A Noninferiority Clinical Trial Comparing Fluconazole and Ketoconazole in Combination With Cephalexin for the Treatment of Dogs With Malassezia Dermatitis*

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This double-blinded noninferiority clinical trial evaluated the use of oral fluconazole for the treatment of Malassezia dermatitis in dogs by comparing it with use of an accepted therapeutic agent, ketoconazole. Dogs presenting with Malassezia dermatitis were treated with either fluconazole or ketoconazole in addition to cephalaxin for concurrent bacterial dermatitis. Statistically significant improvements in cytologic yeast count, clinical signs associated with Malassezia dermatitis, and pruritus were seen with both antifungal treatments. There was no statistical difference between the treatments with regard to the magnitude of reduction in these parameters. These results suggest that fluconazole is at least as effective as ketoconazole for the treatment of dogs with Malassezia dermatitis.

INTRODUCTION

Dermatitis associated with the overgrowth of yeast belonging to the genus Malassezia exacerbates many common primary dermatoses in dogs. A significant degree of pruritus, a rancid odor, and a variety of skin lesions, including erythema, greasy exudate, hyperpigmentation, and lichenification, typically result from Malassezia dermatitis in dogs.1–3 Malassezia pachydermatis is a commensal organism of cutaneous and mucosal sites in dogs, but primary conditions that disrupt the protective barrier of the skin or alter the host animal’s immune system can trigger overgrowth of the yeast.3 Dermatitis results as antigens or products from the Malassezia organisms in the stratum corneum trigger cutaneous inflammation and/or hypersensitivity.1,3

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Many primary conditions have been associated with Malassezia dermatitis in dogs, including allergic dermatitis, ectoparasitism, keratinization defects, endocrine diseases, and metabolic disorders. These primary conditions also predispose affected dogs to bacterial infections; consequently, bacterial pyoderma often exists concurrently with Malassezia dermatitis in dogs. This relationship is supported by the finding that dogs with Malassezia dermatitis have increased numbers of cutaneous staphylococcal bacteria. It has been proposed that a symbiotic relationship may exist between M. pachydermatis and cutaneous staphylococci.

Ketoconazole is an imidazole antifungal agent that has been shown to be efficacious in treating Malassezia dermatitis in dogs when administered orally. Oral absorption of ketoconazole is reported to be extremely variable in dogs and is optimized by an acidic gastric environment and, possibly, by the presence of food. Ketoconazole is extensively metabolized by the liver, and only a small amount is excreted unchanged in the urine. Ketoconazole has been reported to cause adverse effects in approximately 15% of dogs, most commonly lethargy and gastrointestinal effects such as anorexia, vomiting, and diarrhea. Ketoconazole can also cause transient, dose-related suppression of gonadal and adrenal steroid synthesis. Additional rare side effects reported in dogs include elevated liver enzyme activities, idiosyncratic or dose-related hepatic toxicity, thrombocytopenia, neurologic signs, and reversible lightening of the haircoat. Ketoconazole is available in a 200-mg generic tablet formulation.

Fluconazole is a triazole antifungal that has been used successfully as a systemic treatment for cutaneous Malassezia infections in people, including infections caused by M. pachydermatis. Data are lacking regarding the efficacy of orally administered fluconazole for the treatment of Malassezia dermatitis in dogs. Studies evaluating the in vitro antifungal sensitivity of M. pachydermatis have found that the minimum inhibitory concentration (MIC) and minimum fungistatic concentration are higher for fluconazole than for other azoles. However, these MIC data are impossible to interpret clinically without established breakpoint values that take pharmacokinetic data and clinical experience into account. For other Malassezia spp isolated from humans, studies of in vitro sensitivity have also shown that fluconazole has higher MICs than other azoles. Despite this in vitro data, fluconazole has been found to be as effective as ketoconazole and more effective than itraconazole for the treatment of cutaneous Malassezia infection ( pityriasis versicolor) in human clinical trials. This difference between the in vitro and in vivo efficacy of fluconazole may be related to its favorable pharmacokinetic profile.

