Evaluation of Serum *Aspergillus*-Specific Immunoglobulin A by Indirect ELISA for Diagnosis of Feline Upper Respiratory Tract Aspergillosis

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Background: Serological tests for diagnosis of aspergillosis in immunocompetent humans and animals are based on *Aspergillus*-specific IgG (As-IgG). In humans with chronic pulmonary aspergillosis, As-IgA may be detectable even if IgG titers are negative. Cats with upper respiratory tract aspergillosis (URTA) have detectable As-IgG, but their ability to mount an IgA response and its diagnostic utility are unknown.

Objectives: To determine whether serum As-IgA can be detected in cats with URTA and evaluate its diagnostic utility alone or combined with As-IgG.

Animals: Twenty-three cats with URTA (Group 1), 32 cats with other respiratory diseases (Group 2), and 84 nonrespiratory controls (Group 3).

Methods: Serum As-IgA and As-IgG was measured by indirect ELISA. Optimal cutoff values were determined by receiver-operating curve analysis. Sensitivity (Se) and specificity (Sp) for URTA diagnosis were determined.

Results: Serum IgA was detected in 91.3% of Group 1 cats. The Se of IgA detection was 78.3% and Sp was 96.9% for Group 2, 85.7% for Group 3 and 88.8% for Group 2 and 3 combined. Assay Se for IgG was 100% and Sp was 92.2%. Using combined IgA and IgG results at cutoffs optimized for Sp for IgA and Se for IgG and combined controls (Groups 2 and 3), Se for diagnosis was 100% and Sp was 91.4%.

Conclusion and Clinical Importance: Most cats with URTA have serum As-IgA antibodies that can be detected by ELISA. Paired measurement of serum As-IgA and IgG shows no benefit for diagnosis of feline URTA over IgG alone.

Key words: Aspergillosis; *Aspergillus*; Feline; Sino-nasal; Sino-orbital.

Upper respiratory aspergillosis (URTA) in cats is an emerging mycotic infection.[1] Two anatomic forms are recognized: sino-nasal aspergillosis (SNA) and sino-orbital aspergillosis (SOA). By molecular techniques for species identification, an association between the anatomic form of disease and the causative agent has been identified—*Aspergillus fumigatus* and *A. felis* are the most common causes of SNA and SOA, respectively.[2–4] Sino-orbital aspergillosis is an invasive disease with high morbidity and mortality, whereas SNA typically is non-invasive and has a better prognosis.

Abbreviations:

AGID	agar gel double immunodiffusion assay
As-IgA	*Aspergillus*-specific IgA
As-IgG	*Aspergillus*-specific IgG
CNS	central nervous system
CPA	chronic pulmonary aspergillosis
CT	computed tomography
DIA	disseminated invasive aspergillosis
ELISA	enzyme-linked immunoassay
EU	ELISA units
IgA	immunoglobulin A
IgG	immunoglobulin G
Ig	immunoglobulin
ITS	internal transcribed spacer
MRI	magnetic resonance imaging
NLR	negative likelihood ratio
OD	optical density
PBS	phosphate-buffered saline
PBS-T	5% nonfat milk in PBS plus 0.05% Tween-20
PLR	positive likelihood ratio
ROC	receiver operator curve
Se	sensitivity
SNA	sino-nasal aspergillosis
SNP	single nucleotide polymorphism
SOA	sino-orbital aspergillosis
Sp	specificity
URTA	upper respiratory tract aspergillosis
URT	upper respiratory tract
UVTHS	University Veterinary Teaching Hospital Sydney

An active immune response and detection of *Aspergillus*-specific antibodies is central to the diagnosis of chronic and allergic forms of pulmonary aspergillosis in systemically immunocompetent humans.[5] Detection of *Aspergillus*-specific IgG by ELISA in these patients has

a sensitivity and specificity of approximately 90%.[5,6] Many patients with chronic pulmonary aspergillosis (CPA) also have persistent increases of serum *Aspergillus*-specific IgA. Patients who test negative for IgG may test positive for IgA, because *Aspergillus*-specific IgA can bind different fungal antigens than does *Aspergillus*-specific IgG.[5] Testing for *Aspergillus*-specific IgA has been advocated recently to improve diagnostic sensitivity in individuals with clinical signs and imaging changes consistent with CPA, but with negative test results for *Aspergillus*-specific IgG.[5]

