Cryptococcal Infection in Cats: Factors Influencing Treatment Outcome, and Results of Sequential Serum Antigen Titers in 35 Cats

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The relationship between treatment outcome and location of cryptococcal infection, gender, magnitude of pretreatment cryptococcal antigen titers, results of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) serology, and serial changes in antigen titers during and after treatment were evaluated in a prospective and nonrandomized study of 35 cats with cryptococcosis. A commercial cryptococcal latex agglutination kit (CALAS; Meridian Diagnostic Inc, Cincinnati, OH) was used to detect cryptococcal antigen in sera. All cats were treated with itraconazole (Sporanox; Janssen Pharmaceutica Inc, Titusville, NJ). Pretreatment mean log titers for serum cryptococcal antigen were not influenced by location of the infection. Treatment outcome was not influenced by gender, location of the infection, or magnitude of pretreatment serum antigen titer. Treatment outcome was influenced by FeLV and FIV status; cats seropositive for FeLV or FIV had a higher likelihood of treatment failure ($P = .008$). The cryptococcal antigen titers of cats successfully treated decreased with significant linearity over time during treatment ($r = -.64$, $P < .000001$), whereas the corresponding titers for cats not treated successfully did not decrease with significant linearity ($r = -.03$, $P > .9$). For cats in which treatment was successful, antigen titers decreased significantly from pretreatment values by 1.3 orders of magnitude at 2 months after initiation of treatment. By 10 months after initiating treatment, log titers decreased by at least 2 orders of magnitude in all cats successfully treated, and 9 of 16 cats had undetectable titers. In contrast, in 5 of 6 cats in which treatment failed, antigen titers were unchanged or increased in magnitude even after at least 6 months of treatment.

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Cryptococcal polysaccharide capsular antigen is usually detectable in the serum of people and cats with active cryptococcosis.[1,2] Its detection in the serum of cats with commercial kits has been validated, and the efficacy of itraconazole PO for treatment of cryptococcal infection in cats was documented.[2-4] Information regarding pretreatment factors that may predict treatment outcome in cats with cryptococcosis, however, is scarce. In some studies in people,[5] quantification of cryptococcal antigen in serum prior to treatment was shown to have prognostic value; high titers before treatment correlated with higher mortality during treatment. The prognostic value of pretreatment antigen titers in cats has not been reported.

The value of monitoring cryptococcal antigen titers in serum during treatment in people is unclear. Some studies have found that serial monitoring of the antigen titer has a limited role in the management of patients with cryptococcal meningitis and concurrent HIV infection.[1] The role of serial monitoring of cryptococcal antigen titers in serum in cats during treatment for cryptococcosis has not been studied systematically.[4]

Other potential prognostic factors, such as feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV) infection in cats, have been examined with conflicting results.[6,7] Cryptococcus is emerging as a major complication of AIDS[8] and other immunodeficient conditions in people; thus, it follows that the immunosuppressive effects of FeLV or FIV infection in cats might increase susceptibility to cryptococcal infection or alter treatment outcome. Other factors influencing treatment outcome, such as location of infection, have not been reported in cats. In dogs with blastomycosis treated with itraconazole,[9] however, central nervous system or advanced pulmonary infection is associated with increased mortality.

In this study, we explored the relationship between treatment outcome and location of infection, gender, FeLV and FIV status, magnitude of pretreatment antigen titers, and serial changes in antigen titers during and after treatment.

Materials and Methods

Clinical Protocol

The study was conducted as a prospective, nonrandomized trial at 28 veterinary hospitals.[3] Cats (n = 35) with microbiological and histopathological or cytologic evidence of cryptococcal infection were entered into the study. All cats were tested for circulating FeLV p27 antigen, and 27 of the cats were tested for antibodies against FIV. In all cats, blood for serum cryptococcal antigen testing was obtained prior to initiation of treatment, during treatment, and several months after treatment. A commercial cryptococcal antigen latex agglutination kit (CALAS; Meridian Diagnostic Inc, Cincinnati, OH) was used to detect cryptococcal antigen in sera, as previously described.[2,4]

All cats were treated with itraconazole (Sporanox; Janssen Pharmaceutica Inc, Titusville, NJ). The dose of itraconazole was 50 mg PO sid for cats weighing less than 3.2 kg and 100 mg PO sid for cats weighing 3.2 kg or more. Itraconazole administration was continued for 2 months beyond resolution of all clinical signs.

