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Effect of topical medication on the nasomaxillary skin-fold microbiome in French bulldogs

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Background – Host–microbe interactions may influence dermatitis pathogenesis in the nasomaxillary folds of French bulldogs, which is often complicated by secondary bacterial and fungal infections.

Objective – To assess the skin-fold microbiome in systemically healthy French bulldogs and to determine the influence of topical medications on this microbiome.

Animals – Nineteen healthy French bulldogs.

Methods and materials – Next-generation DNA sequencing was applied to characterise the microbiome composition in the nasomaxillary folds of systemically healthy French bulldogs. Subsequently, the effect of two topical products on the fold microbiome was assessed. Seven dogs were treated with a protease product (Kalzyme; enzyme) that inhibits biofilm formation without biocidal activity, six dogs were treated with a 2% chlorhexidine diacetate solution (Nolvasan; CHX) with biocidal activity, and six dogs were untreated. Dogs were randomly assigned to each group, and the investigator was blinded.

Results – The primary skin bacterial phyla inhabiting the folds at inclusion were Firmicutes, Actinobacteria and Proteobacteria. The primary skin fungal phyla were Ascomycota and Basidiomycota. Topical treatment increased the diversity of bacterial and fungal compositions over time (increase in microbial diversity score: enzyme 38%, chlorhexidine 11%, control <5%) and the relative abundance of pathogens reduced significantly (enzyme, $P = 0.028$; CHX, $P = 0.048$). A clear correlation ($r^2 = 0.83$) was observed between the abundance of clinically relevant pathogens and microbial diversity.

Conclusions – The nasomaxillary skin-fold microbiome of healthy French bulldogs contained a high abundance of clinically relevant pathogens (mean 36.4%). Topical therapy with enzyme increased microbial diversity of skin folds and reduced the relative abundance of pathogens.

Introduction

Host–pathogen interactions may play an important role in dermatitis pathogenesis in the nasomaxillary skin folds of brachycephalic dogs. A healthy cutaneous fold microbiota is important to prevent the colonisation of pathogenic organisms, such as methicillin-resistant *Staphylococcus*, and to ensure optimal skin function and modulation of the innate immune response.[1,2] Worldwide concern has arisen regarding the rapid emergence of antibiotic-resistant pathogens and potential implications for human and veterinary patients.[3–5] Thus, alternative and nonantibiotic approaches to reduce *Staphylococcus* overgrowth, normalise the epidermal barrier and lessen disease severity have received increasing attention.[3]

Dermatological diseases arise from numerous aetiologies including host genetics, skin barrier integrity, and immune and inflammatory components. Moreover, these diseases may be worsened by environmental factors and hygiene practices.[6,7] Skin infections with bacteria and/or yeast are common in dogs.[8] The clinical manifestation of these conditions results from an imbalance in a dog's microbiota, including fungi, bacteria, viruses, protozoa and phages.[9,10] Microbial pathogens and fungal organisms can easily overgrow in dense skin folds, as these moist, protected environments are favourable for their proliferation. Vulnerable areas include the nasomaxillary folds. Microbial density and diversity have been shown to vary across specific canine body sites, with the nostril and conjunctiva exhibiting the lowest, and the dorsal nose the greatest density and diversity.[1] Intertrigo (skin-fold dermatitis) is caused by a combination of mechanical trauma and optimum moist conditions for microbial organisms (bacteria and fungi).[11] Brachycephalic breeds, including English and French bulldogs, are predisposed to developing intertrigo as a consequence of the anatomy of their skin folds.[11,12]

Traditionally, conventional culture methods have been utilised to assess bacterial and fungal populations on skin.

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Culture testing has significant limitations in determining the overall microbiota owing to the variability in culturing different bacteria from diverse genera.[13] In addition, some fungi may take several weeks to grow in culture, and in many instances, will not grow at all. Next-generation sequencing (NGS) is a DNA sequencing technology that has enabled a more complex understanding of the composition of the natural skin microbiota, as well as its functional capacity.[14] NGS also provides a method for characterizing the microbiota distributed along specific body-site locations independently of culturing.[7,15,16]

The purpose of this study was to investigate the effect of two topical products, 2% chlorhexidine (CHX), a common biocidal solution used in the veterinary field, and a protease-based product (enzyme) that does not kill bacteria, on the abundance of clinically relevant pathogens and the microbiome inhabiting the nasomaxillary skin folds of systemically healthy French bulldogs.[17] The protease-based product forms a temporary barrier against microorganisms when applied to the skin. The barrier strengthens the protection against infection by reducing or preventing the ability of bacteria and fungi to bind to the cells of the skin and mucous membranes.[18–20] Management of bacterial infections is becoming increasingly difficult as a result of the emergence and increasing prevalence of bacterial pathogens that are resistant to available antibiotics.[21] Targeting pathogenicity functions such as adhesion directly is an attractive alternative to conventional antibiotics. Bacterial adhesion to surfaces is the first step in colonisation, invasion and biofilm formation, and represents the Achilles heel of crucial pathogenic functions.[18] Proteases are believed to be one of the most effective enzymes in biofilm eradication via hydrolysis of both matrix proteins and adhesins.[21] Biofilm is the predominant mechanism of skin microbiota overgrowth and promotes adhesion and persistence in the cutaneous microenvironment.[18,21–25] Therefore, we would expect a therapeutic topical treatment that prevents biofilm formation to improve the composition and health of the cutaneous microbiota.

