

A novel non-azole topical treatment reduces *Malassezia* numbers and associated dermatitis: a short term prospective, randomized, blinded and placebo-controlled trial in naturally infected dogs

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Background – *Malassezia* yeast overgrowth on the skin is a common and often recurrent cause of dermatitis in dogs; it can be an exacerbating factor of atopic dermatitis. Anti-fungal drugs have been a standard treatment, but there is some concern that resistance may be evolving in a spectrum of *Malassezia* species. Safe, efficient and easy-to-use alternatives are needed.

Objectives – To assess if a commercially available topical non-azole solution applied to paws affected by *Malassezia*-associated dermatitis (MAD), could ameliorate *Malassezia* numbers and associated signs over a short term (14 day) trial.

Animals – Eighteen dogs with MAD affecting at least two paws.

Methods – The study design was prospective, randomized, blinded and placebo-controlled, using a split-body protocol. Dogs were treated once daily with the test solution on one paw and placebo on the other. Dogs were examined at days 0 and 14 ± 3. The primary end-point was *Malassezia* numbers assessed cytologically. Secondary end-points were clinical scores for lesion severity and pruritus as assessed by a pruritus Visual Analog Scale (PVAS). Owner compliance and adverse effects were assessed.

Results – There was a statistically significant reduction in *Malassezia* numbers and clinical scores for paws treated with the test solution versus placebo. No statistical difference in PVAS was found.

Conclusion – Daily topical application of the test solution was effective in reducing the *Malassezia* burden, as well as improving clinical scores in dogs with MAD of the paws. No adverse effects were reported and owners described the product as either “easy” or “very easy” to use.

Introduction

Statistical analysis

The diagnosis of *Malassezia*-associated dermatitis (MAD) is based upon the combination of compatible clinical lesions, with cytological or laboratory demonstration of the yeast, alongside both inclusion and exclusion of comorbid dermatopathies.^{1–3} There appears to be a correlation between surface density of *Malassezia* organisms and severity of skin disease in dogs.^{4–7} Antimycotic azoles, such as ketoconazole, itraconazole or miconazole,

have been standard therapies in reducing *Malassezia* numbers.² Drug interactions and adverse reactions have been reported in association with both topical and systemic usage of azoles.^{8,9} Furthermore, there are concerns regarding the evolution of *Malassezia* drug resistance. Some *in vitro* studies have reported that *Malassezia* from lesions in dogs with atopic dermatitis were less susceptible to, or even resistant to, azole anti-fungals as compared to *Malassezia* taken from clinically asymptomatic dogs.^{10–12} This was especially evident in strains producing biofilms.^{13,14} There is an ongoing interest in effective, safe and easy-to-use alternative treatments for *Malassezia* infection.

Given the complicated interaction between the host and this opportunistic organism, successful treatment should not only aim to reduce the number of *Malassezia*, but also assist with skin repair. Aptus® Derma Spot On Concentrate™ (Orion Pharma Animal Health; Sollentuna, Sweden) is an anti-*Malassezia* topical treatment that (Table S1) contains zinc, ethyl lauroyl arginate, laureth-9, urea, panthenol, glycerine and butylene glycol. In human patients, a partly similar solution containing urea, propylene glycol, lactic acid and glycerol has shown good efficacy against *Malassezia* associated seborrhoeic dermatitis.¹⁵

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The aims of this study were to assess if Aptus[®] was able to effectively reduce *Malassezia* burden and associated dermatitis in dogs, as well as to report any adverse effects and ease of compliance. We chose to examine *Malassezia*-associated dermatitis of the paws because this is a common disorder in dogs and often affects paws in a symmetrical manner. In addition, as the comparison is of noncontiguous body areas, it is ideal for concurrent examination of parallel treatments in a split-body protocol. We selected *Malassezia* burden as our primary end-point. This is a surrogate clinical outcome marker, but it is a reasonable end-point both because of the short term nature of this trial (two weeks) and because it is recognized that treatment-induced reductions in *Malassezia* numbers are correlated with improvements in clinical signs, at least in humans.¹⁶ Our first hypothesis was that Aptus[®] reduced *Malassezia* burden compared to placebo. Clinical markers of improvement were used as secondary end-points, because clinical signs could reasonably be expected to take a longer period of time to subside than the allotted two week period of this trial. Our second hypothesis was that Aptus[®] reduced clinical signs of MAD relative to placebo.

Material and methods

Ethics

All owners were informed of the nature of this clinical trial. A signed consent form was obtained prior to inclusion. No further ethical permission was needed for this study given the requirements of the Swedish Board of Agriculture (SJVFS 2015:38, saknr L 150, 15-16 §).