Treatment response was determined by comparing clinical signs prior to, during, and after treatment. Treatment response was recorded as success or failure. Success was recorded if the cat had complete resolution of clinical signs at the time treatment was stopped and if clinical remission was maintained for at least 1 year after treatment. Treatment was considered a failure in cats that had progressive or persistent disease, relapsed within 1 year after treat-

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ment, or if the cat died and cryptococcal organisms were detected in any tissues at necropsy.

Results regarding toxicity and efficacy of itraconazole PO against cryptococcus in these cats have been reported.[3] Also, 8 cats originally classified as clinically improved in the previous report[3] were, with longer follow-up, reclassified in this report as follows: 4 successful and 4 unsuccessful outcomes.

Statistical Analysis

Prior to statistical analysis, all cryptococcal antigen titers were transformed logarithmically (base 10). Pretreatment antigen titers were analyzed to determine the influence of the location of cryptococcal infection with an analysis of variance (ANOVA). The association between FeLV/FIV status and treatment outcome was analyzed by Fisher's exact tests.[10]

The initial cryptococcal titers were grouped according to infection location and to whether the treatment was successful or not. These data were analyzed by a two-factor ANOVA.[10] In this analysis, Factor 1 was location and Factor 2 was treatment classification. Also, cryptococcal titers, for successful and unsuccessful treatments were examined over time by ANOVA and an ANOVA for linear regression.[10]

In addition to the linear regression method, the results of sequential cryptococcal antigen titers were examined in another way. For each cat in which sequential titers were available (n = 22), the change in log titer at months 1, 2, 3, and 6 during treatment compared with the pretreatment value were calculated (eg, a pretreatment titer of 10^5 and 1-month treatment titer of 10^4 represents a change in titer of 1 log). Then, the mean change from pretreatment values at months 1, 2, 3, and 6 was determined for the successfully and then the unsuccessfully treated cats. Results were compared by ANOVA.[10]

Results

Cats (n = 35) with documented cryptococcal infection originally were entered into this study, but 2 cats were lost to follow-up. The median age, gender, breed distribution, and presenting clinical signs have been reported previously.[3] Male cats (intact and neutered) constituted 69% of the cases (n = 24). An association between gender and treatment outcome was not apparent because 12 of 24 (50%) of male cats and 4 of 11 (37%) of female cats were treated successfully.

Three groups of cats were identified on the basis of location of infection: intranasal (15); cutaneous (11); and a group designated as "other," which was composed of cats with infections involving lymph nodes (3), retrobulbar space (1), central nervous system (1), eyes (1), and ears (1). Location of infection had no significant effect on pretreatment titers for cryptococcal antigen (F = .6, df = 2/32, $P < .54$). Also, the location of the infection (F = .000000002, df = 1/26) and the magnitude of pretreatment titers (F = .000000003, df = 2/26) had no significant effect on eventual outcome of treatment. We did note that 9 of 11 cats (82%) with cutaneous infection were treated successfully, whereas 8 of 15 cats (53%) with intranasal infection and 3 of 7 cats (43%) with other sites of infection were treated successfully.

In cats with nasal infections, combined FeLV and FIV seronegative status was significantly (P = .03) associated with successful cryptococcal treatment (Table 1). This was not demonstrated for the other infection locations. When the data were combined, however, an FeLV and FIV seronegative status was highly significant (P = .008) with respect to successful treatment.

Table 1. The Association* Between FeLV/FIV Status and Treatment Success or Failure

	Treatment		
	Success	Failure	*P* Value†
Nasal infection			
FeLV/FIV status			
Positive	0	4	
Negative	8	3	P = .03
Cutaneous infection			
FeLV/FIV status			
Positive	1	0	
Negative	8	2	P = .8
Other			
FeLV/FIV status			
Positive	0	2	
Negative	3	2	P = .29
Combined			
FeLV/FIV status			
Positive	1	6	
Negative	19	7	P = .008

Abbreviations: FeLV, feline leukemia virus; FIV, feline immunodeficiency virus.

* Fisher's exact test.

† Probability of this distribution.

The cryptococcal titers of cats treated successfully regressed with significant (F = 54.7, df = 1/77, $P < .000001$) linearity through time (Fig 1). The correlation coefficient (r) was equal to −.64, and the coefficient of determination ($r^2 \times 100$) was equal to 41.6%. The titers of the cats not treated successfully did not regress with significant (F = .03, df = 1/28, $P < .9$) linearity; r was −.03. The mean change in log titer values for months 2, 3, and 6 during treatment compared with pretreatment values showed a significant decrease (P = .01) in successfully treated cats compared with the unsuccessfully treated cats (Fig 2).