Although invasive mycoses often are associated with immune compromise of the host, the majority of cats with either form of URTA appear to be systemically immunocompetent, based on lack of concurrent disease, production of serum IgG, and low detection rates of the fungal antigen galactomannan in serum.[7,8] Reports of comorbidities that could be associated with systemic immunocompromise are limited to diabetes mellitus in 3 cases and feline leukemia virus or feline immunodeficiency virus infection in 2 others.[8–12] A robust *Aspergillus*-specific IgG response has been identified in cats with URTA,[8] and detection of *Aspergillus*-specific IgG by ELISA was both sensitive (95.2%) and specific (92.7%) for diagnosis.[8] Whether detection of serum *Aspergillus*-specific IgA could be useful in diagnosis of URTA in cats has not been investigated.

Accordingly, the aims of our study were to determine whether serum *Aspergillus*-specific IgA can be detected in cats with URTA, and to evaluate the diagnostic utility of IgA detection alone, or in combination with *Aspergillus*-specific IgG.

Materials and Methods

Animals

Cases of URTA and control cases were recruited prospectively from cats presented to the Valentine Charlton Cat Centre at the University Veterinary Teaching Hospital, Sydney (UVTHS), and private referral hospitals in Australia, USA, Belgium, and UK. Serum samples (1–2 mL per cat) were frozen at −80°C and banked for batch testing. Samples were collected with informed consent according to the guidelines of the Animal Ethics Committee of the University of Sydney (N00/9-2012/5774). Three groups were defined:

Group 1 Cases: URTA. A definitive diagnosis of URTA was made on the basis of all 3 of the following criteria: detection of fungal hyphae in tissues, positive fungal culture, and molecular identification by comparative sequence analyses of the internal transcribed spacer (ITS) regions and partial β-tubulin, partial calmodulin genes or both, except for *A. fumigatus* identification, where consistent phenotypic features and demonstration of growth at 50°C without molecular identification was acceptable.[8] Cats with fungal coinfections were excluded. Classification of anatomic form (SNA or SOA) was based on the presence (SOA) or absence (SNA) of an orbital mass lesion on computed tomography (CT) or magnetic resonance imaging (MRI).[7]

Group 2 Respiratory Controls: Non-Aspergillus Upper Respiratory Tract (URT) Disease. This group included cats presented to the UVTHS for investigation of URT disease in which URTA was excluded after CT and rhinoscopy. In addition to prospectively recruited cases, stored archived sera from 7 cats diagnosed with URT cryptococcosis (consistent clinical signs and positive latex cryptococcal antigen agglutination titer[a]) were included in this group.

Group 3 Nonrespiratory Controls. This group included *healthy cats (3a)* and *sick cats (3b)* with nonrespiratory, nonfungal illness, as previously described.[8] Cases with clinical abnormalities suggestive of URT disease in the preceding 4 weeks were excluded.

Aspergillus-Specific IgA Antibody Detection by Indirect ELISA

An indirect ELISA for detection of *Aspergillus*-specific IgA was developed by modification of assays to detect *Aspergillus*-specific IgG.[8,13] The *Aspergillus* antigen was a commercial aspergillin[b] derived from the mycelial phase of culture of *A. fumigatus, A. niger,* and *A. flavus.* Cross-reactivity to the antigen by feline serum antibodies against other species in *Aspergillus* section *Fumigati,* including *A. felis, A. udagawae, A. lentulus,* and *A. thermomutatus,* has been demonstrated.[8] Positive and negative control sera comprised pooled sera from affected and healthy cats, respectively, that tested positive or negative by agar gel double immunodiffusion assay (AGID)[c] were included, along with a blank, on each plate.[8] Checkerboard titrations were used to determine the optimal concentration of aspergillin (2.5 μg/mL), starting test serum dilution (1 : 100), and secondary antibody dilution (1 : 10,000). To determine inter- and intraplate coefficients of variation, 40 repeat samples of the pooled positive control were run on 4 separate plates with 10 repeat samples on each plate. Test samples were run in duplicate.

