Medical Mycology June 2008, **46**, 355–359

informa
healthcare

Pulmonary *Phialemonium curvatum* phaeohyphomycosis in a Standard Poodle dog

D. A. SUTTON*, B. L. WICKES†, E. H. THOMPSON*, M. G. RINALDI*, R. M. ROLAND‡, M. C. LIBAL§, K. RUSSELL§ & S. GORDON‡

**Fungus Testing Laboratory, Department of Pathology and, †Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, ‡Department of Small Animal Clinical Science, and §Department of Pathobiology, College of Veterinary Medicine and Biomedical Science, Texas A & M University, College Station, Texas, USA*

Phialemonium curvatum, frequently misidentified as an *Acremonium* species, is reported here as a new agent of pulmonary phaeohyphomycosis in a Standard Poodle dog, and added as a new species in the genus to cause mycoses in canines. *In vitro* susceptibility data, for both human and animal isolates, suggests resistance to amphotericin B and susceptibility to the triazole agents itraconazole, voriconazole, and posaconazole.

Keywords *Phialemonium curvatum*, canine, systemic phaeohyphomycosis

Introduction

Systemic phaeohyphomycosis, a disease associated with saprobic dematiaceous fungi, has been reported infrequently in the dog. In humans *Cladophialophora bantiana* (synonyms, *Cladosporium trichoides, Cladosporium bantianum, Torula bantiana, Xylohypha bantiana, Xylohypha emmonsii*) is known to be neurotropic, and animals with systemic phaeohyphomycosis also commonly present with neurologic disease. In four reports of systemic *C. bantiana* infection in dogs the animals presented with a clinical history and/or signs of central nervous disease including tetraparesis, neck stiffness, back pain, circling, opisthotonus, nystagmus, protrusion of the nictitating membrane and/or seizures [1–4]. *Ochroconis gallopavum* [5] and *Aureobasidium pullulans* [6] have been isolated from lesions in dogs presenting with ataxia, seizures or 'neurologic dysfunction' and in another case dematiaceous fungi were demonstrated in the brain of an animal presenting with convulsions [7]. Rarely, animals with phaeohyphomycosis present with other primary clinical disease, as in the pug dog with a chronic skin infection and who had a dual systemic infection caused by *Bipolaris spicifera* and *Candida* (*Torulopsis*) *glabrata* [8]. Most cases of systemic phaeohyphomycosis in the dog have been diagnosed at necropsy. In the case reported here, the etiologic agent of pulmonary disease was detected antemortem.

Case report

A two-year-old male, castrated Standard Poodle was presented to the Texas A&M University Veterinary Medical Teaching Hospital in August, 2005 for definitive surgical repair of an atrial septal defect. The surgery was successful. Several days post-operatively the dog developed vasculitis, pancreatitis, as well as pneumonia, which were treated with palliative therapy in combination with enrofloxacin and ticarcillin/clavulanic acid. In October, 2005 the dog developed a chylous pleural effusion, which was corrected by surgical ligation of the thoracic duct in November 2005. The chylous effusion was thought to represent a complication of his previous open heart surgery. Following this surgery, the dog developed pneumonia again as well as a serosanguinous pleural effusion. The pneumonia was treated with enrofloxacin as well as amoxicillin/clavulanic acid. The pleural effusion became chronic and frequent thoracocenteses were performed to help control the clinical signs associated with the effusion. In December, 2005 a course of somatostatin was given but failed to resolve the effusion.

Received 22 August 2007; Accepted 12 December 2007
Correspondence: Deanna A. Sutton, Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas, USA. Tel: +1 210 567 4032. Fax: +1 210 567 4076. E-mail: suttond@uthscsa.edu