Fluconazole reliably has excellent oral bioavailability, and its absorption from the gastrointestinal tract is not influenced by the presence of food or gastric pH. Fluconazole is not extensively metabolized, and a large portion of the dose is excreted unchanged in the urine. It is generally considered to be well tolerated in dogs; the most commonly reported adverse effect is anorexia. Additional adverse effects reported in humans include vomiting, diarrhea, and, rarely, increased liver enzyme activities and hepatic toxicity. Rare cases of cutaneous drug reactions and thrombocytopenia have also been reported in people. Unlike ketoconazole, fluconazole does not affect hormone synthesis. Fluconazole was recently released in a generic formulation and is available in 50-mg, 100-mg, 150-mg, and 200-mg tablets.

In addition to their antifungal effects, azole antifungicides, including fluconazole and ketoconazole, have been shown to have antiinflamm-
matory properties. These immunomodulatory properties may help to alleviate signs associated with underlying allergic responses. Ketoconazole has been shown to suppress production of T helper 2–type cytokines, such as interleukin (IL)-4 and IL-5, by human T cells in vitro.24 Fluconazole has also been shown to decrease IL-4 production and increase production of interferon γ in Candida albicans–infected mice.24 Modulation of these cytokines may lead to decreased IgE synthesis and reduced differentiation of eosinophils. Ketoconazole may additionally prevent IgE class switching in surface IgE–negative B cells, possibly preventing initiation of IgE-mediated allergic reactions.25 In vitro studies using human keratinocytes have further shown that ketoconazole suppresses the production of inflammatory chemokines released from keratinocytes in lesional atopic skin.26 Fluconazole and ketoconazole have both been shown in vitro to inhibit the production of leukotriene B4, an eicosanoid mediator that may contribute to the inflammatory reaction in canine atopy,27 although the effect is more substantial with ketoconazole.28 Fluconazole (but not ketoconazole) has been shown to inhibit neurogenic inflammation in mouse models, suggesting that it may have additional antipruritic activity.29

This study was designed as a noninferiority trial to compare the clinical efficacy of fluconazole to that of ketoconazole, a currently accepted treatment for Malassezia dermatitis in dogs. As is common clinically, dogs included in this study had both yeast and bacterial dermatitis. Concurrent antibiotic therapy with cephalaxin was used to address the bacterial skin infection. The outcome of the antifungal therapy was assessed by comparing the number of Malassezia organisms seen on cytology, the presence of clinical lesions typical of yeast dermatitis, and an estimate of the dog’s degree of pruritus before and after treatment.

MATERIALS AND METHODS

**Dogs**

Client-owned dogs examined by the Louisiana State University (LSU) School of Veterinary Medicine Dermatology Service were considered for inclusion in the study. The study design was approved by the LSU Clinical Protocol Review Committee. Informed written consent was obtained from the owners. All dogs were required to be in good health based on physical examination and client information, with no history of liver or renal disease. Malassezia dermatitis was diagnosed based on a history of pruritus, compatible clinical findings, and surface cytology from affected areas demonstrating at least 10 Malassezia organisms in 15 random oil immersion fields.5,7,30 Dogs were required to have concurrent bacterial dermatitis as evidenced by the presence of pustules, epidermal collarettes, crusts, or papules on physical examination or abundant bacteria on cytology from affected areas.30,31 Dogs were not included in the study if they had been treated with a systemic antibiotic or antifungal medication within the previous 30 days. In all cases, allergic dermatitis was either known or suspected to be the underlying dermatologic disorder; however, diagnosis and treatment of the primary problem were not pursued as part of this study.