Ninety-six-well polystyrene flat-bottom ELISA plates[d] were coated with 75 μL aspergillin antigen (2.5 μg/mL in 1% phosphate-buffered saline [PBS]) and incubated overnight at 4°C. Plates then were blocked with 75 μL of 1% (w/v) polyvinylpyrrolidone[e] in PBS for 1 hour at room temperature. Doubling dilutions from 1 : 100 to 1 : 12,800 of test serum (50 μL) in 5% nonfat milk in PBS containing 0.05% Tween-20[f] (PBS-T) were used. Plates were incubated at 37°C for 2 hour. Fifty microliters of goat anti-cat IgA (H&L)[g] horseradish peroxidase-conjugated secondary antibody (1 : 10,000) in PBS-T were added to each well, and plates were incubated at 37°C for 1 hour. One hundred microliters of KPL SureBlue Reserve™ [h] was added to each well, and plates were incubated in the dark for 45 minutes. The reaction was stopped with 100 μL TMB Stop solution[i], and optical density (OD) absorbance was read with a 450-nm wavelength filter[j] . All incubations were carried out in a humidified chamber, and plates were washed manually 3 times with 150 μL of PBS between incubations.

Aspergillus-Specific IgG Antibody Detection by Indirect ELISA

Aspergillus-specific IgG antibody titers for most banked frozen sera had been determined in a previous study.[8] The same assay was used to determine IgG titers in 10 additional samples evaluated here (3 in Group 1, 7 in Group 2).

Statistical Analysis

Data were assessed for normality by examination of a histogram and normal probability plots. Age and sex were compared among Groups 1, 2, and 3 by Kruskal–Wallis and chi-squared analysis[k], respectively. For ELISA data, mean optical density (OD) readings obtained for each duplicate sample were converted to ELISA units (EU) in Microsoft Excel.[13] Log$_{10}$ OD values were plotted against log$_{10}$ serum dilutions for positive control and test sera. The curves generated were assessed for parallelism, and a minimum of 3 dilution points was necessary to create a dilution curve. The IgA concentrations were expressed as EU/mL with the

positive control serum standard having a concentration of 100 EU/mL. Serum samples with fewer than 3 dilution points within the linear range of the standard and samples that were not parallel to the standard curve were assigned a value 0 EU/mL. The median IgA ELISA units for Groups 1, 2, and 3 were compared by Kruskal–Wallis tests and the Wilcoxon rank-sum test. Positive or negative test results and median IgA concentrations from cats with SOA versus SNA were compared by Fisher's exact and Mann–Whitney U-tests, respectively.

Optimal cutoff values for the IgA ELISA were determined by receiver operator characteristic (ROC) analysis[l] . The ROC analysis was conducted by assigning the binary outcome of 1 or 0, with URTA Group as 1 and control group as 0. Groups used for controls were Group 2, Group 3, and Group 2 and 3 combined. The optimal cutoff value was determined by Youden's index. Sensitivity (Se) and specificity (Sp) at the determined cutoff value was reported for each control group.[14] Optimal cutoff values for the IgG ELISA have been published previously.[8] The Se and Sp using combined IgG and IgA data, when both tests were positive or where only 1 test was positive, also were calculated using the optimal cutoffs for each assay and combined control groups (Groups 2 and 3).

Results

Animals

Group 1 cases (n = 23): Signalment, fungal species, anatomic form, and ELISA data are presented in Tables 1 and 2. Twenty cats were from Australia, and 1 each was from USA, Belgium, and UK. Twelve cats had SNA and 11 had SOA. Fungal species detected included *A. fumigatus* (n = 7), *A. felis* (n = 12), *A. lentulus* (n = 1), *A. udagawae* (n = 1), *A. flavus* (n = 1), and *A. thermomutatus* (*N. pseudofischeri*; n = 1).

Group 2 respiratory controls (n = 32, Table 2) included cats with a variety of URT diseases: chronic rhinosinusitis (n = 9); nasal neoplasia (n = 10; adenocarcinoma [n = 3], squamous cell carcinoma [n = 2], osteosarcoma [n = 1], lymphoma [n = 4]); cryptococcosis (n = 12); and nasopharyngeal stenosis (n = 1).

