EFFECT OF TOPICAL MEDICATION ON THE NASOMAXILLARY SKIN FOLD MICROBIOME OF FRENCH BULLDOGS

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OBJECTIVE

Host-microbe interactions play an important role in the pathogenesis of dermatitis in the nasomaxillary folds of brachycephalic dogs, as it is often complicated by secondary bacterial and fungal infections[1]. This study investigates the effect of two topical products, 2 % Chlorhexidine[4] (CHX), a broad-spectrum biocide and a Protease based product[2] (Enzyme) without biocidal activity on the microbiome, the abundance of clinically relevant pathogens and on the microbial diversity inhabiting nasomaxillary skin folds of apparently healthy French bulldogs.

STUDY DESIGN

Randomized, single-blinded, prospective study was performed including 19 clinically healthy French Bulldogs (control; n=6, Enzyme; n=7, CHX n=6). Dogs were treated twice a day for 28 days. Skin microbiome and cytological samples were collected by swabbing from bilateral nasomaxillary folds on day 0 (before treatment), 14, 28 and 42 (14 days after treatment discontinued). Bacteria and fungi were profiled by NGS methodology, following the packaging insert instructions provided by MiDOG LLC testing service[3].

RESULTS

NASOMAXILLARY SKIN FOLD MICROBIOME

All 19 dogs at inclusion day, before any topical treatment, showed a low diversity at both bacterial and fungal phylum level, but high bacterial diversity and low fungal diversity at the genus and species level. Table shows most abundant bacterial and fungal genera found in nasomaxillary folds of French bulldogs. *Staphylococcus delphini-intermedius-pseudintermedius* was the most abundant pathogen at day 0 and could be detected in 79% of the subjects.

Genus (Bacteria)*	Average abundance (%)
Staphylococcus	14,1
Streptococcus	9,9
Other	7,0
Corynebacterium	6,0
Proteus	5,3
Cutibacterium	3,7
Arcanobacterium	3,6
Porphyromonas	2,5
Sphingomonas	1,9
Finegoldia	1,9

Genus (Fungi)*	Average abundance (%)
Cladosporium	20,1
k__Fungi_NA	13,0
Other	12,5
p__Ascomycota_NA	6,8
Neoascochyta	6,0
Malassezia	3,7
Alternaria	3,2
o__Pleosporales_NA	2,9
Nigrospora	1,8
Curvularia	1,7

*Identified 29 bacteria and 6 fungi as clinically relevant pathogens
Tables are truncated to demonstrate most significant average abundance

TREATMENT EFFECT ON ABUNDANCE OF CLINICALLY RELEVANT PATHOGENS

Topical treatment increased the diversity of bacterial and fungal compositions over time, shown as an increase in microbial diversity score (Figure 1). Clinically relevant pathogens (e.g. *Staphylococci* and *Malassezia*) were determined by reviewing literature. The relative amount of pathogens in the various cohorts differed at inclusion day. The Control group contained 35%, Enzyme 54% and CHX 23% pathogens. A clear correlation (r^2=0.8) between abundance of clinically relevant pathogens and microbial diversity was seen, indicating that a low abundance of clinically relevant pathogens is correlated with a high microbial diversity score. 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 2). There was no statistical difference (p=0.58) between CHX and Enzyme.

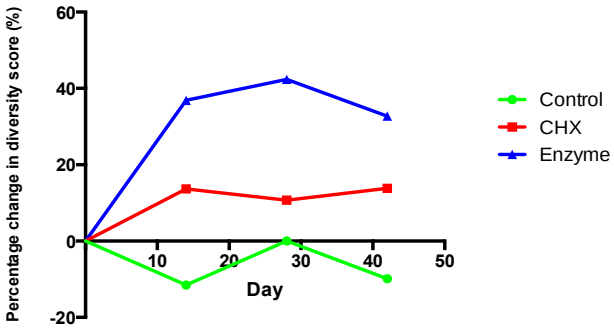

Figure 1 Change in Shannon Diversity Index score during treatment of dogs. Plot displaying the percentage change during 28 days of treatment and 14 days after treatment stop in the Enzyme treated group (n=7) and in the CHX group (n=6).

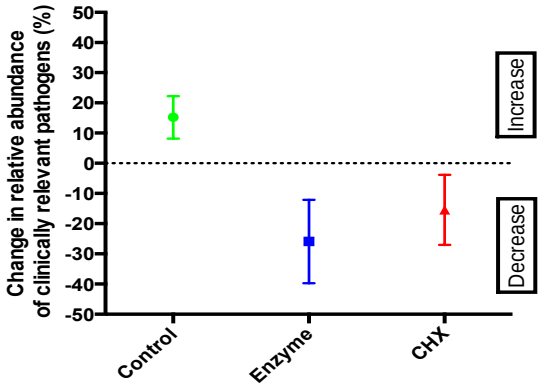

Figure 2. Change in relative abundance of clinically relevant pathogens from Day 0 to Day 28.

CONCLUSION

- The main skin bacterial phyla inhabiting the skin fold of the dogs were *Firmicutes*, *Actinobacteria* and *Proteobacteria*.
- The main skin fungal phyla were *Ascomycota* and *Basidiomycota*
- The topical treatment increased the diversity of bacterial and fungal compositions over time, shown as an increase in microbial diversity score. For the enzyme treatment group the increase was 42%, for the chlorhexidine group 11% and for the control group it was < 5%.
- These findings suggest that antimicrobial therapy may restore diversity of the skin fold microbiome and reduce the abundance of clinically relevant pathogens
- Both CHX and Enzyme treatment, biocidal and non-biocidal effect, decreased the abundance of clinically relevant pathogens.

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