Trabajos de investigación

REVISTA ANALECTA | Facultad de Ciencias VETERINARIAS

Ctrl-X, Ctrl-C and Ctrl-V in Veterinary Dermatology: skin microbiota transplantation as a promising approach for dogs with cutaneous adverse food reactions

Ctrl-X, Ctrl-C y Ctrl-V en Dermatología Veterinaria: el trasplante de microbiota cutánea como un enfoque prometedor para perros con reacciones cutáneas adversas a los alimentos

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Abstract: The probability of influencing the skin microbiome for addressing skin disorders opens a novel aisle of therapy. This study aimed to evaluate the efficacy of skin microbiota transplantation (sMt) for cutaneous adverse food reactions (caFr) in dogs. Ten client-owned dogs with caFr were included in the study. Unenriched heterologous sMt was performed using Nivea Skin Refining Clear-Up Strips (N-cUs). The bacterial microbiota of skin samples was analysed by next-generation sequencing of the 16S rRNA gene. Other relevant biomarkers were involved in VAS (visual analogue scale of pruritus score), CADESI-04 (canine atopic dermatitis extent and severity index) and epidermal corneometric analysis. Increased *Faecalibacterium* (0 to 1.9 %), *Peptoclostridium* (5.49 % to 9.11 %) and *Collinsella* (0.65 % to 8.91 %), and decreased *Fusobacterium* (19.16 % to 9.06 %), *Porphyromona* (8.75 % to 0.13 %), *Streptococcus* (1.63 % to 0.14 %) and *Staphylococcus* (1.09 % to 0.49 %) was evidenced before and after sMt, respectively. Treatment with sMt effectively controlled clinical signs and drastically reduced median VAS pruritus (6.5 vs. 2) and CADESI-04 scores (74.50±22.62 to 19.30±11.30) ($p<0,001$). In addition, skin pH and hydration values were improved ($p<0,001$) after sMt. The heterologous and unenriched sMt with N-cUs could be responsible for the clinical recovery observed in this study.

Keywords: atopic dermatitis, food intolerance, microbiota, skin, transfer.

Resumen: La probabilidad de influir en el microbioma de la piel para abordar trastornos dermatológicos abre una nueva vía de terapia. El objetivo del presente trabajo fue evaluar la eficacia del trasplante de microbiota cutánea (sMt) en diez perros con reacciones en piel, adversas a los alimentos (caFr). Se realizó un sMt heterólogo no enriquecido mediante el uso de tiras clarificadoras Nivea Skin Refining Clear-Up (N-cUs). Se prefirió la secuenciación de próxima generación para investigar la microbiota bacteriana en las muestras de piel de los animales estudiados. Otros biomarcadores relevantes estuvieron involucrados en la puntuación de prurito VAS, CADESI-04 y análisis corneométrico epidérmico (hidratación y pH). El gen 16S rRNA clasificó con éxito la presencia de un aumento de *Faecalibacterium* (0 a 1,9%), *Peptoclostridium* (5,49 % a 9,11 %) y *Collinsella* (0,65 % a 8,91 %), mientras que se observó una disminución de *Fusobacterium* (19,16 % a 9,06 %), *Porphyromonas* (8,75 % a 0,13 %), *Streptococcus* (1,63 % a 0,14 %) y *Staphylococcus* (1,09 % a 0,49 %), antes y después del sMt. El tratamiento con sMt controló eficazmente los signos clínicos y redujo drásticamente la mediana del prurito VAS (6,5 vs. 2) y las puntuaciones CADESI-04 (74,50±22,62 a 19,30±11,30) (p<0,001). Además, tanto el pH de la piel como los valores de hidratación se vieron alterados (p<0,001) después del sMt. El sMt heterólogo y no enriquecido con N-cUs podría ser responsable de la recuperación clínica observada en este estudio

Palabras clave: dermatitis atópica, intolerancia alimentaria, microbiota, piel, transferencia.

Introduction

The “hygiene hypothesis” connected premature vulnerability to microbes to the existence of allergic conditions, including food allergies (Schaub *et al.,* 2006). This phenomenon suggested that microbial exposure during infancy can educate the immune response, resulting in accurate maturation and diminished deviation of the immune response later in life. Arousing interest has been shown in the importance of gut and skin microbiota transplantation (sMt) in health and disease conditions. Considering the hygiene hypothesis, two stages of life (i.e. infancy and untimely childhood) appear as crucial moments for the establishment of microbial colonization, immune response, and potential systemic disease. The skin and gut microbiomes are vital during this process (Hammond *et al.,* 2021). Several cutaneous disorders are related to an unbalanced skin microbiome. Investigation on the integumentary microbiota has gained arousing interest (i.e. therapeutical and cosmetic targeted approach) in which several studies denoted entanglement of the cutaneous ecology of humans, dogs, and cats (Dréno *et al.,* 2016; Liang *et al.,* 2021; Tizard & Jones, 2018). Countless interplay between the cutaneous microbiota and the immune system have been well recognized, in which a diverse and balanced microbiota is crucial for healthy skin (Liang *et al.,* 2021).