Methods and materials

Ethical approval

Study participants were recruited through a private dermatology clinic. Treatments and procedures were reviewed and approved by an internal scientific review board composed of employees from the clinic to ensure that animals were treated under safe and humane conditions. Before study enrollment, written informed consent was obtained from each dog owner.

Animal subjects

Systemically healthy French bulldogs were recruited for this study. The dogs were assigned randomly to control or treatment groups. The French bulldog breed was selected due to their prominent nasomaxillary folds (see Figure S1 in Supporting information).

Owners were able to withdraw consent at any time. Demographic data were collected and analysed with descriptive statistics. No formal hypothesis testing was performed for these parameters.

Study products and dosing

The products evaluated were: CHX – 2% chlorhexidine diacetate solution (Nolvasan Solution, Zoetis; Parsipanny, NJ, USA); and enzyme – an enzyme-based formulation used for treatment of a wide range of common skin disorders in dogs, including bacterial skin infections, containing protease, glycerin, hyaluronate, calcium chloride, water, buffer and a bittering agent (Kalzyme, Lucidian LLC; Chantilly, VA, USA). The control group was left untreated.

One pump stroke (0.18 mL) per 2 cm nose fold of either treatment group was applied twice daily for 28 days utilizing identical spray bottles.

Study design

A randomised, double-blind (investigator and pet owner), prospective study design was employed; 19 of 24 French bulldogs met the inclusion criteria (Appendix S1). None of the enrolled subjects had overt clinical signs consistent with atopic dermatitis as defined by Favrot's criteria.[26] This study was conducted between February 2019 and February 2020. The subjects were assigned randomly to three groups that tested the effect of two topical dermal products (enzyme treatment, n = 7; CHX treatment, n = 6) on systemically healthy canine microbiomes compared to control (n = 6). The objectives of this study were to characterise the microbiome composition of the nasomaxillary folds in systemically healthy French bulldogs and to assess the effect of these two topical products on the fold microbiome. The subjects were assigned to either the enzyme, CHX or control groups at the randomisation visit, Day (D) 0. The study participants returned to the clinic on D14, D28 and D42. Skin microbiome and cytological samples were collected by swabbing the bilateral nasomaxillary folds before, during and after treatment. The pet owner specified the dog's degree of facial pruritus utilizing a provided pruritus Visual Analog Scale (pVAS) (Table S2).[27]

All potential adverse effects were documented. At D28, both treatment groups discontinued the assigned product for an additional 14 days to assess the recolonisation of nasal-fold commensal organisms for each study participant.

Clinical scoring and cytological sampling

Subjective clinical scoring of the nasomaxillary fold appearance and cytological sampling were performed at each visit (D0, D14, D28 and D42). Clinical scoring assessed each nasal fold on a scale of 0–4 (lacking, mild, moderate, strong, very strong) to describe the extent of alopecia, erythema, secretion, hyperpigmentation, papules, pustules, erosions and ulcerations. The inclusion criteria admitted mild pyoderma, and the median intertrigo score (sum of the clinical and cytological scores) at inclusion was 5 (maximum score 48) for all enrolled subjects.

Cytological samples were collected from the nasal folds and semi-quantitatively assessed for bacteria, yeast and neutrophils for each subject at each visit (Table S1). The pet owner rated the dog's facial pruritus with a pVAS scale (Table 1).

Microbiome sampling

Microbiome sampling was performed during each session (D0, D14, D28 and D42). Bacteria and fungi were profiled by an NGS methodology, following the packaging insert instructions (Appendix S2) provided by the MiDOG LLC testing service (Irvine, CA, USA). Microbial DNA was isolated and sequenced using an Illumina HiSeq 1500 apparatus. The microbiota profile of each sample was determined using the bioinformatics analysis pipeline offered by the MiDOG LLC testing service as described previously.[28]

Statistics

Statistical hypotheses were analysed using two-sided tests with a significance level of 5% using PRISM (GraphPad; San Diego, CA, USA). Bacterial and fungal abundances determined from pairwise comparisons between groups were assessed using the Wilcoxon–Mann–Whitney U-test. Pearson correlation and co-occurrence analysis were used to assess microbial interactions with the standard settings of the R package STATS v3.6.1. Linear discriminant analysis (LDA) and effect size (LEfSe) were used to identify taxa that were significantly enriched in each group (QIIME v1.9.1; P-value <0.05 was considered significant). Measurements of alpha-diversity and

Table 1. Demographics and intertrigo scores of study cohorts

Characteristics	Control	CHX	Enzyme
Number of subjects	6	6	7
Median age (range) (years)	2.9 (1.2–6.0)	7.4 (1.2–10.5)	3.5 (1.0–9.0)
Percentage of females	67	50	71
Median intertrigo score* at Day (D)0 (range)	5.0 (0–10)	6.0 (3–10)	5.0 (1–12)
Median intertrigo score* at D28 (range)	6.0 (2–19)	3.5 (0–9)	3.0 (1–17)
Median pVAS score at D0 (range)	1.0 (0–2)	0.5 (0–2)	1.0 (0–2)
Median pVAS score at D28 (range)	1.0 (0–2)	0.0 (0–2)	0.0 (0–2)

CHX, chlorhexidine; pVAS, pruritus Visual Analog Score.
*The intertrigo score is the sum of clinical and cytological scores; see Methods and materials.

evenness were calculated using the Shannon diversity index, with QIIME and a customised Python script.