Group 3 nonrespiratory controls (n = 84, Table 2) comprised 36 healthy cats (Group 3a) and 48 sick cats (Group 3b) presented for nonfungal and nonrespiratory illness. Diagnoses in sick cats included hyperthyroidism (n = 12), enteropathy (n = 11), pancreatitis (n = 2), cholelithiasis (n = 1), chronic kidney disease (n = 7), diabetes mellitus (n = 2), portosystemic shunt (n = 1), chyloabdomen (n = 1), central nervous system (CNS) disease (n = 3), acute kidney injury (n = 1), anemia (n = 1), idiopathic hypercalcemia (n = 1), neoplasia (n = 2), feline infectious peritonitis (n = 1), skin disease (n = 2), and dog bite attack (n = 1).

The median ages were 5 years, 9.8 years, and 8 years for cats in Groups 1, 2, and 3, respectively (Table 2). There was no significant difference among groups with respect to age (P = .36) or sex (P = .78).

Aspergillus-Specific IgA and IgG Antibody Detection by Indirect ELISA

Inter- and intra-assay coefficients of variation for the IgA ELISA were 4.5% and 6.17%, respectively. Serum

Table 1. Fungal species and ELISA serology results for Group 1 cats with URTA. The cutoff for a positive test result on IgA ELISA was 71.9 EU/mL and for IgG ELISA was 5 EU/mL.

	Sino-Nasal Aspergillosis (n = 12)	Sino-Orbital Aspergillosis (n = 11)
Fungal species	*A. fumigatus* (n = 7)	*A. felis* (n = 10)
	A. felis (n = 2)	*A. udagawae* (n = 1)
	A. flavus (n = 1)	
	A. lentulus (n = 1)	
	A. thermomutatus (n = 1)	
IgA ELISA (EU/mL)		
Median	209	84.8
Mean	262.8	167.3
Range	24.2–849.7	0–849.5
IgG ELISA (EU/mL)		
Median	28.6	110.7
Mean	30.9	189.6
Range	5–82.4	26–797.9

Table 2. Composition of Group 1 (cases) and control Groups 2 (respiratory controls) and 3 (nonrespiratory controls).

	Group 1	Group 2	Group 3
Median age (years)	5	9.75	8
Age range (years)	2–15.5	2–16	0.4–19.5
Male intact	0	0	6
Male neutered	13	17	35
Female intact	0	0	6
Female neutered	10	15	37
Domestic short hair	8	17	54
Domestic long hair	1	1	10
Persian	4	2	1
Himalayan	2	1	0
Ragdoll	3	3	3
British shorthair	2	1	1
Scottish shorthair	1	0	0
Other purebred	2	7	15
Total no. of cats	23	32	84

IgA was detected in 91.3% (21/23), 43.8% (14/32), and 50.0% (42/84) of cats in Groups 1, 2, and 3, respectively. Samples from all other cats did not generate a dilution curve with at least 3 dilutions within the range of the standard and were assigned an IgA concentration of 0 U/L.

The IgG and IgA ELISA data for Group 1 cats are listed in Table 1. The optimal cutoff value for the *Aspergillus*-specific IgA ELISA was 71.9 EU/mL (Youden's index = 0.671). At this cutoff, assay Se was 78.3%. Assay Sp was highest using Group 2 as the control group (96.9%; Youden's index = 0.751) compared to Group 3 (85.6%; Youden's index = 0.640) or Groups 2 and 3 combined (88.8%; Youden's index = 0.671; Table 3). Of the 5 Group 1 cats that tested negative for IgA, 4 had SOA caused by *A. felis* and 1 had SNA caused by *A. flavus* (Table 1). False-positive IgA results occurred in 1 of 32 (3.2%) cats in Group 2, a cat with

Table 3. Sensitivity and specificity of IgA ELISA at cutoff values optimized for maximum sensitivity, specificity, or both.

