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Utility of antigen testing for the diagnosis of ocular histoplasmosis in four cats: a case series and literature review

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Abstract

Case series summary This case series describes the clinical utility of antigen testing for the diagnosis of feline ocular histoplasmosis. Four cats with suspected (n = 2) or confirmed (n = 2) ocular histoplasmosis are described: three from Oklahoma and one from California. In one case, serial urine antigen tests, as well as a serum antigen test for *Histoplasma capsulatum*, were negative; however, light microscopy identified microorganisms consistent with *H capsulatum* in ocular tissues at necropsy. In a further two cats with recurrent ocular histoplasmosis following long-term systemic antifungal therapy, *Histoplasma* species urine antigen concentrations were negative, but both cats improved clinically following systemic antifungal therapy and remained in apparent clinical remission after treatment cessation (9–16 months). The final cat displayed profound bilateral endophthalmitis; however, *Histoplasma* species antigen testing of vitreous humor and subretinal fluid from the left eye was negative. Intralesional organisms were detected on histopathology of both eyes, and *H capsulatum* was subsequently isolated and sequenced from tissue of one eye. *Relevance and novel information* These cases highlight the potential difficulty in definitively diagnosing ocular histoplasmosis in cats when conducting antigen testing of serum, urine and even ocular fluids. Although antigen testing has previously proven useful in the diagnosis of disseminated feline histoplasmosis, it may not be adequate in cats with only ocular signs.

Introduction

The thermally dimorphic fungus *Histoplasma capsulatum* exists in hyphal form in the environment and converts to a yeast form in tissues.[1] *H capsulatum* can be found worldwide and is particularly prevalent in soils enriched with bat or bird droppings.[2,3] Although most prevalent in the USA, Central and South America, disease in people and animals has been reported worldwide.[3–5] Within the USA, cases appear most frequently in the Ohio, Missouri, and Mississippi river valleys, as well as Midwestern and southern states such as Oklahoma.[2,4,6–8] Isolated feline cases have also been reported in other countries, but some were associated with immigration from the USA.[9–12] Originally thought to comprise three varieties, instead *H capsulatum* has been found to contain genetically distinct populations that differ based on geographic location.[4,13] Much more is likely to be learned as the phylogeny of this species is further explored and analytical techniques improve. Histoplasmosis results from inhalation or ingestion of spores that then germinate, become engulfed in

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macrophages and spread throughout the body via the bloodstream or lymphatics.[14] This results in either self-limiting infection, localized pulmonary infection, or disseminated disease. Histoplasmosis has been reported in both domestic and wild felids and is the second most common systemic fungal infection in cats.[6,15–17] Younger animals with outdoor access appear to be at the highest risk of infection, although histoplasmosis has been confirmed in cats housed strictly indoors.[6,8,18] Infected cats frequently show non-specific clinical signs, but the most common body systems affected include the respiratory tract, eyes, musculoskeletal system, hemolymphatic organs and skin.[4,19–24] The incidence of ocular disease in cats with histoplasmosis has not been reported, but in one case series all cats with disseminated infection that underwent ophthalmic examination had ocular lesions.[19] In other reports, up to 22–25% of cats with histoplasmosis had ocular signs, although this may be an underestimate as not all cats underwent ocular examination.[7,15,18] Ocular signs include optic neuritis, anterior uveitis, panuveitis, endophthalmitis, choroiditis/chorioretinitis sometimes with retinal detachment and secondary glaucoma.

Histoplasmosis is classically diagnosed via cytologic or histologic identification of the microorganism.[25–26] However, this can be limited by the ability to obtain an adequate diagnostic specimen and the number of microorganisms present may be too few to identify.[27] *H capsulatum* can also be mistaken for other microorganisms such as *Sporothrix*, *Candida* or *Leishmania* species.[27] As an alternative, clinical specimens can be submitted for fungal culture, but this may delay diagnosis owing to prolonged isolation times, has low sensitivity and exposes laboratory personnel to the risk of infection.[27–28] Complement fixation and immunodiffusion antibody detection methods are available, but false-negative results may occur when antibody concentrations are low or absent such as in acute phases of infection, prior to seroconversion or with concurrent immunosuppression.[27] In addition, false-positive tests may result from cross-reaction between other fungal and non-fungal pathogens or owing to detection of circulating antibodies remaining from previous disease exposure.[1,19,27] An assay utilizing the PCR has recently been developed for *Histoplasma* species and used in people, dogs and cats, but cost and availability have limited its clinical use in veterinary patients.[16,29] Use of these tests specifically for cats with ocular histoplasmosis has not been investigated.