To the best of our knowledge, both skin microbiota alterations and manipulation of skin dysbiosis in cutaneous adverse food reactions (caFr) among dogs have not been reported with detailed clinical analysis. This prompted us to perform this study in which heterologous unenriched skin microbiota transplantation (sMt) was applied to 10 dogs with caFr, to change the cutaneous microenvironment, along with searching for alternative strategies for cutaneous disorders.

Materials and methods

Declarations-ethics approval

Dogs participating in this study were all referred to the University of Adnan Menderes, Faculty of Veterinary, Department of Internal Medicine, Small Animal Clinics. All dogs were clinically examined for disease state. Microbiome samples used for this analysis were collected with written owner consent. Owners were aware that sMt was performed for research purposes only. The present study was approved by the local ethics committee of Aydın Adnan Menderes University-HADYEK with number 64583101/2019/022 and the owners provided their consent, having been fully informed, to participate in this study.

Subjects included and study design

A total of 10 skin swab samples were analysed. The timeline included day 0 assigned as before sMt and day 21 as after sMt with N-cUs. All dogs resided in Aydin Municipality where the faculty is located. Ten dogs, from different ages (2 to 6 years old) were included. Relevant data regarding breed, age, gender, and medical records were recorded. All 3 researchers along with assistants (from a group of PhD and Master of Science Students) triaged, examined, and organized the animals eligible to participate in the study. All cases were diagnosed with caFr by i) confirmation through a positive challenge, which was followed by an elimination diet (including both phases of restriction and then provocation) (Martín *et al.,* 2004; Olivry & Mueller, 2019) ii) clinical background and iii) reduction of pruritus following an elimination diet (novel hydrolyzed protein-based diet with low carbohydrate, 17 %) lasting at least 6 weeks (Rondelli et al., 2015). During the study, dogs did not receive any drug or treatment capable of interfering with the results. Allergen-specific serum immunoglobulin (Ig) E was measured using Polycheck in vitro Allergen Testing Cassettes (.Polycheck Allergy Diagnostics, Germany), to support and identify suitable ingredients for an elimination diet trial (Tang *et al.,* 2020). Epidermal corneometric analysis (skin pH and hydration) was evaluated using the Callegari Soft Plus Device (.Callegari, Italy). Other relevant diseases for differential diagnosis were ruled out by routine biochemistry, endocrinology, haematology, skin cytology and dermatological examination.

Microbiota analysis

Sample collection, DNA extraction, library preparation, and sequencing

Samples were analysed by next-generation sequencing to determine both the relative and absolute presence of bacteria before (day 0) and after (day 21) treatment. Samples were collected with a swab collection kit (MiDOG LLC service). Before sMt, swab samples were collected from skin lesions, and thereafter sMt was performed onto the lesional skin. On day 21, samples were collected from the same topographical location with a sterile, DNA-free swab included in the collection kit (twisting off and twirling the swab 10 times over the lesion). Then, the swab tip was broken off into a sterile tube pre-filled with a DNA/RNA preservative (Tang *et al.,* 2020; Ural *et al.,* 2022; Ural *et al.,* 2023). All samples were then shipped for processing to the MiDOG LLC testing center (Irvine, California).

Genomic DNA was obtained through ZymoBIOMICS™-96 DNA kit (Zymo Research Corp.) with a Hamilton Star. liquid handling robot (Hamilton Company, Reno, NV) (Rondelli et al., 2015). Sample library procedures and relevant informatic analytes regarding bacterial profiling were performed by using the Quick-16S NGS Library Prep Kit (Zymo Research Corp.). The 16S rDNA V1-V3 region was targeted for bacterial analysis. Primer sequences were established on the MiDOG LLC service. Other relevant methodologies were as described elsewhere. Profiles of microbiota were established through MiDOG LLC bioinformatics analysis pipeline. Entire phylotyping was computerized through percentage proportions considering the total number of sequences in each skin sample (Tang *et al.,* 2020).

Methods of evaluation of the efficacy of sMt in dogs with cutaneous adverse food reactions