DOI: 10.1080/13693780701861470

Subsequently immunosuppressive prednisone therapy was initiated. After the addition of prednisone, the dog remained asymptomatic for his pleural effusion for eight months, during which the dose was gradually reduced to an anti-inflammatory dose range and the severity of the effusion was monitored with thoracic radiographs and echocardiography. Pleurocentensis was not performed at anytime during this 8 month period, after which the dog developed dyspnea and a severe suppurative pleural effusion. In addition, right rear lameness developed and a right carpal joint tap revealed septic, suppurative effusion. A urinary tract infection was also documented at this time. The dog was treated with thoracocentesis as needed to control clinical signs of dyspnea and a combination of ticarcillin/clavulanic acid, amoxicillin/clavulanic acid, and enrofloxacin. The prednisone was continued although doses were tapered. Over the next few months the dog presented multiple times with dyspnea due to pleural effusion and thoracentesis was performed. The cytologic examinations of pleural fluid samples varied slightly during this time period with the fluid being classified as either a transudate (low cell counts and low total protein concentration) or a modified transudate (mildly increased cell count or total protein concentration), depending on total nucleated cell counts and total protein concentrations. Several bacterial cultures of the pleural fluid yielded no growth. Given the severity of the recurrent pleural effusion in the face of reducing prednisone doses, and the historical response of the pleural effusion to immunosuppressive doses of prednisone, azothioprine was added to the drug regimen in an attempt to further immunosuppress the patient, and potentially allow the dose of prednisone to be reduced. Azothioprine was not well tolerated and was discontinued. Prednisone was continued at immunosuppressive doses and antibiotic coverage with a combination of clavamox and enrofloxacin were continued. Intermittent pleurocentesis for symptomatic pleural effusion continued although the frequency was somewhat reduced. In October, 2006 the animal was presented again with dyspnea and severe pleural effusion. New skin lesions had also developed near the right carpal footpad and over both tarsi. Cytologic examination of pleural fluid revealed a modified transudate/hemorrhagic effusion and cytologic examination of the footpad lesion revealed mild inflammation with intracellular fungal elements present. In addition, multiple lesions suggestive of dermatophytosis developed on the skin of the inguinal area. Three bacterial cultures and one fungal culture were inoculated with pleural fluid samples (collection dates 27 October 2006, 14 November 2006, 22 November 2006 and 3 November 2006 respectively). In addition, fungal cultures were started with samples from the footpad and tarsal lesions (collection date 14 November 2006). Pleural fluid samples for bacteria were inoculated onto trypticase soy agar with 5% sheep blood, MacConkey's agar, and into tryptose broth (Becton Dickinson, Sparks, MD) and incubated at 37°C in 5% CO_2 for up to 5 days. The first two pleural fluid samples yielded no bacterial growth however a fungus was isolated on the blood agar plates inoculated with each of these samples after 3 and 4 days of incubation, respectively. The third pleural fluid sample yielded *Staphylococcus aureus* after one day of incubation and a fungus on day 5 of incubation. The fungal isolates cultured from pleural fluid on three separate occasions appeared identical. One pleural fluid sample inoculated onto only Sabouraud dextrose agar ([SDA], (BD, BBL, Sparks, MD) was negative after 18 days incubation at 25°C. *Microsporum gypseum* was recovered in cultures started with the tarsal skin samples. The footpad culture grew a sterile dematiaceous mould that was subsequently identified as a coelomycete morphologically resembling a *Microsphaeropsis arundinis* (distinct from the fungi isolated from the pleural fluid samples). At this time azathioprine was discontinued and the dog was started on amphotericin B. The dog then developed a methicillin-resistant *Staphylococcus aureus* infection. Due to the poor prognosis and deteriorating condition, the dog was euthanized in December 2006. Necropsy was not performed.

Identification of the etiologic agent

Three fungal isolates from pleural fluid samples and the isolate from the footpad were forwarded to the Fungal Testing Laboratory at University of Texas, San Antonio, TX for identification. The pleural fluid isolates were accessioned into their stock collection as UTHSC 06-4324, 06-4325, and 06-4326. The morphologic features of the isolates were examined on in house prepared potato flakes agar (PFA) incubated at 25°C. Growth rate was moderate and after two weeks incubation colonies were white to cream, floccose, effuse, with centrally raised areas. Discrete, moist, salmon to brownish-yellow sporodochial areas (macroscopically visible cushion-like masses of short conidiophores bearing conidia) formed throughout the cultures after 4 weeks incubation (Fig. 1 – taken at 8 weeks). Microscopically, hyaline hyphae produced numerous coils and complex fasicles (bundles of hyphae). Conidiogenous cells consisted primarily of adelophialides (short phialides lacking a basal septum) produced directly on the hyphae (Fig. 2) and from coils.