	Cutoff (EU/mL)														
	12.4					71.9					97.4				
Control Groups	Se	Sp	PLR	NLR	J	Se	Sp	PLR	NLR	J	Se	Sp	PLR	NLR	J
Group 2[a] (n = 32)	91.3	56.2	2.09	0.15	0.467	78.3	96.9	25.04	0.22	0.751	60.9	100	–	0.39	0.609
Group 3[b] (n = 84)	91.3	50.0	1.83	0.17	0.413	78.3	85.7	5.48	0.25	0.640	60.9	94.0	10.23	0.42	0.549
Groups 2 and 3 (n = 116)	91.3	51.7	1.89	0.17	0.430	78.3	88.8	6.98	0.24	0.671	60.9	95.7	14.12	0.41	0.576

Se, sensitivity; Sp specificity; PLR, positive likelihood ratio; NLR, negative likelihood ratio; J, Youden's index.
[a]respiratory controls—cats with non-*Aspergillus* upper respiratory tract disease.
[b]nonrespiratory controls—healthy controls(n- = 36) and sick controls (n = 48) with nonrespiratory and nonfungal illness.

nasal adenocarcinoma, and in 12 of 84 (14.3%) cats in Group 3, including 2 healthy and 10 sick cats. The median IgA concentration for Group 1 (137.1 EU/mL [0–847.3]) was significantly higher than that of Group 2 (0 EU/mL [0–89.6]) and Group 3 (7.8 EU/mL [0–169.5]), both *P*-values < .001), but was not significantly different between Groups 2 and 3 (*P* = .71; Fig 1). There was no significant difference between test result (positive or negative) and anatomic form (SOA vs SNA; *P* = .155). The median IgA concentration in cats with SNA (209 EU/mL) was higher than that of cats with SOA (84.8 EU/mL), but the difference was not significant (*P* = .079).

Using Groups 2 and 3 combined as the control, the Se and Sp of the IgG ELISA at a cutoff value of 5 EU/mL was 100 and 92.2%, respectively (Table 4). At a cutoff value for the IgA ELISA optimized for Sp (97.4 EU/mL) and a cutoff value for the IgG ELISA optimized for Se (5 EU/mL), the Se of both a positive IgA and IgG result for diagnosis of URTA was 60.9% and Sp was 96.6%. The Se of a positive IgA or a positive IgG result was 100% whereas Sp was 91.4% (Table 3).

Cross-Reactivity with Cryptococcus

Of the 12 cats with nasal cryptococcosis in Group 2, 12 cats had serum IgA and 10 cats had serum IgG concentrations below the cutoff values for diagnosis of URTA.

Discussion

The majority of cats with URTA in our study had detectable serum *Aspergillus*-specific IgA antibodies. Overall, detection of these antibodies by indirect ELISA as a stand-alone test had only moderate Se and Sp, and IgA concentrations were not significantly different between cats with SNA or SOA. The Sp for detection of *Aspergillus*-specific IgA was highest for cats with URT disease other than aspergillosis (Group 2 respiratory controls), the most relevant control group in a clinical setting.[7,8] Detection of serum IgA alone overall was inferior to detection of IgG for diagnosis.[8] This observation is not surprising because IgG is the most abundant serum immunoglobulin (Ig), and its measurement is the most commonly employed diagnostic tool. When considering the Se and Sp of IgA and IgG
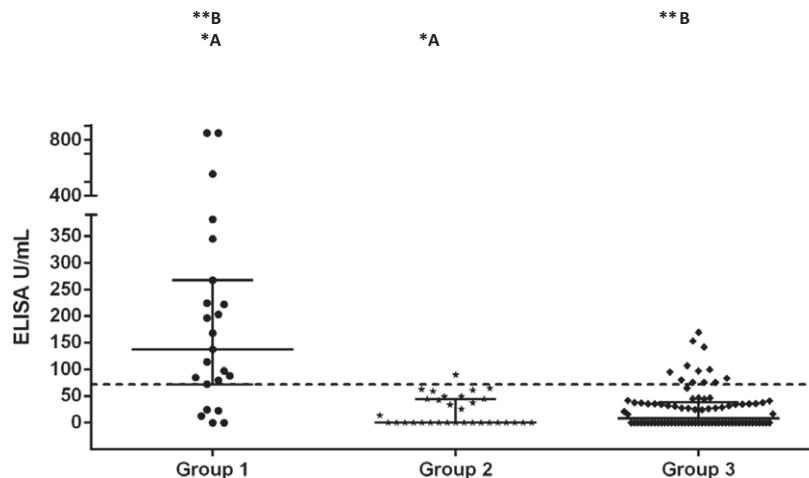
Fig 1. IgA ELISA units/mL of 23 Group 1 cats (black circles), 32 Group 2 cats (black stars), and 84 Group 3 cats (black diamonds). Block lines represent median value, 25th and 75th quartiles are represented by error bars. Dash line represents the optimal cutoff value (71.9 EU/mL). *A—Median EU of Group 1 was significantly different from Group 2 (*P* < .001). **B—Median EU of Group 1 was significantly different from Group 3 (*P* < .001).