**Experimental Design**

At the time of enrollment, cutaneous samples for aerobic bacterial culture and antibiotic sensitivity testing were obtained from each dog and submitted directly to the Louisiana Animal Disease Diagnostic Laboratory. Briefly, the sample for culture was obtained steriley from an intact pustule if one was present or by rolling the culturette swab across a collarette or other skin lesion where cocci were visualized on surface cytology.32,33 Dogs with bacteria demonstrating resistance to cephalothin were
excluded from the study. One dog (#13) was entered into the study before modification of the study protocol to include cutaneous bacterial culture and antibiotic sensitivity. To treat bacterial dermatitis, dogs received cephalaxin at a dose of 22 to 30 mg/kg PO q12h. To allow inclusion of dogs with a wider range of body sizes, both a commercially available generic cephalaxin suspension (50 mg/mL) and generic cephalaxin capsules (250 mg or 500 mg) were used.

For treatment of *Malassezia* dermatitis, dogs were randomly assigned to two treatment groups based on a coin toss. Dogs assigned to the ketoconazole (KETO) group received generic ketoconazole tablets (200 mg) at a dosage of 5 to 10 mg/kg PO q24h, while dogs in the fluconazole (FLU) group received generic fluconazole tablets (100 mg) at a dosage of 5 to 10 mg/kg PO q24h. To avoid excluding dogs on the basis of body weight, a suspension generated from ground tablets of ketoconazole or fluconazole was used in place of tablets for dogs too small to be dosed accurately with the available tablet sizes. All dogs received their medications daily with food for 3 weeks. The primary investigator (L. S.) was blinded to the treatment group and was not involved in dispensing or discussing the medications with the clients. The dog owners were also blinded to the treatment group: the antifungal medication was dispensed in a container marked simply as “antifungal” with prescribing information only.

No other systemic or topical therapy was allowed during the study period, with the exceptions of heartworm prophylaxis and non-corticosteroid-containing otic products. Flea control products, hypoallergenic diets, and allergen-specific immunotherapy were continued, as long as no adjustments had been made to the therapeutic protocol within the previous 6 weeks. Clients were given a log to record administration of the study medications, the incidence of any adverse effects, and clinical observations. Additionally, owners were called weekly to verify that the study protocol was being followed. If persistent or excessive adverse effects were noted, the dermatitis worsened despite treatment, or clients were noncompliant, dogs were removed from the study and the primary investigator was no longer blinded to the treatment so that rescue medications could be given or diagnostics performed as necessary.

**Cytologic Evaluation**

All body areas with lesions suggestive of *Malassezia* dermatitis were sampled with clear adhesive tape strips and examined microscopically. The tape strip cytology was performed using an adaptation of a technique described elsewhere. Any overlying hair was parted, and a strip of tape was placed firmly on the affected skin and then gently removed and reapplied three times in succession. The tape was subsequently placed on a glass slide, stained with modified Wright stain (Quik-Dip Stain; Mercedes Medical, Sarasota, FL), and examined using an oil immersion objective (×1000) to identify yeasts with morphology typical of *Malassezia* spp. For each body area sampled, the primary investigator totaled the number of *Malassezia* organisms in 15 consecutive fields within a 1.25-cm² area of tape. The mean of the four body areas with the highest yeast count was calculated. If a dog had fewer than four affected locations in which *Malassezia* organisms were identified on cytology, then other cutaneous areas most likely to have *M. pachydermatis* identified on cytology were sampled. The alternative sites, listed in the order they were to be sampled if needed, were the lower lip, the chin, and the interdigital region of a front foot. The same four areas used to generate the initial mean yeast count were assessed by the primary investigator at the follow-up visit to determine post-treatment mean
yeast count. The percentage reduction in mean yeast count after treatment was calculated for each dog as a percentage of the initial mean yeast count.

