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Sinonasal and sino-orbital aspergillosis in 23 cats: Aetiology, clinicopathological features and treatment outcomes

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ABSTRACT

Aetiology, clinicopathological findings and treatment outcomes were documented in 23 cats (1.5–13 years of age) with sinonasal (SNA, $n = 6$) or sino-orbital (SOA, $n = 17$) aspergillosis. Cases recruited retrospectively and prospectively were included if fungal hyphae were identified on cytological or histological examination and the fungal pathogen was identified by PCR and DNA sequencing (ITS1 or ITS1-5.8S-ITS2 regions, rDNA gene cluster).

Fungal culture was positive in 22/23 cases. In cases of SNA, the fungal pathogen was *Aspergillus fumigatus* ($n = 4$), *Neosartorya fischeri* or *A. lentulus* ($n = 1$) or a non-speciated *Neosartorya* spp. ($n = 1$). In all cases of SOA ($n = 17$), the fungal pathogen was identified as *Neosartorya* spp. Nine cats had brachycephalic conformation. Cats with SNA were more likely to be infected with *A. fumigatus* and had a better prognosis than cats with SOA.

Introduction

Information about aspergillosis affecting the upper respiratory tract (URT) of cats is restricted to individual case reports and several small case series (Peiffer et al., 1980; Wilkinson et al., 1982; Goodall et al., 1984; Halenda and Reed, 1997; Hamilton et al., 2000; Tomsa et al., 2003; Malik et al., 2004; Whitney et al., 2005; McLellan et al., 2006; Kano et al., 2008; Barachetti et al., 2009; Furrow and Groman, 2009; Karnik et al., 2009; Giordano et al., 2010; Quimby et al., 2010; Smith and Hoffman, 2010). Ten of 25 cats in these reports were Persians or Himalayan Persians, suggesting a possible brachycephalic breed predisposition. Sinonasal aspergillosis (SNA) accounted for approximately half of the reported cases, while the rest had orbital involvement (sino-orbital aspergillosis, SOA). There is limited information on the aetiopathological differences between these two disease presentations, although progression of SNA to SOA has been documented (Hamilton et al., 2000).

The identity of fungal pathogens to species level has been reported in nine affected cats: *Aspergillus fumigatus* ($n = 5$), *A. flavus* ($n = 1$), *A. niger* ($n = 2$) and *A. udagawae* ($n = 1$) (Malik et al., 2004; Whitney et al., 2005; McLellan et al., 2006; Kano et al., 2008; Barachetti et al., 2009; Furrow and Groman, 2009; Smith and Hoffman, 2010; Giordano et al., 2010). However, species identification (*A. udagawae*) was confirmed by molecular studies in only one case; this isolate was initially misidentified as *A. fumigatus* based on phenotypic features (Kano et al., 2008). Members of the *A. fumigatus* complex cannot be identified reliably by phenotypic testing alone (Balajee et al., 2005; Vinh et al., 2009). These findings raise the possibility that pathogens other than *A. fumigatus* could be an underdiagnosed cause of URT aspergillosis in cats.

Treatment of the orbital form of disease is particularly challenging and the prognosis for resolution of infection is generally poor (Hamilton et al., 2000; Kano et al., 2008; Barachetti et al., 2009; Giordano et al., 2010). Few cases have been treated successfully (McLellan et al., 2006; Smith and Hoffman, 2010). The objectives of this study were to document the clinicopathological findings, molecular identity of fungal pathogens and treatment outcomes in cats with URT aspergillosis.

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Materials and methods

Cases and samples

Retrospective cases were identified from the medical records of the University Veterinary Teaching Hospital, Sydney (UVTHS) and Veterinary Pathology Diagnostic Services (VPDS), from January 1998 to December 2006. Cases were recruited prospectively from January 2007 to December 2009 following an Australia-wide call for cases (Barrs et al., 2007). Inclusion criteria were (1) identification of fungal hyphae on cytological or histological examination of tissue biopsies or sinonasal fungal plaques and (2) molecular identification of the isolate from tissue samples (fresh or formalin-fixed paraffin-embedded tissue) and/or culture material. Cats were classified as having SNA or SOA on the basis of absence (SNA) or presence (SOA) of a retrobulbar mass at initial presentation. Clinical data, tissue samples (fresh and/or formalin-fixed paraffin-embedded tissue) and fungal cultures were collected from each case. Data from postmortem examination were included when available.