Table 4. Sensitivity and specificity of IgA and IgG detection by ELISA for the diagnosis of upper respiratory tract aspergillosis in cats.

ELISA[a]	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)
Positive IgA (≥97.4 EU/mL)	60.9% (38.5–80.3%)	95.7% (90.2–98.6%)	6.98 (5.64–35.36)	0.24 (0.25–0.68)
Positive IgG (≥5.0 EU/mL)	100% (85.2–100.0%)	92.2% (85.8–96.4%)	12.89 (6.88–24.14)	0.00 (–)
Positive IgA AND IgG	60.9% (38.5–80.3%)	96.6% (91.4–99.1%)	17.65 (6.38–48.82)	0.41 (0.24–0.68)
Positive IgA OR IgG	100% (85.2–100.0%)	91.4% (84.7–95.8%)	11.60 (6.41–20.98)	0.00 (–)

CI, confidence interval; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

[a]data calculated using 23 cats with aspergillosis in Group 1 and 116 control cats in Group 2 (respiratory controls) and Group 3 (nonrespiratory controls).

ELISA data together, there was no advantage over using IgG results alone in this cohort of cats. However, 1 cat had an IgG titer of 5 EU/mL, the lowest possible for a positive result, but had an IgA titer of 87.6 EU/mL, which was considerably higher than all cutoffs for IgA used in this study. Thus, it is possible that in some cases where IgG titers are negative, IgA could be positive, as described in chronic pulmonary aspergillosis in humans.[5] Future studies of paired IgA and IgG titers in a larger cohort of cats with aspergillosis are warranted.

Differentiation of *Aspergillus*-specific antibody production from the antibody response to other fungi, particularly *Cryptococcus* spp., is important for assay performance in a clinical setting, because cryptococcosis is 1 of the most common mycoses of cats worldwide. Previously, false-positive results on ELISA testing to detect *Aspergillus*-specific IgG antibodies were recorded in 2 of 5 cats with nasal cryptococcosis.[8] We tested an additional 7 cats with cryptococcosis here and found no new false-positive results, confirming that the antibodies detected in cats with URTA were *Aspergillus* specific and that cross-reaction in the ELISA with *Cryptococcus*-specific antibodies does not occur.

Our results support previous findings that cats with URTA produce an active and appropriate humoral immune response to infection, similar to dogs with SNA.[8] The immunopathogenesis of SNA has been investigated in dogs, but not in cats with URTA. In dogs, SNA is a noninvasive mycosis caused by *A. fumigatus*, characterized by lymphoplasmacytic inflammation of the sino-nasal mucosa.[15,16] An active humoral immune response has been demonstrated by identification of *Aspergillus*-specific IgG in serum.[13,17] An effective cell-mediated response mediated by pattern recognition receptors (PRRs), including Toll-like receptors (TLR) 2, 4, and 9 pivotal in T-helper cell development, is essential for protective immunity against fungal infection.[18] Upregulation of mRNA expression of TLR 2, 4, and 9 has been identified in the sino-nasal mucosa of dogs with SNA.[19] However, mutational analysis of these genes in dogs with SNA did not detect single nucleotide polymorphisms (SNPs) in the coding regions,[20] in contrast to studies of invasive[21,22] and chronic pulmonary[23] aspergillosis in humans. Evaluation of mucosal inflammatory cell populations and cytokine expression have identified a predominant Th-1-type adaptive immune response to SNA in dogs.[15,24,25] This response is thought to be responsible for confining the infection to the sino-nasal region, thus preventing systemic spread, but also is implicated in failure to clear disease. In humans, anti-inflammatory activity of T regulatory cells mediated by increased concentrations of IL-10 is proposed to contribute to fungal persistence and decrease host damage in chronic fungal infections.[18] Increases in IL-10 also were detected in dogs with SNA and have been implicated in failure to clear *A. fumigatus*.[25] Direct extrapolation of findings from investigations of SNA in dogs cannot be made; therefore, immunohistochemical, cytokine, and chemokine studies in cats with URTA are warranted to further characterize the immune response and identify defects in the innate or adaptive immune dysfunction underlying disease pathogenesis in this species.