Antigen testing for histoplasmosis detects the fungal cell wall galactomannan in various body fluids, including serum, urine, respiratory tract secretions and cerebrospinal fluid.[30] Galactomannan is secreted by viable *H capsulatum* microorganisms into surrounding tissues and ultimately excreted in the urine. Limitations of the antigen test in people include a variable cross-reactivity rate with other fungi, including *Blastomyces dermatitidis, Paracoccidioides,* *Penicillium marneffei* and *Coccidioides* species.[31–34] Cross reactions have also been reported for *Aspergillus* species, but not for *Cryptococcus* species.[32,35] In one study, cross-reactions were noted in 70% of human beings with other endemic mycoses.[31] Although these data were generated in people, similar concerns might be expected when the test is used on feline body fluids; however, to our knowledge this has not been reported. Although the galactomannan antigen test has proven useful for diagnosis of disseminated histoplasmosis in cats,[31,36] little is known about the sensitivity and specificity of this test in cats that have ocular involvement. This report describes four cats with proven (n = 2) or suspected (n = 2) ocular histoplasmosis but in which antigen testing of various fluid specimens was negative.

Case series description

Case 1

A 12-year-old male castrated domestic shorthair cat residing in Oklahoma had been found by the owner as an adult stray approximately 9 years prior to examination and housed indoors since that time. The cat was referred to Oklahoma State University (OSU) for ocular redness and mydriasis in the left eye (oculus sinister [OS]) of 2 weeks' duration. The cat's only treatment was long-term lysine administration per os (PO). On presentation at OSU, ocular examination revealed panuveitis and a large subretinal granuloma OS. Results of diagnostic tests for infectious agents commonly encountered in Oklahoma are presented in Table 1. As clinical examination and diagnostic test results suggested toxoplasmosis, treatment with clindamycin (15 mg/kg PO q12h), prednisolone (0.35 mg/kg PO q24h), atropine ophthalmic ointment (1/4 inch strip OS q48h) and prednisolone acetate ophthalmic solution (1 drop OS q6h) was instituted.[37] Analgesia provided through topical administration of atropine was deemed sufficient in this case given clinical examination findings.

The result of a *Histoplasma* species antigen test conducted on urine was reported negative. However, the laboratory noted that the antigen level was just below the cut-off for a positive result. To aid interpretation, 12 days after the initial test, serum and a second urine specimen were collected and submitted for antigen testing. *Histoplasma* species antigen was undetectable in urine or serum and the levels were definitively negative at that time. Treatment was continued unchanged. At recheck examination 2.5 weeks following initiation of treatment for toxoplasmosis, signs of panuveitis had improved, but the subretinal granuloma persisted. Systemic treatment for toxoplasmosis was continued unchanged, but topical prednisolone was slowly tapered (1 drop q8h for 1 week then q12h thereafter). After approximately 1 month of therapy, the patient developed inappetence and lethargy; the referring veterinarian diagnosed renal failure and euthanized the cat. The

Table 1 Selected diagnostic test results for cases of suspected or confirmed feline ocular histoplasmosis

Case	CBC and serum biochemistry	FeLV antibody and FIV antigen	*Cryptococcus* species antigen	*Histoplasma* species antigen	*Histoplasma* species antibody	*Toxoplasma gondii* antibody titer
1	Unremarkable	Negative	Negative	Negative (U, S)	Negative	IgM: 1:512 IgG: 1:256
2	Unremarkable	Negative	Negative	Negative (U)	NP	IgM: 1:64 IgG: Negative
3	Unremarkable	NP	Negative	Negative (U)	NP	NP
4	Anemia, thrombocytopenia	Negative	Negative	Positive; below LOQ (U, S)* Negative (S/V)	NP	IgM: 1:64 IgG: Negative