Clinical data

Signalment, history, clinical signs, haematology, biochemistry, retrovirus serology, latex cryptococcal antigen titres (LCAT), agar gel immunodiffusion (AGID) serology for *Aspergillus* spp. antibodies, microbiology, histopathology, treatment and outcome were recorded. Treatment response was categorised as complete remission or treatment failure; complete remission was defined as resolution of all signs $\geqslant$3 months after cessation of therapy; other outcomes were assigned to the treatment failure group. Cats that could not be assessed for treatment response were censored.

Morphological identification

Samples were cultured at 28 °C and 37 °C on Sabouraud's dextrose agar with added gentamicin and chloramphenicol when bacterial contamination was likely. Where available, isolates identified as *Neosartorya* spp. were retrieved and subcultured on malt extract agar in pairs at 30 °C for 30 days in the dark and examined for cleistothecia (fruiting bodies) at the colony junction. Antifungal susceptibility testing was performed at the Australian Reference Laboratory in Medical Mycology, Adelaide.

Molecular identification

DNA extraction, PCR amplification of the ITS1 region (fresh and formalin-fixed paraffin-embedded tissue) and/or ITS1-5.8S-ITS2 region (culture material) of the rDNA gene cluster was performed from clinical specimens as described previously using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers (Chen et al., 2002; Lau et al., 2007). Sequence identity was determined using BLAST against the GenBank[1] and Centraalbureau voor Schimmelcultures (CBS)[2] databases.

Statistical analysis

Statistical analysis was performed using R version 2.6.2. Cats with SNA and SOA were compared with respect to conformation, clinical signs, fungal isolate and response to treatment. Due to the low expected frequencies in some categories, Fisher's Exact Tests were used in preference to χ^2. The fisher.test() function of *R* was used to calculate the *P* value of conditional independence, the conditional Maximum Likelihood Estimate of the odds ratio and the 95% confidence interval of the odds ratio. Significance was ascribed to a *P* value <0.05.

Results

Clinical and clinicopathological findings

Twenty-three cases (4 retrospective, 19 prospective) from New South Wales (n = 10), Queensland (n = 9), Victoria (n = 3) and Western Australia (n = 1) met the inclusion criteria. All cats were neutered (13 female, 10 male). The age range was 1.5–13 years (mean 5.3, median 5 years). There were 12 domestic crossbreds (11 domestic short hair, 1 domestic long hair) and 11 pure breeds comprising one Russian Blue, one Cornish Rex and nine cats with brachycephalic conformation (3 Himalayan Persians, 3 Chinchilla Persians, 2 Ragdolls, 1 Exotic Shorthair) (Table 1).

Six cats had SNA and 17 cats had SOA. Lack of orbital involvement was confirmed by advanced diagnostic imaging (CT; n = 5) or surgical exploration of the affected frontal sinus and ipsilateral orbit (n = 1) in all cases of SNA. In cases of SOA, the presence of a retrobulbar mass was confirmed by advanced imaging (computerised tomography or magnetic resonance imaging; n = 11), at surgery or postmortem examination (n = 4) or by histological confirmation of a fungal granuloma where the retrobulbar mass had perforated into the oral cavity ventrally (n = 2). Four of six cats with SNA and 5/17 cats with SOA were brachycephalic; brachycephalic cats were no more likely to have SNA than non-brachycephalic cats. Five of six SNA and 17/17 SOA cases had a history of sneezing and nasal discharge within the preceding 6 months, whereas 8/17 SOA cases had no signs of sinonasal cavity disease at presentation (Table 2). The only significantly different clinical signs between cats with SNA and SOA were exophthalmos ($P < 0.001$) or presence of a mass or ulcer in the pterygopalatine fossa ($P < 0.05$) (Table 2; Fig. 1).

Retrovirus serology and LCAT were negative in all cats tested (FIV: n = 14; FeLV: n = 11; LCAT: n = 9). *Aspergillus* spp. serology (AGID) was positive in 2/2 cats tested (Table 1). Haematological and serum biochemistry findings for nine cats are presented in Table 3. Five cats with SOA and one with SNA were hyperglobulinaemic; these six cats all had *Neosartorya* spp. infections.

Microbiology

Fungal culture was positive in 22/23 cases. In cases of SNA, the molecular identity of fungal pathogens was *A. fumigatus* (n = 4), *Neosartorya fischeri* or *A. lentulus* (n = 1) and a non-speciated *Neosartorya* spp. (n = 1). Fungal isolates from all 17 cases of SOA were identified as *Neosartorya* spp. by DNA sequencing (Table 1). Fourteen *Neosartorya* spp. were subcultured in pairs. Cleistothecia and ascospores were produced by all isolates from at least one pairing (Fig. 2). No isolates were homothallic. Fourteen of 16 isolates of *A. fumigatus* or *Neosartorya* spp. were resistant to fluconazole (Table 4). Only isolates of *Neosartorya* spp. were resistant to itraconazole (3/13), voriconazole (2/11) and/or posaconazole (1/11). All three isolates of *A. fumigatus* tested were susceptible to voriconazole and posaconazole. No isolates were resistant to amphotericin B.

