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Performance of Chlorhexidine and Ophytrium-Containing Shampoo and Mousse in Dogs with Cutaneous Yeast Imbalance

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Abstract

Superficial microbiota disturbances are common in dogs, with *Malassezia pachydermatis* yeasts often being involved. Topical products are recommended as a first-line management strategy; however, literature regarding specific topical protocols is still scarce. This study aimed to evaluate the performance of a protocol combining chlorhexidine and Ophytrium-containing products in reducing yeast counts in dogs with microscopic evidence of yeast imbalance and dermatological signs. Dogs were shampooed on Day 0 (D0) and then received eight mousse applications at two to three-day intervals. Followed-up parameters on D0, Day 7 (D7), and Day 21 (D21) were microscopic yeast counting, pruritus, and skin irritation scores. At D21, veterinarians' and owners' overall assessments were collected. *Malassezia pachydermatis* counts decreased at D7 (57.3% vs D0, $p < 0.05$) and at D21 (79.9% vs D0, $p < 0.05$). Mean pruritus also decreased at D7 vs D0 (58.3%, $p < 0.05$) and at D21 vs D0 (75%, $p < 0.001$). Efficacy was described by all veterinarians from good to excellent, and the proposed procedures were evaluated as efficient and practical by the owners in 91.7% of the cases. The protocol using chlorhexidine and Ophytrium shampoo and mousse could be a useful option in dogs presenting imbalances in yeast cutaneous microbiota.

Keywords

Chlorhexidine, Ophytrium, *Malassezia pachydermatis*, Topical, Shampoo, Mousse, Dog

1. Introduction

Microbial diversity is critical for maintaining a healthy balance in the high-complexity skin ecosystem. However, environmental condition changes cause frequent commensal flora overgrowth in the canine skin. *Malassezia pachydermatis*, a lipid-dependent yeast that cannot synthesize long-chained fatty acids de novo [1], is the dominant eukaryotic resident yeast that causes secondary skin disorders in dogs [2] [3]. Guidelines privilege topicals as the first management choice [4], stating systemic antimicrobials are often unnecessary and may cause adverse effects. Their misuse may also favor and spread antimicrobial resistance [4]-[6]. Topical formulations offer a local approach, delivering ingredients directly to the stratum corneum, where yeast overgrowth develops. Among the various agents used in dogs, the antiseptic molecule chlorhexidine has the most substantial evidence supporting its antibacterial [2] [3] [7] and yeasticidal [8]-[11] action at various concentrations. A noteworthy add-on to this topical is Ophytrium, a purified isolated natural extract from *Ophiopogon japonicus*, the ornamental plant commonly known as mondo grass. Ophytrium was selected for its high tolerance and threefold action on the skin barrier. Elements such as tight junctions, filaggrin, Natural Moisturizing Factor (NMF) content, and ceramides were restored in a model of the reconstructed epidermis [12]. Ophytrium effectively prevented *in vitro* adhesion and formation of biofilm by *Staphylococcus aureus, Staphylococcus pseudintermedius, Pseudomonas aeruginosa,* and *Streptococcus canis*. It also controlled the secretion of pro-inflammatory cytokines, specifically TSLP, IL-8, and IL-13 [12]. Shampooing has traditionally been the most common way to apply antiseptic topicals to the skin, as it also efficiently removes dirt and debris. However, shampooing is time-consuming, and can eventually increase the burden for pet caregivers [13]. On the other hand, moussing by petting the dog is considered a pleasant act, reinforces the human-pet bond, is quickly performed, and does not imply the need for rinsing [14]. The combined use of the two galenic forms, shampoo and leave-on mousse containing chlorhexidine digluconate and Ophytrium as active ingredients, was considered a convenient approach to control imbalances in yeast cutaneous population, being the hypothesis that it would reduce the yeast charge on the skin by at least 70% in three weeks.

