Mycotic rhinitis caused by *Scedosporium apiospermum* in a dog: first report in Italy

A 4-year-old neutered female Bull Terrier was presented with a 20-day history of unilateral nasal discharge and reverse sneezing. The dog did not respond to antibiotics. Histological examination revealed severe rhinitis with septate hyphae and smooth, oval, pigmented, unicellular conidia. Mycological cultures yielded pure colonies of filamentous fungi, macroscopically and microscopically identified as *Scedosporium apiospermum*. Identification was confirmed by MALDI-TOF analysis. Susceptibility tests were performed. The isolate resulted susceptible in vitro to Econazole and Miconazole, intermediate to Enilconazole and Clotrimazole and resistant to Amphotericin B, Fluconazole, Ketoconazole and Itraconazole. In the dog, S. apiospermum has been previously reported as the causative agent of nasal disease in two cases in Spain, one in New Zealand and one in Australia. To our knowledge, this is the first case of rhinitis caused by S. apiospermum reported in a dog in Italy.

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INTRODUCTION

Scedosporium apiospermum and the sexual state *Pseudallescheria apiosperma* (teleomorph) are ubiquitous ascomycetes with a wide environmental distribution, especially in agricultural soils, muds and slurries[1]. This species of filamentous fungi is a major pathogen for humans, especially in immunocompromised individuals, where it causes a wide variety of localized or disseminated infections. In companion animals, *S. apiospermum* mycoses are rarely described. In the dog, the most frequently reported scedosporosis is the disseminated form, with inauspicious and often fatal prognosis. Predisposition for such infection has been reported in the German Shepherd and in related cross-breeds, in line with the breed's known predisposition for disseminated aspergillosis[2].

Other associated clinical manifestations described in the dog are mycetoma and keratomycosis[3].

To date, *S. apiospermum* has been reported as a causative agent of respiratory disease in the dog in only two cases in Spain[4,5], one in New Zealand[6] and one in Australia[7]. However, an underestimation of cases of rhinitis and sinusitis caused by *S. apiospermum* is plausible, as it can easily be confused with *Aspergillus* spp. because of the similarity of the associated clinical signs.

Scedosporium apiospermum is an important pathogen for both humans and animals. Reports of mycoses caused by this fungus in the dog are rare; the disseminated form is usually more prevalent.

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To the authors' knowledge, this is the first case of rhinitis caused by *S. apiospermum* reported in a dog in Italy.

Histology revealed a severe fungal rhinitis in a 4-year-old Bull Terrier. The mycological examination isolated fungal colonies, of cottony appearance, microscopically characterized by the presence of septate hyaline hyphae and oval shaped, pigmented, unicellular conidia.

CASE REPORT

A 4-year-old female Bull Terrier was presented with a 20-day history of partially haemorrhagic, unilateral, mucopurulent left-sided nasal discharge and reverse sneezing.

The clinical history included non-response to the antibiotic therapy prescribed by the referring veterinarian.

Rhinoscopy was performed; the right nasal cavity presented moderately aedematous turbinates, with no mucus present. The dorsal portion of the left nasal cavity presented a moderate amount of viscous mucus, originating from the dorsal meatus, where a yellowish-white/brown material of rubbery consistency was found.

After partial removal with biopsy forceps, this mucus formation appeared mobile and only partially adherent to the turbinates. The appearance resembled an intranasal fungal colony with cottony traits (Fig. 1A) and partial silver reflections (Fig. 1B). Multiple biopsies were taken from the plaques and the nasal mucosa; these were