Histopathology

Inflammatory infiltrates were lymphocytic (n = 2), histiocytic and eosinophilic (n = 1) or neutrophilic (n = 1) in nasal mucosal biopsies available from 4/6 SNA cases. Biopsies were available from 13/17 SOA cases. Granulomatous (n = 1) or plasmacytic and eosinophilic (n = 1) rhinitis were evident in nasal biopsies from two cases. Retrobulbar (n = 11) and nasopharyngeal (n = 2) masses were characterised by necrosis and well-vascularised granulomatous inflammation. Granulomas contained central areas of coagulative and liquefactive necrosis with abundant periodic acid-Schiff-positive fungal hyphae. Surrounding zones of inflammation comprised epithelioid macrophages interspersed with variable numbers of eosinophils, neutrophils, lymphocytes and plasma cells extending into a peripheral zone of fibrosis.

Complete postmortem examinations were performed in six cats with SOA. All had granulomatous mycotic invasion of the nasal cavities and paranasal sinuses, with variable invasion of the submucosal tissue, invasion of paranasal soft-tissues ipsilateral to the affected orbit and lysis of bone (Fig. 3). Inflammatory lesions effaced the adjacent skeletal muscle and bone in some cases.

Two cats that had surgical exenteration of the right orbit subsequently became blind in the left eye. In one case, there was mycotic involvement of the optic chiasm. In the other case, a retrobulbar mass effaced the left optic nerve. Of nine SOA cases in which ocular

[1] http://www.ncbi.nlm.nih.gov/genbank/.
[2] http://www.cbs.knaw.nl/databases/.

Table 1
Case details, molecular identity of fungal pathogens and treatment outcomes in 23 cases of feline upper respiratory tract aspergillosis.

Case	Sex[a]	Age (years)	Breed[a]	Disease[b]	Fungal culture[c]	*Aspergillus* serology[c]	Fungal species (% identity)[d]	Surgery[g]	Number of topical treatments[h]		Systemic antifungals[h]						Treatment (months)	Outcome[i]	Disease free after cessation of therapy (months)
									Clo	Enil	AMB-D	AMB-L	Itra*	Pos*	Vor*	Ter			
1	F	2.6	Persian	SNA	+	ND	*A. fumigatus*[f] (100%)	R	0	0	+	–	+	+	–	+	7	CR	28
2	M	13	DLH	SNA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	ST	0	0	+	+	–	+	–	+	6	CR	24
3	M	5	DSH	SNA	–	ND	*A. lentulus/N. fischeri*[f] (100%)	ST	0	0	+	–	+	–	–	–	7	CR	50
4	F	11	Persian	SNA	+	+	*A. fumigatus*[e] (100%)	–	0	1	+	–	+	+	+	–	9	TF	–
5	F	6.8	Ragdoll	SNA	+	ND	*A. fumigatus*[e](100%)	ST	1†	0	–	–	–	+	–	–	<1	C	–
6	M	7.4	Persian	SNA	+	ND	*A. fumigatus*[e] (99%)	ST	2‡	0	–	–	–	+	–	–	7	CR	7
7	F	5	CR	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e](99%)	–	0	0	+	–	+	+	+	+	16	CR	19
8	F	8	Exotic SH	SOA	+	ND	*N. pseudofischeri*[e,f] (100%)	Ex	0	0	+	–	+	–	–	–	2	C	–
9	F	3.3	Himalayan	SOA	+	ND	*N. aureola/N. udagawae*[e] (99%)	Ex	0	0	–	–	+	+	–	–	1.3	TF	–
10	M	3.6	DSH	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	Ex	0	0	+	–	–	+	+	+	3	TF	–
11	F	2	DSH	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	Ex	0	0	+	–	–	+	–	+	3	TF	–
12	F	2.7	DSH	SOA	+	+	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	Ex	0	0	–	–	+	–	–	–	3	TF	–
13	F	7	DSH	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	Ex	0	0	–	–	–	+	–	–	4.5	C	–
14	M	4	RB	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	Or	0	0	+	–	+	–	–	–	2	TF	–
15	M	2	Himalayan	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	–	0	0	+	–	–	+	–	+	2	TF	–
16	M	2.3	DSH	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	–	0	0	–	–	–	–	–	–	0	NT	–
17	F	4	DSH	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	–	0	0	–	–	–	+	–	–	4.5	TF	–
18	F	5	DSH	SOA	+	ND	*N. fischeri/N. aureola/N. udagawae*[e] (99%)	–	0	0	–	–	–	+	–	+	10	C	–
19	F	8	Himalayan	SOA	+	ND	*N. aureola/N. udagawae*[f] (99%)	–	0	0	+	–	–	+	+	–	2.5	TF	–
20	F	5.9	DSH	SOA	+	ND	*N. aureola*[e] (99%)	–	0	0	+	–	+	+	–	–	8	TF	–
21	M	1.5	DSH	SOA	+	ND	*N. aureola*[e] (99%)	–	0	0	–	–	–	–	–	–	0	NT	–
22	M	8.2	DSH	SOA	+	ND	*N. aureola*[e] (99%)	–	0	0	–	–	–	+	–	–	1	TF	–
23	M	4.7	Ragdoll	SOA	+	ND	*N. aureola*[e] (99%)	–	0	0	–	–	–	+	–	–	6	TF	–