There was a difference between cryptococcal titers in successfully versus unsuccessfully treated cats at the time of last follow-up. At that time (mean, 10 months; range, 2 to 18 months), serum titers were undetectable (n = 9), between 1:2 and 1:20 (n = 5), 1:100 (n = 1), and 1:1,000 (n = 1), in the successfully treated cats. In the unsuccessfully treated cats, serum titers increased during treatment (n = 2), were unchanged (n = 3), or decreased by one order of magnitude (10^5 to 10^4; n = 1).

Discussion

In this study, male cats had twice the infection rate of female cats, which suggests a gender predilection for males. Gender did not predict successful treatment, however, as approximately equal percentages of infected male and female cats were treated successfully.

Using statistical analyses, location of infection was not a predictor of treatment outcome. Although not statistically significant, we noted that 82% of cats with cutaneous infection were treated successfully, compared with those with

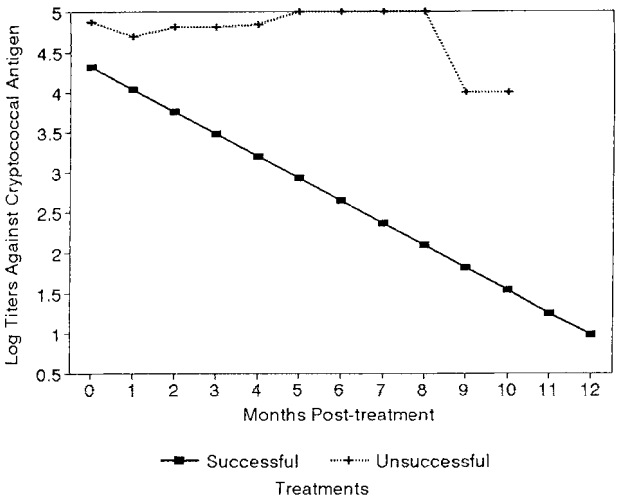

Fig 1. Linear regression of log titers against cryptococcal antigen versus months of treatment for successfully and unsuccessfully treated cats with cryptococcosis. With cats treated successfully for cryptococcosis, log titers against cryptococcal antigen regress with significant ($F = 54.7$, $df = 1/17$, $P < .000001$) negative ($r = -.64$) linearity on months of treatment. The titers of cats not treated successfully do not regress on months of treatment.

intranasal infections (53%) and infections in other sites (43%). Seemingly, the cutaneous form of cryptococcal infection has a high likelihood of being successfully treated with itraconazole. Initial titers were not significantly different based on location of infection, nor were they predictors of treatment outcome.

In this study, the only pretreatment factor examined that predicted a successful outcome was a seronegative test for FeLV and FIV. There is evidence from other studies that FIV infection in cats may influence the host's response to cryptococcal exposure. In one study, the frequency of isolation of *Cryptococcus neoformans* from oropharyngeal swabs was significantly higher in FIV-seropositive cats.[6] Another study concluded that although FIV did not impart an unfavorable prognosis, FIV-seropositive cats required longer courses of treatment and were more likely to have advanced or disseminated disease.[7] The results of our study suggest that FeLV- or FIV-seropositive cats with cryptococcosis have a less favorable prognosis. In people, conditions characterized by immunodeficiency (ie, HIV infection) increase susceptibility to systemic fungal infections and unfavorably influence the prognosis.[8,14-17] Furthermore, in people seropositive for HIV, relapse from sequestered infection after treatment is common. Thus, people with HIV infection typically undergo maintenance treatment with itraconazole after active cryptococcal infection is controlled in an effort to prolong clinical remission. The same strategy may be appropriate for cats with cryptococcosis that are seropositive for FeLV or FIV.

