Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
30 (4): 455-461, 2024
DOI: 10.9775/kvfd.2024.31200

Research Article

Regional Biophysical Variations in Canine Atopic Dermatitis: Non-Invasive Mapping of Skin Parameters

Hasan ERDOGAN [1 (*)] Songul ERDOGAN [1] Kerem URAL [1]

[1] Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Internal Medicine Department, TR-09900 Aydın - TÜRKİYE

(*) **Corresponding author:** Hasan ERDOĞAN
Phone: +90 256 220 6249
Cellular phone: +90 542 805 9355
Fax: +90 256 214 6687
;E-mail: hasan.erdogan@adu.edu.tr

How to cite this article?

Erdogan H, Erdogan S, Ural K: Regional biophysical variations in canine atopic dermatitis: non-invasive mapping of skin parameters. *Kafkas Univ Vet Fak Derg,* 30 (4): 455-461, 2024.
DOI: 10.9775/kvfd.2024.31200

Article ID: KVFD-2024-31200
Received: 13.03.2024
Accepted: 22.06.2024
Published Online: 25.06.2024

Abstract

Mapping the skin with various biophysical properties can demonstrate not only skin condition but also organize a convenient approach to managing diseases. The aim of this study, mapping of the regional alteration for 6 biophysical parameters with non-invasive method. Totally 39 dogs [healthy (n=16) and canine atopic dermatitis (n=23)] were enrolled. Skin pH, hydration, melanin, sebum content and temperature for pinna, perilabial region, hind-fore feet (dorsal metacarpus-dorsal metatarsus), axillae, lateral thorax/flanks, elbows, abdomen/inguinal and ventral tail were measured. Atopic dogs exhibited more acidic skin (P<0.001) compared to healthy dogs, with the thorax/flanks region showing significant regional differences (P=0.037). Hydration levels were consistently lower in atopic dogs (P<0.001), with significant regional differences observed in the abdominal/inguinal region (P=0.037). Skin temperatures were generally higher in atopic dogs, with a significant increase noted in the tail (P=0.037), while other areas showed no significant differences. Melanin and sebum content did not exhibit significant regional variations. In conclusion, this study demonstrated regional variations in biophysical parameters in atopic dogs using non-invasive measurements. These findings provide valuable insights for understanding canine atopic dermatitis and its management.

Keywords: Atopic dog, Non-invasive measurement, Skin-barrier dysfunction, Skin hydration, Skin pH

Introduction

Canine atopic dermatitis (caD) is a chronic inflammatory, multicomplex allergic/pruritic skin disease [1,2] that is frequently encountered in dogs [3]. caD was aroused incidence/spatial distribution recognized for elevated skin permeability [4] and immune response [5,6]. Although the pathophysiology of caD is not fully understood, it was revealed that the skin barrier function is impaired, allergens infiltrate the skin stratum, and the immune system is activated leading to clinical symptoms [7,8].

Various biophysical parameters using noninvasive technics have been evaluated for human skin integrity [8-10]. In dogs skin integrity was mostly evaluated with transepidermal water loss (TEWL) [7,11,12]. Moreover, skin pH and hydration are alternative parameters for assessment [7,13]. A pilot study related to the comparison of skin barrier integrity with different methods among healthy and atopic dogs claimed that pH and skin hydration could be better biomarkers in contrast to TEWL [7].

As the largest organ of the body, the skin has most of its functions including protection by providing a physical barrier, sensation, heat regulation, and excretion in addition to the secretion that controlling water and electrolytes keeping [14]. Mapping of the skin with various biophysical properties can demonstrate not only skin condition but also organize a convenient approach to the management of diseases. In the present study, we aimed to investigate skin hydration, sebum content, melanin, temperature, and pH in the CADESI-4 specified body sites related to caD with mapping of skin.

Material and Methods

Ethical Statement

Dogs presented to the Animal Hospital of the Veterinary Faculty at Aydın Adnan Menderes University and diagnosed with atopic dermatitis were included in this study. Ethical approval was obtained from Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (No: 64583101/2024/22). Additionally, comprehensive

Table 1. *Inclusion/exclusion criteria of the study*

Criteria	Healthy Dog	Atopic Dog	References
Inclusion criteria			
Anamnesis and clinical signs consistent with skin disease	-	at least one-year clinical history	Hensel et al.[16]
Favrot Criteria	-	at least 3 criteria	Favrot et al.[15]
Canine Atopic Dermatitis Extent and Severity Index (CADESI-04)	0	>10	Olivry et al.[1]
Exclusion criteria			
• At least two weeks treatment history with topical and/or systemic antibiotics, glucocorticoids, immunosuppressive and/or antipruritic therapy			
• To be used shampoo or cleaning solution in the last twenty-four hours			
• Concomitant endocrine disorders • Gastrointestinal disease (dehydration)			

information was provided to the owners of all dogs, and written consent was obtained from them.