[a] M, male (neutered); F, female (neutered); DLH, domestic longhair; DSH, domestic shorthair; CR, Cornish Rex; Exotic SH, exotic shorthair; RB, Russian blue.
[b] SNA, sinonasal aspergillosis; SOA, sino-orbital aspergillosis.
[c] + Positive; – negative; ND, not done.
[d] % identity with GenBank and Centraalbureau voor Schimmelcultures (CBS) sequences, rounded to nearest whole integer.
[e] ITS1-5.8S-ITS2 PCR from fungal culture.
[f] ITS1 PCR from paraffin-embedded or fresh tissue.
[g] R, ventral rhinotomy; ST, sinus trephination; Ex, exenteration (orbital); Or, orbitotomy plus orbital debridement; –, not treated surgically.
[h] Clo, clotrimazole; Enil, enilconazole; AMB-D, amphotericin B deoxycholate; AMB-L, liposomal amphotericin B; Itra, itraconazole; Pos, posaconazole; Vor, voriconazole; Ter, terbinafine; + treatment prescribed; – treatment not prescribed; * azole drugs were prescribed sequentially not simultaneously; † topical 1% clotrimazole in polyethylene glycol plus 2% clotrimazole cream (Sissener et al., 2006); ‡ topical 1% clotrimazole in polyethylene glycol.
[i] CR, complete remission; TF, treatment failure (euthanased); NT, not treated (euthanased at diagnosis); C, censored.

histology was performed, none had mycotic invasion of the globe, but one cat had anterior uveitis.

Treatment regimes

Treatment was attempted in all six cases of SNA and 15/17 cases of SOA. Two cats with SOA were euthanased when treatment was declined. All 21 treated cats received systemic antifungal therapy. Treatment regimes included amphotericin B plus itraconazole or posaconazole ($n = 5$), amphotericin B plus itraconazole or posaconazole plus terbinafine ($n = 7$) or monotherapy with itraconazole ($n = 2$), posaconazole ($n = 8$) or voriconazole ($n = 4$) (Tables 1 and 5). Some cats received more than one treatment regime. Debridement of fungal plaques within the SNA was performed in 5/6 SNA cases, of which three were also treated with a topical antifungal azole.

Table 2
Clinical findings at presentation in 23 cats with upper respiratory tract aspergillosis.

Clinical findings	SNA[a] ($n = 6$)	SOA[b] ($n = 17$)
Nasal discharge	4	8
Stertor	4	8
Epistaxis	2	0
Temperature ⩾ 39.2 °C	2/4	5/12
Signs of sinonasal cavity disease at presentation	6	9
History of nasal discharge or sneezing within preceding 6 months	5	17
Discharging sinus	2	0
Unilateral exophthalmos	0	16
Bilateral exophthalmos	0	1
Exophthalmos	0	17
Corneal ulcer	0	6
Pterygopalatine fossa mass	0	13
Pterygopalatine fossa ulcer	0	2
Hard palate ulcer	0	4
Paranasal soft tissue swelling	0	6
Pain on opening mouth	0	2
Mandibular lymph node enlargement	2	8/13
Peripheral vestibular disease	0	1

[a] SNA, sinonasal aspergillosis.
[b] SOA, sino-orbital aspergillosis.