2. Materials and Methods

Inclusion and Non-Inclusion Criteria

Client-owned dogs of any sex, age, and breed, considered healthy based on clinical examination and presenting signs of skin dysbiosis where predominant yeast

imbalance was confirmed by cytology, were recruited from veterinary clinics in France. Pregnant or lactating dogs, as well as those suffering deep pyoderma, parasitic infestation, or other diseases whose management could potentially interfere with the study's objective and results, were not included. Systemic antifungal medication was strictly forbidden; other concomitant treatments were to be recorded.

Products and Application Protocol

Commercially available shampoo and mousse containing Ophytrium and chlorhexidine digluconate 3% (DOUXO® S3 PYO Shampoo, DOUXO® S3 PYO Mousse, Ceva Animal Health, Libourne, France) were used for this study. The application protocol consisted of one shampoo and two mousse applications during the first week, followed by three mousse applications per week for the following two weeks (Figure 1).

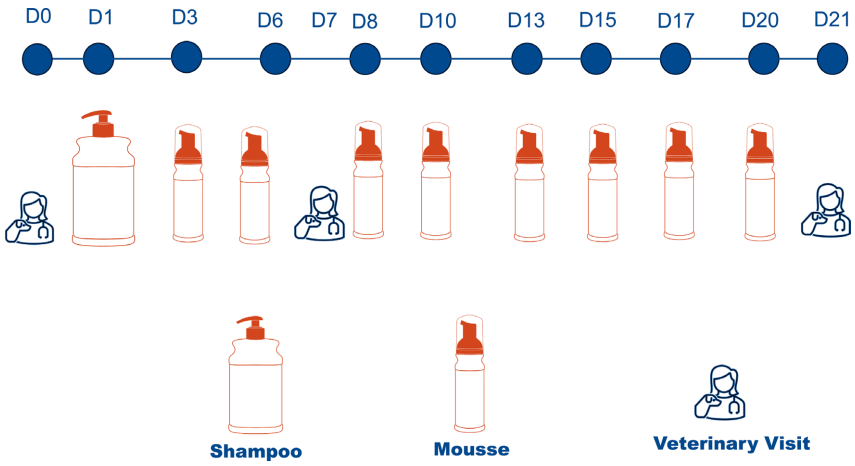

Figure 1. Study protocol.

An interval of 48 to 72 hours was maintained between applications. Depending on the dog's size, the owner shampooed the dog with 5 to 15 ml of the shampoo and warm water, rubbing the fur and skin and leaving in contact with the dog's skin for 10 minutes, before rinsing and removing the excess water with a towel. Temperature permitting, the dog was allowed to air dry. For mousse applications, owners were instructed to brush the coat against the direction of hair growth thoroughly and then dispense the mousse (1 to 2 pumps per kg depending on the hair length and density); gently massaging to allow it to reach the skin.

Study Schedule and Evaluated Parameters

Three visits were scheduled: D0, or inclusion visit; D7, a follow-up visit; and D21, the end-of-study visit. At each one, dogs underwent a physical and dermatological examination. Cytology samples from the most affected site were obtained using clear cellophane-type tape, pressing the sticky portion directly onto the area, and stained using the in-house modified Giemsa stain (RAL 555, Centravet, Dinan, France) technique. The five specific areas for counting *Malassezia pachydermatis* elements per Oil Immersion fields (OIF) were chosen in the densest stained area of each slide and examined as shown in Figure 2.

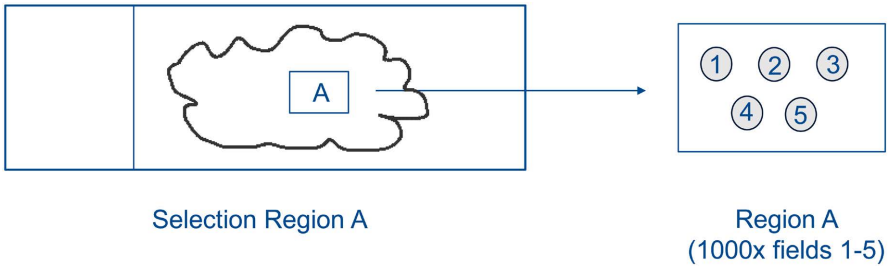

Figure 2. Area selection for yeast counting.