Study Population: Exclusion and Inclusion Criteria

Different ages, breeds and both sexes, to those of dogs 16 healthy and 23 with atopic dermatitis were enrolled in this study.

The inclusion/exclusion criteria of healthy and atopic dogs were presented in *Table 1*. Dogs were composed of any history or clinical signs consistent with skin disease. Within the diagnosis of atopic dermatitis, studies by Favrot et al.[15] and Hensel et al.[16] as well as at least one year of clinical history were considered. The clinical index of atopic dogs was performed according to the canine atopic dermatitis extent and severity index (CADESI-4) [17].

Dogs were excluded if they had undergone at least two weeks of topical or systemic antibiotic, glucocorticoid, immunosuppressive, or antipruritic therapy, or if they had recently used shampoo or cleaning solution. Additionally, dogs with concurrent endocrine problems and gastrointestinal disease related dehydration were eliminated *(Table 1)*.

Clinical Scoring with PVAS and CADESI-4

Pruritis score was recorded using the pruritus visual analog scale (PVAS) as described previously by Hill et al.[18]. Briefly, The PVAS score was assessed by owners to indicating the severity of the itching on the scale that consists to 10 scales with 0 (no pruritus) to 10 (extremely pruritus).

CADESI-4 was used for evaluating skin lesion severity that were based on scaling of skin lesions (erythema, lichenification, and excoriation/alopecia) on each of 20 body parts with none to severe four-point and it was classified as mild (10 point), moderate (35 point) or severe (≥60 point) atopic dermatitis [1].

Biophysical Parameters

Before the beginning of measurement, all parameter cartridges were calibrated according to the manufacturer's instructions, and the calibration process was repeated in the same procedure when necessary. Hydration, sebum content, melanin, temperature, and pH parameters were measured at three times at the body sites specified CADESI-4 (hind-fore feet, axillae, perilabial region, pinna, ventral tail, lateral thorax/flanks, elbows, hindlimbs, abdomen/inguinal) by the same investigators and for each body sites scored according to Olivry et al.[1].

All parameters were assessed using the Corneometer (®Callegari, Soft Plus, Italy). With dogs in lateral recumbency/standing, the device probe was placed on both the right and left body side without the abdomen/inguinal region of the dogs. Measurements were obtained by placing the probes at the specified body sites. The working principle of all parameters was summarized in *Fig. 1*.

Skin hydration is based on a capacitive measurement principle of the dielectric constant that changed with the water of the skin. In addition to hydration, skin melanin measurement is based on strength of the absorbed and the reflected light at λ1=875 nm, λ2=660 nm. Skin surface temperature is based on non-contact infrared measurement.

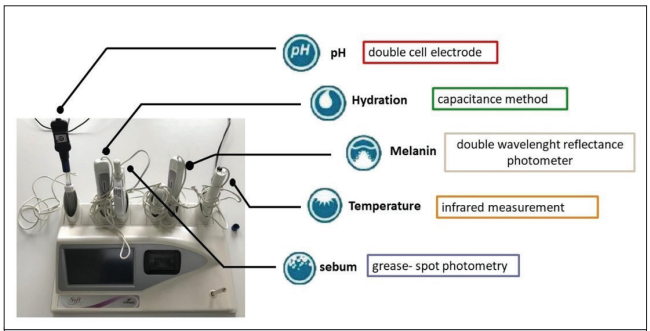

Fig 1. Working principle of all biophysical parameters

The skin surface pH was measured by a device probe that worked with ±1% accuracy based on double cell electrode system. The probe was removed from the buffer solution after it was kept at 24°C with 2 applications (a) activation button and b) sensor. Then, it was contacted with the skin by pushing it slightly forward with the activation button at the same time. Analysis and measurement were carried out in a very short time with a positive warning sound from the device.

Statistical Analysis

Statistical analyses were conducted using SPSS® Software version 26 (IBM SPSS Inc., Armonk, NY, USA). The normality distribution of continuous variables was assessed using the Shapiro-Wilk test, and data were presented as mean±standard error (SE). Mann-Whitney U Test was utilized for comparing groups and Spearman correlation were used for correlation analyses. A P-value of less than 0.05 was statistically significant.

Results

Demographic Data

Encountered total of 39 dogs were grouped as healthy controls (n=16) and caD (n=23). Out of dogs with caD, 18 were pure-bred and other relevant 5 mixed-breed. Pure-breed atopic dogs were consisted of French bulldog (n=4), Pomerian (n=4), Pug (n=3), West Highland white terrier (n=2), Yorkshire terrier (n=1), Golden retriever (n=1), Border collie (n=1), Dogo Argentino (n=1), Toy Poodle (n=1). The mean age was 3.9 years (range 18 month-11 years) of dogs with caD and 3.9 years (range 1-7 years) of healthy.