Treatment outcomes

Five cases had complete remission (4 SNA, 1 SOA). In the treatment failure group, there were 12 cats (1 SNA, 11 SOA). Four cats were censored (1 SNA, 3 SOA). Cats with SNA were significantly more likely to respond to treatment than cats with SOA ($P < 0.05$). The four SNA cases with complete remission had resolution of clinical signs at follow-up 7, 24, 28 and 50 months after cessation of treatment (Table 1). The SNA case with treatment failure was euthanased 13 months after diagnosis with persistent nasal signs and chronic kidney disease. Complete remission was achieved in one SOA case treated with oral itraconazole and amphotericin B (Table 1, Case 7). However, when treatment was stopped after 8 months, the cat developed recurrent disease and was treated with posaconazole (32 weeks) and terbinafine (16 weeks). The cat remained asymptomatic, with resolution of disease on CT examination, until 19 months after cessation of therapy, when it developed recurrent SOA. Clinical signs persisted despite treatment with posaconazole and liposomal amphotericin B. Clinical signs and CT evidence of infection resolved after treatment with caspofungin, then posaconazole monotherapy, which was ongoing 12 months later.

All SOA cases with treatment failure were euthanased due to progressive disease. Five cats treated with systemic antifungal agents (mean treatment period 2.6 months) and orbital exenteration developed neurological signs, including blindness or reduced vision ($n = 4$), circling and pleurothotonus ($n = 1$), facial muscle fasciculation and stargazing ($n = 1$) or hyperaesthesia ($n = 1$). Of six cats treated with medical therapy alone (mean treatment period 3.9 months), one developed ataxia and paresis.

Of the four cases that were censored, one died during anaesthetic recovery and postmortem examination was declined (Table 1, Case 5), one had resolution of signs but died 1 month after treatment stopped (no postmortem examination performed, Case 13), treatment was ongoing in one (case 18) and one was euthanased with heart failure (hypertrophic cardiomyopathy, Case 8).

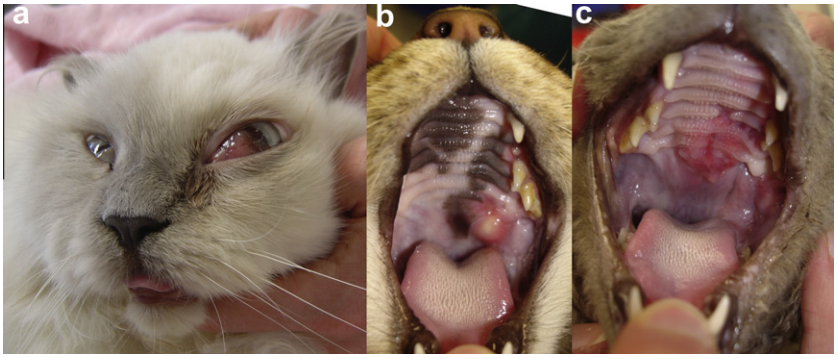

Fig. 1. Exophthalmos (a), mass in the left pterygopalatine fossa (b) and ulceration of the hard palate (c) due to retrobulbar fungal granulomas in cats with sino-orbital aspergillosis.

Table 3
Haematology and serum biochemistry values in nine cats with upper respiratory tract aspergillosis.

Variable	Median (range)	Number with low values	Number with high values	Reference range
Haematocrit (%)	38 (30–48)	0	1	30–45
Total leucocytes ($\times 10^9$/L)	9 (6.5–18.5)	3	2	8–14
Neutrophils ($\times 10^9$/L)	5.2 (2.9–13.7)	1	2	3.8–10.8
Band neutrophils ($\times 10^9$/L)	0	0	0	0–0.4
Monocytes ($\times 10^9$/L)	0.5 (0–1.1)	2	2	0.08–0.6
Lymphocytes ($\times 10^9$/L)	5.3 (0.9–5.0)	1	0	1.6–7
Eosinophils ($\times 10^9$/L)	0.890 (0.1–2.1)	1	1	0.2–1.4
Albumin (g/L)	30 (24–39)	1	0	19–38
Globulin (g/L)	56 (40–106)	0	6	26–51
Urea (mmol/L)	5.5 (4.6–8.1)	0	0	3–10
Creatinine (mmol/L)	119 (100–151)	0	0	90–180
Calcium (mmol/L)	2.5 (2.2–2.8)	0	2	1.75–2.6
Alanine amino transferase (IU/L)	33 (3–64)	0	1	<60
Alkaline phosphatase (IU/L)	20 (13–62)	0	1	<50