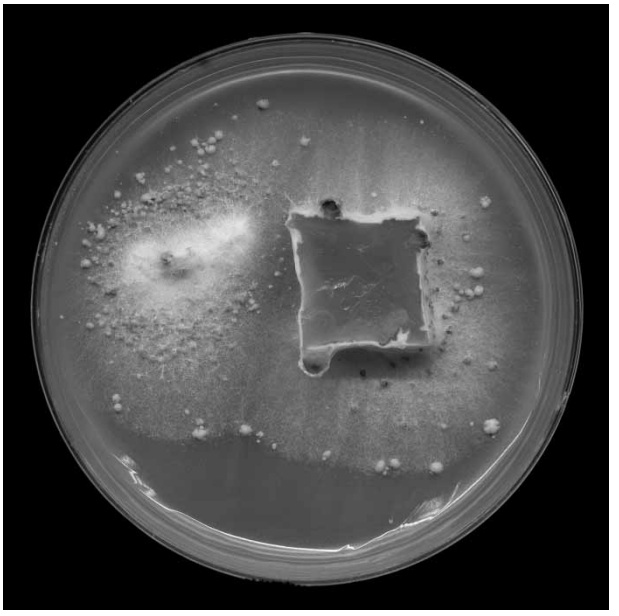

Fig. 1 Potato flakes agar plate, 8 weeks at 25°C, showing area of slide culture preparation on the right, and an undisturbed colony on the left. Salmon to brownish-yellow, moist, raised sporodochial areas are seen throughout the culture.

However longer phialides delimited by a basal septum as seen in *Acremonium* species were also occasionally present. Long, setae-like phialides were also produced from the sporodochia. Slightly allantoid (curved) conidia (1–1.5 × 4.4.5 μm) were borne in mucoid clusters at the apices of these conidiogenous cells. Chlamydoconidia were also present. Based on the features noted above, the isolate was morphologically identified as *Phialemonium curvatum* [9–11].

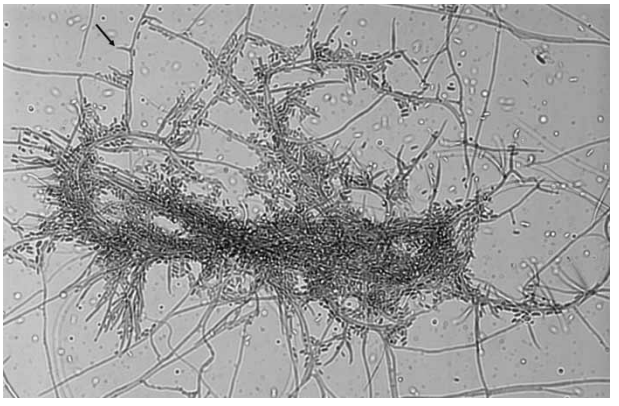

Fig. 2 Microscopic morphology of a young, immature sporodochium after 7 days growth at 25°C on potato flakes agar. Figure depicts short adelophialides (reduced phialides lacking a basal septum), black arrow, as well as longer phialides delimited by basal septa as seen in *Acremonium* species.

One of the isolates, UTHSC 06-4324 (= R-3884) was submitted for molecular characterization to confirm the morphologic identification. DNA was isolated from conidia recovered from a 72 h PDA plate using the Prepman Ultra reagent (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Five microliters each of the supernatant were used in two PCR reactions to amplify the ITS and D1/D2 regions from the rDNA locus. The ITS region was amplified as described using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [12]. The D1/D2 region was amplified using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3) as described [13,14]. Both PCR reactions were performed in a PTC-100 thermocycler (MJ Research, Watertown, MA) using Triple Master Taq polymerase (Fisher Scientific, Pittsburgh, PA). Amplicons were purified using a Qiaquick PCR purification kit (Qiagen, Inc., Valencia, CA) and then sequenced on both strands at the UTHSCSA Advanced Nucleic Acids Core facility. The data obtained from each sequence were then used to perform BLASTn searches at the NCBI website <http://ncbi.nlm.nih.gov/BLAST/> [15]. Identifications were made at a cutoff of ≥ 98% sequence identity.