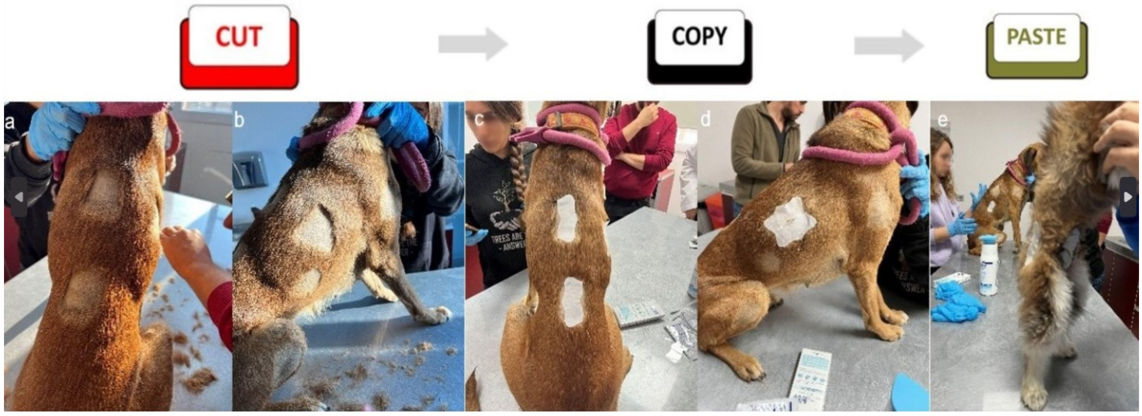
The canine atopic dermatitis extent and severity index fourth version (CADESI-04) was used to evaluate the lesions (Olivry *et al*., 2014). Furthermore, to evaluate the severity of pruritus, the Visual Analogue Scale of pruritus scoring (VAS) was used, composed of the full range of possible values (0 to 10) (Rybníček *et al*., 2009).

Skin microbiota transplantation by use of N-cUs

The same research group developed a previous methodology of both heterologous and autologous origin of sMt based on unenriched sMt (Ural *et al.,* 2022, 2023). Changing the local microbiome (with possible different effects on the whole skin widespread) with a heterologous origin of sMt was hypothesized, and all skin samples for microbiota analysis were withdrawn from the transplant site. Day 0 was planned as referral (initial sampling without any application) whereas day 21 was preferred as second sampling (after sMt data). Allocation days (days 0, 5, and 12) were selected based on several transplantations, based on our previous experience (Ural *et al.,* 2022, 2023). The evidence-based data indicated that tape-stripping removal of skin produced a newly existing skin microbiome with similarities to deeper stratum corneum layers for up to 2 weeks (Zeeuwen *et al.,* 2012). Briefly, five of 10 dogs received three sMt on days 0, 5 and 12, four dogs received two sMt on days 0 and 5, and one dog received un sMt on day 0. The number of sMt procedures was based on algorithmic decision and observation of researchers through evidence of limited novel hair growth (via DermLite DL4 dermatoscopy) and decreased erythema and supported by previous studies (Ural *et al.,* 2022, 2023).

A total of three healthy dogs were selected as sMt donors after screening for disease status, serum biochemistry, endocrinology, and haematology. The dogs were also checked for skin and gut microbiota analysis. No clinically relevant pathogen was detected, nor disease state was evident during the trial. In each case, at least one of the N-cUs was unboxed, and every single strip was detached. Four apparent healthy areas of skin with normal hair growth and no visible lesions were selected (Figures 1a and 1b). Strips moistened in lactated Ringer's solution (with a skin-appropriate pH of 6.5) were placed over the partially clipped areas (CUT) and allowed to adhere (COPY) for twelve minutes (Figure 1c and 1d). All strips were then transferred to the diseased and moistened skin (Figure 1e) and allowed to adhere (PASTE) for at least 15 minutes (Ural *et al.,* 2022, 2023). Finally, all strips were removed. None of the dogs were bathed during the trial. Unpleasant side effects involving urticaria and suddenly appearing erythema were monitored for possible existence.

Figure 1



Skin microbiota transplantation (sMt) for cutaneous adverse food reactions in dogs. Three different stages of unenriched sMt: a-b) clipping of donor (CUT), c-d) heterologous origin of copying skin microbiota through N-cUs (COPY), and finally e) recipient transplantation of unenriched skin microbiota through N-cUs (PASTE).

Statistical analysis

Quantities of α-diversity and evenness and quantity of observed species were estimated using the Shannon index. CADESI-04, VAS pruritus, skin pH and hydration were reported as mean and standard deviation. Statistical comparisons before and after treatment were evaluated using the Mann-Whitney U test (GraphPad Prism, Version 9).

Results

Demographic data

Demographic data including sex, breed, age, and number of sMt procedures are shown in Table 1. The number of sMt procedures depended on clinical scoring, epidermal corneometric analysis and response to the first sMt procedure. Five out of 10 cases received a maximum of three sMt procedures, after which clinical response was evident, and no more applications were deemed necessary. CADESI-04 scores particularly served as a guide for further sMt procedure; if the latter decreased, sMt was not continued.