Regional Variation of the Biophysical Parameters

The overall results for the biophysical parameters were summarized in *Table 2* and the heatmap for the visualization of group differences was shown in *Fig. 2*.

The mean pH, hydration, melanin, sebum and temperature (°C) overall were 5.66±0.18, 27.60±1.44, 27.20±3.04, 16.92±1.77 and 34.11±0.46 in atopic dogs versus 7.04±0.04, 43.38±2.73, 12.50±0.56, 38.52±1.75 and 32.61±0.23 in healthy ones, respectively. Considering the total mean variable, all parameters of atopic dogs were found to be significant compared to healthy (P<0.001). It was revealed that skin pH was more acidic in atopic dogs (P<0.001). Additionally, reduced hydration (P<0.001) and sebum (P<0.001) but increased melanin levels (P<0.001) and temperature (P<0.001) were observed in atopic versus healthy dogs.

Hydration levels were consistently lower in atopic dogs compared to healthy ones at all sites (P<0.001), but the regional statistical difference was only obtained for the abdominal/inguinal region (P=0.037). Considering

Table 2. *Regional skin pH, hydration, melanin, sebum and temperature values of healthy and atopic dogs*

Region	pH		P Value	Hydration		P Value	Melanin		P Value	Sebum		P Value	Temperature		P Value
	Atopic	Control		Atopic	Control		Atopic	Control		Atopic	Control		Atopic	Control	
Pinna	5.47±0.33	6.43±0.17	0.857	25.0±4.5	53.31±6.8	0.111	22.39±5.6	12.62±3.4	0.403	19.7±4.0	21.87±5.1	0.638	33.7±1.3	32.68±0.6	0.538
Axillae	5.93±0.28	6.60±0.1	0.491	21.65±4.4	38.75±5.8	0.403	29.95±6.5	10.12±2.7	0.363	18.88±4	35.75±4.5	0.857	33.31±1.3	34.57±0.4	0.691
Dorsal Metacarpus	5.66±0.3	7.19±0.1	0.403	30.30±5.4	32.00±4.5	0.290	18.08±4.8	17.50±2.9	0.745	16.65 ±4.1	36.75±3.4	0.174	33.80±0.6	29.30±0.4	0.691
Dorsal Metatarsus	5.38±0.3	7.46±0.1	0.857	20.78±5.8	27.50±3.7	0.446	20.65±5.7	9.31±1.5	0.801	11.30 ±3.1	42.56±3.1	0.691	34.76±0.4	29.03±0.3	0.174
Abdominal/ Inguinal	5.88±0.3	7.44±0.1	0.446	24.73±5.9	68.31±6.7	0.037	32.3±7.2	8.87±1.6	0.914	15.95±4.2	43.75±3.1	0.446	33.83±0.4	33.1±0.7	0.801
Perilabial region	5.64±0.3	7.07±0.1	0.257	33.47±7.1	34.00±3.1	0.971	34.26± 7.8	19.43±2.7	0.257	15.30±3.5	43.75±3.7	0.587	34.49±0.3	33.23±0.7	0.080
Tail	5.92±0.3	7.12±0.1	0.691	25.08±5.3	25.00±3.8	0.055	35.86±6.4	12.43±2.9	0.691	18.30±4.3	37.00±3.3	0.801	32.55±1.6	32.4±0.6	0.037
Elbow	5.06±0.2	6.97±0.1	0.403	29.8±7.6	42.87±5.1	0.587	26.39±7.0	12.37±2.8	1.00	19.73±4.2	40.81±4.1	0.638	35.22±0.5	34.13±0.4	0.363
Thorax/ Flanks	5.90±0.2	70.7±0	0.037	37.47±7.9	68.75±5.6	0.325	24.86±4.8	9.87±2.3	0.587	16.30±4.4	44.50±4.0	0.691	35.17±0.3	35.00±0.4	0.691

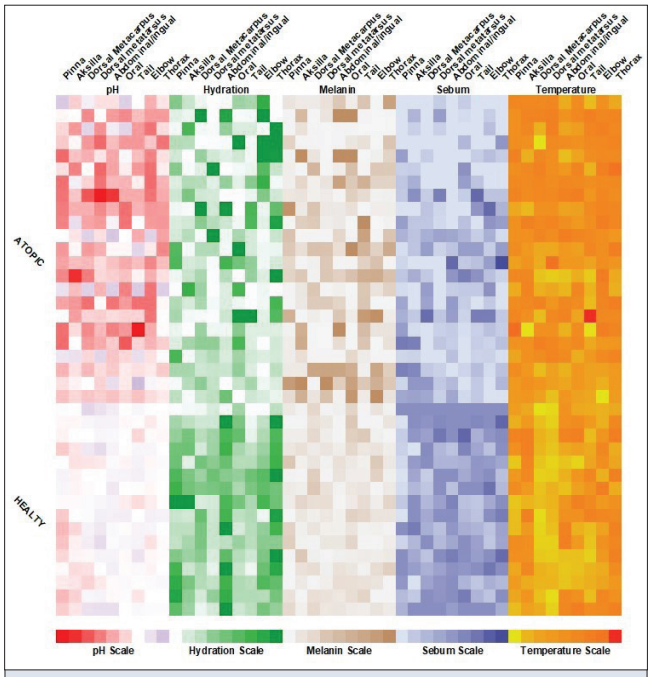

Fig 2. Heatmap colour visualization providing to the regional alteration of the biophysical parameters by compared group