The results of the two BLAST searches showed the greatest identity with other sequences deposited from *P. curvatum*. The top three hits for the D1/D2 sequence were *P. curvatum* sequences, each at 99% identity. The top three hits for the ITS search were *P. dimorphosporum*, *P. curvatum*, and *P. curvatum*, each at 99% identity. Since *P. dimorphosporum* is a synonym of *P. curvatum* [16], the sequence identity of the isolate was assigned as *P. curvatum*. The case isolate UTHSC 06-4323 (= R-3884) has been deposited in the University of Alberta Microfungus Collection under the accession number UAMH 10825. The nucleotide sequence data has been deposited into GenBank under the accession numbers EU035984 (ITS) and EU035985 (D1/D2).

The footpad isolate was accessioned as UTHSC 06-4327. After one month incubation at 25°C on a variety of media prepared in-house including PDA, V-8 agar, and carnation leaf agar [17], rare pycnidial structures developed. Conidia were narrow-cylindrical, 1–1.5 × 4 μm, individually subhyaline, but dark in mass. Based on these features the isolate morphologically resembled the coelomycete *Microsphaeropsis arundinis*. The recovery of this organism from the footpad, while potentially significant for localized infection at this site [18,19], was not contributory to systemic fungal

disease. No additional testing was performed on this isolate.

In vitro antifungal susceptibility testing

Retrospective antifungal susceptibility testing of *P. curvatum* was accomplished in a macrobroth dilution format in essential agreement with the previously published Clinical and Laboratory Standards Institute document M38-A [20]. Amphotericin B (AMB, Bristol-Meyers, Squibb, New York, NY) and caspofungin (CAS, Merck, Rahway, NJ) were tested in Antibiotic Medium 3 (Difco, Sparks, MD) while 5-fluorocytosine (5FC, Valient, Irvine, CA), fluconazole, voriconazole (FLC, VRC, Pfizer, Inc., New York, NY), itraconazole (ITC, Janssen Pharmaceutica, Piscataway, NJ) and posaconazole (PSC, Schering Plough, Galloping Hill, NJ) were tested in RPMI-1640 (Hardy Diagnostics, Santa Maria, CA). Tubes were incubated at 35°C with endpoints read at 24 and 48 h. The endpoints for AMB were the lowest concentration that inhibited visual growth, while those for 5FC and the triazoles were 80% inhibition compared to the growth control. Caspofungin endpoints were read as minimum effective concentrations (MECs) [21,22]. Results at 24/48 h were as follows in μg/ml: AMB 2/4; CAS 0.25/0.5; 5-FC >64; FLC 8/16; ITC 0.06/0.25; VRC 0.125/0.25; PSC 0.03/0.125.

Discussion

The genus *Phialemonium,* having morphologic features between the genera *Acremonium* and *Phialophora,* currently contains two species, *P. obovatum* and *P. curvatum* [9,16]. *Phialemonium obovatum* produces a distinct, pale green diffusing pigment, has obovate conidia (like an upside-down egg), and has been previously reported in German shepherd dogs causing osteolytic [23] and disseminated disease [24]. To our knowledge, this is the first report of *P. curvatum* in the veterinary literature. As the use of SDA as a sole primary isolation medium is less than optimal for the recovery of filamentous fungi, this and other etiologic agents may be under-diagnosed. Sporodochial-forming *Phialemonium curvatum* isolates were initially recognized in 2004 in human cases of hemodialysis-associated endovascular infection [10]. They have subsequently been seen in cases of endocarditis and endophthalmitis stemming from intracavernous penile autoinjections of contaminated fluids [11,25], and from intra-articular injection of corticosteroids [26]. Isolates are often misidentified as *Acremonium* species based on the overall macroscopic and microscopic similarities of the two genera.

In humans, the formation of phialides and phialoconidia within tissues in the host, termed 'adventitious' conidia by Liu *et al.* [27], appear to facilitate hematogenous dissemination inciting fungemia [16], endocarditis [28,29], and peritonitis [30]. Disseminated disease usually occurs in the setting of immune compromise. The same scenario presumably occurs in dogs. On occasion dogs operated for chylous effusion can develop non-chylous effusion post operatively that responds to immunosuppression suggesting an underlying inflammatory etiology [31]. However, dogs receiving chronic immunosuppressive agents are, like humans, at risk for infections, particularly from a variety of potential fungal pathogens [32,33]. Secondary infections may also be present and, in this case, likely contributed to the severity of the pleural effusion in the later stages of the disease.

