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Disseminated *Scedosporium prolificans* infection in a Labrador retriever with immune mediated haemolytic anaemia

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ABSTRACT

Disseminated scedosporiosis is rare in dogs and is usually reported in German Shepherds with suspected heritable immunodeficiency. This is the first report of disseminated scedosporiosis due to *Scedosporium prolificans* in a Labrador retriever dog that was receiving immunosuppressive drug therapy for treatment of immune-mediated haemolytic anaemia. Despite cessation of immunosuppressive medications and an initial response to aggressive treatment with voriconazole and terbinafine the dog developed progressive disease with neurological signs necessitating euthanasia six months from diagnosis.

1. Introduction

Infections caused by fungi belonging to the *Scedosporium/Pseudallescheria* complex (SPCF) in dogs are restricted to a small number of cases including localised infections involving the skin, upper respiratory tract and eyes and disseminated disease [1]. *Scedosporium prolificans* and *Scedosporium apiospermum* (teleomorph *Pseudallescheria boydii*) are the medically significant species in the SPCF [2]. We report the first case of disseminated mycosis due to *S. prolificans* in a Labrador retriever dog that was receiving immunosuppressive drug therapy for treatment of immune mediated haemolytic anaemia (IMHA).

2. Case

A four-year-old male desexed Labrador retriever was referred to the University Veterinary Teaching Hospital, Sydney (UVTHS) with a four week history of right hind limb lameness and a one week history of multiple ulcerated skin lesions. Ten months previously the dog had been diagnosed with primary immune mediated haemolytic anaemia (IMHA) and was treated with immunosuppressive medication (prednisolone 1.5 mg/kg PO every 12 h; azathioprine 1.25 mg/kg PO every 24 h), and antiplatelet therapy (aspirin 0.6 mg/kg PO every 24 h). Following tapering of immunosuppressive medication, the dog represented to the UVTHS three months after initial diagnosis (day −176) with severe recurrent IMHA. Prednisolone (1 mg/kg PO every 12 h) and azathioprine (1.25 mg/kg PO every 24 h) were again prescribed with the addition of cyclosporine (5.5 mg/kg PO every 24 h). The dog responded well and all drug dosages were tapered slowly over the following seven months (prednisolone 0.125 mg/kg PO every second day; azathioprine 1.25 mg/kg PO every other day). Cyclosporine was discontinued three months (day −97) prior to presentation.

On presentation to the UVTHS on day 0 the dog weighed 39.7 kg, was in good body condition and had a normal heart rate, respiratory rate and rectal temperature. It was lame (grade III/V) on the right hind limb and had multiple ulcerated skin lesions with draining sinus tracts over the head, neck and body (Fig. 1). Pain was elicited on manipulation of the right coxo-femoral joint and mid thoracic spine. Pitting oedema was present distal to the right hock. Mild bilateral elbow effusion was detected. The left pre-scapular and popliteal lymph nodes were palpably enlarged.

Given the clinical presentation and previous history of IMHA, differential diagnoses included disseminated bacterial or fungal infection, immune mediated polyarthropathy and dermatopathy as an indication of multisystemic autoimmunity, or systemic lupus erythematous.

A complete blood count (CBC) and serum biochemistry were performed. There was a mild non-regenerative anaemia (haematocrit 0.32 L/L; reference interval: 0.37–0.55 L/L), moderate leucocytosis (23.4×10^9 /L; reference interval: $7–12 \times 10^9$ /L) primarily due to a neutrophilia (18.95×10^9 /L; reference interval: $4.06–9.36 \times 10^9$ /L) with a left shit (bands 0.23×10^9 /L; reference interval: $0–0.24 \times 10^9$ /L) and a mild monocytosis (1.64×10^9 /L; reference interval: $0.21–0.96 \times 10^9$ /L). Mild elevations in alkaline phosphatase

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(ALP 261 U/L; reference interval: < 110 U/L), alanine transaminase (ALT 86 U/L; reference interval: < 60 U/L), total protein (72.8 g/L; reference interval: 50–70 g/L) and phosphate (2.02 mmol/L; reference interval: 0.8–1.6 mmol/L) were noted on serum biochemistry. In addition, total serum calcium (3.43 mmol/L; reference interval: 2.1–2.9 mmol/L) was elevated and ionised calcium was normal (1.28 mmol/L; reference interval: 1.25–1.5 mmol/L). An antinuclear antibody (ANA) titre was negative (< 1:25).