Study design and considerations

The study was prospective, placebo-controlled, randomized, blinded and analysed on an intention-to-treat basis. All cases were prospectively recruited across an 11 month period (January to November 2015). Although there was a risk of seasonal influence on pododermatitis, each individual dog in a split-body protocol acted as its own control, and with assessment taking place in a short time frame (days 0 & 14 ± 3), any seasonal impact was unlikely to be significant. The split-body design increases the likelihood that active and placebo solutions were administered in the same way for each dog. It also has the potential advantage of tending to ensure that any co-morbidities and environmental factors are identical for the concurrently treated areas.

Study population

Dogs meeting the inclusion criteria (see below) were consecutively recruited via online mailshot of the hospital client base.

Clinical assessment

Dogs were examined on Day 0 ("start date"), received approximately two weeks of treatment (14 ± 3 days: the "end date") and were then reassessed the following day (12–24 h after the end of treatment). Pruritus was assessed by the same owner for each paw, using a pruritus Visual Analog Scale (PVAS) with clinical descriptions provided alongside a ten-point scale.¹⁷ The following dermatological signs were rated, using a nonvalidated scoring method, on a four-point scale by the same veterinarian (first author), generating a lesion score: (i) greasy exudation, (ii) scaling, (iii) hyperpigmentation, (iv) erythema and (v) excoriation.

Cytological assessment

The *Malassezia* score was a semi-quantitative assessment of skin surface burden. In brief, for clinically affected paws only, an adhesive tape strip was applied three times to an area approximately

1.5 × 2 cm² on the interdigital skin. The same location was used for start and end dates, with an elapse of 12–24 h between last treatment application and cytological re-sampling. Tapes were Romanowsky stained with Hemacolor[®] (Merck KGaA; Darmstadt, Germany), slide-mounted and evaluated cytologically. *Malassezia* numbers were counted per high power oil immersion field (HPOIF, at ×1,000 total magnification). Sampling and cytological evaluation were performed by the same veterinarian (first author).

Inclusion criteria

Interested owners of adult dogs were screened by telephone to check that their dogs were reported to have paw problems with the following characteristics: current active signs such as pruritus and/or skin lesions affecting at least two paws, combined with chronicity (>12 weeks). Selected dogs were invited to consultation, where a general dermatological history and examination was conducted. Dogs could have any PVAS score. Dogs had to have clinical evidence of pododermatitis lesions on at least two paws. It has been reported previously that MAD is more commonly associated with greasy exudation and scale.^{2,18} In our study, lesions had to comprise at a minimum, either greasy exudation and/or scale to a cumulative minimum lesion score of two in each paw. Affected paws had to be affected by *Malassezia*, as evidenced by tape strip demonstration of the highest level *Malassezia* score. Owners had to report compliance with treatments via a recorded daily diary, along with any problems over the 14 ± 3 day treatment period. Finally, included dogs had to be re-evaluated from 12 to 24 h following the end of treatment.

Exclusion criteria

Dogs that had received any anti-fungal systemic or topical treatments up to 14 days prior to study entry were excluded. Dogs that were already receiving any medication (whether dermatological or not) were not permitted to have undergone any change to that treatment within 14 days of enrolment until to the end of their assessment period. Dogs could not receive any other pododermatitis topical interventions during the study time frame. Dogs with either highly asymmetric skin lesions or noncongruent *Malassezia* counts between two different paws (a clinical subjective assessment) were excluded. Dogs could not have any bacteria evident on any interdigital tape strips. In the event of a suspected adverse drug reaction, the pododermatitis treatment solutions were to be stopped and the affected dog's data at that time recorded for an intention-to-treat analysis. In the event of noncompliance to the study protocol, the affected dog would remain in the intention-to-treat analysis.

Treatment

All dogs were treated with both Aptus[®] Derma Spot On Concentrate[™] (Orion Pharma Animal Health; Sollentuna, Sweden) and a placebo (Table S1), supplied in two spray bottles, each containing 50 mL of liquid. One affected paw was treated with one bottle only, according to a colour code (see treatment blinding below). Each interdigital space received at least one spray pump, with a minimum of three pumps delivered per affected paw. At 0.2 mL per pump, a minimum of 0.6 mL of solution was applied per paw, with the owners instructed to apply a sufficient amount of solution to make each affected paw surface damp (achieved by the owner spreading the applied solution around the paw and thus onto the skin surface). We provided disposable gloves and instructed owners to change gloves between paws, thus avoiding cross-contamination of treatments. Paws were left to air dry and the dog was prevented from licking its paws by wearing an E-collar for at least 15 min post-application. Treatments were applied once daily for 14 ± 3 days by one owner.