Results

Summary of study participants

A total of 24 systemically healthy French bulldogs were enrolled in the study; 19 participants met the inclusion and exclusion criteria. The female:male ratio was 9:10, and the median age at inclusion was 4.6 years (range 1.0–10.5 years). The median pruritus score (pVAS) at the start of the study was 1.0 (range 0–2). As the pVAS score was not 0, dogs with mild atopic dermatitis may have been included in the study. Six dogs were included in the control group, six dogs in the CHX group and seven dogs in the enzyme group. The demographics of the three groups were recorded (Table 1). The age in the control group ranged from 1.2 to 6 years (median 2.9 years) with 67% female. The ages in the CHX group ranged from 1.2 to 10.5 years (median 7.4 years), with 50% female. The ages in the enzyme group ranged from 1.0 to 9.0 years (median 3.5 years), with 71% female. There were no significant differences between the three groups for age or sex; the median age ranged from 2.9 to 7.4 years.

Nasomaxillary skin-fold microbiome

5Before topical treatment, all 19 dogs showed low diversity at both the bacterial and fungal phylum levels, and high bacterial diversity and low fungal diversity at the genus and species levels. The primary bacterial phyla inhabiting the nasal folds were Firmicutes, Actinobacteria and Proteobacteria. The primary fungal phyla were Ascomycota and Basidiomycota. At the genus and species levels, greater variability was observed between subjects. A taxonomy abundance heat map for bacteria and fungi genera revealed sample clustering patterns of microbial distribution among the subjects at the genus level (Figure S2). No specific clustering of the three cohorts was observed. The predominant bacterial and fungal genera were recorded for all subjects at inclusion (Table 2). The most abundant bacteria genera observed in the nasomaxillary folds of the subjects were Staphylococcus, Streptococcus and Corynebacterium. The most abundant fungal taxa observed were the genus *Cladosporium*, two taxa that could only be assigned to the kingdom level, one taxon assigned to the phylum Ascomycota and one taxon of the genus *Neoascochyta* (Table 2). The average abundance of the genus *Malassezia* was 3.7%.

Table 2. Most abundant bacterial and fungal genera found in the nasomaxillary folds of French bulldogs at Day 0 (n = 19)

Bacteria		Fungi	
Genus (Bacteria)	Average abundance (%)	Genus (Fungi)	Average abundance (%)
Staphylococcus	14.1	*Cladosporium*	20.1
Streptococcus	9.9	k__Fungi_NA (1)	13.0
k__bacterium	7.0	k__Fungi_NA (2)	12.5
Corynebacterium	6.0	p__Ascomycota_NA	6.8
Proteus	5.3	*Neoascochyta*	6.0
Cutibacterium	3.7	*Malassezia*	3.7
Arcanobacterium	3.6	*Alternaria*	3.2
Porphyromonas	2.5	o__Pleosporales_NA	2.9
Sphingomonas	1.9	*Nigrospora*	1.8
Finegoldia	1.9	*Curvularia*	1.7
Rothia	1.5	*Bipolaris*	1.6
Fusobacterium	1.4	*Trichoderma*	1.4
Methylobacterium	1.2	*Cochliobolus-Curvularia*	1.2
Conchiformibius	1.0	*Vishniacozyma*	1.2
f__*Moraxellaceae*_NA	1.0	*Fusarium*	0.9
Pseudomonas	0.9	*Aureobasidium*	0.8
f__*Pasteurellaceae*_NA	0.8	o__Trechisporales_NA	0.8
Microbacterium	0.7	o__Hypocreales_NA	0.7
Peptostreptococcus	0.7	f__Didymellaceae_NA	0.7
o__Rhizobiales	0.7	*Wallemia*	0.7

NA, known classifiable taxa (to the following level: o, order; f, family; k, kingdom; p, phylum) currently without an assigned nomenclature.

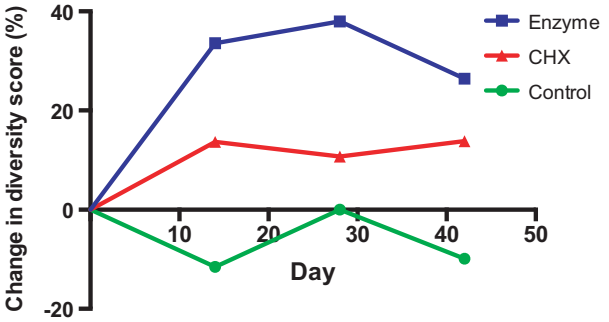

Figure 1. Change in the Shannon diversity index score during topical treatment of nasomaxillary folds of French bulldogs.
The percentage change during 28 days of treatment and 14 days post-treatment are shown for the enzyme-treated group (n = 7) and the chlorhexidine (CHX)-treated group (n = 6). The control group (n = 6) did not receive any treatment.

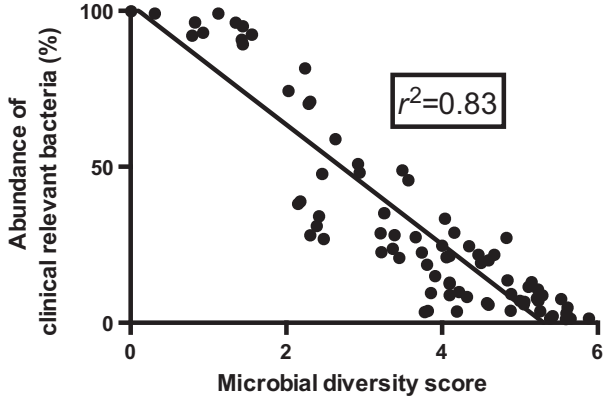

Figure 2. Correlation between abundance of clinically relevant bacteria and microbial diversity in nasomaxillary folds of French bulldogs.
The abundance of clinically relevant bacteria is shown as a function of the microbial diversity score (i.e. the Shannon index, a quantitative measure that reflects the number of different species in a dataset). A clear correlation (r^2 = 0.83) was observed between the two parameters, indicating that a low abundance of clinically relevant pathogens is correlated with a high microbial diversity score.