the regional distribution of pH in atopic dogs, it was seemingly more acidic for all the regions (P<0.001), however, a significant difference was only obtained for the thorax/flanks with lower than compared to healthy dogs (P=0.037). Although there was a statistical difference for the mean temperature of atopic dogs as like others (P<0.001), considering regional distribution, it was only higher for the tail with 32.55±1.6 versus 32.4±0.6 at the healthy (P=0.037). There was no significant regional variation of melanin and sebum content in comparison to the healthy group (P<0.05) *(Table 2)*.

Clinical Scoring

Overall, moderate to severe clinical signs were observed in the caD group according to CADESI-4 with the mean score measured as 49.4 (11-180) with mild (n=18) to severe (n=5). Additionally, the mean PVAS was obtained at 7.6 (4-10). For the healthy group, CADESI-4 and PVAS scores were obtained as 0. No correlation (P>0.05) was found between biophysical parameters and the CADESI-4 severity or PVAS in a selected region of atopic dogs.

Discussion

Canine atopic dermatitis is a complicated disease originated from interactive relation between multiple factors mostly related of allergy. All of factors induce caD with disrupting the skin barrier function and leading immunological response. There is increasing evidence, primarily in humans but more recently in dogs, indicating that an impaired skin barrier plays a significant role in the pathophysiology of atopic dermatitis [7,19,20]. There are many clinical and immunological similarities including the distribution of clinical lesions and exposure to allergens through the skin between humans and dogs that ensured the encouragement to research of skin barrier distribution in caD [21]. For this purpose, we aimed to presented various skin biophysical properties including hydration, sebum content, melanin, temperature, and pH to being CADESI-4 specified body sites of dogs with atopic dermatitis for evaluation of skin barrier integrity. We indicated to alteration in the biophysical properties of caD with mapping.

Skin barrier function with biophysical properties of atopic dogs have been investigated by a few studies [7,22,23]. Zając et al.[22] was observed that skin erythema intensity, hydration, and pH might be used for the evaluation of skin lesion severity, although the correlation is limited. In a more recent study performed by Cobiella et al.[7], they compared three consecutive measurements of skin hydration, pH, TEWL, skin absorbance and erythema in the pinna, axillae, interdigital and inguinal region, and they observed that those parameters were used for evaluating skin barrier dysfunction. Conversely, in the study essentially aimed to treatment efficiency of probiotic containing spray on clinical assessment and skin microbiota, they evaluated to skin barrier function. They found to hydration for pinna was reduced after 28 days treatment [23]. In our data was shown that although mean pH, hydration and sebum were downward tendency at all sites (P<0.001), regional variation was not the same for all parameters. The hydration for abdominal/inguinal, pH for thorax/flanks and temperature for tail were differently significant in caD compared with health dogs (P<0.05), however, there was no importance at regional values of sebum and melanin. In line with the investigations, our research suggests that the primary non-invasive parameters that serve as indicators of skin barrier dysfunction are skin pH, hydration, and temperature.

Disrupted skin integrity is having a role on the pathophysiology of caD [7,21]. Skin barrier homeostasis is mostly closely related to the network of microbiota, immune, and chemical stimulant [25]. In human atopy, pH is known to play a regulatory role in this communication [26]. caD was mostly found to be associated with increased skin pH [7,27] due to filaggrin dysregulation and bacterial dysbiosis [28]. Controversially, it has been stated that there are regional differences, as well [7]. In cited study, pH was measured usually higher in atopic dogs for only 4 body sites (inguinal, axillae, pinna and interdigital), however difference was found only for axillae and the inguinal region (P<0.05). Moreover, Oh and Oh [29] was reported to skin pH generally increased in 14 body sites without footpad, interdigital areas, and ears of healthy dog before

and after anesthesia. In our study skin pH was totally measured as acidic of atopic dogs compared to healthy (P<0.001) and only had statistical significance for thorax/flanks (P=0.037). Similar to our study, it is stated that the relationship between the decrease in pH and the high abundance of *S. aureus* was influenced in a physiological, microbiome, and clinical outcomes conducted on cases with atopic dermatitis [30]. Also, there are variable functional discrepancy between species including dermal cells and glands, enzymes or microbiome for maintaining optimal skin function [21,31]. Epidermal gland type [32,33], hairless [29], lesion [7,29] and enzyme secretion [21] play role on the pH regulation as cited by previous studies. Breed, age, time, excitement as other factors also have to be affected on skin pH [34,35].