Note that some dogs were diagnosed with quadrupedal MAD. In these dogs, their owners wished to treat all four paws. Randomized treatment in both fore and hind paws was maintained; however, for statistical purposes, only one pair of paws per dog was included in the analysis.

The placebo contained ingredients that were also constituents of the Aptus[®] solution (Table S1) and have no known antimicrobial effect. Randomization of treatment bottles (Aptus[®] and placebo) was

performed by a computerized generator (Random.org; Dublin, Ireland). The owner, the assessing clinician (first author) and data analysts (first and co-authors) were blind to treatment ("triple blind"). Aptus[®] and placebo spray bottles were identical, except for the label with its randomized code and a colour mark (red mark for right paw, green mark for left paw). The liquid volumes and physical characteristics were the same, and there was no significant odour difference between the two solutions. The randomization code was unmasked following the completion of data entry of all results.

Compliance

Owners were closely assisted over the short time frame of this trial. During consultation, a plan diagram of the dog was provided and the right affected paw was highlighted in red pen, and left with green pen (to match the red and green labelled treatment bottles). Furthermore, a claw on the dog's affected right paw was painted with red nail varnish to provide an easy visual aide-mémoire. In addition, we collected compliance data, with owners asked to record their usage of the solutions and any problems encountered in their diary. At reassessment, we collected all treatment bottles and visually estimated the remaining contents (to exclude gross noncompliance). Finally, we asked the owners to judge the ease of using the two solutions, on a scale of very easy/easy/intermediate/difficult/very difficult.

Statistical analysis

Statistical analyses were performed with SAS[®] EG for Windows v9.4 (SAS Institute Inc.; Cary, NC, USA). *A priori* end-points were *Malassezia* score and the following secondary end-points: (1) clinician-appraised Lesion Score and (2) owner-appraised pruritus (PVAS).

With reference to our primary end-point (*Malassezia* score), previous studies have shown that *Malassezia* surface density varies with location, with higher numbers observed, for example, in the interdigital space versus glabrous inguinal skin in normal dogs.^{4,6,7} A finding of <1 organism per HPOIF is considered to be normal in asymptomatic dogs.^{4,6} Various studies have proposed differing cut-off points for abnormal *Malassezia* numbers in clinical cases, varying from ≥ 1 to >10 organisms per HPOIF.^{19,20} In our study, five HPOIF were counted and averaged to generate an empirical *Malassezia* score as follows: 0, zero *Malassezia* per HPOIF; 1, one to four *Malassezia* per HPOIF; 2, five to nine *Malassezia* per HPOIF; 3, ≥ 10 *Malassezia* per HPOIF. All paws commenced on Day 0 with a high score of 3 (see inclusion criteria) and for this surrogate outcome marker, we set clinical significance as an improvement by ≥ 2 score categories for the same paw between the trial start and end dates (we refer to these dogs as "*Malassezia* responders"). For the purposes of data analysis, the *Malassezia* score has the following characteristics: it is dependent data (results from within the same dog), paired (categories assessed on the same days and across two feet in the split-body protocol), the score is ordinal data (a four-point scale), but finally assessed as categorical dichotomous data using a 2×2 contingency table [treatment versus placebo, *Malassezia* responder (change in score ≥ 2) or not (change in score < 2)]. To derive a *P*-value, the percentage of dogs with an improvement in *Malassezia* score, in the Aptus[®] versus placebo treated paws was examined using a nonparametric McNemar's chi-square test. In addition, an indicative confidence interval for a true underlying proportion was estimated, based upon an assumption of normal distribution. This is a reasonable presumption based upon a sample size of $n > 30$, for although the number of dogs was 18, the number of samples was $n = 36$ (2×18 paws).²¹

Defined dermatological lesions (greasy exudation, scaling, hyperpigmentation, erythema and excoriation) were each given a severity on a four-point ordinal scale (0, none; 1, mild; 2, moderate; 3, severe) and an empirical Lesion Score obtained by summing all scores per paw. The percentage difference between each paw from start to end date was calculated, and then the mean of this difference obtained for each treatment group. The difference in means between Aptus[®] and placebo was examined via a Wilcoxon signed rank test. In addition, an indicative confidence interval was calculated.

The PVAS was assessed, for each paw, on an interval scale ranging from 0 (no pruritus) to 10 (most severe pruritus). PVAS could be at any level on entry to the study. The percentage difference between each paw from start to end date was calculated, and then the mean of this difference obtained for each treatment group. Therefore, this difference in means between Aptus[®] and placebo was examined using a nonparametric method for matched samples, namely, the Wilcoxon signed rank test.