Abdominal ultrasound demonstrated increased, but homogeneous echogenicity of the pancreas, consistent with chronic pancreatitis and mild bilateral renal pelvis dilation. On urinalysis short septate branching fungal hyphae were detected. Whole body computed tomography (CT) on day 2 revealed multiple productive bone lesions in the distal portion of the third and eight ribs; lysis of the opposing endplates at thoracic vertebrae 5–6 and sternebrae 2–3 with surrounding bone sclerosis; and irregular periosteal new bone formation associated with the metatarsal and tarsal bones of the right hind limb. The right liver lobes had irregular borders and diffusely heterogeneous post-contrast enhancement in the arterial phase. In addition, generalised abdominal, sternal and peripheral lymphadenopathy was seen (Fig. 2). Cytological examination of joint fluid from the right hock and carpus was unremarkable. Fine-needle aspirate biopsies from rib and sternebrae lesions, and impression smears of multiple skin lesion biopsies showed marked pyogranulomatous inflammation. Fungal hyphae were identified in skin lesions.

Itraconazole (5 mg/kg PO twice daily) was prescribed on day 3 whilst awaiting fungal culture results and the dog was discharged from hospital. Immunosuppressive medication doses were reduced then stopped after 5 days.

A suede-like black pigmented fungus was cultured on Sabouraud's dextrose agar on day 7 at 28 °C from urine, bone and skin, but not from joint fluid. On microscopic examination, septate hyphae had basally inflated flask shaped conidiophores with small clusters of conidia consistent with *S. prolificans*. A PCR targeting the partial β-tubulin gene was performed using DNA extracted from fungal culture material, as previously described [3]. The molecular identity was *S. prolificans* (99% homology with GenBank accession number AJ889591.1) [4]. The isolate was submitted to the Australian Reference Laboratory in Medical Mycology, Adelaide for susceptibility testing (Table 1).

Histopathology of skin revealed multifocal pyogranulomatous dermal inflammation with brown pigmented periodic acid-Schiff (PAS) positive irregularly septate hyphae occasionally branching at 90° within inflammatory foci.

Over a three week period the skin lesions improved. Terbinafine (30 mg/kg PO once daily) was added to the treatment regimen on day 16. Following antifungal susceptibility testing results, voriconazole (5 mg/kg PO twice daily), obtained from a veterinary compounding pharmacy was substituted for itraconazole (day 35). The dog was continued on voriconazole and terbinafine with no clinical side effects noted. After 6 weeks of treatment, all skin lesions had resolved and the hind limb lameness had improved. The dog's haematocrit had increased from 0.31 to 0.37 L/L indicating that the IMHA was in remission. Repeat serum biochemistry was unremarkable.