Cytological Parameters

- Semi-quantitative cytology *M. pachydermatis* score [15]

An evaluation was performed by the veterinarian to semi-quantitatively assess (10× and 40× magnification) the presence and number of yeasts (0 = no yeast, 1+ occasional yeast, 2+ yeasts present in low numbers but detectable rapidly without difficulties, 3+ yeasts present in more significant numbers and noticeable rapidly without difficulties, 4+ massive amounts of yeasts present and detectable rapidly without problems).

- Number of *M. pachydermatis* organisms per OIF [16]

After identifying each dog's most affected location (A in **Figure 2**), a yeast count was performed by the veterinarian under oil immersion using a 100× objective in five distinct, non-overlapping areas of the slide (Region A (1000× fields 1 - 5, **Figure 2** [17]. The mean count was then used and reported as the representative value for tracking the progression for *M. pachydermatis* counts.

Each case was classified as "success" or "failure" based on its count at D21 achieving or not a mean number of yeasts ≤ 1 per OIF [16].

Clinical Parameters

- Pruritus score [18]

The owner assessed the pruritus level using a Pruritus Visual Analogue scale (PVAS). This consisted of a 10-cm line on which the pet owner put a mark to indicate the level of itching of his pet. The pruritus score was collected by measuring the distance between the beginning of the line (corresponding to a normal dog) and the owner's mark.

- Irritation scores *[19]*

Those were measured by the veterinarian in terms of severity and extent.

-Severity ranging from 0 to 4: 0, absence of irritation; 1, mild; 2, moderate; 3, severe; 4, very severe.

-Extent ranging from 0 - 5: 0, absent; 1: only one or two lesions; 2, <5% affected body surface, 3, 5% - 50% affected body surface; 4, >50% affected body surface.

Owners' and Veterinarians' Questionnaires

At D21, pet owners' and veterinarians' evaluations were collected. Veterinarians were asked to appraise the clinical evolution vs D0 and assess the product efficacy, ranging from 0 (very poor) to 5 (excellent) and the need for further management for the initial yeast imbalance. Owners were asked to assess the performance and practicality of the protocol and the likeliness of the product's organoleptic

characteristics using a four-point scale: from 0 (disagree); 1 (partially disagree), 2 (partially agree), 3 (agree), to 4 (totally agree). They were also asked to evaluate the overall response to the product from 0 (no response) to 5 (excellent response).

Adverse Events

If an adverse event occurred between the visits or was reported by the owner since the last visit, an Adverse Event Form was filled in. The severity and causal relationship of the adverse event were evaluated, and the outcome was recorded.

Data Analysis

Data were analyzed using SAS 9.4 (SAS Institute). Efficacy data were summarized per visit, and the normality of the data was graphically checked.

The mean number of yeasts per OIF and the pruritus score were summarized using the mean with standard deviation. The median ± Q1 - Q3 was used for the figures and the statistical tests. A Wilcoxon paired test was used to compare the absolute change from baseline.

A p-value < 0.05 was considered significant.

Owners' and veterinarians' satisfaction were detailed as categorical variables by frequency distributions and number of responses.

3. Results

Demographic Characteristics of the Population

Among twenty recruited patients (safety population = 20), twelve dogs finished the study and were included in the analysis (efficacy population = 12, Table 1).

Table 1. Demographic characteristics of the population.