*Samples held longer than 14 days
CBC = complete blood count; FeLV = feline leukemia virus; FIV = feline immunodeficiency virus; U = urine; S = serum; NP = not performed; LOQ = limit of quantification; S/V = subretinal/vitreal fluid
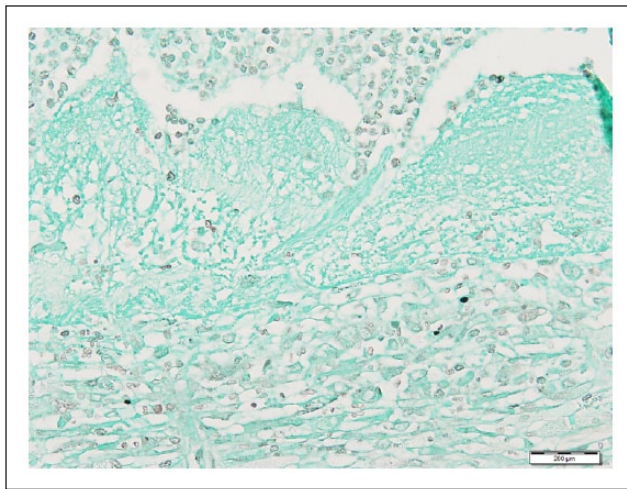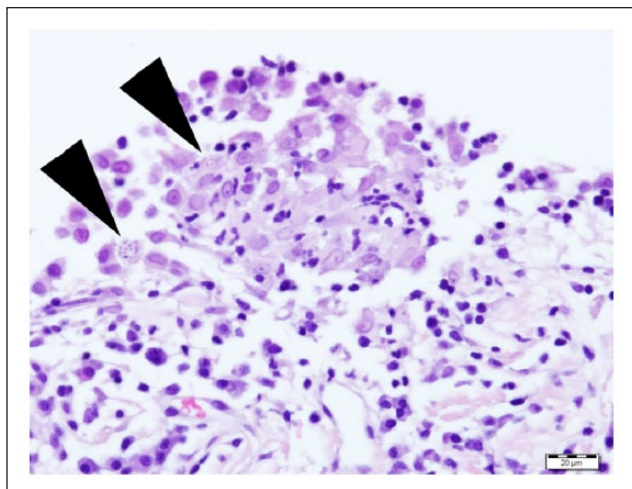

Figure 1 Photomicrograph of the choroid of the left eye of a 12-year-old male castrated domestic shorthair cat (case 1). Note the darkly staining, round yeast organisms consistent with *Histoplasma capsulatum*. Grocott's methenamine silver staining. *Courtesy of the Comparative Ocular Pathology Laboratory of Wisconsin*

left globe was submitted for histopathologic analysis, which revealed severe, focally extensive pyogranulomatous and lymphoplasmacytic chorioretinitis with marked epichoroidal fibrosis and intralesional microorganisms compatible with *H capsulatum* (Figure 1). A full necropsy was not performed and there was no attempt to further characterize the microorganisms.

Case 2

A 7-year-old male castrated domestic shorthair cat residing in Oklahoma and housed indoors had been diagnosed with and treated for conjunctivitis, posterior uveitis and a facial abscess due to histoplasmosis at OSU approximately 2 years earlier. At that time, the urine

Histoplasma species antigen concentration was markedly elevated at 10.74 ng/ml. Systemic fluconazole therapy (12.5 mg/kg PO q24h) was continued for 4 months until *Histoplasma* species antigenuria could no longer be detected. At that time, all ocular signs of active inflammation had resolved and the cat was visual despite having multiple chorioretinal scars. Approximately 15 months after ceasing therapy, the cat was again evaluated by the OSU Ophthalmology Service for apparently acute reduction in vision at home. Several subretinal granulomas and an extensive non-rhegmatogenous retinal detachment were noted in the right eye (oculus dexter [OD]). Chorioretinal scars without evidence of active disease were confirmed OS. Owing to clinical suspicion of recurrent histoplasmosis, fluconazole (10 mg/kg PO q24h) and prednisolone (0.5 mg/kg PO q24h) were initiated prior to obtaining diagnostic test results (Table 1). Owing to the posterior nature of disease and lack of overt discomfort or miosis, atropine and systemic analgesics were deemed unnecessary. *Histoplasma* species antigen was not detected in urine; however, serum *Toxoplasma gondii* titers were positive. Therefore, clindamycin (14 mg/kg PO q24h) was added. Within 1 month, all clinical evidence of chorioretinal inflammation resolved, leaving chorioretinal scars. At that time, *T gondii* titers were unchanged and clindamycin therapy was discontinued. Oral fluconazole was continued for 5 months beyond clinical resolution of disease. The patient remained stable but minimally visual in both eyes (oculus uterque [OU]) 16 months following cessation of therapy until the cat developed frequent coughing episodes, lethargy, decreased appetite and weight loss. A relapse of disseminated histoplasmosis was confirmed via positive urine antigen test (5.82 ng/ml), but imaging was not performed. After 6 months of fluconazole therapy (12.5 mg/kg PO q12h), the urine antigen level was again negative and clinical signs had resolved.