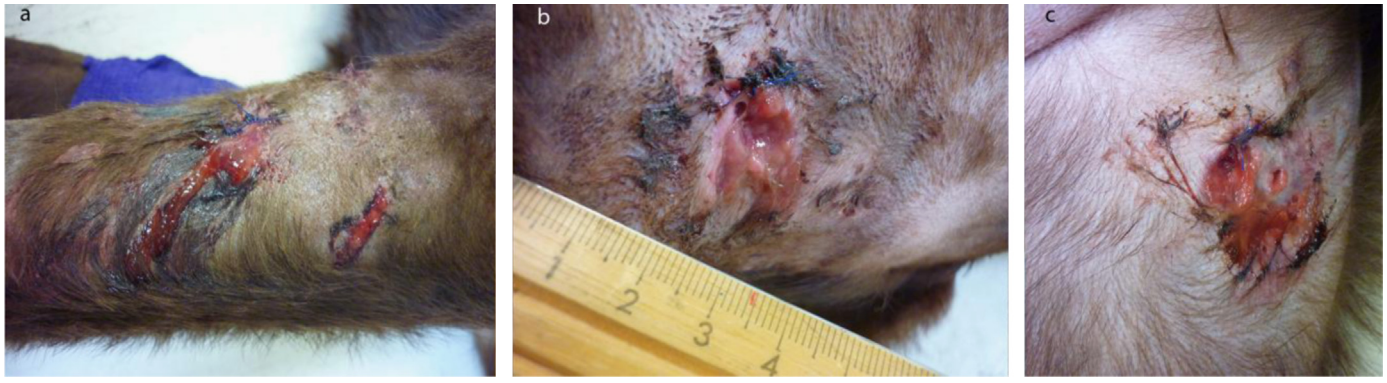

Fig. 1. Ulcerated skin lesions with draining sinus tracts. (a) Left antebrachium. (b) Right lateral aspect of the neck. Similar lesions were also seen on the left side of the neck, at the base of the right ear and at the lip commissures; and (c) medial aspect of right hind limb.

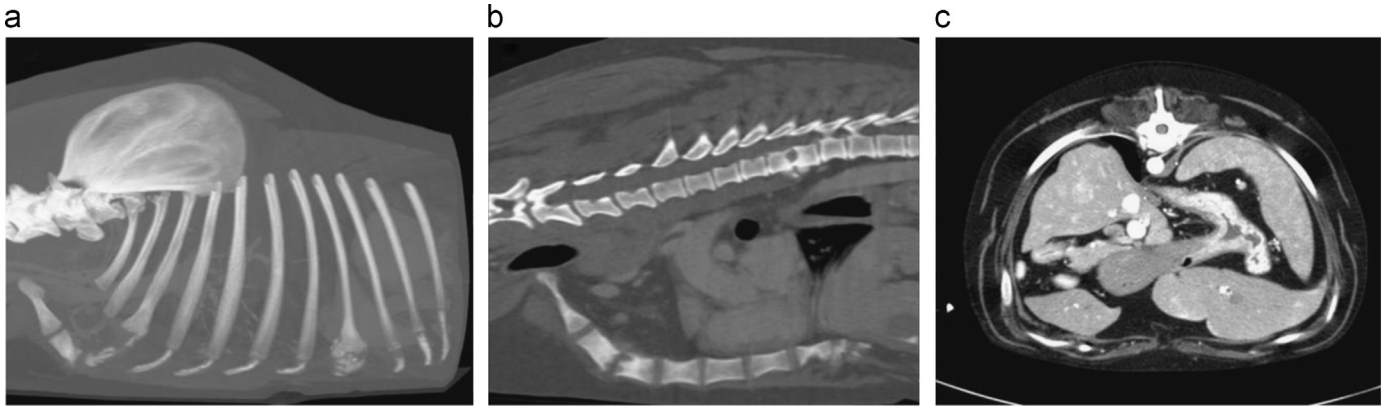

Fig. 2. CT images. (a) Sagittal volume rendering maximum intensity projection reconstruction of the thorax. On the right side of the thorax, a productive lesion, associated with irregular new bone formation can be seen affecting the distal aspect of the third and eight rib at the level of the costochondral junction. (b) Sagittal reconstructed image of the thorax. Lysis of the opposing end-plates of thoracic vertebrae 5–6 and sternebrae 2–3. There is increased bone opacity surrounding the lytic end-plates. Spondylosis deformans can be seen associated with the T5–6 lesion. (c) Axial image of the abdomen at the level of the liver acquired during the arterial phase. Right side of the liver presents heterogeneous enhancement and irregular contours. A 15 mm hypoattenuating lesion can be seen in the caudate lobe during the arterial phase only.

Table 1
Antifungal susceptibility testing results.

Antifungal	MIC (mg/L)	R/S/I/SDD[a]
Amphotericin B	4.0	R
5-Fluorocytosine	> 64	R
Fluconazole	> 256	R
Itraconazole	> 16	R
Voriconazole	4.0	R
Posaconazole	> 8.0	R
Caspofungin	> 8.0	R
Anidulafungin	> 8.0	R
Micafungin	> 8.0	R

R = resistant.
I = intermediate.
SDD = susceptible dose dependent.
[a] S = susceptible.

The dog was readmitted to hospital approximately 3 months (day 101) later for a repeat CT evaluation, CBC, serum biochemistry and urine culture. At this time, despite resolution of the right hind limb lameness, the dog had lost 6.7 kg since diagnosis. The haematocrit (0.49 L/L) and leucocyte count (9.0×10^9 /L) were normal. There was moderate hypoalbuminaemia (19.7 g/L; reference interval: 23–43 g/L) with normal total protein (61.5 g/L; reference interval: 50–70 g/L) and globulins (41.8 g/L; reference interval: 27–44 g/L). On repeat CT, bone, liver and lymph node lesions were unchanged. Repeat urine culture was positive for *S. prolificans*.