Parameter		Statistics	Total (n = 12)
Age	Years	Mean (SD)	6.1 (3.7)
Bodyweight	Kg	Mean (SD)	12.9 (8.4)
Breed	Purebred	% (n)	83.3% (10)
	Crossbred	% (n)	16.7% (2)
Sex	Male	% (n)	33.3% (4)
	Female	% (n)	66.7% (8)
Neutered	No	% (n)	25.0% (3)
	Yes	% (n)	75.0% (9)
Coat length	Short	% (n)	25.0% (3)
	Long	% (n)	75% (9)
Dense coat	No	% (n)	66.7% (8)
	Yes	% (n)	33.3% (4)
Housing	Outdoor only	% (n)	0.0% (0)
	Indoor only	% (n)	50% (6)
	Indoor/Outdoor	% (n)	50% (6)

Three dogs of the efficacy population were receiving concomitant treatments at inclusion: an ear drop solution containing florfenicol, terbinafine hydrochloride and mometasone furoate; a dog chew containing fluralaner; and chlorhexidine wipes for local management of vulvar intertrigo. Female dogs represented 66.7% of the efficacy population, with a mean weight of 12.9 ± 8.4 kg and a mean age of 6.1 ± 3.7 years. More than half (58.3%) were long-haired and not dense coated.

Cytological Parameters

- Semi-quantitative *M. pachydermatis* score

The semiquantitative cytology score decreased by 77.4% (3.1 at D0 vs 0.7 at D21). Missing data did not permit the statistical analysis of this parameter at D7.

- Number of *M. pachydermatis* in the most affected site per OIF

Table 2 summarizes individual data.

Since the data were not normally distributed, the evolution of the median was used for statistical assessment instead of the mean (Figure 3(a) and Figure 3(b)). After 7 days, a reduction of 57.3% (±SD) of the mean *Malassezia* count was observed, and the reduction reached 79.9% (± SD 44.1) after 21 days Median of the reduction was −96.6% (−100% (−100,0; −83.2) (see Table 3).

Significant reduction in median is observed at D7 ($p < 0.05$) and at D21 ($p < 0.01$). The number of yeasts decreased significantly from 9.5 (median Q1; Q3 8.2; 13.8) per OIF on D0 to 0.5 (0.0; 2.4) on D7 ($p = 0.0342$) to 0.0 (0.0; 1.4) on D21 ($p = 0.0015$).

Table 2. Summary of the mean *Malassezia* count per OIF.

Animal ID	*Malassezia* D0	*Malassezia* D7	*Malassezia* D21
02_05	17	0	0.2
02_06	18	0	0
02_07	8	0	0.6
02_08	8.4	0	0
04_01	14.6	1	0
05_01	5.2	2.2	3
05_02	13	0	0
05_04	8.4	2.6	2.2
05_05	7.4	0	0
06_02	10	4	0
10_01	9	34	13.4
11_01	12.2	1.8	0
Mean *Malassezia* per OIF	**10.9**	**3.8 (−57.3%)**	**1.64 (−79.9%)**
Median *Malassezia* per OIF	**9.5**	**0.5 (−96.6%)**	**0.0 (−100%)**
≤1 *Malassezia* OIF	**0%**	**58.30%**	**75.0%**