Using the linear regression model, we found that the correlation of log titers versus time after initiation of treatment was significantly different between the "successful" and "unsuccessful" treatment groups. In the successfully treated group, the serum titers decreased significantly from pretreatment values, whereas for the group in which treatment was

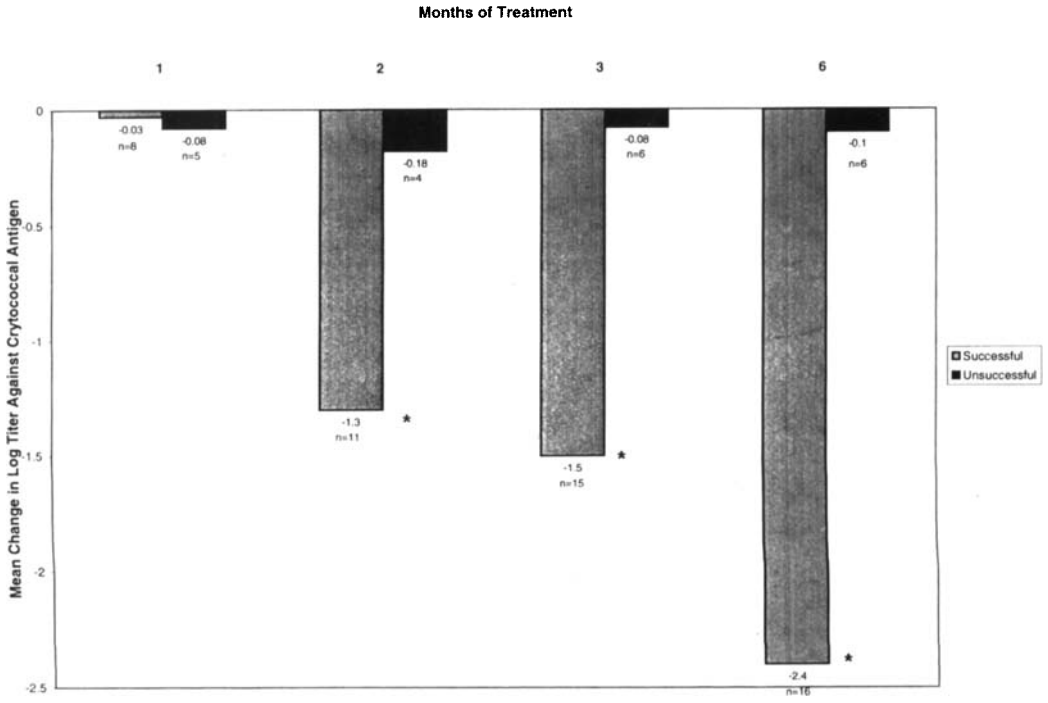

Fig 2. Mean changes in cryptococcal antigen log titers from pretreatment values at 1, 2, 3, and 6 months after the start of treatment in successfully and unsuccessfully treated cats with cryptococcosis. Asterisk (*) denotes significant difference ($P < .01$) from pretreatment values.

unsuccessful, the serum titers either were unchanged or increased during treatment in 5 of 6 cats (Figs 1, 2). Because the cats in our study did not have the same initial pretreatment titers and because there was variation in their serial titer values, the pooled data might not have accurately reflected individual responses of serum titers to treatment. Accordingly, we explored the change in log titers at treatment months 1, 2, 3, and 6 compared with the initial log titer for each cat. The results showed that in the group of cats that were successfully treated a significant decrease in titer was demonstrated by the end of the second month of treatment, whereas in the cats that were unsuccessfully treated a significant decrease in titer was never demonstrated (Fig 2).

In a study of experimentally infected cats, a progressive decline in serum titer during treatment was observed.[4] At the end of 3 months of treatment two thirds of the infected cats had a negative titer, and serum titers remained negative for a 3-month period of observation after completion of treatment. In our study, the log titers decreased to an undetectable level in most instances, or decreased by at least 2 orders of magnitude. The highest residual titer was 1:1,000 (n = 1, initial pretreatment titer = 1:100,000) after 10 months of treatment. In the group of cats in which treatment was unsuccessful, a decrease in log titer was detected in only 1 cat at any time during the course of treatment. In that cat, the maximum decrease in titer was 1 log after 10 months of treatment (1:100,000 to 1:10,000).

From the results of our study, we concluded that a seropositive test result for FeLV or FIV imparts an unfavorable influence on treatment outcome. Also, serial monitoring of serum cryptococcal antigen titers during treatment is valuable in the ongoing assessment of patient progress and prognosis and in optimizing the duration of treatment. A favorable prognosis is associated with a progressive decrease in antigen titer of at least 1 order of magnitude at the end of 2 months of treatment. We recommend that treatment continue for 1 month not only after resolution of clinical signs but also after a decrease in antigen titer by at least 2 orders of magnitude, preferably until serum antigen is undetectable. Furthermore, our results suggest that, similar to other systemic fungal infections, a long course of treatment often is required for a successful outcome.[4,11-13]

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