Figure 2 Photomicrograph of the choroid of the right eye of a 9-year-old male castrated domestic shorthair cat (case 3). Note the macrophages laden with round yeast organisms consistent with *Histoplasma capsulatum* (arrowheads). Hematoxylin and eosin stain. *Courtesy of the Comparative Ocular Pathology Laboratory of Wisconsin*

Case 3

A 9-year-old male castrated domestic shorthair cat found as an adult stray approximately 8 years prior and housed indoors since that time had been diagnosed with bilateral ocular histoplasmosis at 4 years of age by another veterinary ophthalmologist using antigen testing (antigen concentration and sample type could not be confirmed). Clinical diagnoses at that time included anterior uveitis OD, a subretinal granuloma OD and chorioretinitis OU. The cat's signs resolved within a few months of oral fluconazole therapy, which was continued for 2.5 years until the cat began vomiting and fluconazole therapy was stopped. Within 6 weeks of cessation of therapy, the right eye became red and cloudy and the cat was presented to OSU about 2 weeks later. On presentation, the cat was blind OD owing to anterior uveitis and secondary glaucoma. Assessment of the posterior segment was limited as a result of changes in the anterior segment. Clinical examination of the left eye was normal other than multiple chorioretinal scars. As the right eye was irreversibly blind and painful, it was enucleated and submitted for histopathologic assessment, which revealed glaucoma, marked lymphoplasmacytic anterior uveitis and chronic, multifocal, granulomatous and lymphoplasmacytic choroiditis with intralesional microorganisms consistent with *H capsulatum* (Figure 2). *Histoplasma* species antigen testing was not conducted and antifungal therapy was not instituted. Postoperative analgesia was provided through sublingual buprenorphine administration (0.01 mg/kg q8–12h as needed). Four months following surgery, the left eye had no evidence of active disease and the cat remained clinically healthy. However, 1 year later

(16 months after enucleation), a routine recheck examination revealed a subretinal granuloma OS. Results of diagnostic tests for infectious agents, including *Histoplasma* species antigen testing, were unremarkable (Table 1). However, owing to high suspicion of fungal disease, systemic therapy with fluconazole (7 mg/kg PO q24h) and prednisolone (0.35 mg/kg PO q24h) was instituted. The granuloma improved within 1 month of starting therapy and became a chorioretinal scar by 3 months later. Fluconazole was continued for 7 months. The cat was clinically stable and visual when evaluated approximately 9 months after stopping therapy.

Case 4

A 16-year-old female spayed domestic shorthair cat that had resided in California for the past 8 years after relocating from Ohio was evaluated by the University of California, Davis Veterinary Ophthalmology Service for a 10 day history of mydriasis and apparent blindness OU. In addition, the cat had progressive weight loss over the previous 6–12 months and progressive pelvic limb weakness of 2 years' duration. On ophthalmic examination, endophthalmitis with exudative retinal detachment was detected OU (Figure 3). Diagnostic testing included a complete blood count, serum biochemistry, serologic testing for *T gondii* antibodies and *Cryptococcus* species antigens (Table 1), and abdominal and thoracic imaging. Ultrasonographic findings included hepatomegaly, splenomegaly, mesenteric lymphadenopathy and thickened bowel wall. Splenic aspirates revealed erythrophagia. Urine culture revealed one colony of *Aspergillus* species believed to be a contaminant.

