



Case Report

Scedosporium apiospermum infection presenting as a mural urinary bladder mass and focal peritonitis in a Border Collie

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ABSTRACT

Scedosporium apiospermum is an opportunistic mold that is an emerging disease in humans and animals. This report describes a case of *S. apiospermum* infection inciting a mural urinary bladder mass and focal peritonitis in a dog that had a history of multiple traumatic events several years prior. For diagnosis, culture followed by MALDI-ToF, PCR, and sequencing was performed to accurately identify the species. Susceptibility testing was also performed due to the inherent resistance of *S. apiospermum* to numerous antifungal agents.

1. Introduction

Pyogranulomatous peritonitis occurs uncommonly in dogs and is most often associated with trauma to the gastrointestinal tract, skin, female reproductive tract, or urinary bladder causing direct implantation of infectious opportunistic organisms [1]. The most commonly isolated organisms include filamentous bacteria such as *Actinomyces* spp. and *Nocardia* spp., or fungal organisms such as zygomycetes [1,2]. Rarely reported causes of pyogranulomatous peritonitis in dogs include ascomycete mold/fungi of the genus *Scedosporium* [3–5]. *Scedosporium* spp. are opportunistic and saprophytic organisms that are commonly found in soil, sewage, and polluted water in temperate moist climates [6]. Following implantation, *Scedosporium* can induce the formation of eumycotic mycetomas [7]. Diagnosis of *Scedosporium* hyphae cannot be made by histologic features alone due to its morphologic similarities to other more common causes of fungal infection, such as *Aspergillus* [5,8]. A combination of culture and molecular characterization is necessary to confirm the diagnosis. Treatment is often difficult and prolonged because it requires surgery followed by long term antifungal treatment. Judicious selection of antifungals often requires susceptibility testing because *Scedosporium* is frequently resistant to numerous antifungal agents [6]. Only one report in the literature describes a case of *Scedosporium apiospermum* causing ureteral and bladder granulomas in a dog [9]; however, the portal of entry was not identified. This report documents a unique case of *Scedosporium apiospermum* infection presenting as

a urinary bladder wall mass in addition to a second mass within the cranial peritoneal cavity in a Border Collie that had a history of multiple traumatic injuries over the course of several years.

2. Case presentation

In October 2020, a 10-year-old male intact Border Collie dog presented to a specialty veterinary hospital for a two-month history of stranguria, tenesmus, and weight loss. Thoracic radiographs were unremarkable. Complete bloodwork demonstrated mildly decreased albumin and increased globulins. Abdominal ultrasound revealed a large caudal abdominal mass measuring 7.7 × 3.5 × 7.9 cm of unknown origin and marked medial iliac lymphadenopathy. A mass was also noted in the right testicle. Fine needle aspiration of the abdominal mass was consistent with pyogranulomatous inflammation. Exploratory laparotomy revealed a large mass adhering to the ventral body of the urinary bladder and abdominal wall, which was excised by partial cystectomy. An additional small mass was removed from the mesentery adjacent to the spleen. Both specimens were submitted to the Veterinary Diagnostic Laboratory at Michigan State University for analysis. This dog had a previous history of septic abdomen in 2013 due to perforation associated with the use of nonsteroidal anti-inflammatory drugs, a right femoral head and neck osteotomy in 2013, several pelvic fractures (date unknown), and was an outdoor, sheep herding dog.

The urinary bladder mass and mesenteric mass were fixed in 10%

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neutral buffered formalin, embedded in paraffin, serial sectioned at 5 μm , and stained with hematoxylin and eosin (H&E), Periodic acid-Schiff stain (PAS), and Grocott's methenamine silver stain (GMS). Additional fresh urinary bladder tissue was submitted for aerobic and fungal culture (see below).