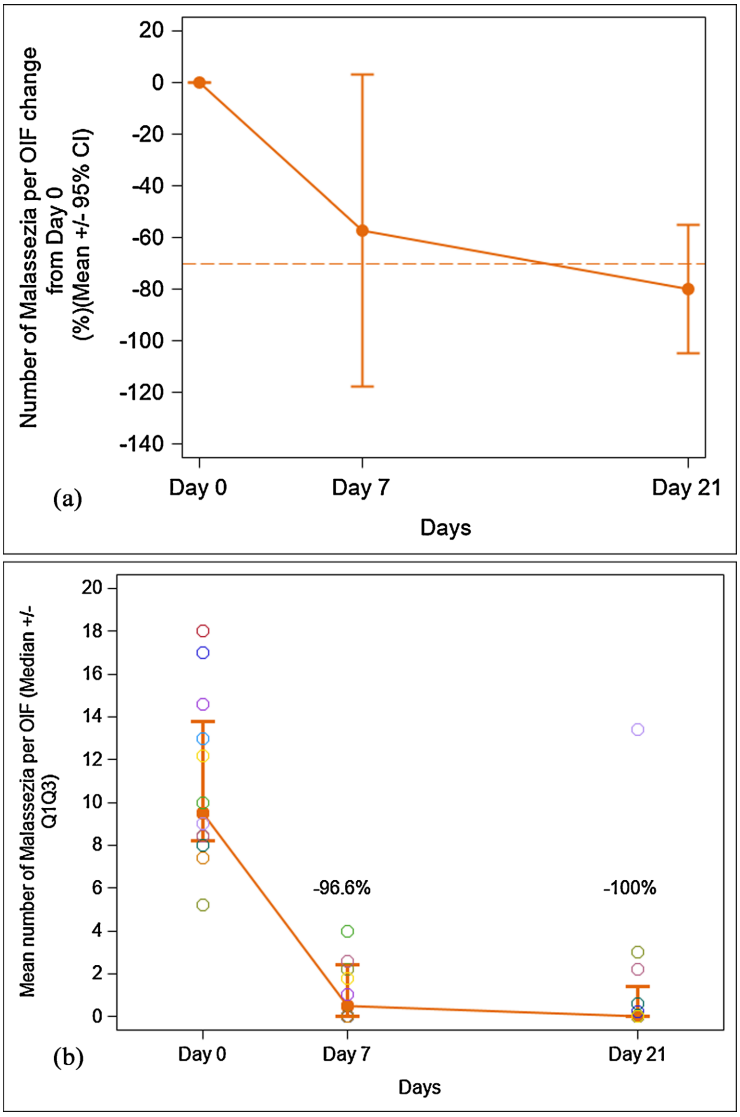

Figure 3. (a) Evolution of mean number of *Malassezia* per OIF evolution (Efficacy population, n = 12). (b) Evolution of mean number of *Malassezia* per OIF evolution (Efficacy population, n = 12).

Table 3. Summary of the reduction *Malassezia* count per OIF.

Parameter	Statistics	Day 0 (n = 12)	Day 7 (n = 12)	D21 (n = 12)
Mean number of *Malassezia* per OIF	Mean (SD)	10.9 (4.0)	3.8 (9.6)	1.6 (3.8)
	Median (Q1; Q3)	9.5 (8.2; 13.8)	0.5 (0.0; 2.4)	0.0 (0.0; 1.4)
Mean number *Malassezia* per OIF change vs D0 (%)	Mean (SD)		−57.3 (106.8)	−79.9 (44.1)
	Median (Q1; Q3)		−96.6 (100.0; 64.5)	−100.0 (−100.0; −83.2)

At the end of the study, 9/12 (78.5%) dogs had achieved counts ≤ 1 per OIF and were considered back to normal.

Clinical Parameters

- Pruritus score.

Table 4 shows individual data.

At inclusion, PVAS was 5.0 (median ± 2.5; 5.5 Q1Q3). Mean PVAS scores decreased to 1.4 (±1.0; 4.1 Q1Q3) on D7 ($p = 0.002$) and then to 0.9 (±0.1; 2.3 Q1Q3) on D 21 ($p < 0.001$). The mean PVAS change between D0 and D21 was −71.9% (Figure 4 in the text). Nine dogs out of twelve (75.0%) reached PVAS < 2 on D21. The evolution of the PVAS score is detailed in Table 5.