Empirical therapy with 0.1% dexamethasone ophthalmic solution (1 drop OU q6–8h) and doxycycline (19.2 mg/kg PO q24h) was initiated pending test results. Based upon *T gondii* testing (IgM = 1:64; IgG = negative) and poor clinical response, doxycycline was replaced with clindamycin (9.6 mg/kg PO q12h), and serum and urine were submitted for *Histoplasma* species antigen testing. Results were below the limit of quantification, although specimens had been refrigerated for >14 days prior to testing. Owing to progressive endophthalmitis OU (OS worse than OD) despite 4 weeks of clindamycin therapy, the left eye was enucleated. Analgesia was provided through buprenorphine administration (0.01 mg/kg sublingually and on gingiva q8–12h as needed). Immediately following enucleation but prior to formalin fixation, fluid from the vitreous body and subretinal space was obtained and submitted for *Histoplasma* species antigen testing, which was negative. Histopathology of the left eye revealed severe, chronic, fibrinous, pyogranulomatous and plasmacytic endophthalmitis and optic neuritis with fibrinonecrotizing choroidal vasculitis (Figure 4). There were many intralesional microorganisms that morphologically resembled *Sporothrix*
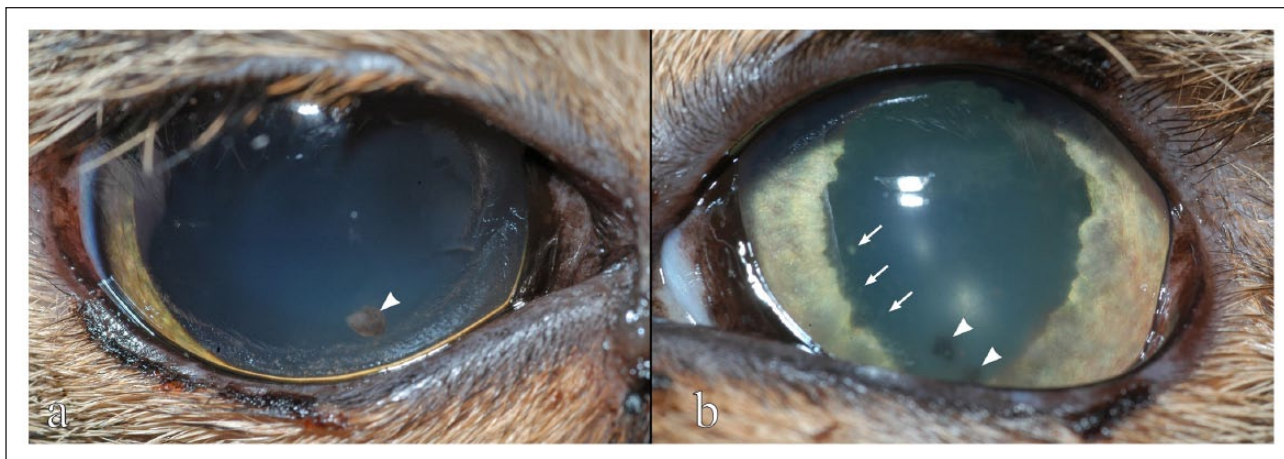
Figure 3 (a) Right and (b) left eyes of a 16-year-old female spayed domestic shorthair cat (case 4) with a 5 week history of bilateral blindness. Note the generally fine (arrows) but occasionally clumped (arrowheads) keratic precipitates on the ventral corneal endothelium OU and rubeosis iridis laterally OS. The right pupil is mydriatic and the left pupil is mid-range and irregular owing to posterior synechia. Other ophthalmic findings (not visible in this figure) included aqueous flare, anterior chamber cells, dense nuclear sclerosis, vitreous cells and hemorrhage, and retinal separation with hemorrhage and intraretinal cellular infiltrate OU
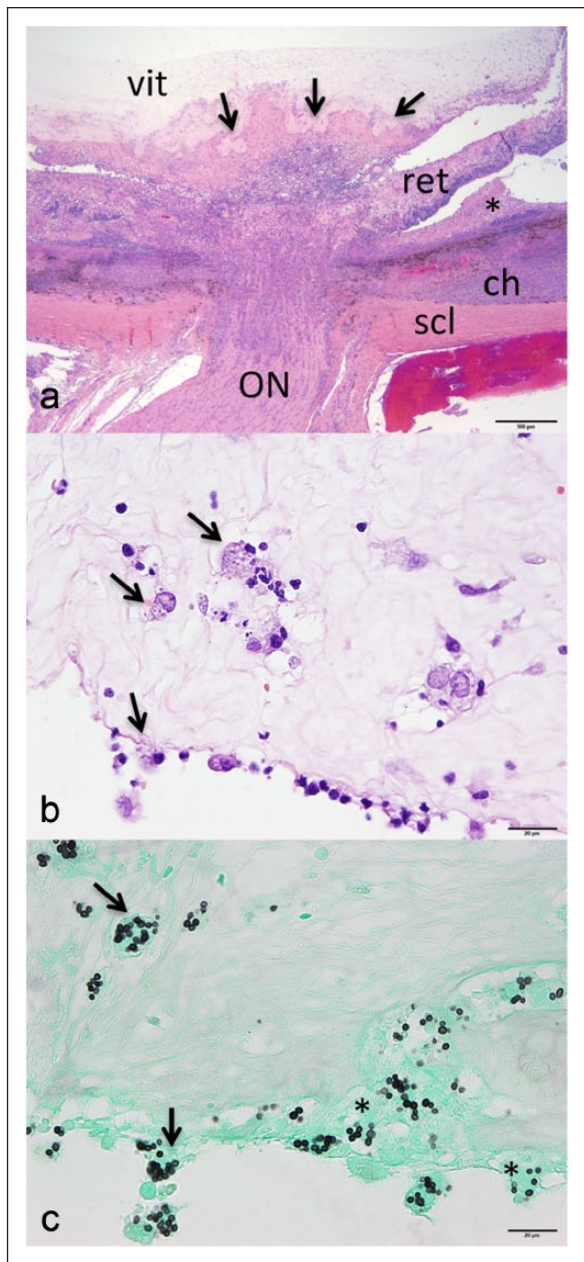
species, and many of which were extracellular. The organisms were strongly argyrophylic when stained with Grocott's methenamine silver (GMS) technique, and weakly positive with periodic acid–Schiff techniques. Additionally, immunohistochemistry using the pan-fungal and mycobacterial antibody against the Bacillus Calmette–Guérin antigen labeled the capsule of organisms strongly, and highlighted their predominantly intracellular location, which was less apparent with hematoxylin and eosin and GMS techniques. A few extracellular organisms were similarly identified. Tissue scrolls from the paraffin-embedded whole globe were submitted for PCR analysis using primers directed at *Sporothrix* and *Cryptococcus* species DNA (University of California Davis Real-time PCR Research and Diagnostics Core, Davis, CA, USA); both were negative. Owing to the histologic appearance of the microorganisms and the results of multiple *Histoplasma* species antigen tests, PCR assessment for *Histoplasma* species was not conducted.