Topical treatment of nasomaxillary folds: effect on microbiome

There was no statistical difference among the intertrigo scores for each group at D0 (enzyme–control, P = 0.56; CHX–control, P = 0.54; and enzyme–CHX, P = 0.95) (Table 1). Large individual variations within the groups was observed, with Intertrigo scores ranging from 0 to 12. The topical treatment increased the diversity of the bacterial and fungal compositions over time (Figure 1). The diversity increased by 38% for the enzyme treatment group, by 11% for the CHX group, and by <5% for the control group.

Clinically relevant pathogens were identified by a literature review, which yielded 29 clinically relevant bacterial pathogens and six fungal pathogens. The 20 most abundant bacterial pathogens found at inclusion were identified (Table S3). *Streptococcus halichoeri*, *Staphylococcus delphini-intermedius-pseudintermedius* and *Proteus mirabilis* were the most abundant pathogens at D0, detected in 26%, 79% and 5% (respectively) of the subjects (Table S3). One dog (Case 18) revealed a high average abundance (99%) of *Proteus* (Figure S2); however, *Proteus* was not detected in the other subjects. Case18 had a more diverse abundance of fungi. The relative number of pathogens in the various cohorts differed at the day of inclusion, with 35% pathogens in the control group, 54% in the enzyme group and 23% in the CHX group. A clear correlation (r^2 = 0.83) was observed between the abundance of clinically relevant pathogens and microbial diversity. This finding indicates that a low abundance of clinically relevant pathogens is correlated with a high microbial diversity score on the Shannon index, a quantitative measure that reflects the number of different species in a dataset (Figure 2). In both treatment groups, the abundance of clinically relevant pathogens decreased significantly (enzyme, P = 0.028; CHX, P = 0.048) compared to the control (Figure 3).

A statistical analysis (LefSE) was conducted to identify those species that were significantly impacted and enriched by the treatments over time. The analysis showed that 30 species were significantly enriched either during or after the treatments (i.e. time points D14, D28 and D42; Table S4). Of those 30 species, six species are known canine skin bacterial pathogens (*Myrmecridium* sp., *Filifactor villosus, Tilletiopsis lilacina, Pelomonas saccharophila, Nocardia takedensis* and *Stenotrophomonas maltophilia* in the CHX group, and *Staphylococcus hominis* in the enzyme group) and four are fungi (*Myrmecridium* sp., *Fusarium* sp., *Tilletiopsis lilacina* in the CHX group, *Didymella glomerata* in the enzyme group).[29–34] Interestingly, CHX had the biggest impact on increasing specific taxa of the microbiome (80% of species identified), followed by enzyme (10%) and control (10%).

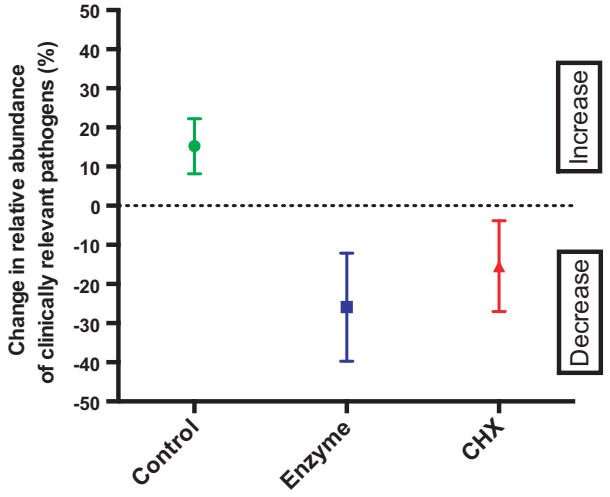

Figure 3. Change in relative abundance of clinically relevant pathogens in nasomaxillary folds of French bulldogs from day (D)0 to D28 for groups treated with enzyme and chlorhexidine (CHX) or untreated (control).
The abundance of clinically relevant pathogens decreased significantly in both treatment groups, while the control group displayed an increase in clinically relevant pathogens. The enzyme-treated group displayed a slightly larger decrease (P = 0.028) than the chlorhexidine (CHX)-treated group (P = 0.048). Data are plotted as means with error bars (standard error).