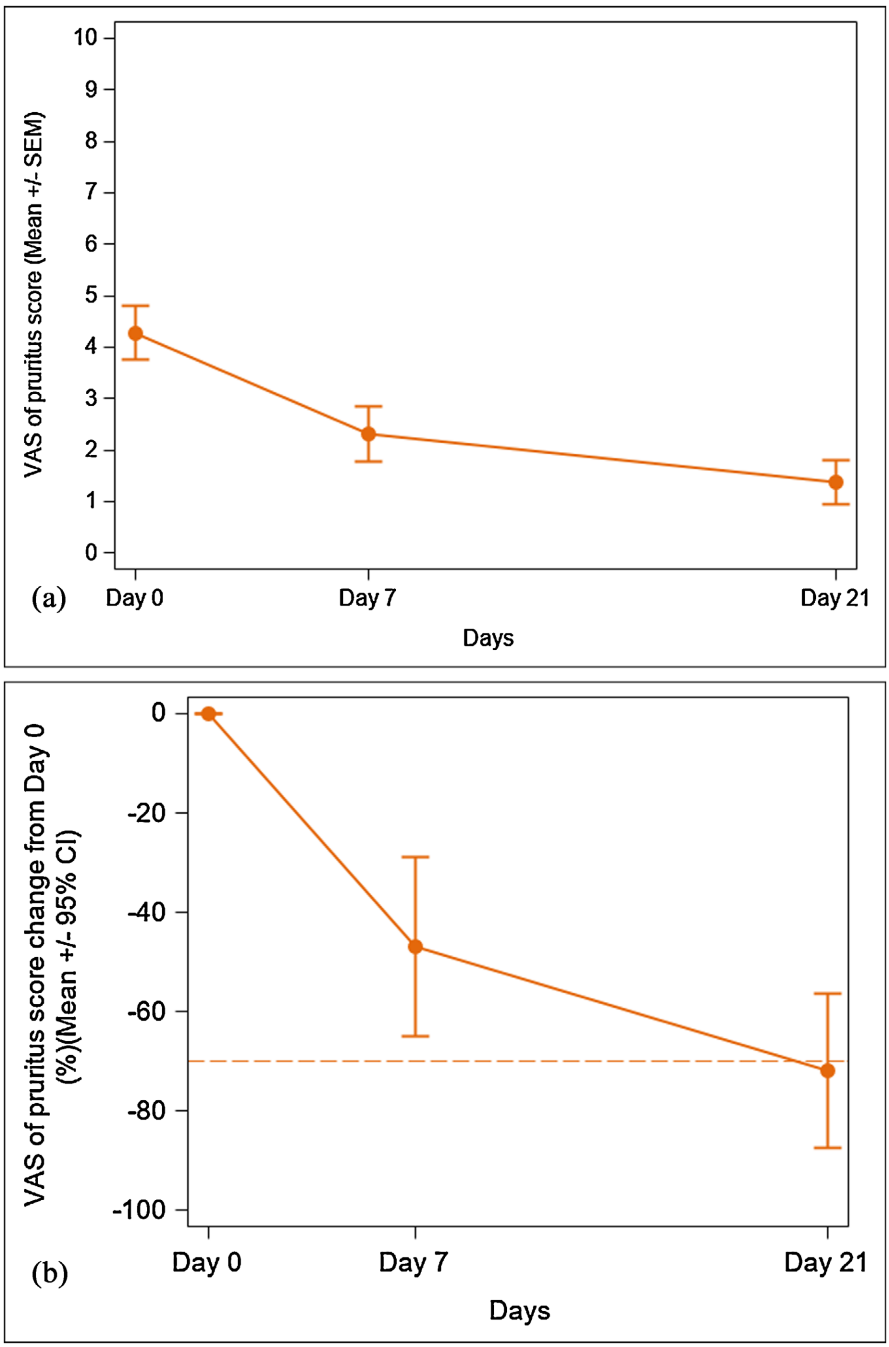

Figure 4. (a) Evolution of mean VAS scores (Mean ± SD, Efficacy population, n = 12); (b) Evolution of mean VAS scores (Median ± Q1Q3, Efficacy population, n = 12).

Table 4. Summary of the mean pruritus scores.

Animal ID	Pruritus D0	Pruritus D7	Pruritus D21
02_05	2	1.4	0.1
02_06	3	1.5	1.5
02_07	7.3	4	3.8
02_08	6	4.1	1.7
04_01	2.7	0	0
05_01	5.3	4.8	2.8
05_02	1.9	1	0.3
05_04	4.8	Missing data	4.1
05_05	5.2	1.1	0.1
06_02	5.2	5.2	1.8
10_01	2.2	1.3	0.2
11_01	5.7	1	0.1
Mean	**4.3**	**2.3**	**1.4**
Median	**5**	**1.4**	**0.9**
VAS pruritus < 2	**8%**	**58.3%**	**75%**

Table 5. PVAS score evolution (Efficacy population, n = 12).