Pending PCR results, clindamycin was discontinued and fluconazole (5 mg/kg PO q12h) was initiated. The endophthalmitis continued OD and secondary glaucoma along with corneal ulceration developed (Figure 5). Because the right eye was considered to be painful and permanently blind, and a possible nidus of infection, it was enucleated approximately 5 weeks after the left eye so as to relieve pain and pursue a definitive diagnosis. Analgesia was provided through administration of buprenorphine as previously. Immediately following enucleation and prior to formalin fixation, vitreous and subretinal fluid along with a section of peripapillary retina and choroid were submitted for fungal, as well as anaerobic and aerobic bacterial, cultures. Histologic findings OD were similar to those OS, although fewer intralesional
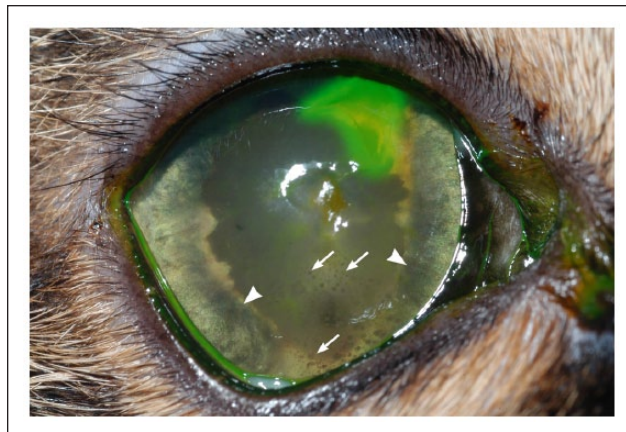
microorganisms were seen. Bacterial cultures were negative. Fungal culture conducted on inhibitory mold agar (Hardy Diagnostics) at 30°C yielded very small numbers of *H capsulatum* whose identity was confirmed by sequencing of the internal transcribed spacer region (University of Texas Health Science Center Fungus Testing Laboratory, San Antonio, TX, USA). Fluconazole was continued for 8 months. At a recheck examination at that time the cat appeared clinically well and had gained 1.4 kg body weight; however, thrombocytopenia and anemia persisted. The cat was then lost to follow-up.