On histologic examination, the urinary bladder wall including the muscularis propria was regionally transmurally obscured by multifocal to coalescing pyogranulomas (Fig. 1A and B). Pyogranulomas were characterized by central masses of 3–5 μm wide, nonpigmented, parallel walled, irregularly branching, septate hyphae with bulbous dilatations, surrounded by degenerate neutrophils, epithelioid macrophages, and multinucleated giant cells, surrounded by a dense rim of lymphocytes, plasma cells, hemosiderin-laden macrophages, and dense bands of fibrosis. Hyphae were highlighted using PAS and GMS stains (Fig. 2A–D). The urothelium was widely ulcerated and lined by fibrin, sheets of degenerate neutrophils, and karyorrhectic debris. The underlying lamina propria contained dense perivascular infiltrates of lymphocytes and plasma cells. Inflammation extended into the surrounding omental adipose tissue. The second submitted specimen arising from the mesentery adjacent to the spleen was characterized by adipose tissue extensively replaced by multifocal to coalescing pyogranulomas that surrounded mats of fungal hyphae surrounded by dense bands of fibrosis, as described in the urinary bladder wall. The morphologic

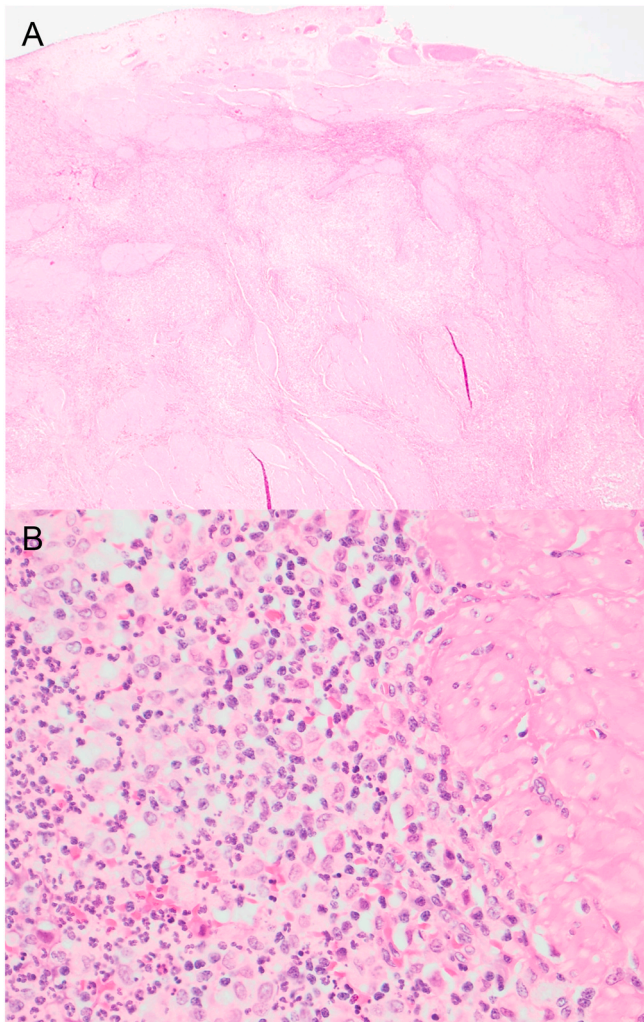


Fig. 1. A) H&E section. Photomicrograph illustrates the pyogranulomatous inflammation that effaces the entire urinary bladder wall. B) Higher magnification of the pyogranulomatous inflammation partially obliterating the muscularis propria.

diagnosis of the bladder wall mass was severe, chronic, multifocal to coalescing pyogranulomatous cystitis and peritonitis with intralumenal fungal hyphae.

Fungal culture was performed on the urinary bladder mass. The sample was inoculated onto Sabouraud dextrose agar with chloramphenicol and Mycobiotic agar plates (Remel, Lenexa, KS). The plates were incubated at 25 °C and fungal growth was observed after 24 h incubation. The rapidly growing colonies were pink at 48 h (Fig. 3A) and turned to greyish-white as the colonies aged. Microscopically, the branching hyphae were septate and numerous single celled ovoid conidia were observed on lactophenol cotton blue mount (Fig. 3B). The fungus was identified as *Scedosporium apiospermum* (*Pseudoallescheria-Scedosporium boydii* complex) on MALDI-ToF (Microflex LT, Bruker Daltonics, Billerica, MA) using the National Institute of Health fungal reference database [10]. Since the MALDI-ToF score (1.64) was low, a PCR based identification was attempted. Briefly, fungal DNA was extracted using Soil/Fecal DNA miniprep kits (Zymo, Irvine, CA) and PCR using the primers ITS1 and ITS4 was performed [11]. The PCR amplicon was cleaned up with ExoSAP-IT Express (Thermo Fisher Scientific) and the DNA was sequenced (Eurofins Genomics, Louisville, KY) and submitted to GenBank (accession no. MW 554911). The sequence was analyzed using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and confirmed 100% sequence similarity to *Scedosporium apiospermum*. The fungal culture was sent to Animal Health Diagnostic Laboratory (Cornell University, Ithaca, NY) for antifungal susceptibility testing. Susceptibility testing revealed that this fungal isolate had elevated MIC for 5-Flucytosine and Fluconazole, moderate MIC for Amphotericin B, Anidulafungin, Caspofungin, and Micafungin, and low MIC to Itraconazole, Posaconazole, and Voriconazole (Table 1). This dog was placed on Sporonox (Itraconazole; 5mg/kg PO q 24hrs) for 6 months.