Table 1

Skin microbiota transplantation sMt for cutaneous adverse food reactions in dogs

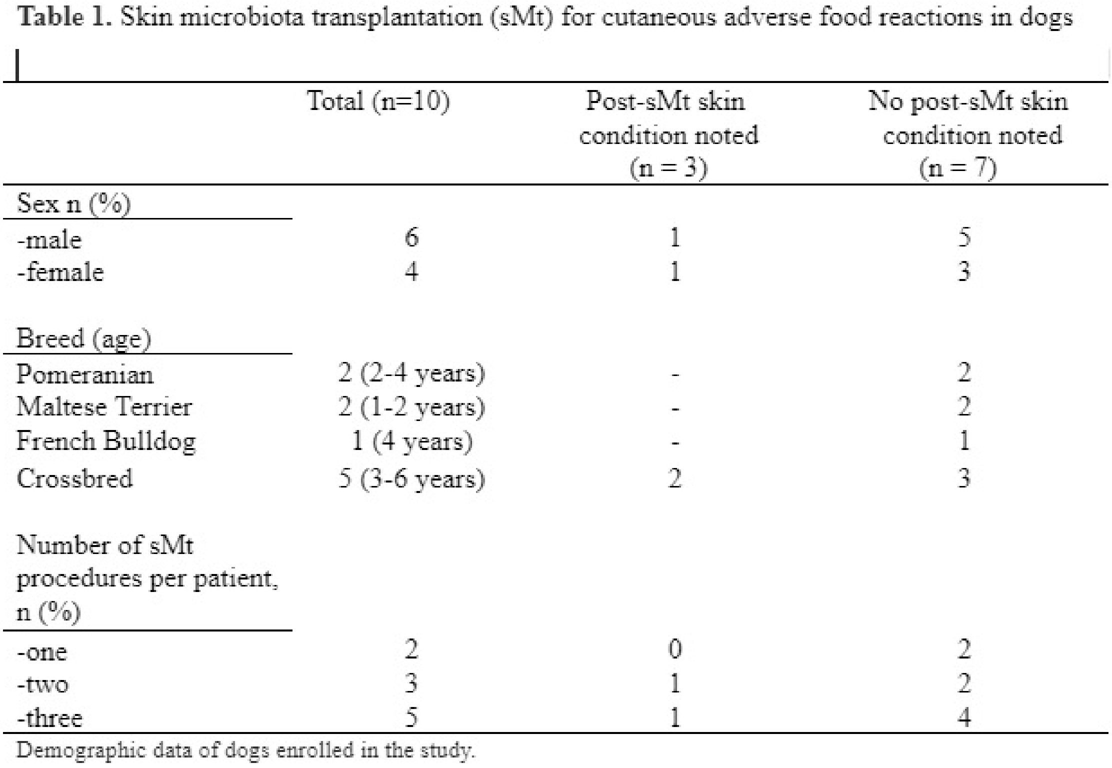


Table 1. Skin microbiota transplantation (sMt) for cutaneous adverse food reactions in dogs

	Total (n=10)	Post-sMt skin condition noted (n = 3)	No post-sMt skin condition noted (n = 7)
Sex n (%)			
-male	6	1	5
-female	4	1	3
Breed (age)			
Pomeranian	2 (2-4 years)	-	2
Maltese Terrier	2 (1-2 years)	-	2
French Bulldog	1 (4 years)	-	1
Crossbred	5 (3-6 years)	2	3
Number of sMt procedures per patient, n (%)			
-one	2	0	2
-two	3	1	2
-three	5	1	4

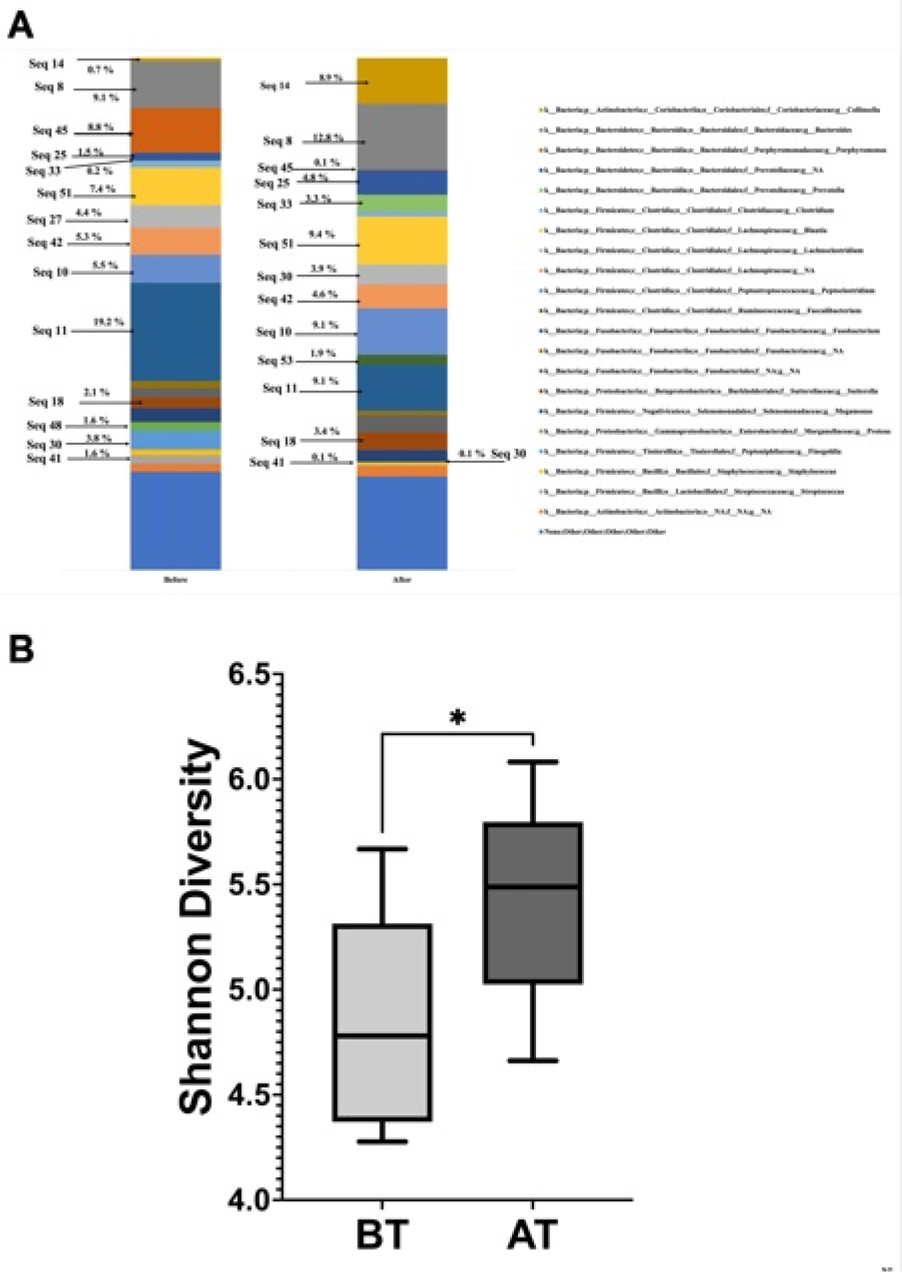
Demographic data of dogs enrolled in the study.