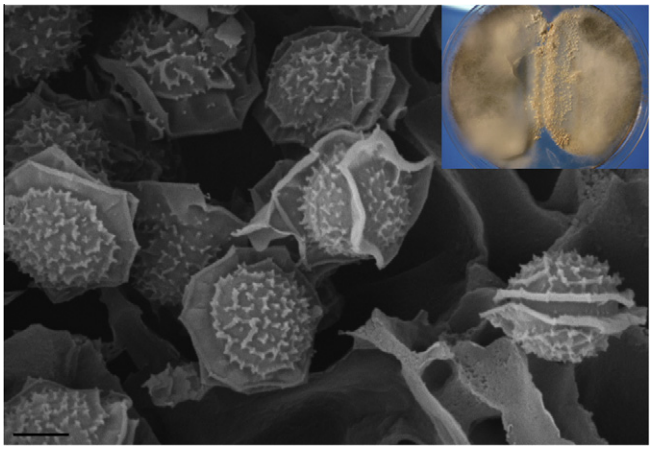

Fig. 2. Cleistothecia (small spherical structures) at the colony junction in paired cultures of *Neosartorya* spp. isolates from two cases (inset). *Neosartorya* spp. ascospores with roughened side walls and two axial crests. Scanning electron micrograph (Zeiss EVO LS15). Scale bar = 2 μm.

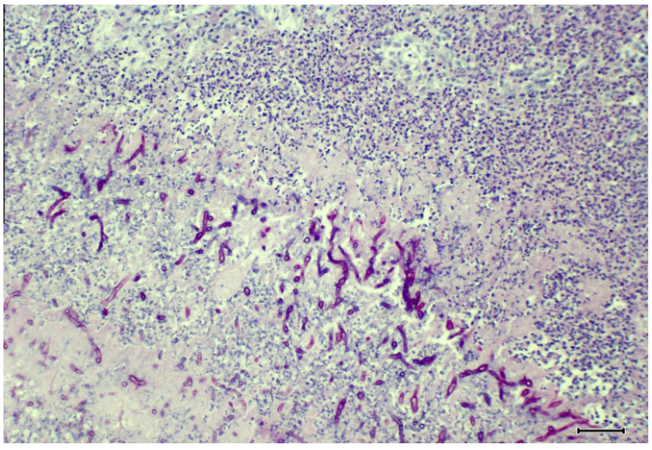

Fig. 3. Periodic acid-Schiff-stained section of frontal sinus epithelium from a cat with invasive sino-orbital aspergillosis. Fungal hyphae are present deep within the sinus epithelium, demonstrating the invasive nature of this mycosis. Scale bar = 60 μm.

Discussion

URT aspergillosis is an emerging infection in cats (Barrs et al., 2007). In our study, all cats were infected with fungi within the complex designated *Aspergillus* section *Fumigati* subgenus *fumigati*, also termed the *A. fumigatus* complex. This complex contains asexual members (anamorph, *Aspergillus* spp.), some of which also have sexual forms (teleomorph, *Neosartorya* spp.). *N. fumigata*, the teleomorph of *A. fumigatus*, has only been induced in vitro after an incubation period of 6 months (O'Gorman et al., 2009). Our ability to induce teleomorphs with relative ease in vitro within 30 days provides additional phenotypic evidence that species other than *A. fumigatus* cause feline SOA and that these fungal pathogens are not strict anamorphs. However, induction of teleomorphs is impractical for routine identification, since clinical isolates may not produce fruiting bodies in the laboratory setting and complementary mating strains are required for heterothallic species (Balajee et al., 2007; Kano et al., 2008).

For comparative purposes, we performed ITS1 and/or ITS1-5.8s-ITS2 PCR and sequencing of archival tissues from the sinonasal cavity of seven dogs with SNA; similar to other reports, the molecular identity of all seven canine mycoses was *A. fumigatus* (Peeters et al., 2005; Windsor et al., 2006). *Neosartorya* spp. has not been identified in URT aspergillosis of dogs; however, in canine SNA where fungal pathogens were identified by phenotypic features alone, it is possible that some cases were caused by members of the *A. fumigatus* complex and mistakenly identified as *A. fumigatus*. There is growing evidence that infections with *Neosartorya* spp. are more common in humans with invasive pulmonary aspergillosis than earlier thought, having been misidentified previously as *A. fumigatus* (Balajee et al., 2005; Vinh et al., 2009; Sugui et al., 2010). A polyphasic taxonomic approach is the gold standard for species identification within the *A. fumigatus* complex. This involves a combination of macro- and micromorphological traits, growth temperature regimes, extrolite profiles and PCR-based detection methods (Balajee et al., 2007; Samson et al., 2007).

