not the first case in which this specific misdiagnosis has occurred [7,8]. For accurate diagnosis in a clinical setting, culture is recommended concurrently with cytologic and histopathologic investigations, providing the pathologist/clinician familiarizes themselves with the pathological potential of SPCF and their features [6,7]. Colonies are generally cottony and greyish–white [11], later becoming dark grey to smoky brown [18]. In contrast, cultures of *Aspergillus* spp. are usually white with green patches, or, in the case of *A. fumigatus*, a solid blue–green colour [11]. Various other ancillary methods of diagnosis have been described such as histochemical staining, serology, immunohistology, selective media and PCR [2], however components of these techniques are not available in veterinary laboratories (and even some medical reference laboratories) and/or have not been validated clinically [4,6,7].

Although microscopic morphology is sufficient for identification to genus level, this requires familiarity with the complex [7]. Species-level identification should be routinely performed to ensure a definitive diagnosis and an appropriate treatment plan [4,7]. Molecular methods are essential for this [2], for example, the new matrix-assisted laser desorption ionization time-of-flight/mass spectrometry (MALDI-TOF/MS) technology [3,4]. Although PCR techniques are yet to be validated clinically and may not be able to differentiate between every species of the complex, they too are shown to be promising tools [3,4].

Given the absence of the use of molecular diagnostics, it is fair to hypothesise that the aetiological agents involved in previous cases may have been any species of the *Scedosporium/Pseudallescheria* complex. Species-level identification is essential for epidemiological investigation of the complex, such that we can further understand and prevent transmission from the environment to susceptible patients [4]. Considering the presence of fungal rhinitis in dogs is, by default, almost exclusively assumed to be caused by *Aspergillus* spp. [19], the difficulty in diagnosis, unfamiliarity with the complex, and emerging nature of the SPCF, two questions must be asked: Are cases of nasal scedosporiosis regularly misdiagnosed as aspergillosis and are these cases being treated appropriately?

While systemic SPCF infections are generally refractory to treatment and most clinical cases in veterinary species are fatal, this does not appear to be the case for nasal infections in dogs [2]. Treatment of nasal scedosporiosis in previous cases has varied in invasiveness from endoscopic debridement of fungal mass without systemic or topical antifungal treatment [10] through to antifungal infusion via surgically-placed catheters and sinus trephination at the most aggressive [8], however all resolved clinically. Three cases [1,10] (including this one) achieved treatment success *without* the need for topical antifungal administration. Of the other three, two [8,15] were treated topically without attempting sole systemic therapy first. Based on these previous cases, there is evidence to suggest aggressive topical therapy as first-choice treatment, as is typical for cases of nasal aspergillosis [19], may be excessive and unnecessary for nasal scedosporiosis.

Although 'in vitro' antifungal susceptibility testing does not always correlate with 'in vivo' efficacy, it is indicated regardless of whether species-level identification is achieved to help guide therapeutic management [20]. Voriconazole has been shown to have the most activity against SPCF, however variation in antifungal susceptibility between species, as well as strain-specific variation within species, has been demonstrated [9,20]. This gives more evidence of clinical differences among species of the complex and further questions the validity of the complex for all *Scedosporium spp*. [5] Accurate diagnosis is required to save clinicians, clients and patients from unnecessary procedures e.g. sinus trephination.

Due to an increase in reports of infection with the *Scedosporium/Pseudallescheria* complex fungi in veterinary patients, research into appropriate diagnostics and treatment has increased in recent years [2]. Ideally, culture should be performed for all cases of fungal rhinitis to complement microscopic diagnosis [7]. Molecular identification should be attempted, facilitating better understanding of the epidemiology, pathogenicity, prognosis and antifungal susceptibility of each species of the SPCF. Further research is needed to determine optimum treatment protocols, however evidence from this case report, as well as that in other literature, suggests that systemic treatment, with an appropriate anti-fungal, and debridement of the lesion where possible, should be attempted before use of topical treatment, to avoid subjecting the patient to invasive, expensive therapy.

Although the 'species complex' model, described by de Hoog et al. [16], is still applicable to clinically relevant fungi, variation in

antifungal susceptibility and pathogenicity between species of the *Scedosporium/Pseudallescheria* complex warrants a review into the validity of the 'complex' status for this genus.

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Conflict of interest

There are none.

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