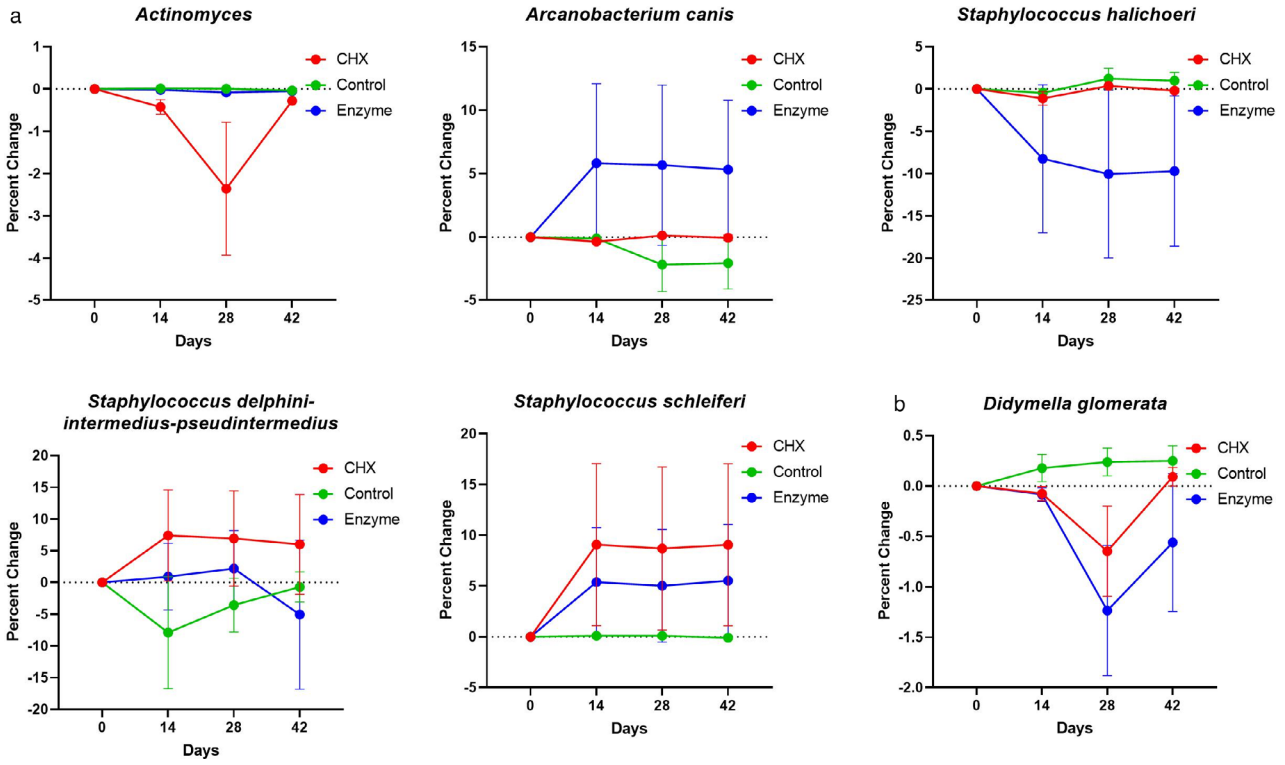

Figure 4. A comparative analysis (LefSE) was conducted to identify those species that were significantly impacted by the treatments from day 0 to day 42.
Of those taxa identified by the LefSE analysis, the changes in their relative abundance per subject over time were calculated using the relative abundance of each given taxa on day 0 as the reference. Shown here are those taxa that had at least a 1% change in their relative abundances on average per group and are taxa of interest, i.e. known bacterial pathogens (a) or rare fungi (b).

The percentage change of microbial species per subject over time (delta) was calculated for pathogens and those taxa identified by the LefSe analysis. Six taxa not driven by a single subject were identified that had a delta of ≥1% at a given time point per group. This included five bacterial taxa (Figure 4a) and one fungal species, *D. glomerata* (Figure 4b). The control group had the fewest changes over time compared to the two treatments. The genus *Actinomyces* was reduced by CHX treatment; as soon as the treatment was stopped, the abundance of this genus returned to the initial value. A similar phenomenon was seen for *D. glomerata*. For all other species analysed, the impact of the treatment was immediate. For *A. canis*, only the enzyme treatment had an effect on the abundance, where the change was immediate, then stabilizing between +5.6% and +5.3% until the end of the study. The enzyme treatment also induced an immediate decrease of *S. halichoeri* that stabilised at –10.0% and –9.7%. This was the largest delta seen of all taxa. CHX appeared to have no effect on this species. For *S. delphini-intermedius-pseudintermedius*, only CHX had a consistent effect, stabilizing the abundance at approximately +6.7% compared to the beginning of the study. This species showed the largest range of delta, ranging from +7.4 to –7.9. For *S. schleiferi* CHX had the largest effect, increasing the relative abundance of the species by 8.7% to 9.1% over time, which was approximately 55–60% higher than the enzyme effect (5.0% to 5.4%). Overall treatment effects were very individualised for both the subject and the taxa. In most cases, changes occurred immediately and then either stabilised or returned to the initial value.

Discussion

This study aimed to investigate the bacterial and fungal nasomaxillary fold microbiome of systemically healthy French bulldogs. Variations were recorded in the microbiome composition after topical treatment with either 2% CHX or an enzyme-based product without biocidal activity. Before topical therapy, analysis of the microbiome composition showed low diversity at both the bacterial and fungal phylum levels, and high bacterial diversity and low fungal diversity at the genus and species levels.

In this study, the most abundant bacterial genera observed in the nasomaxillary folds of the subjects were *Staphylococcus*, *Streptococcus* and *Corynebacterium*. The most abundant fungal genera were *Cladosporium* and two fungal taxa that could not be assigned more closely than to the kingdom level. The average abundance of *Malassezia* was 3.7%. Previous studies have described the most dominant canine cutaneous organisms as *S. pseudintermedius*, *S. schleiferi* and *M. pachydermatis* across all samples and phenotypes.[3,28,35] One study showed that haired skin on the dorsal nose had a high diversity and yielded Proteobacteria as the most abundant phylum, followed by Firmicutes, Actinobacteria and Bacteroidetes.[4] Previous studies also have reported that an increased

abundance of *Corynebacterium* is positively correlated with staphylococcal dermatitis in atopic dogs.[3,36]