Results

A total of 72 dogs were initially recruited and forwarded for assessment. In 31 dogs, clinical lesions were asymmetrical between paws and these dogs were excluded, as were nine dogs with asymmetrical *Malassezia* counts between paws, eight dogs with concurrent cytological evidence of visible surface bacteria and five dogs with insufficient *Malassezia* counts. One eligible dog was withdrawn by the owners during consultation as it was not randomized and did not commence treatments. Finally, 18 consecutively recruited dogs were enrolled and randomized to treatment.

The signalment of enrolled dogs is described in Table S2. Our inclusion criterion was that adult dogs should have chronic pododermatitis, but in fact 17 of 18 dogs had additional dermatological problems affecting other body areas (ears, perianal area, ventral or lateral abdomen/thorax, lips, axillae and/or groin). Chronicity of dermatological disease ranged from four months to more than 12 years.

All 18 dogs completed treatment of both paws. No adverse effects were reported. Compliance was 100% based on diary entries and visual assessment of returned treatment bottles did not suggest noncompliance. The mean treatment period was 14.56 days (range 11–17 days).

Seven of 18 dogs had quadripedal MAD, with a *Malassezia* score of three for all four paws. The owners treated all four paws while maintaining the randomized treatment side in both fore and hind paws. Assessment of all paws in these dogs yielded the same *Malassezia* scores on each side of treatment at start and end dates. However, as per study design, only one pair of paws per dog was included.

Malassezia score

At Day 0, inclusion criteria for the study dictated that at least two affected paws both had high *Malassezia* burdens (100% symmetric score three paws). By the end date, for placebo-treated paws, only one of 18 dogs (5.6%) was responder (i.e. had an improvement by ≥ 2 *Malassezia* score categories), whereas for Aptus[®]-treated paws, 17 of 18 dogs (94.4%) were responders, a statistically significant difference (McNemar's $P < 0.0001$). The absolute difference in *Malassezia* responders between Aptus[®] and placebo was $94.4 - 5.6\% = 88.9\%$ (95% confidence interval 74–104%). The magnitude of treatment benefit as represented by the % difference in change between Aptus[®] versus placebo was $(94.4 - 5.6\%) / 5.6\% = 1,600\%$. For 16 of the above 17 responders, Aptus[®] treatment resulted in complete cytological cure (score 0; Figure 1).

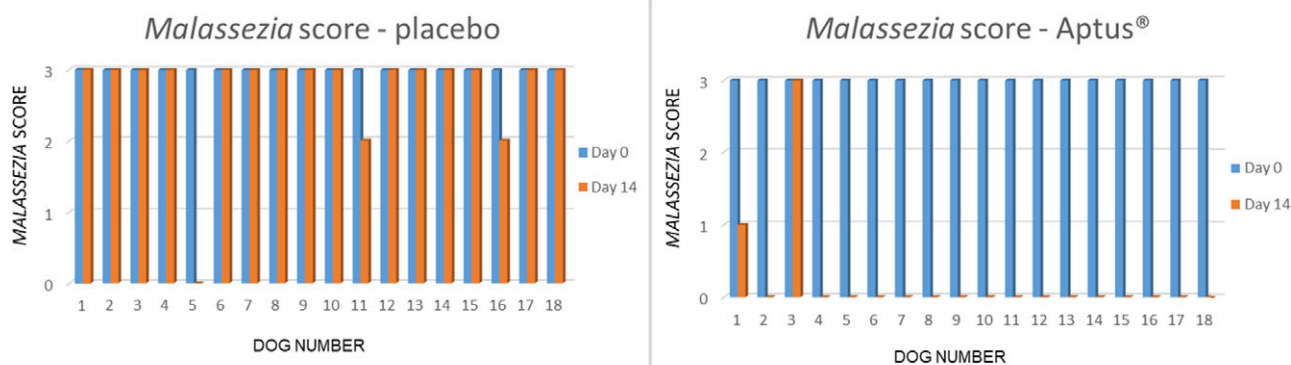


Figure 1. *Malassezia* scores for each treated paw, (placebo versus Aptus®) at start date and end date (day 14).

Scores generated by counting mean number of *Malassezia* organisms (M), from tape strip impressions, for five high power microscopy fields (HPF) using a 0–3-severity scale (0 = none, 1 = 1 to 4 M/HPF, 2 = 5 to 9 M/HPF, 3 = ≥ 10 M/HPF).