The pan-mycotic PCR used in this study can be used to identify a diverse range of fungal genera from clinical specimens, including both filamentous fungi and yeasts (Lau et al., 2007). However, as demonstrated here, the ITS1 and ITS2 regions flanking the 5.8SrDNA may contain insufficient variation to enable identification of some individual species within the *A. fumigatus* complex. After ITS sequence analysis, comparison of partial β-tubulin gene sequences, alone or together with partial rodlet A gene sequences, is recommended for species identification (Balajee et al., 2007; Sugui et al., 2010).

In our study, SOA ($n = 17$) was more frequent than SNA ($n = 6$). Only cats with SNA were infected with *A. fumigatus*, while cats with SOA were infected with other species from the *A. fumigatus* complex. The reason for such a high proportion of SOA cases in this study could in part be due to increased recognition of the disease (Barrs et al., 2007). The ecological niche of species within the *A. fumigatus* complex is the soil and *A. fumigatus* is distributed worldwide (Latge, 1999; Samson et al., 2007). Some *Neosartorya* spp. have a worldwide distribution, whereas others are geographically restricted.

Cases of SOA have been reported in cats from the USA, Italy and Japan (Peiffer et al., 1980; Halenda and Reed, 1997; Hamilton et al., 2000; McLellan et al., 2006; Barachetti et al., 2009; Quimby et al., 2010; Smith and Hoffman, 2010). The molecular identity of the causative agent was confirmed in one case as *A. udagawae* (Kano et al., 2008).

SOA is an invasive mycosis of apparently immunocompetent cats. All cats in this study had URT signs, supporting the sinonasal cavity as the primary site of infection. Furthermore, there was involvement of the sinonasal cavity in all of the cases submitted for postmortem examination. Erosions were found in the orbital lamina, adjacent to the sinonasal cavity, suggesting that extension

Table 4
Results of in vitro antifungal susceptibility tests.

Fungal species	Voriconazole			Posaconazole			Itraconazole			Fluconazole		Amphotericin B	
	S	I	R	S	I	R	S	I	R	S	R	S	I
Aspergillus fumigatus	3	0	0	3	0	0	1	2	0	0	3	2	1
Neosartorya spp.	5	4	2	7	3	1	3	7	3	2	11	10	3

Numbers of isolates susceptible (S), with intermediate/dose-dependent susceptibility (I) or resistant (R).

Table 5
Drugs and dosages used in treatment of 21 cats with upper respiratory tract aspergillosis.

Drug	Number of cats treated	Dose	Dose comments[a]	Adverse effects
Amphotericin B deoxycholate	12	0.5 mg/kg in 350 mL 0.45% NaCl by SC infusion 2–3 times weekly	MCD 9 mg/kg (range 5.5–14 mg/kg)	
Liposomal amphotericin B	2	1–1.5 mg/kg IV every 48 h	MCD 5 mg/kg	
Caspofungin	1	1 mg/kg IV every 24 h	MCD 22 mg/kg	
Voriconazole	4	5–12 mg/kg PO every 24 h	MD 8.3 mg/kg/day. Treatment duration 4–21 days	Anorexia, dilated pupils, hind limb ataxia (3/4 cats)
Itraconazole	9	5–20 mg/kg PO every 24 h	MD 12 mg/kg PO 24-hourly	
Posaconazole	15	2.5–4.5 mg/kg PO every 12 h	MD 3 mg/kg PO 12-hourly	1.1–2× elevations in alanine amino transferase activity (2/10 cats)
Clotrimazole	2	1% topical preparation in polyethylene glycol	Sinonasal instillation (via sinus trephination)	
Enilconazole	1	1% topical preparation	Sinonasal instillation	
Terbinafine	7	12.5–20 mg/kg PO every 12–24 h	15 mg/kg PO 12-hourly	

[a] MCD, mean cumulative dose; MD, mean dose.

of disease occurs via this route. Lysis of the orbital lamina has been identified on CT in cats with SOA and progression from SNA to SOA has been documented (Hamilton et al., 2000; Barachetti et al., 2009).

More than one-third of cats in our study were pure-bred and had a brachycephalic conformation, suggesting that brachycephalic cats are predisposed to URT aspergillosis. Since cats were recruited from around Australia, comparison with local demographic data was not possible. However, data from two Australian states show that there are 2–3 times more domestic cross-bred cats than pure-bred cats and that Burmese cats are the most popular pure-bred cat, comprising up to 25% of the pure-bred population (Lederer et al., 2009; Toribio et al., 2009; NSW Cat Fancy Association kitten registration data 2001–2009, unpublished data). In our study, brachycephalic cats were no more likely to have SNA than non-brachycephalic cats. Furthermore, we identified URT aspergillosis in 13 females and 10 males, which does not support the preponderance of males reported previously (7 females, 14 males).