3. Discussion

In the literature, the organism *Scedosporium apiospermum* was previously commonly referred to as *Pseudoallescheria/Scedosporium boydii*; however, currently *S. apiospermum* and *P. boydii* are considered separate species [12]. *Pseudoallescheria* refers to the sexual reproductive stage, while *Scedosporium* refers to the asexual stage of the ascomycete [6]. There are ten known species of *Scedosporium* with *S. apiospermum* being one of the most pathogenic [13,14]. For simplicity, many laboratories report these pathogens as *Scedosporium/Pseudoallescheria* Complex Fungi (SPCF) despite the fact that individual species show different susceptibilities to antifungal agents [15]. In humans, *S. apiospermum* is an important emerging pathogen for immunocompromised as well as immunocompetent individuals. In one review, 18 out of 20 cases of *S. apiospermum* infection occurred in immunocompromised patients, 16 cases had localized infection, while 4 cases had disseminated disease. Interestingly, only 4 cases reported minor trauma (associated with gardening or IV catheter site), 2 of which led to disseminated disease [16].

In dogs, there has recently been an increasing number of reports of *S. apiospermum*, which has been implicated in cases of fungal rhinitis [17–20], osteomyelitis and discospondylitis [8,21], keratomycosis [22–24], enteritis and peritonitis [4], and systemic disease [3,5,25–28]. In the only report describing a case of *S. apiospermum* causing ureteral and bladder granulomas in a dog, they were unable to identify the portal of entry [9]. In this case, given the clinical history of multiple traumatic events and an outdoor living style, there were many opportunities for opportunistic infection by *S. apiospermum*. The incubation period for mycetomas in general may vary from weeks to years [7]. Given that severe inflammation obliterated large portions of the full thickness of the urinary bladder wall, it is unclear whether the infection arose from within the urinary bladder (as an ascending infection) and extended into the peritoneal cavity or whether the gastrointestinal perforation several years prior was the inciting event.