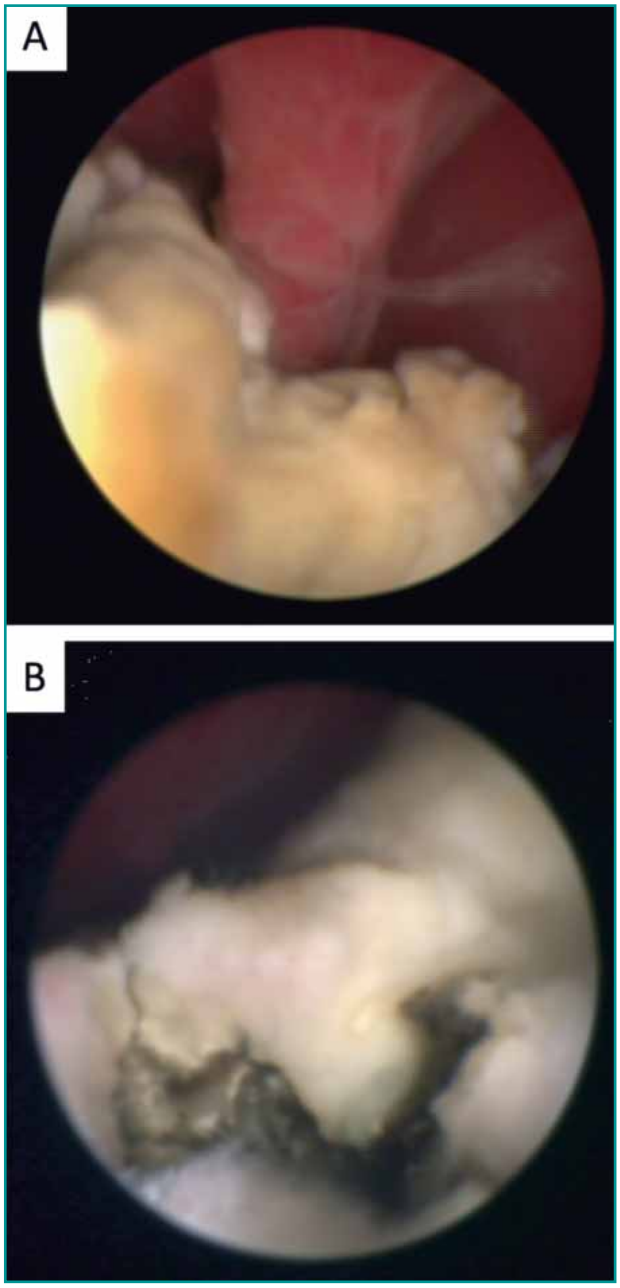

Figure 1 - Rhinoscopy of the left nasal cavity. Presence of amorphous, white-yellowish/brown coloured material adhering to the nasal mucosa. The material is compatible with a whitish, cottony fungal colony, with grey/silvery areas.

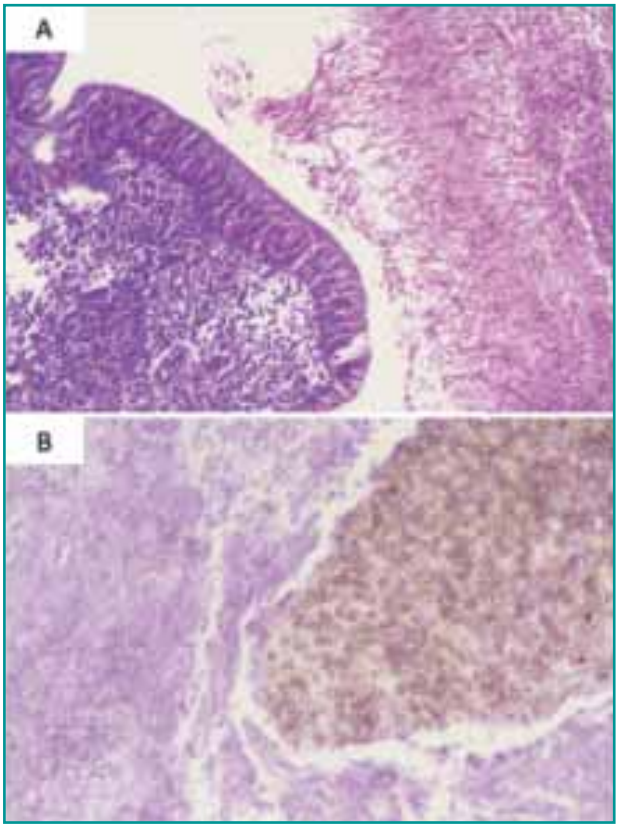

Figure 2 - Histological examination of nasal biopsies. 1A: The mucosa is characterised by an abundance of inflammatory infiltrate and large accumulations of septate hyphae positive to PAS staining (PAS, 20X). 1B: Detail of the large fungal clusters, with evident aggregates of pigmented conidia (Hematoxylin-Eosin, 40X).

fixed in 10% neutral buffered formalin and examined histologically.

Multiple sections were analysed with routine haematoxylin-eosin staining and PAS (Periodic acid-Schiff) reaction. The histological samples of the nasal mucosa showed a marked inflammatory pyogranulomatous process.

S. apiospermum was found to be sensitive to Econazole and Miconazole, intermediate to Enilconazole and Clotrimazole and resistant to Amphotericin B, Fluconazole, Ketoconazole and Itraconazole.

The mucous surface presented large clusters of fungal hyphae (Fig. 2A) characterised by irregularly parallel, septate walls and the presence of oval conidia, at times of brownish colour (Fig. 2B).

The masses were intensely eosinophilic, confluent, surrounded by degenerated neutrophils and necrotic debris. The mucosa presented a dense, mixed inflammatory infiltrate, consisting of neutrophils, macrophages, lymphocytes and plasma cells.