Parameter	Statistics	Day 0 (N = 12)	Day 7 (N = 12)	D21 (N = 12)
VAS score	Mean (SD)	4.3 (1.8)	2.3 (1.8)	1.4 (1.5)
	Median (Q1; Q3)	5.0 (2.5; 5.5)	1.4 (1.0; 4.1)	0.9 (0.1; 2.3)
VAS score change vs D0 (%)	Mean (SD)		−46.9 (30.5)	−71.9 (27.3)
	Median (Q1; Q3)		−45.2 (−78.80; 30.0)	−77.9 (−96.5; −49)

- Irritation scores.

The severity and extent scores decreased from D0, with 75% of the cases with no or mild irritation and 58.3% with absence or only one or two areas affected at D21. Individual values are shown in Table 6.

Owners' and Veterinarians' Questionnaires

In 9 out of 12 (75.0%) cases, the veterinarian considered improvement good or excellent, being satisfactory in two cases (16.7%).

Overall, pet owners considered the response to the product application as "good" or "excellent "in 10 out of 12 (83.3 %) cases. Two owners considered the response "fair." All of them appreciated the performance and practicality of the protocol and the products' sensory characteristics (fragrance and texture).

Adverse Events

Five adverse events that could be related to the products' application were

Table 6. Summary of the irritation scores.

Parameter	Scores	D0 % (n)	D7 % (n)	D21 %(n)
Skin irritation severity	None	0.0% (0)	8.3% (1)	33.3% (4)
	Mild	8.3% (1)	66.7% (1)	41.7% (5)
	Moderate	66.7% (8)	16.7% (2)	25.05% (3)
	Severe	25.0% (3)	8.3% (1)	0.0% (0)
	Very severe	0.0% (0)	0.0% (0)	0.0% (0)
Skin irritation extent	Absent	0.0% (0)	8.3% (1)	25.0% (3)
	one or two areas affected	25.0% (3)	25.0% (3)	33.3% (2)
	<5% of body surface affected	41.7% (5)	41.7% (5)	16.7% (2)
	5% - 50% of body surface affected	33.3% (4)	25.0% (3)	25.0% (3)
	>50% of body surface affected	0.0% (0)	0.0% (0)	0.0% (0)

noted in the safety population, n = 20.

-One dog displayed sticky hair due to excessive mousse application.

-Three dogs showed increased pruritus after applying the product, with spontaneous recovery.

-One dog developed a localized urticarial reaction on D2, spontaneously ending on Day 5 without treatment and without stopping the products application.

Two other adverse events were reported, although they could not be linked to the protocol or the products used:

-One dog showed tiredness on Day 2 (D2).

-One dog developed *Bacillus* sp otitis on D21.

4. Discussion

Previous studies performed with similar products in small cutaneous areas [17] showed that, since yeasts are located on the *stratum corneum,* the external layer of the epidermis, topical management may be sufficient to control overgrowth. The results of this field study provide further *in vivo* evidence of the effectiveness of this local approach against yeast imbalance of cutaneous microbiota without using azole derivatives. The good tolerance of topical chlorhexidine products is indeed an advantage over systemic treatment such as ketoconazole, for which adverse reactions such as vomiting, anorexia, lethargy, and diarrhoea, are common [20]. The scientific consensus, supported by various *in vitro* studies, is that *M. pachydermatis* is typically susceptible to chlorhexidine concentrations between 2 and 4% [3] [21] [22]. No reduced susceptibility to this molecule has been reported so far [1] [23], although it is currently under evaluation [23]. On the other hand, chlorhexidine has been identified as having some undesirable side effects, ranging from mild allergy contact reactions to fixed eruption drug reactions and anaphylaxis [22] [23]. A previous study had shown good shampoo and mousse tolerance

[24]. In the present work, there were events for which a relationship with the tested protocol was evaluated as "possible". Although they resolved spontaneously without treatment, this protocol should be considered for yeast management only after dysbiosis cytological confirmation [1] [9].