A urine protein creatinine ratio was normal (0.2; reference interval: 0.2–0.5), as were pre- and post-prandial bile acids. Hypoalbuminaemia was attributed to gastrointestinal losses due to mycotic involvement or concurrent inflammatory bowel disease. The owner was reluctant to pursue further diagnostic investigations. Antifungal drugs were continued and the dog was transitioned to a hypoallergenic diet.

The dog remained clinically well for another seven weeks before representing three times over a five week period for recurrent inappetence, pyrexia, dehydration and altered behaviour. On each occasion, the dog was treated conservatively with intravenous fluids and discharged within 24 h. Repeat biochemistry indicated worsening hypoalbuminaemia (13.8 g/L). During this time the owner sought a second opinion at another small animal specialist practice. A rectal scape demonstrated the presence of fungal hyphae, further mycotic involvement of the gastrointestinal tract.

Six months (day 183) following diagnosis, the dog represented with inappetence and lethargy. Twelve hours following admission, the dog's neurological status rapidly deteriorated with abnormalities suggestive of a C1–T2 spinal cord segment or brain stem lesion. It became obtunded, hyperthermic, tachycardic and developed an erratic breathing pattern. Given the rapid deterioration and poor prognosis, the owners elected to humanly euthanase the dog. A full post mortem was not conducted. Cerebrospinal fluid collected immediately following euthanasia indicated a mild mononuclear pleocytosis. Fungal culture was negative.

3. Discussion

This is the first report of disseminated mycosis due to *S. prolificans* in a Labrador retriever dog. Previous reports of disseminated scedosporiosis in dogs have been restricted to German Shepherd dogs (GSD) [5–9], and were due to *S. apiospermum* infection [5,6,8] except in one case of *S. prolificans* infection [7]. There is a single case reported of disseminated *S. prolificans* (previously *S. inflatum*) infection in another breed; a Beagle with osteomyelitis and pulmonary involvement [9].

Scedosporiosis is considered an emerging disease in human medicine. In contrast to *S. apiospermum*, which is commonly found in soil, sewage and polluted waterways, *S. prolificans* is more commonly isolated from soil and potted plants and has a more restricted geographical distribution with the majority of cases in humans reported in Australia and Spain [2]. Interestingly, one of the two previously reported cases of disseminated *S. prolificans* infection in dogs also occurred in Australia [7], with the other occurring in New York [9]. Scedosporiosis in humans represents a broad spectrum of clinical disease including local cutaneous and subcutaneous infection (eumycetoma) that occur secondary to penetration wounds; saprobic involvement of a previously damaged bronchopulmonary tree; invasive pulmonary disease; sinopulmonary and central nervous system infection associated with near drowning's in polluted water ways contaminated with *P. boydii*; and disseminated infection. Except for eumycetomas and near drowning's infection occurs in people with immune suppression or immune dysfunction. More specifically, disseminated infections are most commonly reported in humans with disorders in innate immunity such as chronic granulomatous disease or in those receiving immunosuppressive drug therapy as a result of haematopoietic malignancies and bone marrow or solid organ transplants [2].

GSD have an increased susceptibility to disseminated fungal infections, including aspergillosis and scedosporiosis. Abnormalities in IgA regulation and function, and dysfunction of cell mediated immunity is suspected [10]. No such immune deficiency has been identified in Labrador retrievers. In the Beagle with disseminated scedosporisosis, no underlying immune deficiency or history of immune suppressive medication was noted. In our case, it is likely that immune suppression induced by treatment for IMHA allowed for opportunistic infection with *S. prolificans*. The use of glucocorticoids is a well-documented risk factor for developing invasive fungal infections [2]. Glucocorticoids therapy results in global immune suppression and the risk of infection increases with increasing dose and duration of treatment beyond 21 days [11]. A history of corticosteroid use and persistent profound neutropenia have been identified as risk factors in developing disseminated *S. prolificans* infection in humans [2]. Cyclosporine also has potent immunosuppressive activity, acting by interrupting intracellular signalling, resulting in significant negative effects on T-cell function and cell medicated immunity. Invasive fungal infections are a common complication in humans treated with cyclosporine following solid organ transplant [12]. In our case, the dose of prednisolone was low at the time of presentation; however the prolonged duration of therapy, initial high doses and combination therapy with azathioprine and cyclosporine, likely contributed to significant immunosuppression. A similar recent case report from Australia describes a 2 year male desexed Rhodesian Ridgeback dog that developed lymphocutaneous lesions due to *S. apiospermum* infection whist receiving cyclosporine, azathioprine and prednisolone for IMHA and immune mediated polyarthritis [13]. In contrast to our case, once immunosuppressive medication was discontinued, the dog was able to clear the infection.