Demographic data of dogs enrolled in the study

Microbial composition before and after sMt

The microbial composition at different time points, before and after sMt is shown in Figure 2a. Microbiota analysis before and after sMt treatment, respectively, revealed that, at the phylum level, Firmicutes were the most abundant (32.98 % vs. 32.90 %) followed by *Fusobacteria* (22.28 % vs. 13.25 %), *Bacteroidetes*(19.54 % vs. 21.09 %), *Proteobacteria* (3.74 % vs. 3.50 %) and *Actinobacteria* (2.24 % vs. 10.92 %). Unless stated otherwise average relative abundances are reported. Full-length analysis of the 16S rRNA gene successfully classified the dominant bacteria at species level. The taxa comprising the canine skin microbiota studied included unclassified *Actinobacteria* (1.59 %), an unclassified, unknown species belonging to the family *Fusobacteriales* (3.11 %), an unclassified species belonging to the family *Lachnospiraceae* (5.3 %) and *Prevotellaceae* (1.54 %) (Figure 2a). In addition, the presence of *Fusobacterium* (19.16 %), *Peptoclostridium* (5.49 %), *Blautia* (7.39 %), *Porphyromonas* (8.75 %) and *Bacteroides* (9.1 %) were determined at the genus level in the pre-treatment period. Remarkably, in the genus-based evaluation following sMt, evidenced presence of increased *Faecalibacterium* [0 to 1.9 %], *Peptoclostridium* [5.49 % to 9.11 %] and *Collinsella* [0.65 % to 8.91 %], and decreased *Fusobacterium* [19.16 % to 9.06 %], *Porphyromonas* [8.75 % to 0.13 %], *Streptococcus* [1.63 % to 0.14 %] and *Staphylococcus* [1.09 % to 0.49 %] was evidenced. Microbiota variation (median observed species) changed from 25 to 42.5 (Figure 2b). Hence, to differentiate disease and health status of each dog enrolled, categorization was based on their bacterial diversity. 110 long-read DNA sequencing was applied to a subset (n = 11) of the clinical samples, in which following sMt, healthier dogs exhibited greater species diversity. Compared to initial values (before sMt) there was a significant loss inα-diversity detected by using the Shannon Index mean values (4.847 vs. 5.43, $p < 0.01$) (Figure 2b).

Figure 2

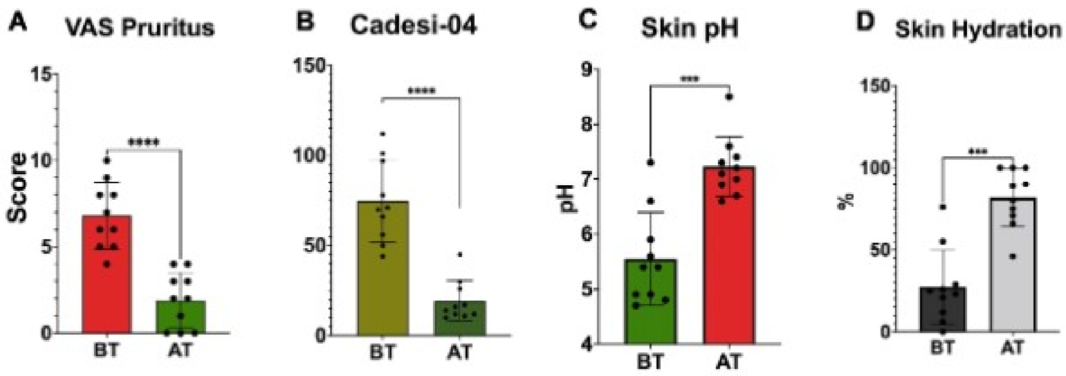


a) Distribution of bacterial species before and after skin microbiota transplantation (sMt) for treatment of adverse skin reactions to food in dogs. The bacterial species represented by sequence code are colour-coded and are valid across columns. b) Shannon index (mean) for measurements of α-diversity before (BT) and after (AT) sMt.