The fungal elements visible at histology did not appear morphologically compatible with A*spergillus* spp. New samples were therefore collected for mycological cultural examination.

The samples were cultured on Sabouraud dextrose Agar with the addition of chloramphenicol 0.05 g/l and incubated for seven days at 28°C. After 3 days, a pure culture was observed, with fast-growing colonies of cottony appearance. The colonies appeared brown on the reverse and initially white on the surface (Fig. 3A); with

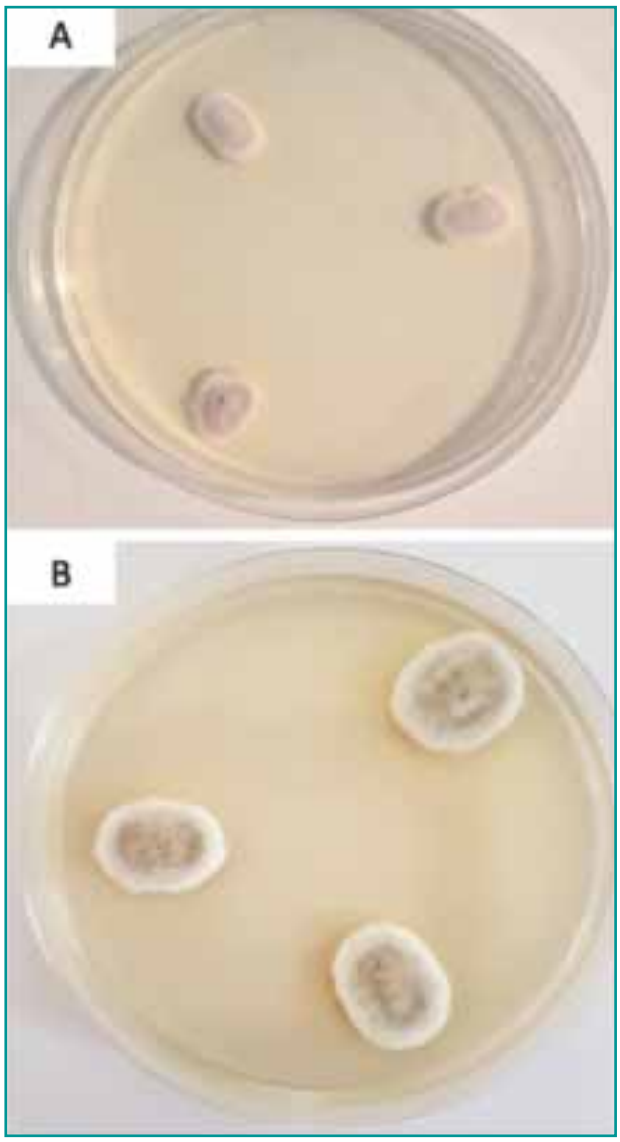

Figure 3 - Macroscopic presentation of the colonies. 2A: *Scedosporium apiospermum* on Sabouraud dextrose culture medium at day 3 of incubation. 2B: *Scedosporium apiospermum* on Sabouraud dextrose culture medium at day 7 of incubation. The pigmentation of the front side of the colonies turns dark grey.