Unlike cats, in which SOA is now the most common form of invasive aspergillosis, SOA is rare in dogs. Disseminated invasive aspergillosis (DIA) is the most common form of invasive aspergillosis in dogs.[26] German shepherd dogs are overrepresented for both this disease and for selective IgA deficiency.[27] Although definitive evidence is lacking, systemic immunodeficiency caused by alterations in regulation of IgA production, defective synthesis, defective release, or both has been proposed in the pathogenesis of DIA in German Shepherd dogs.[28] We did not detect a deficiency in serum *Aspergillus*-specific IgA in cats with URTA; however, studies in healthy dogs have confirmed that serum IgA concentrations do not correlate with mucosal IgA concentrations at a variety of sites.[29,30] Therefore, assessment of IgA competence cannot be based on serum IgA concentrations alone, and measurement of mucosal immunoglobulin concentrations is warranted in cats with URTA to more fully evaluate IgA competence.

Brachycephalic, pure bred cats, especially those of Persian lineage, are at increased risk of developing URTA.[8] The basis for susceptibility is unknown. A heritable immunogenetic defect is possible, especially because Persian cats also are susceptible to development of invasive dermatophyte infections (pseudomycetomas).[31] Altered skull conformation may contribute to this susceptibility. The skull of brachycephalic cats is rounded and has a decreased face length and brain case due to shortening and dorsal rotation of bones of the

face. This results in narrowing of the nasal cavity and airways as well as deformation and displacement of the ethmoid and ventral nasal conchae.[32] These anatomic abnormalities may result in increased mucosal edema, decreased turbinate airflow, and decreased mucociliary clearance of nasal secretions and have been suggested as possible factors increasing the risk of mycotic infection.[2,33,34] However, because in dogs with SNA, dolichocephalic and mesaticephalic breeds are more commonly affected, anatomic abnormalities alone are unlikely to be responsible for the increased risk of disease in brachycephalic cats. Other factors that have been proposed to increase the risk of sino-nasal mycotic colonization include previous viral upper respiratory tract infection, chronic rhinosinusitis, and the use of antibiotics.[2,33]

A limitation of our study is the relatively small sample size of the affected group. However, all cats had naturally occurring disease and, despite the small numbers, statistically significant differences were identified. Positive and negative predicative values could not be determined because the true prevalence of URTA is not known.

In conclusion, most cats with URTA have serum *Aspergillus*-specific IgA antibodies that can be detected by ELISA. However, as a stand-alone test, serum IgA has only moderate sensitivity and specificity for diagnosis of URTA in cats. Although paired measurement of serum *Aspergillus*-specific IgA and IgG was of no diagnostic benefit over use of serum IgG alone, exploration of paired titers in a larger cohort of cats is warranted to determine the diagnostic utility of serum IgA in cases where serum IgG is below the cutoff value for diagnosis.

Acknowledgments

Footnotes

[a] CALAS, Meridian Biosciences Cincinnati, Ohio
[b] Aspergillus Immunodiffusion Antigen ref 100501, Meridian Bioscience
[c] Fungal Immunodiffusion Kit, Meridian Bioscience
[d] Costar 3590, Corning Inc. Corning, New York
[e] Simga-Aldrich, St Louis Missouri
[f] Simga-Aldrich, St Louis Missouri
[g] Abcam ab112795, Abcam, Level 16,414 La Trobe Street, Melbourne, VIC 3000, Australia
[h] 3.3′,5,5′ tetramethylbenzidine, KPL SureBlue Reserve™ TMB Microwell Peroxidase Substrate (1-Component), KPL, Gaithersburg, MD
[i] KPL, Gaithersburg, MD
[j] FLUROstar OMEGA, filter-based multimode microplate reader, BMG Labtech, Offenburg Germany
[k] GraphPad Prism® version 6, GraphPad Software, Inc. 2014
[l] SPSS statistical software, version 22, 64-bit edition

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