Identification of *Scedosporium* species based on culture morphology

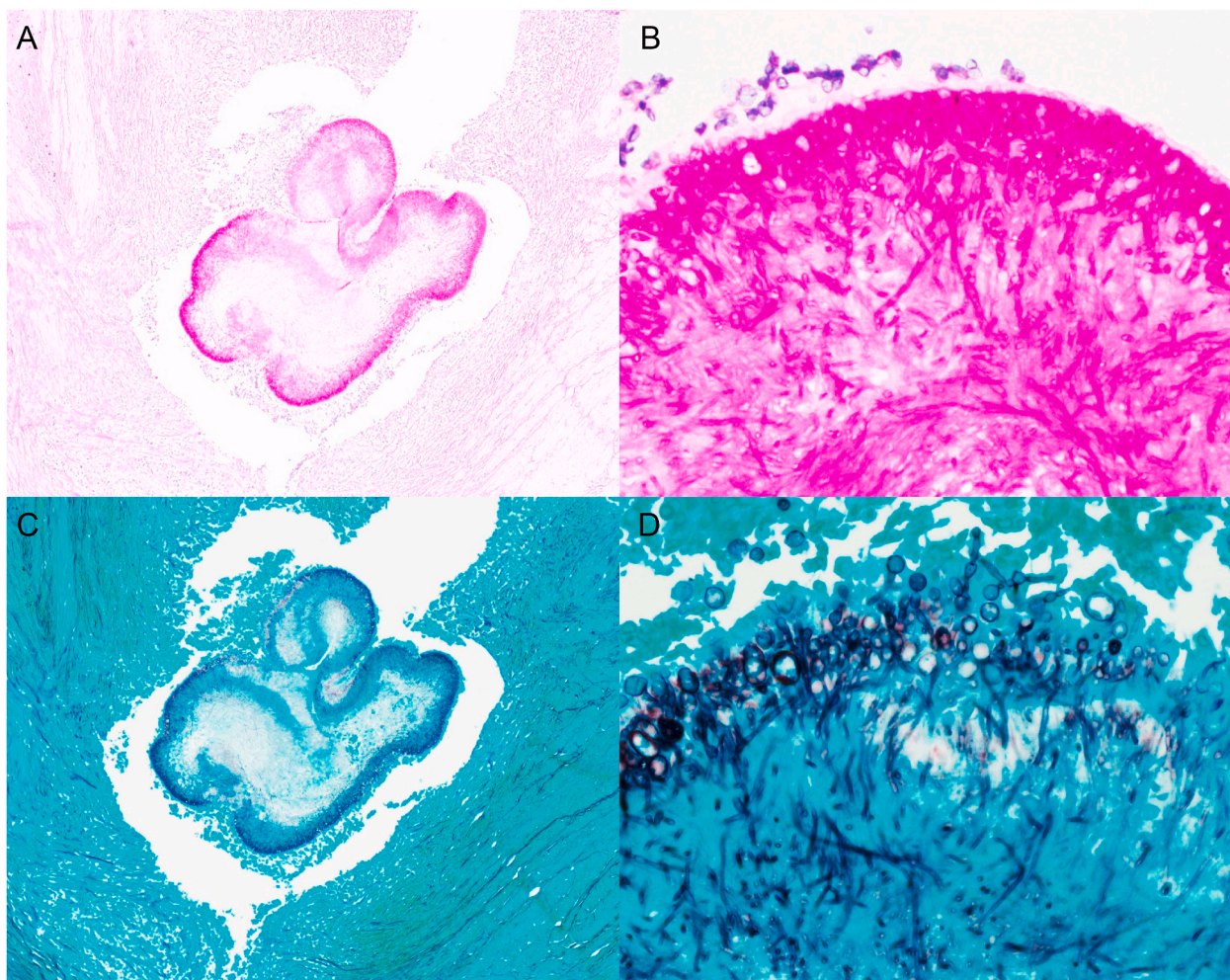


Fig. 2. A) PAS stain highlights the mats of fungal hyphae. B) Higher magnification of A showing fungal hyphae and conidia. C and D) GMS stain as in A and B.

alone is challenging. Molecular methods including PCR and MALDI-ToF are preferred for an accurate diagnosis. Both ITS sequencing and MALDI-ToF can identify *S. apiospermum* and *P. boydii* as *S. apiospermum* species complex [12,29]. These two genetically related species have similar pathology and antifungal susceptibilities and so identification to species complex should be adequate for clinical purpose [12]. Partial sequencing of the beta-tubulin gene is preferred for differentiation to a particular species [30].

Luckily in this case, susceptibility testing identified that this fungus was susceptible to Itraconazole, Posaconazole, and Voriconazole, which is consistent with what is documented in the literature. In one report, Itraconazole had variable *in vitro* activity against *S. apiospermum* ranging from 0.03 to 16 µg/ml (presented as minimal inhibitory concentrations), Posaconazole had 0.03–0.25 µg/ml, and Voriconazole had 0.01–0.25 µg/ml [31]. The recommended course of treatment is several months to a few years and requires careful follow up including liver enzyme and abdominal ultrasound monitoring every 1–2 months for at least 6 months (*recommendations of A Conkling*).

This dog was doing well at his two week recheck post-surgery with an unremarkable physical exam other than moderate incision inflammation with urine dribbling. Abdominal ultrasound demonstrated no notable inflammation around the bladder or mass effect. According to the owner in May 2021, the patient had two days left of the prescribed 6 months of antifungal treatment and was doing really well with normal urination habits and return to sheep herding activities.