Discussion

Antigen testing for diagnosis of histoplasmosis has been studied extensively since it became available for use in humans. False-negative results have previously been reported in cats, similar to the cases presented herein, potentially owing to low antigen concentrations, antigenic differences among fungal strains, poor specimen handling and laboratory error.[24,36] In one report, *Histoplasma* species urine antigen testing was negative in a cat, despite identification of *H capsulatum* on cytologic examination of rectal mucosa.[36] It is possible that *H capsulatum* sequestration at a distant site or presence of low microorganism burden precludes disease detection by urine antigen testing, as studies in people have shown the test to be less reliable in cases of focal or mild disease.[32,38] The eye may represent a site of focal infection and therefore be less likely to result in detectable antigenuria. In cases of localized disease, collection of body fluids nearest the site of infection, such as bronchoalveolar lavage fluid for diagnosis of focal pulmonary histoplasmosis, has been recommended as the most reliable means of antigen detection.[32] However, as evidenced by

Figure 5 Right eye of a 16-year-old female spayed domestic shorthair cat (case 4) with a 3 month history of blindness. Note the fluorescein-stained corneal ulcer with loose epithelial edges and <5% stromal loss dorsomedially, the central corneal facet, extensive keratic precipitates (arrows), early iris bombé (arrowheads) and subtle rubeosis iridis. The pupil is mid-range, irregular and fixed owing to posterior synechia. Other ophthalmic findings (not visible in this figure) included aqueous flare and anterior chamber cells, dense nuclear sclerosis and diffuse anterior capsular cataract. Examination of the posterior segment was not possible owing to anterior segment changes. The left eye had previously been enucleated.

Figure 4 Photomicrographs of the left eye of a 16-year-old female spayed domestic shorthair cat (case 4). (a) Low-magnification photomicrograph of the ocular posterior segment. There is variably cellular inflammatory exudate (arrows) in the vitreous (vit). The retina (ret) is detached, with cellular exudate in the subretinal space (*). The choroid (ch) is markedly expanded by mixed inflammatory infiltrate. Similar infiltrate extends through the optic nerve head and into the optic nerve parenchyma (ON). There is hemorrhage resulting from the enucleation external to the sclera (scl). Hematoxylin and eosin, × 2; bar = 500 μm. (b) Higher magnification of intracellular *Histoplasma capsulatum* organisms. Punctate, amphophilic bodies are present in some foamy macrophages (arrows) in the posterior vitreous. Hematoxylin and eosin, × 40; bar = 20 μm. (c) Grocott methenamine silver technique (× 40) highlighting, in black, intracellular (arrows) and fewer extracellular (*) *H capsulatum* organisms. Bar = 20 μm

case 4 of the present series, ocular fluid may not yield positive results, even when the microorganism can be cultured from closely adjacent tissues. To our knowledge, the quantitative antigen test has yet to be validated on ocular fluid and further research in this area is warranted. Sensitivity of the urine antigen test has most frequently been assessed using immunocompromised people with a large microorganism burden, which may not reflect the clinical scenario in all veterinary patients.[1] Studies in humans have demonstrated that antigen testing of both serum and urine can increase test sensitivity when the disease is focal.[39] However, as evidenced by cases 1 and 4 in the present series, this may still fail to identify disease, particularly when it appears limited to or to predominantly involve the eye. A recent case report described antigenemia in the absence of antigenuria in a cat with renal histoplasmosis.[40] The absence of antigenuria in that case was particularly interesting given the direct involvement of the urinary system in the infectious process. Cases such as these demonstrate the need for research to determine the most appropriate sample(s) to submit and test(s) to perform to maximize disease detection in cats with suspected focal histoplasmosis.

Monitoring of antigenuria can be used to aid decisions regarding treatment cessation and for detection of relapses.[30,41] This case series suggests use of the test in this manner may not be appropriate for feline ocular histoplasmosis as low antigen burden or sequestration of the