Clinical Evaluation

The four body areas sampled for the yeast count were evaluated by the primary investigator at the initial and follow-up visits to generate pre- and post-treatment clinical index scores (CISs). The clinical scoring system used was adapted from previous studies. Each body area was assigned a value from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe) for each of five clinical features typical of Malassezia dermatitis: erythema, greasy exudate, scaling, hyperpigmentation, and lichenification. The score for each location was tallied (with a maximum score of 15 for each location), and the total score for the four sites was the CIS. Additionally, each dog was subjectively evaluated at the follow-up visit to determine if there was clinical resolution of the Malassezia and bacterial dermatitis. Clinical resolution of Malassezia dermatitis was based on resolution of the seborrheic dermatitis with improvement of erythema and hyperpigmentation. Bacterial dermatitis was considered clinically resolved based on the absence of compatible clinical lesions, such as pustules, epidermal collarettes, crusts, or papules. Clinical resolution did not require complete cytologic resolution and was the basis for deciding if continued systemic antifungal or antibacterial treatment was required.

At both the initial and follow-up visits, the client was asked to mark a visual analogue scale (VAS) to represent the dog’s current severity of pruritus. The VAS was an adaptation from a previous study and was a nonscored, 100-mm line with brief descriptions of the severity of pruritus. The left end of the line represented no pruritus, and the right end represented constant pruritus; the level of severity described increased from left to right. A score (from 0 to 100) was generated from the VAS by measuring the distance from the left side of the line. For both the CIS and the VAS score of each dog, the percentage reduction in posttreatment score from the initial to the follow-up visit was calculated as a percentage of the initial score.

Statistical Evaluation

The primary objective of this noninferiority trial was to show that fluconazole is as effective as ketoconazole when combined with cephallexin for the treatment of dogs with Malassezia dermatitis. While standard statistical testing was performed to support a conclusion of no difference and thus noninferiority, the margin of noninferiority (± δ) was established such that any difference within this margin was considered clinically unimportant and supportive.
of a conclusion of noninferiority. A treatment at least 80% as effective as the standard treatment of ketoconazole was required and was used to set the margin of noninferiority (δ = 20%) for reduction in mean yeast count, VAS score, and CIS. The 95% confidence interval (CI) of the difference between the groups was reported and compared with the 20% noninferiority (lower) margin of the ketoconazole result. If the CI of the difference was to the right of the lower end of the noninferiority margin, inferring that the difference was at least 80% of the standard treatment, then the conclusion of noninferiority could be supported.

Continuous data (age, body weight, yeast count, VAS score) were tested for normality using E6.

TABLE 1. Yeast Count and Percentage Reduction in Yeast Count, Clinical Distribution of Malassezia Dermatitis, and Dose of Antifungal Administered for Each Dog

<table>
<thead>
<tr>
<th>Dog</th>
<th>Dose (mg/kg)</th>
<th>Clinical Distribution</th>
<th>Mean Yeast Count</th>
<th>% Reduction Yeast Count</th>
<th>% Reduction Yeast Count (Mean ± SD)</th>
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<tbody>
<tr>
<td></td>
<td>FLU Initial</td>
<td>Follow-up</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>6.6</td>
<td>Paws, face</td>
<td>46.3</td>
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<td>100.0</td>
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<tr>
<td>2</td>
<td>7.3</td>
<td>Generalized</td>
<td>30.3</td>
<td>2.8</td>
<td>90.9</td>
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<tr>
<td>3</td>
<td>7.4</td>
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<td>19</td>
<td>84.5</td>
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<tr>
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<td>671.3</td>
<td>125</td>
<td>79.8</td>
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<td>Follow-up</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
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<td>195.3</td>
<td>5.3</td>
<td>97.3</td>
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</tr>
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<td>99.0</td>
</tr>
<tr>
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<td>0.8</td>
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<td>Paws, face</td>
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<tr>
<td>25</td>
<td>9.5</td>
<td>Paws, face</td>
<td>85.8</td>
<td>2.3</td>
<td>97.4</td>
</tr>
</tbody>
</table>

*Mean percentage reduction in yeast count for each treatment group and mean percentage reduction in yeast count subdivided based on clinical distribution.
ing the Shapiro–Wilk test with the null hypothesis of normality rejected at $P < .05$. Normal data were compared between groups using a $t$ test with significance set at $P < .05$ (two-sided). Ordinal score data (CIS) and nonnormal data (age, body weight, yeast count, VAS score) were compared between groups using the Wilcoxon signed rank test (WR) with significance set at $P < .05$ (two-sided). The frequency of clinical resolution of pyoderma and Malassezia dermatitis (yes versus no) was compared between groups using the Fisher exact test (FET) with a two-sided hypothesis at $P < .05$.