Prognosis for disseminated disease in dogs is poor due to natural death associated with the disease [5], or euthanasia due to poor quality

of life [25,27,32]. Localized disease, such as that of the nasopharynx or cornea, treated with surgical debridement followed by appropriate antifungal treatment has resulted in complete remission in several cases [18,20,22]. Overall, good prognosis appears to be largely dependent on infection that is localized and that is amenable to surgery and appropriate antifungal treatment. In this case, since the bulk of the disease was excised surgically and this dog was treated as recommended with a full 6 month course with an appropriate antifungal, prognosis is expected to be good. In the only other reported case of *S. apiospermum* involving the urinary bladder in a dog, the patient was doing well 1.5 years after presentation with resolution of the fungal granuloma after long-term antifungals [9]. In this case, continued monitoring via abdominal ultrasound to identify any new masses or lesions was recommended as a precaution.

This report highlights a unique presentation of *Scedosporium* infection in a dog, and the importance of accurate fungal identification by culture followed by advanced molecular diagnostic techniques and susceptibility testing due to its histologic resemblance to other more common fungal agents (eg. *Aspergillus*) and its inherent resistance to many antifungal agents.

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There are none.

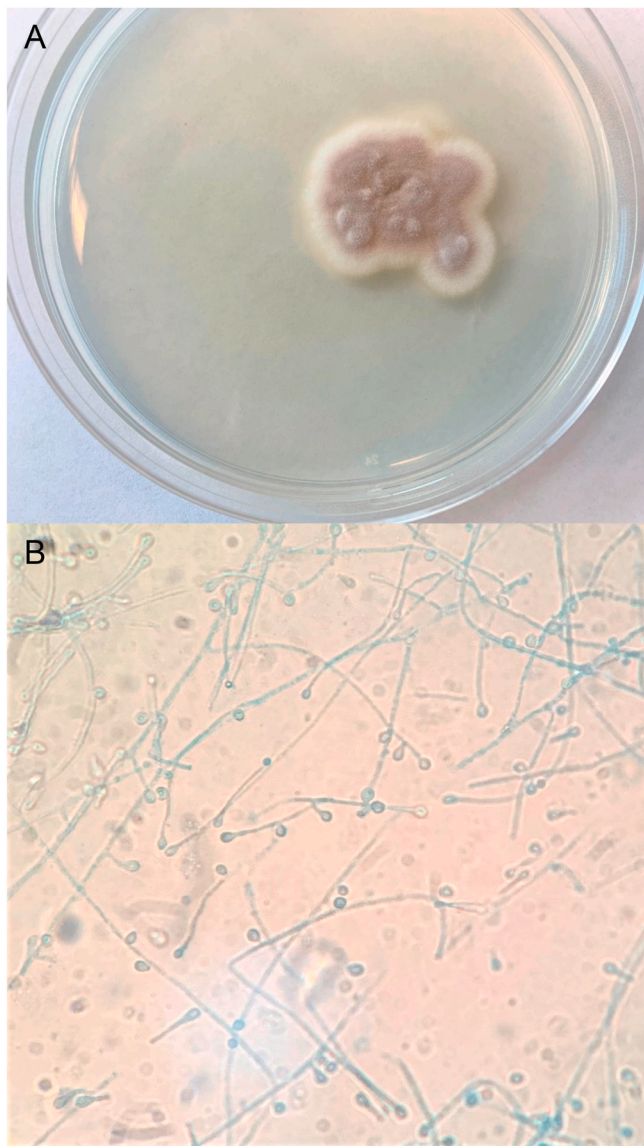


Fig. 3. A) Pink colonies grew rapidly by 48 h. B) Microscopically the branching hyphae were septate and numerous single celled ovoid conidia were observed on lactophenol cotton blue mount. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Fungal susceptibility results presented as minimal inhibitory concentration (ug/ml).

Antifungal agent	MIC (ug/ml)
5-Flucytosine	>64
Amphotericin B	4
Anidulafungin	>8
Caspofungin	>8
Fluconazole	32
Itraconazole	0.12
Micafungin	>8
Posaconazole	0.12
Voriconazole	0.12

Consent

Written informed consent was obtained from the patient or legal guardian(s) for publication of this case report and accompanying

images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Declaration of competing interest

There are none.

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