Topical therapy as sole treatment should be considered for cases of superficial pyoderma. The lack of effective antimicrobial agents and emerging antimicrobial resistance have led to diminished patient prognoses.[37–39] Looking to a future of nonantimicrobial therapeutic options, including pre- and probiotics, proteases and other antibiofilm products, more detailed studies of microbial relationships and longitudinal studies are needed to confirm gain or loss of taxa.[36] This study highlighted how treatment decisions that impact upon the microbiome might not be approached by a one-fits-all approach.[40] The skin microbiome was highly variable between subjects and the treatment effects of both enzyme and CHX seemed to be partly species-specific. Even species of the same genus responded to the treatments differently (e.g. *S. halichoeri* abundance was reduced 10% by the enzyme treatment, yet the abundance of *S. delphini-intermedius-pseudintermedius* was relatively stable). Interestingly, the resilience of the microbiome profile also was species-specific, with some members (i.e. *Actinomyces* and *D. glomerata*) returning to their start value immediately after the treatment was finished, and others (i.e. *A. canis, S. halichoeri*, *S. delphini-intermedius-pseudintermedius* and *S. schleiferi*) remaining at the value of the last treatment time point.[41] Depending on the dominant pathogen in each case, the results can differ largely and even show increases in the relative abundance of certain pathogens.

In conclusion, we showed that topical treatment (enzyme and CHX) increased the overall diversity of bacterial and fungal compositions in some dogs over time. These findings suggest that antimicrobial therapy in general may increase the diversity of the skin-fold microbiome and that the effects may be reduced once treatment is withdrawn. Our findings support results published previously.[4] Larger studies on dogs with clinically evident nasomaxillary fold pyoderma are warranted to confirm these findings.

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Author contributions

Alissa Rexo: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing-original draft, Writing-review & editing. **Bruce Hansen:** Conceptualization, Data curation, Investigation, Project administration, Resources, Supervision, Validation, Writing-review & editing. **Mats Clarsund:** Formal analysis, Resources, Software, Writing-original draft. **Janina Krumbeck:** Formal analysis, Funding acquisition, Software, Writing-review & editing. **Joseph Bernstein:** Conceptualization, Supervision, Writing-review & editing.

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Supporting Information

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

Appendix S1. Collection from the nasomaxillary fold via a MiDog next-generation sequencing swab.
Appendix S2. Bacterial and fungal pathogens determined via a literature scan[1,28,42–45]
Table S1. Cytological criteria for normal versus infected dogs. Classification of the semiquantitative scale classification description.[46]
Table S2. Dog's degree of facial pruritus as determined by the owner via the scraping scale (PVAS). The owner objectively assessed the level of facial pruritus using a 0–5 behavior-based pruritus score developed at the Royal (Dick) School of Veterinary Studies.[27]
Table S3. Most abundant bacterial pathogens at inclusion day (n=19).
Table S4. Microbial species which were significantly enriched in a group during or after the treatments. Highlighted in bold are those time points that were significantly higher compared to the other two groups at the same time point. Shown are the average abundances for each day for the group that was significantly enriched. Abbreviations: Abund.: Abundance.
Figure S1. Clinical image of a study participant on day 0, revealing the prominent nasomaxillary folds that predispose the French bulldog breed to intertrigo. This patient had a PVAS of approximately 1 on each of 4 visits and an average cytological bacterial assessment of approximately 3. The yeast and neutrophils were 0.
Figure S2. Heat map showing the microbial composition of the samples at the genus level with the top fifty most abundant taxa identified. (A) Bacteria and (B) fungi. Each row represents the abundance for each taxon, with the taxonomy ID shown on the right. Each column represents the abundance for each subject. Group information is indicated by the colored bar located on the top of each column. Hierarchical clustering was performed for the samples based on the Bray–Curtis dissimilarity. Hierarchical clustering was also performed for the taxa such that taxa with similar distributions are grouped together.

Résumé
Contexte – Les interactions hôte-microbe peuvent influencer la pathogénie de la dermatite dans les plis naso-maxillaires du bouledogue français, souvent compliquée par des infections bactériennes et fongiques secondaires.
Objectifs – Evaluer le microbiome des plis cutanés chez les bouledogues français sains et déterminer l'influence des médications topiques sur ce microbiome.
Sujets – Dix neuf bouledogues français sains.

Matériels et méthodes – Le séquençage d'ADN de dernière génération a été appliqué pour caractériser la composition du microbiome des plis naso-maxillaires de bouledogues français sains. L'effet de deux topiques sur le microbiome des plis a été évalué. Sept chiens ont été traités avec protéase (Kalzyme; enzyme) qui inhibe la formation de biofilm sans activité biocide, six chiens étaient non traités. Les chiens ont été répartis au hasard dans chaque groupe et l'investigateur était en aveugle.
Résultats – Les phyla bactériens cutanés primaires inhibant les plis à l'inclusion étaient Firmicutes, Actinobacteria et Proteobacteria. Les phyla fongiques primaires cutanés étaient Ascomycota et Basidiomycota. Le traitement topique a augmenté la diversité des compositions bactériennes et fongiques dans le temps (augmentation du score de diversité microbienne : enzyme 38%, chlorhexidine 11%, contrôle <5%) et l'abondance relative des pathogènes a diminué significativement (enzyme, $P = 0.028$; CHX, $P = 0.048$). Une corrélation nette ($r^2 = 0.83$) a été observée entre l'abondance des pathogènes cliniquement importants et la diversité microbienne.
Conclusions – Le microbiome des plis cutanés naso-maxillaires des bouledogues français sains contient une abondance élevée de pathogènes cliniquement significatifs (moyenne 36,4%). Le traitement topique avec enzyme augmente la diversité microbienne des plis cutanés et réduit l'abondance relative des pathogènes.