Concerns have also been raised in humans about fixed drug reactions when chlorhexidine has been used as a mouthwash [25], but no such effect was seen in the study.

The protocol performed well in improving cytology outcome; nine out of twelve dogs achieved cytological return to normality status after 21 days of management. Cytological success threshold was set at one organism per OIF, as it is always possible to see a very low number of yeasts in a normal dog [26] [27]. At each visit, a standardized sampling and counting cytological method was used to evaluate *M. pachydermatis* counts, which is quick and easy with reasonable specificity [28] [29].

Typical manifestations in superficial yeast imbalance are malodour, erythema, alopecia, greasy exudation, scaling, and, in chronic cases, lichenification and hyperpigmentation. Pruritus is usually present and can range from mild to severe. Yeast hypersensitivity contributing to pruritus has also been reported [1] [30]. It was consequently selected an essential clinical parameter, and the owner evaluated it using a validated visual analogue scale [31]. Pruritus scores rapidly improved in the study, with owners reporting a mean decrease of 71.5% after 21 days of application. Those results are consistent with the 72.2% reduction of pruritus reported by Maynard *et al.* [9] after 2 to 6 weeks of treatment with 3% chlorhexidine shampoo treatment protocol and the 80.0% reported in the same study after twice weekly for as long as needed up to 6 weeks 2% miconazole −2% chlorhexidine shampooing. Irritation severity and extent were also evaluated in the present study to follow the overall development of the *M. pachydermatis* overgrowth, with 88% of the cases returning to normal or having only mild irritation at D21. This encouraging reduction can be linked to the antiseptic effect of chlorhexidine digluconate at 3% and the soothing properties of Ophytrium, which has been shown to decrease the production of pro-inflammatory cytokines *in vitro* [1].

Clinical and cytological evolutions concur in this trial. However, some publications report that clinical cure may precede the cytological one [29] [32] while some other authors declare the opposite [9]. It has been recommended to continue topical application until seven days beyond the clinical resolution of all signs [26] [33].

There is no control group in this study, which, together with the reduced sample size, constitutes its main limitation. On similar clinical conditions, studies with a placebo or a vehicle group indicated very few spontaneous resolutions in the placebo group compared with the treated group [34]-[36], even with shampoos where a mechanical effect on microbial count is expected. Therefore, as spontaneous reduction of the yeast load is likely to be low if left untreated, providing a placebo to some dogs was considered arguable. Dogs were their own controls, and improvement was judged versus their basal values. However, a group following a

different protocol could have been considered to support the efficacy of the one chosen.

Besides being overall efficient, this protocol was considered to be practical, which was much appreciated by most owners. Regular shampooing is reported to be fastidious and time-consuming, as it involves letting the shampoo contact the dog's skin, and then rinse and dry [13]. The fragrance was also positively evaluated and helped compliance, as some owners find the chlorhexidine smell unpleasant. In addition, no difference was observed depending on the coat's length or density, including some of the most common atopic dermatitis predisposed breeds [9], which suggests that this protocol can be used regardless of the fur's length and density.

5. Conclusion

Topical management with chlorhexidine and Ophytrium-based shampoo and mousse was successful in this trial. Those results are in concordance with the guidelines that privilege topical products versus the use of azole-derivative systemic products. In addition, using a mousse formula enhances owners' compliance, as massage applications are pleasant for the pet and reinforce the owner-pet bond while allowing the actives more in-contact time. The product's scent successfully overcomes the unpleasant chlorhexidine odour issue, which is probably an added value for adequate use and adherence to the protocol. To conclude, the protocol combining applications of chlorhexidine and Ophytrium containing shampoo and mousse was shown to be well tolerated and resulted in a quick and significant improvement of pruritus and cytological features in dogs with yeast cutaneous microbiota imbalance.

Author Contributions

EO, MG, EC, GP, LS, TB, and XdJ contributed to the study's conception and design. AC, NS, and MA wrote the final manuscript. EI, FP, PF, CP, J.F. R and VV recruited dogs for the study and performed clinical and cytological examinations. NS participated in elaborating on the results and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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