Lower skin hydration and TEWL were caused by the preventing of water flowing to the epidermis, otherwise increased TEWL and decreased skin hydration resulted from epidermal dehydration [36]. In both scenarios, reducing stratum corneum hydration results in dryness of skin, and dry skin has been linked to pruritus [37]. So, it is seen that the high TEWL and low hydration state has gained in characteristic features for atopic lesions [38,39]. Although a decrease in skin hydration and lower TEWL have traditionally been regarded as markers of skin integrity [36], Cobiella et al.[7] discovered that skin pH and hydration were superior biomarkers. Decreased skin hydration demonstrates to loss of cutaneous water capacitance that is not desired for physiological activity including epidermal cell proliferation and differentiation [40]. We observed more dehydrated skin compared with healthy dogs (P<0.001). Also, the abdominal/inguinal region indicated lower hydration compared to healthy dogs (P=0.037). This finding was compatible with a forementioned previous studies. It has been stated that TEWL decreases in different animal species such as horses [41], cats [42], and dogs. There are even statistical differences between dog breeds [34,43,44]. In our study, regional water loss was examined with skin hydration in 16 dogs with atopic dermatitis of different breeds. The effect of dog breed could not be investigated due to the small number of animals.

Skin water loss is controlled by passive diffusion through the epidermis, but at high temperatures, this transition is greatly affected by sweat penetrating and diffusing into the epidermis in mammalians [45]. Body temperature and age affect skin hydration and pH in humans. These parameters are also influenced by current skin temperature, blood flow and immunity, as well as ambient temperature [46]. Indeed, skin temperature and TEWL are closely related to the stimulation of sweat glands for secretion [47]. The skin temperature in dogs is generally ranged between 34.5 and 35.5°C [48]. In the present study, the mean skin temperature was measured as 34.11±0.46 in atopic dogs and it was only higher for tail compared to the healthy group (P=0.037). According to Breathnach et al.[44] there were no differences in mean skin temperatures between dogs with pododermatitis and healthy dogs. Our findings indicate that while the mean skin temperature in atopic dogs does not significantly differ from healthy dogs in most regions, the higher temperature observed in the tail area suggests a localized variation that may be relevant to the pathophysiology of atopic dermatitis.

Considering the lesion of dogs with caD, affected body sites are classically distributed at head, extremities, axillae, and ventral abdomen [49]. Within years, typical lesions and affected regions of caD have been evaluated for both diagnosis and disease various clinical scorings have been developed for following [17,50,51]. In our study, body site lesions and pruritus evaluation and scoring were performed with CADESI-4 [17] and PVAS [18]. These were used for assessing the relationship between clinical findings and skin barrier at a given site. Observed by Marsella et al.[31] CADESI-03 was found to be correlated with TEWL and PVAS (P<0.05) without hydration. Also, it was found that skin barrier evaluated parameters (capacitance, pH, TEWL, erythema and absorbance) had a low correlation with the CADESI-4 [7]. Contrary to expectations, our study found no correlation between biophysical parameters and clinical scores (CADESI and PVAS) in atopic dogs.

There are some limitations to this study, such as a limited number of atopic dogs, differences in age, breed and diet of dogs included in the study, as well as the inability to demonstrate a clear relationship between the selected biophysical parameters and the specific site of the lesion.

We generated a regional map of atopic dogs by non-invasively measuring a range of biophysical parameters. These fundamental measurements provide a foundation to build upon for offering a practical approach to the disease. Our findings suggest that these parameters could be utilized to identify skin barrier dysfunction in dogs affected by atopic dermatitis.

Declarations

Availability of Data and Materials: The authors declare that data supporting the study findings are also available to the corresponding author (H. Erdogan)

Funding Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics Approval: Ethical approval was obtained from Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (No: 64583101/2024/22). Additionally, comprehensive information was provided to the owners of all dogs included in this study, and written consent was obtained from them.

Competing Interest: Authors declare that have no relevant financial or non-financial interests to disclose.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the article and/or tables and figures were not written/created by AI and AI-assisted technologies.

Author Contributions: HE, SE and KU designed and conceived of the study, HE, SE and KU performed corneometer analysis and collected data, HE performed the statistical analysis, HE and SE reviewed the literature and wrote the article. KU wrote and supervised all part of the study. All authors revised and approved the final version of the manuscript.