To examine if the distribution of the lesions modified the treatment effect on the outcome, a mixed-effect analysis of variance (ANOVA) was performed on the percentage reduction in yeast count, CIS, and VAS score. The model included the fixed effects of treatment and distribution, their interaction, and the random variance of dog nested within treatment. A significant interaction effect was considered at $P < .05$. PROC UNIVARIATE, PROC MIXED, TTEST, and NPAR1WAY (SAS v 9.1, SAS Institute, Cary, NC) were used for the analysis.

## RESULTS

### Dogs

A total of 32 dogs was included in the study. After bacterial culture results were obtained, four dogs were excluded based on the finding of methicillin-resistant *Staphylococcus*. Of the remaining 28 dogs, three did not complete the study. All three dogs were in the FLU group. Two were lost to follow-up; the third was withdrawn after 1 week of therapy because of increased pruritus.

A total of 25 dogs completed the study (13 in the FLU group, and 12 in the KETO group): three intact male dogs, 14 castrated male dogs, and eight spayed female dogs. The following breeds were represented: mixed breed (four dogs); pug (three dogs); shih tzu, Labrador retriever, golden retriever, miniature schnauzer, Chihuahua, and beagle (two of each); and cocker spaniel, Newfoundland, French bulldog, dachshund, blue heeler, and Scottish terrier (one of each).

Dogs in the FLU group ranged in age from 1.25 to 12 years (mean: 7.5 years) and in weight from 1.1 to 34 kg (mean: 14.8 kg). Dogs in the KETO group ranged in age from 1 to 10 years (mean: 4.9 years) and in weight from 5 to 52.3 kg (mean: 24.2 kg). There was no significant difference in age ($t$ test; $P = .07$) and weight ($t$ test; $P = .12$) between groups.

*Malassezia* dermatitis had a generalized distribution in 14 dogs (7 of 13 in the FLU group, and 7 of 12 in the KETO group). The remaining 11 dogs (6 of 13 in the FLU group, and 5 of 12 in the KETO group) had *Malassezia* pododermatitis, most with additional areas of dermatitis in intertriginous regions of the face, such as the lip fold and facial fold. The distribution of lesions for each dog is presented in Table 1 along with the mean percent reductions in yeast count for dogs with (1) generalized *Malassezia* dermatitis or (2) more localized *Malassezia* dermatitis. There was no significant effect of location of *Malassezia* dermatitis (generalized versus localized) on the percentage reduction in mean yeast count (ANOVA; $P = .68$), CIS (ANOVA; $P = .88$), or VAS score (ANOVA; $P = .63$).

Adverse effects were reported in six of 13 dogs (46%) in the FLU group and included vomiting (five dogs; #1, 5, 10, 12, and 13), soft stool (one dog; #12), and diarrhea (one dog; #2). In the KETO group, adverse effects were seen in 6 of 12 dogs (50%) and included anorexia (two dogs; #23, 25), vomiting (four dogs; #17, 18, 22, 24), and soft stool (one dog; #22). In all cases, the adverse effects seen were mild and transient and did not require symptomatic treatment or cessation of therapy.
Mean Yeast Count

There was no significant difference in the mean yeast counts at the initial \((t \text{ test}; P = .14)\) and follow-up visits \((t \text{ test}; P = .98)\) between groups. The percentage reduction in mean yeast count for each dog is presented in Table 1 along with the mean percentage reduction for each group. For both groups, there was a significant decrease \((t \text{ test}; P < .001)\) in mean yeast count at the follow-up visit (week 3) compared with the count at the initial visit. There was no significant difference in the percentage change in yeast count \((t \text{ test}; P = .43)\) between groups. The mean reduction in yeast count for the KETO group was 97.8%, with an 80% noninferiority margin of −19.6%. There was a difference between treatments of −1.9%. The 95% CI of the difference was −3% to 7%, thus to
the right of the inferior margin (−19.6%), supporting a conclusion of noninferiority.