Lesion Score

From the inclusion criteria, the minimum Lesion Score a dog could start with was two. The maximum Lesion Score obtainable was 15. At outset (Day 0), Lesion Scores were very similar for placebo- versus Aptus®-treated paws [median (range): 10 (6–15) and 10 (6–15) respectively]. By end date, Lesion Scores were reduced in both placebo- and Aptus®-treated paws [8 (3–13) and 7.5 (2–12), respectively]. Examining the absolute differences between paired paws, Aptus® succeeded in reducing Lesion Scores relative to placebo by a mean difference of 1.3 points (95% confidence interval 0.1–2.6), which was statistically significant (Wilcoxon signed rank $P = 0.041$). The magnitude of treatment benefit as represented by the % difference in change between Aptus® versus placebo was 62%.

PVAS

At outset (Day 0), PVAS was very similar for placebo- versus Aptus®-treated paws [median (range): 4.4 (0–8.4) and 5.1 (0–8.4), respectively]. By end date, PVAS was reported to be reduced in both placebo- and Aptus®-treated paws [1.8 (0–8.2) and 1.8 (0–8.8), respectively]. The Wilcoxon signed rank test showed no statistical difference between Aptus®- and placebo-treated paws with respect to PVAS when analysing change from Day 0 to end date ($P = 0.5723$).

Compliance and ease of use

Our owner compliance assessment showed that 22% of owners found the solution “very easy”, and 78% found the solution “easy” to apply. No adverse effects were reported in any dog.

Discussion

To the best of the authors’ knowledge, this is the first prospective, randomized, blinded and controlled study in dogs assessing the efficacy of the Aptus® product for the treatment of MAD.

The use of azole drugs to reduce *Malassezia* numbers has been extensively reported, although few randomized controlled trials have been conducted.^{2,22–24} A variety of non-azole treatments for MAD have been reported. In an

uncontrolled study, 20 dogs with pododermatitis were treated with a gel containing ethanol, triclosan and glycerine (PawCare®).²⁵ The authors found that *Malassezia* counts estimated immediately following application were significantly decreased with the conclusion that the reduction was attributed to mechanical cleansing and possibly the ingredient triclosan. In a 30 day, single-blinded study, ten dogs received an oral azole plus topical chlorhexidine compared with 20 dogs that were treated with a topical non-azole treatment only (Malacalm® containing mixed herbal oils).²⁶ Both treatment arms resulted in successful reduction in *Malassezia* burden across the body. However, in terms of complete cytological resolution of the interdigital skin, this was achieved in only 40% of the traditional arm versus 55% of Malacalm®-treated cases. These results contrast with the current study, where Aptus® resulted in significant reduction in *Malassezia* burden in 94% of treated paws (and in fact for 89% of Aptus®-treated paws, there was complete cytological resolution). Our first hypothesis; that Aptus® reduces *Malassezia* burden compared to placebo, was demonstrated with statistical significance, along with a very high magnitude of difference between the treatments (1,600%). *Malassezia* burden has been reported to be a good surrogate clinical outcome marker: reducing burden has been correlated with reductions in clinical signs.¹⁶

We assessed the clinical signs of MAD via two direct clinical outcome markers: (i) a clinician assessment of scored dermatological lesions and (ii) an owner assessment of pruritus. Aptus® succeeded in reducing Lesion Scores relative to placebo, and this was statistically significant. The magnitude of treatment benefit was moderate (62% change for the difference in treatments) over the course of this short term trial. However, there was no statistically significant change in pruritus for the Aptus® versus placebo arm. Our second hypothesis, that Aptus® reduces clinical signs of MAD compared to placebo was therefore demonstrated in part. It is possible that the pruritus score may not have achieved significance because of insufficient case numbers, or insufficient trial duration. Note also that owners found it difficult to assess relative pruritus in one paw versus another despite the rigour of maintaining a daily diary.

We had anticipated greater ease in discrimination of noncontiguous dermatological regions (split-paw) than might be achieved with contiguous dermatological areas.

The product used in our study was a leave-on solution and thus application was easy and efficient (compared to the use of traditional shampoos for example). The owner's assessment of ease of use was in agreement with this presumption.

In conclusion, once daily application with the non-azole topical Aptus® Derma Spot on Concentrate™ was effective in reducing yeast counts and signs of *Malassezia*-associated dermatitis in dogs. It was reported to be easy to use by pet owners and no adverse events were observed in this study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Ingredients of the solutions tested in this study.

Table S2. Details of dogs included in this study.

Résumé

Contexte – La prolifération de *Malassezia* sur la peau est une cause fréquente et souvent récidivante de dermatite chez le chien; elle peut être un facteur aggravant de dermatite atopique. Les antifongiques en sont des traitements standards mais il existe des inquiétudes concernant le développement de résistances à certaines espèces de *Malassezia*. Des alternatives sûres, efficaces et faciles d'emploi sont nécessaires.