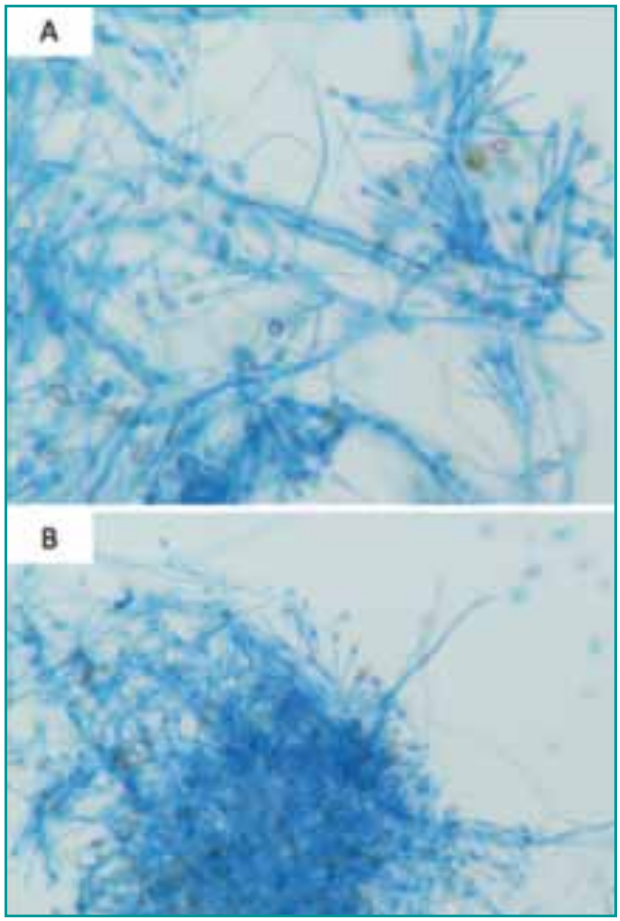

Figure 4 - Microscopic presentation of *Scedosporium apiospermum* (staining with Lactophenol blue, 40x). 3A: Septate and branched vegetative hyphae. Bundles of conidiophores of various lengths, bearing single, oval, unicellular conidia, truncated at the base. 3B: hyphae, conidiophores and conidia. Conidia present different degrees of pigmentation.

the aging of the culture the colonies turned dark grey (Fig. 3B).
The isolated fungal strain was microscopically analysed by sampling a portion of the aerial mycelium with tape and staining it with Lactophenol Blue. Septate hyaline hyphae, of irregular diameter and with ramifications, were found. The ends of each hypha bore conidiophores with single, oval conidia, truncated at the base and variably pigmented (Fig. 4A; Fig. 4B).
Due to the macroscopic and microscopic characteristics and the absence of ascocarps, which are proper of the teleomorphic sexual state, the isolate was identified as *S. apiospermum*. Identification at species level was confirmed by MALDI-TOF mass spectrometry. The antimycotic susceptibility of the identified fungal strain was tested following the Clinical Laboratory Standards Institute (CLSI) guidelines for non-dermatophyte filamentous fungi[8].

This is the first case of nasal mycosis caused by *Scedosporium apiospermum* reported in a dog in Italy. Identification is essential for the selection of therapy, as susceptibility to antimycotic agents varies with the species.

The *S. apiospermum* strain was found to be sensitive to Econazole and Miconazole, intermediate to Enilconazole and Clotrimazole and resistant to Amphotericin B, Fluconazole, Ketoconazole and Itraconazole. In view of the results obtained, the nasal cavities were infused with Miconazole 2% in saline solution.
At the follow-up visit, two weeks after the start of the antimycotic therapy, no clinical symptoms of rhinitis were found.
At rhinoscopy, the turbinates in the rostral tract of the left nasal cavity appeared normal, while caudo-dorsally the turbinates were absent, a secondary consequence of the erosive effect of the mycosis. No additional lesions or altered nasal mucosa attributable to mycosis were observed at this site.

DISCUSSION

The case described represents the first case of mycotic rhinitis caused by *S. apiospermum* reported in a dog in Italy; this fungus is rarely reported as a cause of rhinitis in dogs and, to the authors' knowledge, only four[4,5,6,7] similar cases have been reported in literature as this fungus is more commonly associated with visceral forms secondary to penetrating traumas or wound contamination.
The clinical presentation and the lesions associated with *S. apiospermum* are not pathognomonic and are similar to those caused by other filamentous fungi, in particular *Aspergillus* spp. Similarly, at histology, the morphology of the hyphae may not be useful in distinguishing unambiguously the aetiological agent of the mycosis.
However, in the case described, the observation of oval conidia in the histological sections of nasal biopsies allowed a preliminary presumptive identification. A mycological culture was in any case necessary for the final discrimination between *Aspergillus* spp. and *Scedosporium* spp.; this allowed an accurate morphological identification of the fungal strain, although confirmation of the morphological identification of the species by molecular methods or mass spectrometry is advisable.
The accurate identification of the causative agent is a fundamental requirement for the selection of the most effective antimycotic agent, as a certain variability in the susceptibility of the different species of Scedosporium[9] to various molecules has been reported. However, to date, the interpretation criteria for antimycotic-susceptibility tests for *Scedosporium* spp. have not yet been standardised. In view of the resistance of the species to numerous antimycotic drugs, the treatment of *S. apiospermum* mycoses is complex. A high percentage of patients with scedosporosis do not survive.
Localised infections such as keratomycosis and upper airway infections have a better prognosis[3]. The case described suggests the importance of considering *Scedosporium* spp. as a possible emerging pathogen responsible for rhinitis and/or sinusitis in the dog. Although the incidence of scedosporosis in veterinary medicine appears to be lower than in human medicine it is worth considering that the actual number of cases could be higher.
In fact, cases of scedosporosis are very often not diagnosed, or are not correctly evaluated and misinterpreted for aspergillosis.
When possible, mycological cultures should therefore be carried out, even when the main suspect for the mycosis is an *Aspergillus* spp. Fungal culture remains the gold standard for the diagnosis of Scedosporium infections[10]. In addition, the univocal differentiation of the fungal agent involved in the lesion is necessary for the selection of the most appropriate antimycotic therapy.

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