Retrospective antifungal susceptibility results for the case isolate were similar to those seen for human isolates. Although there are no defined breakpoints for this organism, elevated MICs for AMB and 5FC suggested resistance. Clinical deterioration while on AMB therapy may support lack of efficacy for this agent. Itraconazole, as well as the newer triazoles PCZ and VCZ, demonstrated low MICs, while the FLZ MIC was somewhat elevated at 16μg/ml. Minimum effective concentrations for CAS were also low at 0.5 μg/ml; a departure from MEC values seen in human isolates.

In conclusion, *Phialemonium curvatum* is reported as a new agent of pulmonary phaeohyphomycosis in a Standard Poodle dog, and is added as a new species in the genus to cause disease in canines. Based on limited data, the triazole drugs ITZ, VRZ, and PCZ would appear appropriate for empiric therapy pending susceptibility test results.

Acknowledgements

B.L.W. is supported by grant PR054228, from the U.S. Army Medical Research and Materiel Command, Office of Congressionally Directed Medical Research Programs. We thank Dora McCarthy of the Fungus Testing Laboratory for performing the antifungal susceptibility testing.

References

1 Anor S, Sturges BK, Lafranco K, *et al.* Systemic phaeohyphomycosis (*Cladophialophora bantiana*) in a dog – clinical diagnosis with sterotactic computed tomographic-guided brain biopsy. *J Vet Intern Med* 2001; **15**: 257–261.