Objectifs – Déterminer si une solution topique disponible dans le commerce, non-azolée, appliquée sur les espaces interdigités atteints de dermatite à *Malassezia* (MAD) pourrait améliorer le nombre de *Malassezia* et les signes associés sur un essai à court terme (14 jours).

Sujets – Dix-huit chiens atteints de MAD sur au moins deux pattes.

Méthodes – L'étude est prospective, randomisée, en aveugle et contrôlée contre placebo, utilisant un protocole en corps séparé. Les chiens ont été traités une fois par jour avec une solution test sur une patte et par un placebo sur l'autre. Les chiens ont été examinés à jours 0 et 14 $\pm$ 3. Le premier point était le nombre de *Malassezia* estimé par cytologie. Les autres points observés étaient les scores cliniques pour la sévérité des lésions et du prurit et évalués par PVAS (pruritus Visual Analog Scale). La compliance des propriétaires et ses effets secondaires ont également été évalués.

Résultats – Il y a eu une diminution significative du nombre de *Malassezia* et des scores cliniques pour les pattes traitées à la solution test contre le placebo. Aucune différence significative n'a été trouvée pour les PVAS.

Conclusion – L'application quotidienne de la solution test a été efficace dans la diminution de *Malassezia* ainsi que pour l'amélioration des scores cliniques chez les chiens atteints de MAD des pattes. Aucun effet secondaire n'a été rapporté et les propriétaires ont décrit le produit comme facile ou très facile d'utilisation.

Resumen

Introducción – El crecimiento excesivo de la levadura *Malassezia* en la piel es una causa común y a menudo recurrente de dermatitis en perros; puede ser un factor que empeore los signos clínicos de la dermatitis atópica. Los fármacos antifúngicos han sido un tratamiento estándar, pero existe cierta preocupación de que la resistencia pueda estar evolucionando algunas especies de *Malassezia*. Se necesitan alternativas seguras, eficientes y fáciles de usar.

Objetivos – Evaluar si una solución tópica comercial sin azol aplicada a extremidades distales afectadas por dermatitis asociada con *Malassezia* (MAD), podría disminuir el número de organismos de *Malassezia* y los signos asociados en una prueba a corto plazo (14 días).

Animales – Dieciocho perros con MAD afectando al menos dos extremidades distales.

Métodos – El diseño del estudio fue prospectivo, al azar, ciego y controlado con placebo, utilizando un protocolo de cuerpo dividido. Los perros se trataron una vez al día con la solución de prueba en una extremidad y placebo en la otra. Los perros fueron examinados en los días 0 y 14 $\pm$ 3. El punto final primario fue el número de organismos de *Malassezia* evaluados citológicamente. Los criterios de valoración secundarios fueron las valoraciones clínicas de la gravedad de las lesiones y el prurito evaluado mediante una Escala Análoga Visual (PVAS) de prurito. Se evaluaron el seguimiento del protocolo por el propietario y los efectos adversos.

Resultados – Hubo una reducción estadísticamente significativa en el número de organismos de *Malassezia* y las valoraciones clínicas de las extremidades tratadas con la solución de prueba versus placebo. No se encontró diferencia estadística en PVAS.

Conclusión – la aplicación tópica diaria de la solución de prueba fue eficaz en la reducción de la carga de *Malassezia*, así como la mejora de las puntuaciones clínicas en perros con MAD de las extremidades. No se observaron efectos adversos y los propietarios describieron el producto como "fácil" o "muy fácil" de usar.

Zusammenfassung

Hintergrund – Eine Überwucherung durch *Malassezien* auf der Haut ist eine häufige und oft wiederkehrende Ursache für eine Dermatitis bei Hunden; es kann sich dabei um einen Faktor handeln, der die atopische Dermatitis schlimmer macht. Antimykotika sind eine Standardtherapie, aber es bestehen Bedenken, dass sich im Spektrum der *Malassezien* Spezies Resistenzen entwickeln könnten. Sichere, wirksame und leicht anwendbare Alternativen sind gefragt.

Ziele – Eine Untersuchung, ob eine kommerziell verfügbare topische Nicht-Azol-Lösung, die auf Pfoten mit *Malasseziendermatitis* (MAD) aufgetragen wird, die Anzahl der *Malassezien* verändern und entsprechende Zeichen über einen kurzem Zeitraum (14 Tage) beeinflussen kann.

Tiere – Achtzehn Hunde mit MAD, die zumindest zwei Pfoten betraf.