**Clinical Index Score**

There was no significant difference in the CIS at the initial (WR; \( P = .89 \)) and follow-up visits (WR; \( P = .48 \)) between groups. The percentage reduction in CIS for each dog is presented in Table 2. There was a >50% reduction in CIS for eight of 13 dogs (62%) in the FLU group and seven of 12 dogs (58%) in the KETO group. The mean percentage reduction in CIS was 56.2% (±11.7%) for the FLU group and 51.6% (±19.4%) for the KETO group. For both groups, there was a significant decrease in CIS at week 3 compared with the CIS at presentation (WR; \( P < .001 \)). There was no significant difference in the percentage reduction in CIS between groups (WR; \( P = .91 \)). With a mean reduction in CIS of 51.6% for the KETO group, the 80% noninferiority margin was −10.3%. There was a difference between treatments of 4.6%. The 95% CI of the difference was −8% to 17.7%, thus to the right of the margin of inferiority (−10.3%), supporting a conclusion of noninferiority.

Clinical resolution of the *Malassezia* dermatitis was obtained in 10 of 13 dogs (77%) in the FLU group and 10 of 12 dogs (83%) in the KETO group during the 3 weeks of the study. The clinical outcomes of the *Malassezia* and bacterial dermatitis for each dog are presented in Table 2. There was no significant difference in the frequency of resolution of the *Malassezia* or bacterial dermatitis for either group (FET; \( P = 1 \)). Several fleas were noted on one dog (#2) at the initial visit, but with continuation of the dog’s flea control regimen, no fleas were found at the follow-up visit. Fleas were not noted on any other dogs at either visit.

**Visual Analogue Scale Score**

There was no significant difference in the VAS scores at the initial (WR; \( P = .96 \)) and follow-up visits (WR; \( P = .81 \)) between groups. The percentage reduction in VAS score for each dog is presented in Table 2. For the FLU group, 11 of 13 dogs (85%) had a >50% reduction in VAS score; this degree of response was seen in 10 of 12 dogs (83%) in the KETO group. The mean percentage reduction in VAS score was 62.6% (±34.9%) for the FLU group and 64.3% (±34.8%) for the KETO group. For both groups, there was a significant decrease (WR; \( P < .001 \)) in VAS score at week 3 compared with the score at presentation. There was no significant difference in the percentage reduction in VAS score (WR; \( P = .85 \)) between groups. With a mean reduction in VAS score of 64.3% for the KETO group, the 80% noninferiority margin was −12.9%. There was a difference between treatments of −1.7%, thus to the right of the margin of inferiority (−12.9%), supporting a conclusion of noninferiority. The 95% CI of the difference was −30% to 27%, which spanned beyond the inferior and superior margins.

**DISCUSSION**

In the present study, ketoconazole was considered an accepted standard of treatment for *Malassezia* dermatitis in dogs, against which fluconazole was compared. We did not detect a significant difference between treatment with fluconazole or ketoconazole when assessed by quantitative (yeast count), semiquantitative (CIS, VAS score), and subjective (clinical resolution) parameters. These results suggest that fluconazole is at least as effective as ketoconazole for the treatment of *Malassezia* dermatitis in dogs. Further support for the efficacy of both treatments comes from the finding that for both groups, there was a statistically significant decrease in yeast count, CIS, and VAS score after 3 weeks of treatment.