Clinical biomarkers for remission/recovery

Clinical parameters evaluated are reported in Figures 3 and Table 2. Treatment with sMt positively/effectively diminished clinical signs associated with caFr and drastically reduced median VAS pruritus (6.5 vs. 2 p<0,001) and mean ± SD) CADESI-04 scores (74.50±22.62 to 19.30±11.30) (p<0,001). In addition, median values for skin pH (5.4 vs. 7.1) and hydration (24 vs. 84.5) denoted altered skin barrier functioning, before and thereafter sMt treatment, respectively, (p<0,001) (Table 2). Clinical records of selected cases are shown in figures 4, 5A and 5B.

Figure 3



Epidermal corneometric interpretation before and after sMt for dogs with cutaneous adverse food reactions. A: VAS pruritus; B: Cadesi-04; C: Skin pH; D: Skin hydration.

Table 2

Skin microbiota transplantation sMt for cutaneous adverse food reactions in dogs

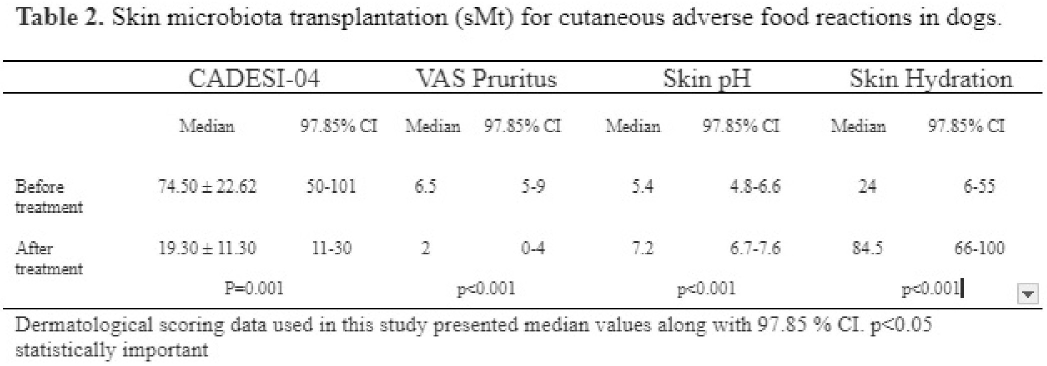


Table 2. Skin microbiota transplantation (sMt) for cutaneous adverse food reactions in dogs.

	CADESI-04		VAS Pruritus		Skin pH		Skin Hydration	
	Median	97.85% CI	Median	97.85% CI	Median	97.85% CI	Median	97.85% CI
Before treatment	74.50 ± 22.62	50-101	6.5	5-9	5.4	4.8-6.6	24	6-55
After treatment	19.30 ± 11.30	11-30	2	0-4	7.2	6.7-7.6	84.5	66-100
	P=0.001		p<0.001		p<0.001		p<0.001	

Dermatological scoring data used in this study presented median values along with 97.85 % CI. p<0.05 statistically important

Dermatological scoring data used in this study presented median values along with 97.85 % CI. p<0.05 statistically important

Figure 4

Skin microbiota transplantation (sMt) for cutaneous adverse food reactions in dogs. Appearance of case 1 before and after sMt (week 3).

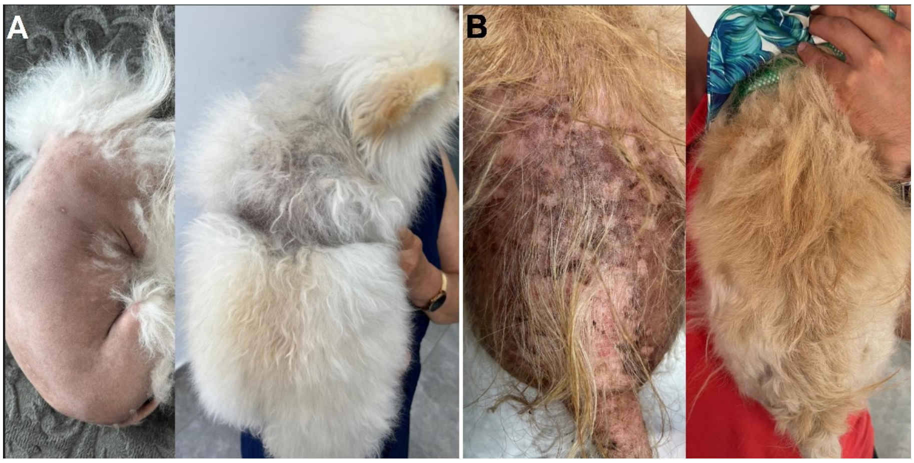


Figure 5

Baseline and treatment characteristics of two cases (A y B) at day 0 and week 6.