Methoden – Es handelte sich bei dieser Studie um eine prospektive, randomisierte, blinde und Placebo-kontrollierte Studie, die ein Split-Body Protokoll verwendete. Die Hunde wurden einmal täglich mit der Testlösung an einer Pfote und mit Placebo an der anderen behandelt. Die Hunde wurden an den Tagen 0 und 14 $\pm$ 3 untersucht. Der primäre Endpunkt erfasste *Malassezien*zahlen zytologisch. Sekundäre Endpunkte waren klinische Beurteilungswerte für die Schwere der Läsionen und des Pruritus mittels Pruritus Visual Analog Scale (PVAS). Es wurden die BesitzerInnen Compliance und die Nebenwirkungen erfasst.

Ergebnisse – Es bestand eine signifikante Reduzierung der *Malassezien*zahlen und der klinischen Bewertung für die mit der Testlösung behandelten Pfoten im Vergleich zu Placebo. Es wurde kein statistisch signifikanter Unterschied in Bezug auf die PVAS gefunden.

Schlussfolgerung – Eine tägliche Applikation der Testlösung war sowohl für eine Reduktion der *Malassezien*belastung, sowie für eine Besserung der klinischen Werte der Hunde mit MAD der Pfoten hilfreich. Es

wurde von keinen Nebenwirkungen berichtet und die BesitzerInnen beschrieben das Produkt entweder als „leicht“ oder „sehr leicht“ anwendbar.

要約

背景 – 皮膚におけるマラセチア酵母の過剰増殖は、犬の皮膚炎の原因として一般的に認められ、しばしば再発を繰り返す。アトピー性皮膚炎の悪化要因となる可能性もある。抗真菌薬は一般的な治療法であるが、マラセチアが耐性を獲得する可能性が懸念される。安全で効率的、かつ、使用しやすい治療選択肢が必要とされている。

目的 – マラセチア関連皮膚炎(MAD)に罹患した肢端に、市販の局所非アゾール溶液を塗布することによって、マラセチア数およびそれに関連した臨床徴候が改善されるかどうかを短期間の試験(14日間)にて評価すること。

供与動物 – 少なくとも2つの肢端がMADに罹患した18頭の犬。

方法 – 本試験は、スプリットボディプロトコルを用いた前向き無作為化盲検プラセボ対照試験である。犬は1日1回、一方の肢端に試験溶液を、もう一方の肢端にはプラセボが塗布され、0日目および14±3日目に評価された。主要エンドポイントは細胞学的に評価したマラセチア数とした。二次のエンドポイントは、臨床スコアで評価された病変重症度および掻痒の視覚アナログスケール(PVAS)によって評価された痒みとした。飼い主のコンプライアンスと有害事象も評価された。

結果 – 試験溶液を塗布された肢端はプラセボと比較して、マラセチア数および臨床スコアが統計的に有意に減少した。PVASに統計的な有意差は認められなかった。

結論および臨床的な重要性 – 試験溶液の連日局所塗布は、肢端のMADを有する犬のマラセチア量を軽減し、臨床スコアを改善する効果があった。本治療による副作用は報告されておらず、飼い主による本製品の使い易さに対する評価は「簡単」または「非常に簡単」であった。

摘要

背景 – 马拉色菌过度增殖是犬常见皮肤疾病和皮炎复发的常见原因;此外,也是加重异位性皮炎的因素。标准的治疗方案包括使用抗真菌药物,但目前担心,多种马拉色菌可能产生耐药性。因此,需要安全、有效和易于使用的替代方案。

目的 – 发生了马拉色菌皮炎(MAD)的脚爪外用市售的非唑类溶液,评估其是否可以在短期(14天)试验中减少马拉色菌数量和改善相关症状。

动物 – 18只患犬,每只犬至少有两只脚爪发生MAD。

方法 – 本研究设计具有前瞻性、随机、双盲和安慰剂对照特性,并使用分体试验方案。在患犬的一只脚爪上使用试验溶液,每日一次,而在另一只脚爪上使用安慰剂。在第0天和第14±3天对犬进行检测,主要检测指标是通过细胞学评估马拉色菌数量。次要检测指标是对病变的严重程度进行临床评分,并使用瘙痒视觉模拟量表(PVAS)对瘙痒进行评估。同时评估宠主的服从性和不良反应。

结果 – 与安慰剂相比,使用试验溶液治疗的脚爪,其马拉色菌数量和临床评分都显著降低。PVAS上未见统计学差异。

总结 – 每日外部使用试验溶液能有效降低马拉色菌载量,也能改善MAD患犬爪部的临床评分。未见不良反应报道,宠主评价该产品使用“方便”或“非常方便”。

Resumo

Contexto – O supercrescimento de leveduras do gênero *Malassezia* na pele é uma causa comum de dermatite, frequentemente recorrente em cães, que pode ser um fator exacerbador de dermatite atópica. Drogas antifúngicas tem sido o tratamento de escolha, entretanto, há o receio de que a resistência possa estar sendo desenvolvida em um espectro de espécies de *Malassezia*. São necessárias alternativas de tratamento seguras, eficazes e de fácil utilização.