Previous in vitro studies evaluating *M. pachy-
dermatitis have found that the MIC of fluconazole is higher than that of other azoles, including ketoconazole. 

Despite this in vitro data, the results of this clinical study suggest that fluconazole is not inferior to ketoconazole in vivo. This discrepancy may be explained in part by the favorable pharmacokinetics of fluconazole. After oral administration, fluconazole distributes well throughout most of the body due to its very low protein binding and solubility in water. Studies in people have shown that fluconazole rapidly accumulates in the stratum corneum via direct diffusion from capillaries and also by transfer from eccrine sweat and sebum. Compared with levels in plasma, fluconazole can reach concentrations 12 to 37 times higher in human stratum corneum when administered daily for 5 or 12 days, respectively. Fluconazole is detectable in the human stratum corneum for at least 12 days after discontinuing therapy because it is eliminated from the skin about two to three times more slowly than from serum or plasma. Although data are not available for dogs, and despite the fact that dogs lack eccrine sweat glands in haired skin, it seems likely that fluconazole also accumulates in the stratum corneum of dogs. Given that peak plasma levels in dogs have been shown to be approximately 10 µg/mL following a 10 mg/kg dose of fluconazole, it could be extrapolated that at this dose, fluconazole levels in the stratum corneum should well exceed published MIC values for M. pachydermatis (4 to 16 µg/mL). Further, given that fluconazole plasma concentrations show good linearity in proportion to dose, lower doses of fluconazole should, theoretically, lead to effective levels in the stratum corneum of dogs. In this study, the favorable results seen with fluconazole were achieved using doses lower than 10 mg/kg.

Unlike fluconazole, ketoconazole does not appear to concentrate in the stratum corneum. Ketoconazole is a lipophilic drug that is highly protein bound and reaches its highest levels in the liver and adrenal glands. Studies in people have shown that it is rapidly transferred to the stratum corneum via eccrine sweat and by passive diffusion, but drug levels are typically lower in the stratum corneum than in plasma.

Secondary Malassezia and bacterial dermatitis are often present concurrently in dogs, as was the case for the dogs enrolled in this study. To address the bacterial infection, it was deemed necessary to include an antibiotic in the therapeutic protocol. Cephalexin was selected as the antibiotic, and in vitro antibiotic sensitivity testing was performed to exclude dogs with bacteria resistant to cephalaxin as assessed by disk sensitivity to cephalothin. In the one case in which bacterial culture was not performed, the pyoderma resolved completely with cephalexin therapy.

The significant reduction in the number of cutaneous Malassezia organisms, along with treatment of the bacterial dermatitis, were most likely the major factors leading to the improvements in CIS and VAS score seen with both treatment groups. However, fluconazole and ketoconazole have immunomodulatory properties that may have reduced cutaneous inflammation caused by either the secondary infections or (perhaps more importantly) underlying allergy. These antiinflammatory actions may have led to clinical improvements that contributed to the reductions in CIS and VAS score. Fluconazole appears to have additional antipruritic properties that may have further contributed to the reduction in VAS score in that group.

Although the adverse effects seen during treatment were mild, the incidence of adverse effects in both groups was relatively high. Given the small number of dogs enrolled in the study, it is difficult to draw conclusions regard-
ing the frequency of adverse effects. Additionally, it is difficult to determine whether the cephalexin, the antifungal, or the combined effect of both was responsible for any given adverse event. Cephalexin therapy is generally associated with a low rate of adverse effects, but it has been reported to cause adverse gastrointestinal effects, including anorexia, vomiting, and diarrhea.\textsuperscript{9} In a similar study by Rosales et al,\textsuperscript{30} no adverse effects were seen in a group of dogs treated with a combination of ketoconazole and cephalexin, whereas diarrhea and/or vomiting was seen in 29\% of dogs treated with cephalexin alone. It is possible that in our study, a significant proportion of the adverse effects were due to the cephalexin therapy rather than to either antifungal medication. In a large-scale retrospective study, ketoconazole was reported to cause adverse effects in approximately 15\% of dogs.\textsuperscript{30} Limited data are available regarding the frequency of adverse effects attributable to fluconazole use in dogs, but the drug is generally thought to be well tolerated.\textsuperscript{9}