The route of infection in our case is not clear. Humans with disseminated *S. prolificans* have a high rate of fungaemia that results in fungal embolization of multiple organs. Haematogenous spread from primary pulmonary infection is the most common cause of fungaemia, although cutaneous lesions have been noted to precede dissemination in some cases [2]. No clear route of infection has been identified in previously reported cases of disseminated disease in dogs. In several cases with osteomyelitis [5,6,9], a penetrating wound or foreign body was suspected but unproven. In our case, the dog had ulcerated skin lesion noted five months prior to presentation. The lesions were noted on the forelimbs associated with areas of pressure or wear and were considered likely due to the effects of prednisolone therapy on the skin namely thinning and increased fragility. The skin lesions had

healed six weeks prior to initial presentation however this break in integument may have been the route of entry for the fungal infection. The dog had no clinical sings attributable to pulmonary disease and mild changes noted on the CT scan of the thorax where more consistent with atelectasis rather than pneumonia. It seems less likely that dissemination occurred from primary pulmonary disease however this cannot be excluded as previous case reports have only noted disease involving the lungs at the time of post mortem examination [7,9]. Polyphagia and pica are common side effects seen in dogs receiving prednisolone. Given this, entry through the gastrointestinal tract as a result of indiscriminate eating patterns is also possible in this case. Gastrointestinal fungal involvement was demonstrated on rectal scape that would support this.

There is little available information regarding successful treatment of disseminated scedosporiosis in dogs. Treatment with itraconazole was attempted in three previous cases with no success; two dogs were euthanaised within a few weeks of treatment due to lack of improvement [6,9] and the third dog died acutely 5 days after initiation of treatment [7]. The case of lymphocutaneous infection due to *S. apiospermum* was successfully treated with itraconazole and terbinafine and cessation of immunosuppressive medication. Success in this case may be due to several reasons; removal of the cause of immunosuppression in an otherwise immunocompetent individual; restriction of infection to the skin; and a less virulent, more anti- fungal responsive isolate, *S. apiospermum*.

Mortality rates as high as 90% are reported in human with disseminated *S. prolificans* infection [14]. *S. prolificans* has a high degree of antifungal resistance with high minimum inhibitory (MICs) values to the majority of available antifungal agents. However, combination therapy using terbinafine with voriconazole, itraconazole or miconazole has been shown to result in synergy in vitro against human clinical isolates of *S. prolificans* such that MIC values were reduced to clinically achievable levels [15]. In vitro synergy has also been reported with combinations of amphotericin B and micafungin [16], and voriconazole and micafungin [17]. Successful treatment has been reported in vivo in clinical cases using combination antifungal therapy with terbinafine and voriconazole [18–20]. However aggressive surgical debridement and/or immune modulation therapy was often used to complement antifungal therapy. Combination antifungal susceptibility testing is not easily available in Australia. Thus information was extrapolated from studies in humans and used to guide the choice of combination antifungal treatment prescribed in our dog. Despite high in vitro MIC ($>$ 16 mg/L) for itraconazole, significant and sustained clinical improvement occurred for six months after treatment with combination therapy using itraconazole then voriconazole, combined with terbinafine. It is also possible that voriconazole, sourced from a compounding pharmacy, was not as efficacious as the non-generic formulation of the drug.

In conclusion, we present a novel case of a Labrador retriever that developed disseminated *S. prolificans* infection secondary to immunosuppressive drug therapy and demonstrate a partial clinical response to combination therapy with voriconazole and terbinafine.

Conflict of interest statement

None.

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