Objetivos – Avaliar se uma solução comercial tópica contendo um ativo não azólico aplicada nas patas afetadas por dermatite associada à *Malassezia* (MAD), poderia reduzir o número de *Malassezia* e os sinais clínicos associados em um experimento de curta duração (14 dias).

Animais – Dezoito cães com MAD afetando ao menos duas patas.

Métodos – O delineamento do estudo foi prospectivo, randomizado, placebo-controle cego, utilizando um protocolo de divisão do corpo. Os cães foram tratados uma vez ao dia com a solução teste em uma pata e placebo na outra, e foram examinados nos dias 0 e 14 ± 3. O parâmetro primário analisado foi a contagem de *Malassezia* por citologia e os secundários foram os escores clínicos para severidade de lesões e prurido avaliado pela escala analógica visual de prurido (PVAS). Foram avaliados também o comprometimento dos proprietários e os sinais adversos.

Resultados – Houve uma redução significativa na contagem de *Malassezia* e nos escores clínicos para as patas tratadas com a solução teste, quando comparado ao placebo. Não houve diferença significativa no PVAS.

Conclusões – A aplicação diária da solução teste foi eficaz na redução da quantidade de *Malassezia*, bem como na melhora dos escores de sinais clínicos em cães com MAD nas patas. Não foram observados efeitos adversos e os proprietários descreveram o produto como “fácil” ou “muito fácil” de ser aplicado.

Supporting information

Table S1. Ingredients of the solutions tested in this study.

Ingredients	Placebo	Aptus®
Aqua	↓	↓
Sodium Lactate	↓	↓
Polyquaternium 10	↓	↓
Zinc sulfate		↓
Urea		↓
Laureth-9		↓
Panthenol		↓
Butylene glycol		↓
Propanediol		↓
Glycerin		↓
Caprylyl glycol		↓
Ethyl lauroyl arginate		↓
Sodium Acetate		↓

Table S2. Details of dogs included in this study.

No.	Breed	Age	Gender	Age at time of first dermatological signs	Concurrent regions with dermatological signs at time of inclusion	Medication at time of inclusion	Diagnosed with atopic dermatitis
1	Welsh springer spaniel	6	FI	10 months	Ears, lips	ASIT 0.5 ml every other week Ciclosporin 4.7 mg/kg once daily	↓*
2	German shepherd	5	MN	1 year	Ears	-	↓
3	Softcoated Wheaten terrier	8	FI	4 months	Ears, perianal area	-	
4	English toy terrier	1	FI	6 months	Lips, ventral/lateral	-	↓

					abdomen/thorax, legs		
5	American Staffordshire terrier	1	MI	9 months	Axilla, groin, chin, legs	-	↓
6	French bulldog	6	MI	5 years	Ears	-	
7	Cocker spaniel	3	FI	4 months	Ears, perianal area	-	↓
8	German shepherd	8	FI	10 months	Ears, perianal area, lips, ventral abdomen/thorax	-	
9	French bulldog	4	FI	1 year	Perivulvar and perianal area	-	↓
10	Douge De Bordeaux	1	MI	3 months	Ears, lateral abdomen/thorax	-	
11	Mongrel	6	FI	1 year	Axilla, groin	-	↓
12	Bracco Italiano	7	FI	10 months	Chin, groin, ventral abdomen,	-	
13	Flat-coated retriever	13	MI	4 months	Ears	-	
14	Jack Russell terrier	6	FI	1.5 years	Ears		
15	Bedlington terrier	6	MI	4 months	Ears, ventral/lateral abdomen/thorax		↓
16	Staffordshire bull terrier	3	FI	10 months	Ears		
17	Miniature schnauzer	4	FI	10 months	Ears		
18	Douge de Bordeaux	3	MI	1.5 years	-		

Abbreviations: MI, male intact; MN, male neutered; FI, female intact; ASIT, allergen specific immunotherapy

* Dog 1 was diagnosed with atopy prior to study (and was thus on treatments for atopy at the time of inclusion). Other dogs with atopic dermatitis were diagnosed immediately after the conclusion of this study.