Resumen
Introducción – las interacciones huésped-microbio pueden influir en la patogenia de la dermatitis en los pliegues nasomaxilares de los Bulldogs Franceses, que a menudo se complica por infecciones bacterianas y fúngicas secundarias.
Objetivo – evaluar el microbioma del pliegue cutáneo en Bulldogs Franceses sistémicamente sanos y determinar la influencia de los medicamentos tópicos en este microbioma.
Animales – diecinueve Bulldogs Franceses sistémicamente sanos.
Métodos y materiales – se aplicó la secuenciación de DNA de próxima generación para caracterizar la composición del microbioma en los pliegues nasomaxilares de Bulldogs Franceses sistémicamente sanos. Posteriormente, se evaluó el efecto de dos productos tópicos en el pliegue del microbioma. Siete perros fueron tratados con un producto de proteasa (Kalzyme; enzima) que inhibe la formación de biopelículas sin actividad biocida, seis perros fueron tratados con una solución de diacetato de clorhexidina al 2% (Nolvasan; CHX) con actividad biocida y seis perros no fueron tratados. Los perros se asignaron al azar a cada grupo y el investigador fue cegado.
Resultados – los filos de bacterias cutáneas primarias que habitaban los pliegues en el momento de la inclusión fueron Firmicutes, Actinobacteria y Proteobacteria. Los principales filos fúngicos de la piel fueron Ascomycota y Basidiomycota. El tratamiento tópico aumentó la diversidad de composiciones bacterianas y fúngicas con el tiempo (aumento en la puntuación de diversidad microbiana: enzima 38%, clorhexidina 11%, control <5%) y la abundancia relativa de patógenos se redujo significativamente (enzima, $P = 0{,}028$; CHX, $P = 0{,}048$). Se observó una clara correlación ($r^2 = 0{,}83$) entre la abundancia de patógenos clínicamente relevantes y la diversidad microbiana.
Conclusiones – el microbioma del pliegue cutáneo nasomaxilar de los Bulldogs Franceses sistémicamente sanos contiene una gran abundancia de patógenos clínicamente relevantes (media del 36,4%). La terapia tópica con enzimas aumenta la diversidad microbiana de los pliegues de la piel y reduce la abundancia relativa de patógenos.

Zusammenfassung
Hintergrund – Die Interaktion von Wirt und Mikroben könnte die Pathogenese der Dermatitis in den nasomaxillären Falten von Französischen Bulldoggen, welche oft durch sekundäre bakterielle Infektionen oder durch Pilzinfektionen verkompliziert wird, beeinflussen.
Ziel – Eine Erfassung des Mikrobioms der Hautfalten bei systemisch gesunden Französischen Bulldoggen und eine Feststellung, welchen Einfluss eine topische Behandlung auf dieses Mikrobiom hat.
Tiere – Neunzehn systemisch gesunde Französische Bulldoggen.
Methoden und Materialien – Next-Generation DNA-Sequenzierung wurde angewendet, um die Zusammensetzung des Mikrobioms in den nasomaxillären Falten von systemisch gesunden französischen Bulldoggen zu charakterisieren. In der Folge wurde die Auswirkung zweier topisch angewendeter Produkte auf das Mikrobiom der Falten erfasst. Sieben Hunde wurden mit einem Protease Produkt (Kalzyme; Enzym) behandelt, welches ohne eine biozide Wirkung Biofilmbildung verhindert, sechs Hunde wurden mit 2%iger Chlorhexidindiacetatlösung (Nolvasan; CHX) mit biozider Aktivität behandelt und sechs Hunde wurden nicht behandelt. Die Hunde wurden zufällig in die Gruppen eingeteilt, die UntersucherInnen wurden geblindet.
Ergebnisse – Die bakteriellen Primärstämme, die die Falten bewohnten, waren Firmicutes, Actinobacteria und Proteobacteria. Die mykotischen Primärstämme waren Ascomycota und Basidiomycota. Durch die topische Behandlung vergrößerte sich mit der Zeit die Diversität der bakteriellen Zusammensetzung sowie der Zusammensetzung der Pilze (Zunahme des mikrobiellen Diversitätswerts: Enzym 38%, Chlorhexidin 11%, Kontrolle <5%) und die relative Häufigkeit der Pathogene war signifikant reduziert (Enzym, $P = 0{,}028$; CHX,

P = 0,048). Eine deutliche Korrelation (r^2 = 0,83) wurde zwischen der Häufigkeit der klinisch relevanten Pathogene und der mikrobiellen Diversität beobachtet.
Schlussfolgerungen – Die Mikrobiome der nasomaxillären Hautfalten von systemisch gesunden Französischen Bulldoggen beinhalten eine große Anzahl an klinisch relevanten Pathogenen (Durchschnitt 36,4%). Eine topische Therapie mit Enzymen erhöht die mikrobielle Diversität der Hautfalten und reduziert die relative Häufigkeit der Pathogene.