Discussion

The present study herein describes the successful treatment of primary and/or secondary skin lesions due to caFr in dogs using unenriched sMt of heterologous origin, as evidenced by a) decreased pruritus VASand

CADESI-04 scores and b) altered skin microbiota as shown by increased mean observed species and changes in skin microbiota composition. Following sMt, *Firmicutes, Fusobacteria* and *Proteobacteria* abundances were decreased, whereas *Bacteriodetes* and *Actinobacteria* relative abundances were elevated. Interestingly *Actinobacteria*abundance was strikingly high after sMt (2.24 % vs. 10.92 %), in contrast to previous reports. Similarly, to human cutaneous ecology, the most abundant phyla detected among dogs were *Proteobacteria, Firmicutes, Actinobacteria*, and *Bacteroides*. On the other hand, given that *Actinobacteria* prevails in humans whilst it is slightly more abundant among dogs (Costello *et al.,* 2009; Grice *et al.,* 2008; Grice & Segre, 2011; Rodrigues Hoffmann *et al.,* 2014), in the present study, following sMt, recovered dogs showed greater abundance of Actinobacteria and mildly increased abundance of Bacteriodetes. Although not shown, Corynebacteriales and Coriobacteriales orders were the vast majority of Actinobacteria phylum following sMt application. Interestingly, although not surprisingly, at the genus level average relative abundances of Streptococcus (1.63 % to 0.14 %) and Staphylococcus (1.09 % to 0.49 %) were all decreased following sMt. This could be related to the recovery of skin among dogs, with withdrawal of clinical signs.

Given the microbial composition in different skin regions among humans, with Staphylococcus and Corynebacterium spp. predominating in moist areas (Griece *et al.,* 2009), and Corynebacteria being members of the healthy cutaneous microecology, it is quite strenuous to differentiate among infection, colonization, and contamination even if the latter agents are isolated from purulent material (Esteban *et al.,* 1999). Further studies are necessary to clarify the role of Corynebacteria in healthy skin. Another possible explanation is that the movement of unenriched microbiota by use of N-cUs possibly transferred and relocated the entire cutaneous microenvironment from donor to diseased dogs in the present study.

Skin microbiota transplantation in dogs is still in its infancy, with little published evidence and few clinical trials reported, which unfortunately limits its use, and the extent of application by veterinarians remains unknown. In the present research, treatment with sMt was found to effectively control/manage signs of caFr and drastically reduce median VAS pruritus (Table 2, Figure 3a) in the absence of any immunosuppressive drug and/or antibiotic usage. Furthermore, clinical recovery was also supported by epidermal corneometric analysis Median values for skin pH and hydration selectively denoted altered skin barrier functioning, before and after sMt treatment. This efficacy was also related to sMt application. Cutaneous adverse food reactions negatively affected skin barrier functioning, as before treatment median pH and hydration values were improved after treatment (Table 2). Since the association between abnormal pH and skin diseases has been reported, all clinical analyses observed in this study should at least partly explain the role of sMt in supporting skin barrier integrity.

To the authors' knowledge, no previous research to date has investigated the likelihood of conducting a skin microbiota transplant capable of moving the entire integumentary bacterial community, along with its complex network of metabolic interactions (Perin *et al.,* 2019). The importance of relocating a community is based on the condition that several members of the microbiome require their community partner (selected microbes counteract with obligately mutualistic metabolism, oftentimes denoted as syntropy, or cross-feeding fashion of living) (Morris *et al.,* 2013). According to the human gut microbiome analysis, there is emerging proof of cross-feeding in commensal bacteria to produce bioactive short-chain fatty acids among healthy hosts (D'hoe *et al.,* 2018; Hoek & Merks, 2017; Louis & Flint, 2017).