2 Dillehay DL, Ribas JL, Newton JC, Kwapien RP. Cerebral phaeohyphomycosis in two dogs and a cat. *Vet Path* 1987; **24**: 192–194.
3 Schroeder H, Jardine JE, Davis V. Systemic phaeohyphomycosis caused by *Xylohypha bantiana* in a dog. *J S Afr Vet Assoc* 1994; **65**: 175–178.
4 Fiske RA, Choyce PD, Whitford HW, Martin EC. Phaeohyphomycotic encephalitis in two dogs. *J Am Anim Hosp Assoc* 1986; **22**: 327–330.
5 Singh K, Flood J, Welsh RD, *et al*. Fatal systemic phaeohyphomycosis caused by *Ochroconis gallopavum* in a dog (*Canis familaris*). *Vet Path* 2006; **43**: 988–992.
6 Perkins MC, Martin P, Krockenberger MB, Weeks K, Malik R. Cerebral phaeohyphomycosis in a young female German shepherd. *Australian Vet Prac* 2004; **34**: 110.
7 Migaki G, Casey HW, Bayles WB. Cerebral phaeohyphomycosis in a dog. *J Am Vet Med Assoc* 1987; **191**: 997–998.
8 Waurzyniak BJ, Hover JP, Clinkenbeard KD, Welsh RD. Dual systemic mycosis caused by *Bipolaris spicifera* and *Torulopsis glabrata* in a dog. *Vet Path* 1992; **6**: 566–569.
9 Gams W, McGinnis MR. *Phialemonium*, a new anamorph genus intermediate between *Phialophora* and *Acremonium*. *Mycologia* 1983; **75**: 977–987.
10 Proia LA, Hayden MK, Kammeyer PL, *et al*. *Phialemonium* species: Emerging mold pathogens that caused 4 cases of hemodialysis-associated endovascular infection. *Clin Infect Dis* 2004; **39**: 373–379.
11 Weinberger M, Mahrshak I, Keller N, *et al*. Isolated endogenous endophthalmitis due to a sporodochial-forming *Phialemonium curvatum* acquired through intracavernous autoinjections. *Med Mycol* 2006; **44**: 253–259.
12 White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis N, Gelfand D, Sninsky J, White TC (eds). *PCR-Protocols and Applications – A Laboratory Manual*. New York, NY: Academic Press, 1990: 315–322.
13 Kurtzman CP, Robnett CJ. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. *J Clin Microbiol* 1997; **35**: 1216–1223.
14 Peterson SW, Kurtzman CP. 1990. Phylogenetic relationships among species of the genus *Issatchenkia* Kudriavzev. *Antonie Van Leeuwenhoek* 1990; **58**: 235–240.
15 Altschul SF, Madden TL, Schäffer AA, *et al*. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997; **25**: 3389–3402.
16 Guarro J, Nucci M, Akiti T, *et al*. *Phialemonium* fungemia: two documented nosocomial cases. *J Clin Microbiol* 1999; **37**: 2493–2497.
17 Nelson PE, Toussoun TA, Marasas WFO. 1983. *Fusarium species. An Illustrated Manual for Identification*. Pennsylvania State University Press: University Park, PA.
18 Pendle S, Weeks K, Priest M, *et al*. Phaeohyphomycotic soft tissue infections caused by the coelomycetous fungus *Microsphaeropsis arundinis*. *J Clin Microbiol* 2004; **42**: 5315–5319.
19 Kluger EK, Della Torre PK, Martin P, Krockenberger MB, Malik R. Concurrent *Fusarium chlamydosporum* and *Microsphaeropsis* arundinis infections in a cat. *J Feline Med Surg* 2004; **6**: 271–277.
20 Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard. CLSI document M38-A. 2002; Clinical and Laboratory Standards Institute, Wayne, PA.
21 Arikan S, Lozano-Chiu M, Paetznick V, Rex JH. *In vitro* susceptibility testing methods for caspofungin against *Aspergillus* and *Fusarium* isolates. *Antimicrob Agents Chemother* 2001; **45**: 327–330.
22 Kurtz MB, Heath IB, Marrinan J, *et al*. Morphological effect of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-beta-D-glucan synthase. *Antimicrob Agents Chemother* 1994; **38**: 1480–1489.
23 Lomax LG, Cole JR, Padhye AA, *et al*. Osteolytic phaeohyphomycosis in a German shepherd dog caused by *Phialemonium obovatum*. *J Clin Microbiol* 1996; **23**: 987–991.
24 Smith AN, Spencer JA, Stringfellow JS, Vygantas KR, Welch JA. Disseminated infection with *Phialemonium obovatum* in a German shepherd dog. *J Am Vet Med Assoc* 2000; **216**: 708–712.
25 Strahilevitz J, Rahav G, Schroers H-J, *et al*. An outbreak of *Phialemonium* infective endocarditis linked to intracavernous penile injection for the treatment of impotence. *Clin Infect Dis* 2005; **40**: 781–786.
26 Dan M, Yossepowitch O, Hendel D, Shwartz O, Sutton DA. *Phialemonium curvatum* arthritis of the knee following intra-articular injection of a corticosteroid. *Med Mycol* 2006; **44**: 571–574.
27 Liu K, Howell DN, Perfect JR, Schell WA. Morphologic criteria for the preliminary identification of *Fusarium*, *Paecilomyces*, and *Acremonium* species by histopathology. *Am J Clin Pathol* 1997; **109**: 45–54.
28 Schonheyder HC, Jensen HE, Gams W, *et al*. Late bioprosthetic valve endocarditis caused by *Phialemonium curvatum* and *Streptococcus sanguis*: a case report. *J Med Vet Mycol* 1996; **34**: 206–214.
29 Gavin PJ, Sutton DA, Katz BZ. Fatal endocarditis caused by the dematiaceous fungus *Phialemonium obovatum*: case report and review of the literature. *J Clin Microbiol* 2002; **40**: 2207–2212.
30 King D, Pasarell L, Dixon DM, McGinnis MR, Merz WG. A phaeohyphomycotic cyst and peritonitis caused by *Phialemonium* species and a re-evaluation of its taxonomy. *J Clin Microbiol* 1993; **31**: 1804–1810.
31 Smeak DD, Gallagher L, Birchard SJ, Fossum TW. Management of intractable pleural effusion in a dog with pleuroperitoneal shunt. *Vet Surg* 1987; **16**: 212–216.
32 Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. *The Lancet* 2003; **362**: 1828–1838.
33 Grooters AM. Weird fungi: Making sense of emerging and unusual mycoses. In: Proceedings of the 21st Annual American College of Veterinary Internal Medicine Forum; Charlotte, NC, 2003.