要約
背景 – フレンチブルドッグの鼻上顎ヒダにおける皮膚炎の病態には、宿主と微生物の相互作用が影響している可能性があり、二次的な細菌や真菌感染を伴うことが多い。
目的 – 本研究の目的は、健常フレンチブルドッグの皮膚表面のマイクロバイオームを評価し、このマイクロバイオームに対する外用薬の影響を明らかにすることであった。
供試動物 – 19頭の健常フレンチブルドッグ。
材料と方法 – 次世代DNAシーケンサーを用いて、健常フレンチブルドッグの鼻上顎ヒダにおけるマイクロバイオームの構成を明らかにした。その後、2種類の外用剤がヒダのマイクロバイオームに及ぼす影響を評価した。7頭の犬には殺菌性のないバイオフィルム形成阻害プロテアーゼ製品 (カルザイム; 酵素) を、6頭の犬には殺菌性のある2%クロルヘキシジンジアセテート溶液 (ノルバサン; CHX) を、6頭の犬には無処置を施した。犬は各グループに無作為に割り当てられ、調査者は盲検化された。
結果 – 研究包含時に、ヒダに生息していた主要な皮膚細菌門は、Firmicutes、Actinobacteria、Proteobacteriaであった。また、皮膚の真菌類は、AscomycotaおよびBasidiomycotaであった。外用療法により、細菌および真菌の組成の多様性は時間の経過とともに増加し (微生物多様性スコアの増加: 酵素38%、クロルヘキシジン11%、対照5%未満) 、病原菌の相対的な存在量は有意に減少した (酵素、P = 0.028、CHX、P = 0.048) 。臨床的に重要な病原菌の存在量および微生物の多様性間に明確な相関関係 (r2 = 0.83)を認めた。
結論 – 健常フレンチブルドッグの鼻上顎ヒダのマイクロバイオームには、臨床関連病原体が多く含まれていた (平均36.4%) 。酵素を用いた外用療法は、鄒襞部の微生物の多様性を高め、病原菌の相対的な存在感を低下させる。

摘要
背景 – 宿主-微生物相互作用可能影响法国斗牛犬鼻上颌皱襞皮炎的发生, 通常并发继发性细菌和真菌感染。
目的 – 评估全身健康的法国斗牛犬的皮褶微生物组, 并确定外用药物对该微生物组的影响。
动物 – 19只全身健康的法国斗牛犬。
方法和材料 – 应用新一代DNA给全身健康的法国斗牛犬鼻上颌皱襞微生物组测序, 描述其特征。随后, 评估了两种外用产品对微生物组折叠的影响。7只犬接受抑制生物膜形成而无杀菌活性的蛋白酶产物 (Kalzyme; 酶) 治疗, 6只犬接受具有杀菌活性的2%双乙酸氯己定溶液(Nolvasan;CHX)治疗, 6只犬未接受治疗。将犬随机分配至各组, 并对研究者设盲。
结果 – 常驻褶皱处的皮肤细菌门包括厚壁菌门、放线菌门和变形菌门。主要皮肤真菌门包括子囊菌门和担子菌门。随着时间的推移, 外部治疗增加了细菌和真菌组成的多样性 (微生物多样性评分增加: 酶38%, 氯己定11%, 对照 < 5%) , 病原体的相对丰度显著降低 (酶, P = 0.028;CHX,P = 0.048) 。观察到临床相关病原体的丰度与微生物多样性之间存在明确的相关性(r2 = 0.83)。
结论 – 全身健康法国斗牛犬的鼻上颌皮褶微生物组含有高丰度的临床相关病原体 (平均36.4%) 。酶外部治疗增加了皮肤皱襞的微生物多样性, 降低了病原体的相对丰度。

Resumo
Contexto – As interações entre hospedeiro e microrganismo podem influenciar a patogênese da dermatite na dobra nasomaxilar de buldogues franceses, que é frequentemente complicada por infecções bacterianas e fúngicas secundárias.
Objetivo – Avaliar o microbioma de dobras cutâneas em buldogues franceses sistêmicamente saudáveis e determinar a influência de medicações tópicas neste microbioma.
Animais – Dezenove buldogues franceses sistêmicamente saudáveis.
Métodos e materiais – Sequenciamento de DNA de última geração foi aplicado para caracterizar a composição do microbioma nas dobras nasomaxilares de buldogues franceses sistêmicamente saudáveis. Subsequentemente, o efeito de dois produtos tópicos no microbioma da dobra foi avaliado. Sete cães foram tratados com um produto a base de protease (Kalzyme; enzima) que inibe a formação de biofilme sem atividade biocida, seis cães foram tratados com uma solução de diacetato de clorexidina a 2% (Nolvasan; CHX) com ação biocida, e seis cães não receberam tratamento. Os cães foram aleatoriamente divididos entre os grupos, e o investigador era cego.
Resultados – Os principais filos bacterianos habitantes das dobras cutâneas na inclusão no estudo foram Firmicutes, Actinobateria e Proteobacteria. Os principais filos de fungos foram Ascomycota e Basidiomycota. O tratamento tópico aumentou a diversidade das composições bacterianas e fúngicas ao longo do tempo (aumento no escore de diversidade microbiana: enzima 38%, clorexidine 11%, controle < 5%) e a abundância relativa de patógenos reduziu significativamente (enzima, *P* = 0,028; CHX, *P* = 0,048).

Observou-se uma correlação clara (r^2 = 0,83) entre a abundância de patógenos clinicamente relevantes e diversidade microbiana.
Conclusões – O microbioma da dobra cutânea nasomaxilar de buldogues franceses sistêmicamente saudáveis contêm grande abundância de patógenos clinicamente relevantes (media 36,4%). Terapia tópica com enzima aumenta a diversidade microbianae reduz a abundância relative de patógenos.