References

1. Olivry T, DeBoer DJ, Griffin CE, Halliwell RE, Hill PB, Hillier A, Sousa CA: The ACVD task force on canine atopic dermatitis: Forewords and lexicon. *Vet Immunol Immunopathol,* 81 (3-4): 143-146, 2001. DOI: 10.1016/s0165-2427(01)00343-9

2. Marsella R: Advances in our understanding of canine atopic dermatitis. *Vet Dermatol,* 32 (6): 547-e151. 2021. DOI: 10.1111/vde.12965

3. Hillier A, Griffin CE: The ACVD task force on canine atopic dermatitis (I): Incidence and prevalence. *Vet Immunol Immunopathol,* 81 (3-4): 147-151, 2001. DOI: 10.1016/S0165-2427(01)00296-3

4. Santoro D, Marsella R, Pucheu-Haston CM, Eisenschenk MN, Nuttall T, Bizikova P: Pathogenesis of canine atopic dermatitis: Skin barrier and host-micro-organism interaction. *Vet Dermatol,* 26 (2): 84-125, 2015. DOI: 10.1111/vde.12197

5. Sinke JD, Rutten VP, Willemse T: Immune dysregulation in atopic dermatitis. *Vet Immunol Immunopathol,* 87, 351-356, 2002. DOI: 10.1016/S0165-2427(02)00066-1

6. Mazrier H, Vogelnest LJ, Taylor RM, Williamson P: Altered plasma cytokines in dogs with atopic dermatitis. *Vet Dermatol,* 33 (2): 131-138, 2022. DOI: 10.1111/vde.13044

7. Cobiella D, Archer L, Bohannon M, Santoro D: Pilot study using five methods to evaluate skin barrier function in healthy dogs and in dogs with atopic dermatitis. *Vet Dermatol,* 30 (2): 121-134, 2019. DOI: 10.1111/vde.12723

8. Song Y, Pan Y, Wang H, Liu Q, Zhao H: Mapping the face of young population in China: Influence of anatomical sites and gender on biophysical properties of facial skin. *Skin Res Technol,* 25 (3): 325-332, 2019. DOI: 10.1111/srt.12652

9. Montero-Vilchez T, Segura-Fernández-Nogueras MV, Pérez-Rodríguez I, Soler-Gongora M, Martinez-Lopez A, Fernández-González A, Arias-Santiago S: Skin barrier function in psoriasis and atopic dermatitis: Transepidermal water loss and temperature as useful tools to assess disease severity. *J Clin Med,* 10 (2):359, 2021. DOI: 10.3390/jcm10020359

10. Seo JI, Ham HI, Baek JH, Shin MK: An objective skin-type classification based on non-invasive biophysical parameters. *EADV,* 36 (3): 444-452, 2022. DOI: 10.1111/jdv.17793

11. Shimada K, Yoshihara T, Yamamoto M, Konno K, Momoi Y, Nishifuji K, Iwasaki T: Transepidermal water loss (TEWL) reflects skin barrier function of dog. *J Vet Med Res,* 70 (8): 841-843, 2008. DOI: 10.1292/jvms.70.841

12. Ohmori K, Tanaka A, Makita Y, Takai M, Yoshinari Y, Matsuda H: Pilot evaluation of the efficacy of shampoo treatment with ultrapure soft water for canine pruritus. *Vet Dermatol,* 21 (5): 477-483, 2010. DOI: 10.1111/j.1365-3164.2010.00900.x

13. Bradley CW, Morris DO, Rankin SC, Cain CL, Misic AM, Houser T, Grice EA: Longitudinal evaluation of the skin microbiome and association with microenvironment and treatment in canine atopic dermatitis. *J Invest Dermatol,* 136 (6): 1182-1190, 2016. DOI: 10.1016/j.jid.2016.01.023

14. Garland M: Introduction to canine skin and dermatology. *Vet Nurs J,* 28 (4): 131-133, 2013. DOI: 10.1111/vnj.12025

15. Favrot C, Steffan J, Seewald W, Picco F: A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. *Vet Dermatol,* 21 (1): 23-31, 2010. DOI: 10.1111/j.1365-3164.2009.00758.x

16. Hensel P, Santoro D, Favrot C, Hill P, Griffin C: Canine atopic dermatitis: Detailed guidelines for diagnosis and allergen identification. *BMC Vet Res,* 11 (1): 1-13, 2015. DOI: 10.1186/s12917-015-0515-5

17. Olivry T, Saridomichelakis M, Nuttall T, Bensignor E, Griffin CE, Hill PB, International Committee on Allergic Diseases of Animals (ICADA): Validation of the Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4, a simplified severity scale for assessing skin lesions of atopic dermatitis in dogs. *Vet Dermatol,* 25 (2): 77-e25, 2014. DOI: 10.1111/vde.12107

18. Hill PB, Lau P, Rybnicek J: Development of an owner-assessed scale to measure the severity of pruritus in dogs. *Vet Dermatol,* 18, 301-308, 2007. DOI: 10.1111/j.1365-3164.2007.00616.x