The use of unenriched sMt in this study may potentially be considered a matter of concern. Elevated amounts of bacterial DNA were present in hair follicles in contrast to the epidermis. Bacterial DNA was also present in the dermis and adipose tissue, although the viability of those bacteria remains unclear (Bay et al., 2013; Nakatsuji *et al.,* 2013;). Taking this into account, it was hypothesized that the microbiome belonging to deeper layers of skin is the core skin microbiome (Callewaert *et al.,* 2021). We clipped a limited portion of skin among donor dogs, to prevent probable bacterial DNA existing in hair follicles. N-cUs produced and launched for confiscation of blackheads on the T-Zone were specifically used for sMt, like what has been described elsewhere (Ural *et al.,* 2022, 2023). The latter strips are capable of effectively removing waste material, through activation with water, attaching with dirt, and therefore cleaning superficial skin. Its content of citric acid, capable of trimming superficial layers of old skin tissue, may have helped recovery observed in this study, like what has been reported previously (Ural *et al.,* 2023). Citric acid was responsible for inhibiting *Pseudomonas ceramidase* which consequently suppressed inflammation in atopic dermatitis (Inoue *et al.,* 2010). Given this data, citric acid involved in N-cUs used in this study may have helped the regression of clinical signs through this mechanism. Moreover, citric acid supplementation played a pivotal role in lowering the pH of the intestinal tract, by reducing the population of pathogenic bacteria, specifically E.coli (as it is highly sensitive to acidity), through mechanisms of penetrating pathogenic cell walls leading to suppression of their growth/ reproduction (Russel & Diez-Gonzalez, 1998), whilst elevating the number of beneficial bacteria (Gunal et al., 2006), capable of modulating bacterial cell cytoplasmic enzymes and transport systems preparing cells being resistant to osmotic pressure (Cho & Finocchiaro, 2010). Furthermore, in the present study, a relative abundance of *E. coli* was 2.8 to 8.2 % in 6 out of 10 dogs and shifted to 0 to 0.1 % following sMt. We might conclude that the N-cUs might probably display antimicrobial behaviour against pathogens, whereas support the growth of beneficial bacteria.

A previous study reported by our group (Ural *et al.,* 2022) involving two clinical cases evidenced the beneficial usage of transferring unenriched skin microbiota niches between two heterologous hosts, defined as heterologous skin microbiota transplantation (hSmT). In that study, hSmT involved transfer by use of N-cUs between healthy donors to two different dogs, with scabies. VAS pruritus scores were diminished markedly from day 0 (initial hSmT day) to day 21. Skin scrape was negative after day 2 of hSmT and remained negative throughout the study. Unenriched skin microbiota transplantation from a healthy donor to the dogs with scabies, resulted in clinical and parasitological recovery through modification of the cutaneous microenvironment, without any drug application. Another study (Ural *et al.,* 2023) examined the feasibility of sMt via N-cUs for transferring unenriched skin microbiota communities, to investigate its influence on erythema scores. In this study, four different healthy anatomical locations in each case (autologous) were selected, where the strips were placed (Ctrl C), and then moved (Ctrl V) to the skin tissue with erythema, repeating the procedure on days 5 and 12. The skin erythema severity and atopic dermatitis area and severity index were all statistically decreased throughout the study, likely due to the efficacy of sMt.

However, some limitations may be mentioned in our study. We do not know (still at the time of writing) the viability of transferred bacteria on N-cUs. Furthermore, the longevity of sMt strips is also unknown. However, given that the tape-stripping method for collecting viable skin bacteria previously described resulted in accurate skin microbiome composition (Ogai *et al.,* 2018) and higher alpha diversity (Rungjang *et al.,* 2022), N-cUs could have helped transfer beneficial bacteria or bacterial DNA immediately in the present study. As this was not the primary objective of this study, test strips were not sent for microbiome analysis. As clinicians we prioritized to transfer test strips immediately, so we found no opportunity to divide test strips for interpretation. Hence, we also could not show the movement of DNA from donors to diseased dogs. In a previous study conducted in humans by Perin *et al.* (2019), the DNA of several of the unique, rare-arm bacteria remained in their novel back environment for a day, and an abrupt drop in this signal was observed. The researchers could not identify the likely causes of the collapse of these bacteria for colonizing the recipient site. On the other hand, although we did not recognise transfer material microbiome, in the present study, we showed evidence of transfer dynamics as skin microbiome analyses were performed. Furthermore, increased median observed species as detected by skin microbiota analysis, along with clinical recovery without any usage of drug or nutraceuticals partially showed evidence that beneficiary bacteria should have relocated to treated areas. This limitation should be further investigated, and future research could improve the interpretation of the viability of transferred bacteria.

Conclusions

This study provides evidence that it is possible to relocate the cutaneous microenvironment from donor to diseased dogs (Ctrl X, Ctrl C and Ctrl V). Future studies should be aimed at analysing the viability and colonization success of transferred skin microbiomes between the different sites of two dissimilar individuals.

Unfortunately, due to insufficient project financial support, we were unable to analyse the microbiota evident on N-cUs, which remains the aim of our next study. It is possible to suggest that citric acid could have helped lower cutaneous pH and consequently caused bacterial death due to suppression of the NADH, or, alternatively, modified local epidermal pH (Su *et al.,* 2014) which could be responsible for antimicrobial behaviour and microbiota changes observed at this study. The Shannon index values and median observed species, before and after sMt, likely support this hypothesis.

Acknowledgements

Collected samples were shipped for processing to the MiDOG LLC testing centre (Irvine, California), profiles of microbiota were established through MiDOG LLC service, and all data and relevant fields were received in electronic transformation and through email.

Author contribution

Kerem Ural: Conceptualization, data curation, methodology; supervision; visualization; and writing; original draft preparation and writing; review and editing. Hasan Erdogan: Methodology; data curation; visualization; writing; review and editing. Songul Erdogan: Data curation; writing; review and editing.

Conflict of interest

There is no conflict of interest, including financial, personal, or other relationships, with other persons or organizations.

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