To help account for the impact of cephalexin therapy on the antifungal treatment, the inclusion of a negative control group consisting of dogs treated exclusively with cephalexin would have been ideal. In the study by Rosales et al, when cephalexin was administered to dogs with concurrent bacterial and Malassezia dermatitis, there was little improvement in the Malassezia dermatitis following therapy.\textsuperscript{30} The use of cephalexin in the dogs in that study resulted in a mean reduction in yeast count of only 28.8\%.\textsuperscript{30} Thus, for our study, we assumed that cephalexin therapy would have minimal effects on the Malassezia dermatitis. The use of a control group would have additionally helped to account for any placebo effects. However, in clinical trials where a standard, proven treatment exists, conducting the trial with a placebo is often viewed as unethical; therefore, a placebo group was not included in this study.\textsuperscript{41}

A limitation of this study was the small number of dogs evaluated. In an attempt to include as many dogs as possible, steps were taken not to exclude dogs on the basis of body weight. A cephalexin suspension was used for some dogs in order to meet the antibiotic dose requirement, and for two dogs (#1, 5), a fluconazole suspension was formulated in order to administer the required antifungal dose.

Without a large sample size, it is always difficult to demonstrate confidence in a conclusion of no difference between treatments. If the treatments are in fact not different, the power of standard statistical testing will always be low because the objective of that testing is to prove a difference. This can only be rectified by having a comparison group that is different (i.e., a negative control). Thus, without the opportunity to include a placebo group to demonstrate the effectiveness of both antifungal treatments, the most appropriate evaluation is through a noninferiority trial with the objective of demonstrating that the novel treatment (fluconazole) is no less effective than the standard treatment (ketoconazole). Noninferiority trials are typically designed with a null hypothesis that the experimental treatment is inferior to the standard treatment by a certain clinically meaningful result.\textsuperscript{37} For our study, it was proposed that fluconazole would be considered noninferior if it was at least 80\% as effective as ketoconazole, that is, with a margin of noninferiority (\(\delta\)) set at no more than 20\%. This was applied to the assessment of mean yeast count reduction, VAS score reduction, and CIS reduction to show that fluconazole appears to be as effective as ketoconazole.

An additional limitation of this study is that the primary dermatologic condition may have affected the CIS and VAS score results. One dog in the FLU group was withdrawn from the study after 1 week of therapy because of increased pruritus that was most likely attributable
to underlying atopic dermatitis. Two dogs (#4, 22) had an increase in VAS score despite improvements in yeast count and CIS; this was also attributed to exacerbation of underlying allergy. One dog (#22) received food substances known to result in cutaneous adverse food reaction 3 days before the follow-up visit. The other dog (#4) also had increased pruritus in the final week of the study that was suspected to be due to an acute exacerbation of atopic dermatitis.

To better characterize the use of oral fluconazole for Malassezia dermatitis in dogs, additional studies with larger numbers of dogs are needed. Further work may include studies evaluating different therapeutic doses, pulse therapy regimens, variable treatment duration, and the inclusion of adjunctive topical therapy.

CONCLUSION

Ketoconazole has recognized clinical efficacy for Malassezia dermatitis in dogs. The results of this study suggest that fluconazole is at least as effective as ketoconazole when administered concurrently with cephalixin to dogs with Malassezia and bacterial dermatitis. Based on these findings, fluconazole may be considered as an alternative to ketoconazole for the treatment of Malassezia dermatitis in dogs. With generic formulations of both drugs available, the cost of fluconazole and ketoconazole is similar. Fluconazole is available in a wider variety of tablet sizes, which makes dosing more convenient in small dogs.

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REFERENCES