19. Hagströmer L, Kuzmina N, Lapins J, Talme T, Emtestam L: Biophysical assessment of atopic dermatitis skin and effects of a moisturizer. *Clin Exp Dermatol,* 31 (2): 272-277, 2006. DOI: 10.1111/j.1365-2230.2005.02054.x

20. Yazdanparast T, Yazdani K, Humbert P, Khatami A, Nasrollahi SA, Firouzabadi LI, Firooz, A: Biophysical measurements and ultrasonographic findings in chronic dermatitis in comparison with uninvolved skin. *Indian J Dermatol.,* 64:90, 2019. DOI: 10.4103/ijd.IJD_464_17

21. Marsella R, Olivry T, Carlotti DN, International Task Force on Canine Atopic Dermatitis: Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. *Vet Dermatol,* 22 (3): 239-248, 2011. DOI: 10.1111/j.1365-3164.2011.00967.x

22. Zając M, Szczepanik MP, Wilkołek PM, Adamek ŁR, Pomorski ZJ, Sitkowski W, Gołyński M: Assessment of a correlation between Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) and selected biophysical skin measures (skin hydration, pH, and erythema intensity) in dogs with naturally occurring atopic dermatitis. *Can J Vet Res,* 79, 136-140, 2015.

23. Santoro D, Fagman L, Zhang Y, Fahong Y: Clinical efficacy of spray-based heat-treated lactobacilli in canine atopic dermatitis: A preliminary, open-label, uncontrolled study. *Vet Dermatol,* 32 (2): 114-e23, 2021. DOI: 10.1111/vde.12915

24. Kanwal S, Singh SK, Soman SP, Choudhury S, Kumari P, Ram PK, Garg SK: Expression of barrier proteins in the skin lesions and inflammatory cytokines in peripheral blood mononuclear cells of atopic dogs. *Sci Rep,* 11:11418, 2021. DOI: 10.1038/s41598-021-90992-z

25. Kobayashi T, Imanishi I: Epithelial–immune crosstalk with the skin microbiota in homeostasis and atopic dermatitis - A mini review. *Vet Dermatol,* 32, 533-e147, 2021. DOI: 10.1111/vde.13007

26. Ali SM, Yosipovitch G: Skin pH: From basic science to basic skin care. *Acta Derm Venereol,* 93, 261-267, 2013. DOI: 10.2340/00015555-1531

27. Bakker DS, Nierkens S, Knol EF, Giovannone B, Delemarre EM, van der Schaft J, Thijs JL: Confirmation of multiple endotypes in atopic dermatitis based on serum biomarkers. *J Allergy Clin Immunol,* 147, 189-198, 2021. DOI: 10.1016/j.jaci.2020.04.062

28. Hoffmann AR: The cutaneous ecosystem: The roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. *Vet Dermatol,* 8, 71-83, 2017. DOI: 10.1111/vde.12408

29. Oh WS, Oh TH: Mapping of the dog skin based on biophysical measurements. *Vet Dermatol,* 21, 367-372, 2010. DOI: 10.1111/j.1365-3164.2009.00841.x

30. Hülpüsch C, Tremmel K, Hammel G, Bhattacharyya M, de Tomassi A, Nussbaumer T, Traidl-Hoffmann C: Skin pH-dependent *Staphylococcus aureus* abundance as predictor for increasing atopic dermatitis severity. *Allergy,* 75, 2888-2898, 2020. DOI: 10.1111/all.14461

31. Marsella R, Ahrens K, Wilkes R, Trujillo A, Dorr M: Comparison of various treatment options for canine atopic dermatitis: A blinded, randomized, controlled study in a colony of research atopic beagle dogs. *Vet Dermatol,* 31 (4): 284-e69, 2020. DOI: 10.1111/vde.12849

32. We R: Role of the sweat gland in eczema of dogs; a preliminary report. *J Am Vet Med Assoc,* 124, 51-54, 1954.

33. Meyer W, Neurand K: Comparison of skin pH in domesticated and laboratory mammals. *Arch Dermatol Res,* 283, 16-18, 1991. DOI: 10.1007/BF01207245

34. Young LA, Dodge JC, Guest KJ, Cline JL, Kerr WW: Age, breed, sex and period effects on skin biophysical parameters for dogs fed canned dog food. *Nutr J,* 132, 1695-1697, 2002. DOI: 10.1093/jn/132.6.1695S

35. Campbell KL, LaFlamme DP, Harrison J: Seasonal variations in skin surface pH, skin surface hydration and hair epilation of normal dogs in Midwestern United States climate. *Vet Dermatol,* 11 (Suppl. 1): 14-40, 2000. DOI: 10.1046/j.1365-3164.2000.00001.x

36. Lodén M: The skin barrier and use of moisturizers in atopic dermatitis. *Clin Dermatol,* 21, 145-157, 2003. DOI: 10.1016/S0738-081X(02)00373-5