The basis for an association between brachycephalic conformation and URT aspergillosis in cats is not clear. In humans, decreased sinus aeration and drainage of respiratory secretions secondary to infection, polyps and allergic rhinosinusitis are risk factors for invasive SNA (Siddiqui et al., 2004). Reduced drainage of URT secretions due to brachycephalic conformation could be a risk factor in cats. However, since brachycephalic dogs are under-represented for SNA, it is likely that additional risk factors are present in cats. These could include heritable defects in mucosal immunity, previous viral URT infection and previous antibiotic treatment favouring fungal colonisation (Tomsa et al., 2003).

There is no evidence in our study or in previous reports of an association between retrovirus infection and URT aspergillosis, with only one FeLV positive case reported (Goodall et al., 1984). Hyperglobulinaemia, the most common biochemical abnormality in cats in our study, was documented in four previous cases of SOA (Hamilton et al., 2000; McLellan et al., 2006; Smith and Hoffman, 2010). Chronic antigenic stimulation may explain this hyperglobulinaemia, although electrophoresis was not carried out to determine whether specific immunoglobulin peaks were evident.

The majority of isolates in this study were resistant to fluconazole, which is typical of fungi in the *A. fumigatus* complex (Alcazar-Fuoli et al., 2008). *A. fumigatus* isolates in our study were susceptible to triazole antifungals and amphotericin B, which is also typical for this species (Alcazar-Fuoli et al., 2008). Elevated minimum inhibitory concentrations of voriconazole for *Neosartorya* spp. have been described previously (Balajee et al., 2005).

Presence or absence of orbital involvement in cats with URT aspergillosis has important prognostic significance. Complete remission was significantly more likely in cats with SNA than SOA. Further studies are required to investigate whether the infecting fungal species is a major determinant of treatment outcome. While SOA was only caused by species other than *A. fumigatus*, these cases may be more responsive to treatment if diagnosed before orbital extension. Two out of three SNA cases with complete remission had infections with *Neosartorya* spp. or *N. fischeri*/*A. lentulus*.

Due to the potential for SNA to progress to SOA, systemic antifungal treatments were used in all cases. In all SNA cases with complete remission, gross fungal plaques within the sinonasal cavity were surgically debrided, which could have contributed to treatment success, as reported for canine SNA (Zonderland et al., 2002). Favourable outcomes have resulted from the use of single topical clotrimazole treatment alone ($n = 2$) or in combination with systemic antifungal agents ($n = 1$) in cats with SNA (Tomsa et al., 2003; Furrow and Groman, 2009). Two cats were disease free 2 and 4 years post-infusion. In our study, one case of SNA resolved after a second topical clotrimazole treatment. Multiple topical clotrimazole treatments can improve outcomes in refractory cases of canine SNA (Pomrantz and Johnson, 2010). Whether SNA due to *A. fumigatus* in cats is more likely to respond to topical antifungal therapy than infections due *Neosartorya* spp., which have an increased propensity for invasion, is not yet clear.

Pharmacological data for posaconazole and echinocandins in cats is lacking. Posaconazole was used in our study because it is fungicidal against *Aspergillus* spp. and was reported to cure a feline SOA case that did not respond to itraconazole and amphotericin B (McLellan et al., 2006). Posaconazole was well tolerated, with infrequent mild transient liver enzyme elevations. Use of echinocandins in humans with invasive aspergillosis is restricted to salvage therapy or combination antifungal therapy in refractory disease (Patterson, 2006). In our study, caspofungin was well tolerated and efficacious in the single cat receiving this agent. Kano et al. (2008) documented unsuccessful treatment using micafungin in a cat with SOA.

Adverse effects seen in three cats receiving voriconazole in our study were similar to those reported in five other cats (Quimby et al., 2010; Smith and Hoffman, 2010). The pharmacology of voriconazole has not been studied in cats and caution is urged in the continued use of this drug given the apparent high frequency of serious neurological adverse effects.

Conclusions

Cats with SNA are significantly more likely to be infected with *A. fumigatus* and have a better prognosis than cats with SOA. SOA is

an invasive mycosis in cats and is caused by fungal species within the *Aspergillus* complex other than *A. fumigatus*, such as *Neosartorya* spp.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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