fungus within the eye may preclude ability to confirm complete infection resolution. This is particularly important, as work in spontaneously infected human beings, as well as animal models of disease, has suggested that *H capsulatum* can remain dormant in tissues for extended periods.[1,14] *H capsulatum* organisms within macrophages can evade immunologic responses and persist as small foci within various organs, resulting in chronic, subclinical infection.[1,14] Typically, the immune system prohibits these microorganisms from proliferating, but does not kill them, resulting in the formation of granulomatous lesions as described in 3/4 cases in this report.[1] Reactivation of the disease may result when there is immunocompromise such as during severe illness, chemotherapy or other immunosuppressive treatments.[14,42] This phenomenon is well-documented in people who have not traveled to areas of endemic *H capsulatum* in many years but develop severe disseminated disease,[43,44] and has been suggested to occur in cats.[8] This also seems likely in case 4 of this series, as the cat had lived in California for 8 years, where this disease appears to occur less commonly.[45] In a research setting, exposure of immunocompetent mice to a low inoculum of *H capsulatum* resulted in persistent fungal burden (microorganisms cultured from organ samples) even 90 days after exposure.[46] Half of these mice went on to develop progressive fatal infection or evidence of chronic infection within 1 year following inoculation. The remaining mice appeared to clear the disease spontaneously and had no culturable fungal burden at the time of their demise.[46] This observation supports the persistence of *H capsulatum* in the body even when urine antigen concentration is low and there is absence of clinical disease.

The eye is a sequestered organ normally protected from the rest of the body via the blood–aqueous and blood–retinal barriers. However, these barriers become reversibly more permeable in eyes affected with uveitis. This has potential implications for detection of disease, spread of disease to the rest of the body and treatment. However, to our knowledge, sequestration of *H capsulatum* in ocular tissues and its potential to act as a nidus for infection, especially once antifungal therapy is discontinued, has not been definitively determined. Work with *Blastomyces* species, another dimorphic fungus with potential to spread to the eye, suggests that persistence of fungal elements in ocular tissues can suppress the immune response and delay clearance.[47] Although this reduced immune response decreases ocular inflammation and improves vision retention, it may pose risk to the rest of the body. Therefore, enucleation of blind eyes in animals with confirmed blastomycosis (and possibly other fungal diseases such as histoplasmosis) may be in the patient's best interest.[37] One study looking at treatment of feline histoplasmosis reported relapse in only 2/8 patients within 6–10 months of cessation of therapy; however, both had ocular involvement. The only other cat in that study with ocular disease

had the globe enucleated during treatment and had not suffered relapse at last follow-up 3 months after stopping therapy.[48] Data from the current study reveals that serial urine and/or serum antigen testing may not be helpful for confirming complete resolution of infection, and suggests the need to remove blind eyes in such patients. Alternatively, lifelong maintenance of antifungal therapy may be indicated to control disease.[49]

Three of the four cases in the present study had elevated *T gondii* titers with two of these animals having only an elevated IgM titer suggestive of acute infection, reactivation or slow release of antigen from bradyzoite cysts.[50] Although it is possible these animals were immunodeficient as a direct result of histoplasmosis or from an unrecognized cause that made them susceptible to infection with, or reactivation of, *Histoplasma* species and *T gondii*, *Toxoplasma* titers are exceedingly difficult to interpret. Although immunosuppression due to concurrent feline leukemia virus or feline immunodeficiency virus infection is reported, evidence of retroviral infection was not found in the three cats tested in the present series.[19,20,48,51] In case 2, serial *T gondii* titers did not change despite this cat improving clinically while receiving clindamycin and antifungal therapy. Thus, it seems likely that fungal infection was the primary agent in this case. Similarly, case 4 received clindamycin for 4 weeks without clinical improvement; however, *T gondii* titers were not repeated. Both cats improved once long-term antifungal therapy was introduced. These cases are similar to a cat from Missouri that had an elevated *Toxoplasma* species titer but failed to improve clinically on antiprotozoal therapy until an antifungal drug was also initiated.[51]

Conclusions

The present report underscores the fact that negative urine *Histoplasma* species antigen test results cannot be used alone to rule out a diagnosis of histoplasmosis in cats.[1,36,39,40] If initial urine testing is negative, but clinical suspicion of infection remains high (especially within the eye or other sequestered location), additional antigen testing of alternate fluid samples could be considered, but direct methods of organism detection such as PCR, culture, cytology or histology should also be pursued.[39] Further research is needed to determine the most appropriate test(s) to perform to maximize disease detection in cats with focal histoplasmosis. Our series also suggests that further research is warranted to improve interpretation of *Histoplasma* species antigen testing on ocular fluid. Antifungal therapy may be indicated in cases where clinical suspicion of histoplasmosis is high based on previously confirmed infection, but antigen testing fails to yield a positive result. Lastly, the current report suggests that *Histoplasma* species antigen testing should be used very cautiously to determine duration of therapy if signs appear limited to the eye, as fungal

elements may be sequestered and undetectable in this manner.

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