37. Jensen JM, Fölster-Holst R, Baranowsky A, Schunck M, Winoto-Morbach S, Neumann C, Proksch, E: Impaired sphingomyelinase activity and epidermal differentiation in atopic dermatitis. *J Invest Dermatol,* 122, 1423-1431, 2004. DOI: 10.1111/j.0022-202X.2004.22621.x

38. Jung JY, Nam EH, Park SH, Han SH, Hwang CY: Clinical use of a ceramide-based moisturizer for treating dogs with atopic dermatitis. *J Vet Med Sci,* 14, 199-205, 2013. DOI: 10.4142/jvs.2013.14.2.199

39. Panzuti P, Videmont E, Fantini O, Fardouet L, Noel G, Cappelle J, Pin D: A moisturizer formulated with glycerol and propylene glycol accelerates the recovery of skin barrier function after experimental disruption in dogs. *Vet Dermatol,* 31, 344-e89, 2020. DOI: 10.1111/vde.12859

40. Proksch E, Holleran WM, Menon GK, Elias PM, Feingold KR: Barrier function regulates epidermal lipid and DNA synthesis. *Br J Dermatol,* 128, 473-482, 1993. DOI: 10.1111/j.1365-2133.1993.tb00222.x

41. Szczepanik MP, Wilkolek PM, Pluta M, Adamek LR, Pomorski ZJH: The examination of biophysical parameters of skin (transepidermal water loss, skin hydration and pH value) in different body regions of ponies. *Pol J Vet Sci,* 15 (3): 553-559, 2012. DOI: 10.2478/v10181-012-0081-8

42.Szczepanik MP, Wilkołek PM, Adamek ŁR, Pomorski ZJ: The examination of biophysical parameters of skin (transepidermal water loss, skin hydration and pH value) in different body regions of normal cats of both sexes. *J Feline Med Surg,* 13, 224-230, 2011. DOI: 10.1016/j.jfms.2010.11.003

43. Lau-Gillard PJ, Hill PB, Chesney CJ, Budleigh C, Immonen A: Evaluation of a hand-held evaporimeter (VapoMeter) for the measurement of transepidermal water loss in healthy dogs. *Vet Dermatol,* 21, 136-145, 2010. DOI: 10.1111/j.1365-3164.2009.00738.x

44. Hester SL, Rees CA, Kennis RA, Zoran DL, Bigley KE, Wright AS, Kirby NA, Bauer JE: Evaluation of corneometry (Skin Hydration) and transepidermal water-loss measurements in two canine breeds. *J Nutr,* 134, 2110-2113, 2004. DOI: 10.1093/jn/134.8.2110S

45. Jenkinson DM: The topography, climate and chemical nature of the mammalian skin surface. *Proc R Soc B: Biol Sci,* 179, 3-22, 1980. DOI: 10.1017/S0269727000010265

46. Montero-Vilchez T, Segura-Fernández-Nogueras MV, Pérez-Rodríguez I, Soler-Gongora M, Martinez-Lopez A, Fernández-González A, Arias-Santiago S: Skin barrier function in psoriasis and atopic dermatitis: Transepidermal water loss and temperature as useful tools to assess disease severity. *J Clin Med Res,* 10:359, 2021. DOI: 10.3390/jcm10020359

47. Mathias CT, Wilson DM, Maibach HI: Transepidermal water loss as a function of skin surface temperature. *J Invest Dermatol,* 77, 219-220, 1981. DOI: 10.1111/1523-1747.ep12479939

48. Breathnach RM, Quinn PJ, Baker KP, McGeady T, Strobl E, Abbott Y, Jones BR: Association between skin surface pH, temperature and *Staphylococcus pseudintermedius* in dogs with immunomodulatory-responsive lymphocytic-plasmacytic pododermatitis. *Vet Dermatol,* 22, 312-318, 2011. DOI: 10.1111/j.1365-3164.2010.00946.x

49. Griffin CE, DeBoer DJ: The ACVD task force on canine atopic dermatitis (XIV): Clinical manifestations of canine atopic dermatitis. *Vet Immunol Immunopathol,* 81, 255-269, 2001. DOI: 10.1016/S0165-2427(01)00346-4

50. Olivry T, Marsella R, Iwasaki T, Mueller R, International Task Force On Canine Atopic Dermatitis: Validation of CADESI-03, a severity scale for clinical trials enrolling dogs with atopic dermatitis. *Vet Dermatol,* 18, 78-86, 2007. DOI: 10.1111/j.1365-3164.2007.00569.x

51. Plant JD, Gortel K, Kovalik M, Polissar NL, Neradilek MB: Development and validation of the Canine Atopic Dermatitis Lesion Index, a scale for the rapid scoring of lesion severity in canine atopic dermatitis. *Vet Dermatol,* 23, 515-e103, 2012. DOI: 10.1111/j.1365-3164.2012.01113.x