UNIVERSITY OF THESSALY

SCHOOL OF HEALTH SCIENCES

FACULTY OF VETERINARY SCIENCE

CLINIC OF MEDICINE

ULTRASONOGRAPHIC BIOMICROSCOPY OF CANINE SKIN

PANAGIOTIS MANTIS DVM, DIP ECVDI, FHEA, FRCVS

DOCTORAL THESIS

KARDITSA 2022

ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ

ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΤΜΗΜΑ ΚΤΗΝΙΑΤΡΙΚΗΣ

ΠΑΘΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ

ΥΠΕΡΗΧΟΤΟΜΟΓΡΑΦΙΚΗ ΒΙΟΜΙΚΡΟΣΚΟΠΙΑ ΤΟΥ ΔΕΡΜΑΤΟΣ ΤΟΥ ΣΚΥΛΟΥ

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CLINIC OF MEDICINE

ULTRASOUND BIOMICROSCOPY OF CANINE SKIN

PANAGIOTIS MANTIS DVM, DIP ECVDI, FHEA, FRCVS

DOCTORAL THESIS

KARDITSA 2022

DOCTORAL THESIS CONDUCTED AT THE CLINIC OF MEDICINE, FACULTY OF VETERINARY SCIENCE, SCHOOL OF HEALTH SCIENCES, UNIVERSITY OF THESSALY

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ΤΡΙΜΕΛΗΣ ΣΥΜΒΟΥΛΕΥΤΙΚΗ ΕΠΙΤΡΟΠΗ

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Καθηγητής	David Church	Μέλος της Επιτροπής

To my teachers for creating bridges for me to cross

To my students that continue to teach me

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ABBREVIATIONS

AD: Atopic Dermatitis

AD-L: Lesional AD skin

AD-NL: non-lesional AD skin

H-NL: Healthy non-lesional skin

CADESI-4: Canine Atopic Dermatitis Extent and Severity Index-4

CADLI: Canine Atopic Dermatitis Lesional Index

H-NL: non-lesional skin of healthy dogs

PVAS: Pruritus Visual Analogue Scale

SLEB: subepidermal low echogenic band

TGF-b: transforming growth factor beta

UB: Ultrasound biomicroscopy

PREFACE

This research that comprises my PhD Thesis, was contacted at the Clinic of Medicine of the Faculty of Veterinary Science, School of Health Sciences of the University of Thessaly. The aim of this research was to describe the ultrasonographic appearance of normal canine skin from various anatomical locations, using a 50MHz transducer, to identify ultrasonographic abnormalities in the lesional skin of dogs with atopic dermatitis and to evaluate the ultrasonographic changes observed during treatment of canine atopic dermatitis.

I would like to express my deep appreciation to the members of my advisory committee and more specifically:

to Professor Manolis Saridomichelakis, Professor of Medicine at the Faculty of Veterinary Science of the University of Thessaly, and supervisor of my PhD, for trusting me to undertake this research, for supporting me throughout this project by offering advice and direction whenever that was needed and for always urging me to do better,

to Professor Apostolos Galatos, Professor of Animal Surgery and Anaesthesia at the Faculty of Veterinary Medicine of the University of Thessaly, for always checking for my wellbeing and that I continue diligently with the research and publications and for always offering to help when needed,

to Professor David Church, Deputy Principal and Acting Vice Principal (Clinical Affairs) at the Royal Veterinary College of the University of London, for always supporting me throughout my career in every endeavour I have undertaken and for always being available when needed,

to Professor David Lloyd, Emeritus Professor of Dermatology at the Royal Veterinary College of the University of London, for introducing me to the exciting research of ultrasound biomicroscopy of the skin, for teaching me the ropes of research and for assisting me with my ultrasound biomicroscopy of the skin research even after his retirement,

to Professor Pagona Gouletsou, Associate Professor of Reproduction-Obstetrics and Surgery of Companion Animals at the Faculty of Veterinary Science of the University of

Thessaly, for supervising the performance of the ultrasound examinations and making sure the images provided where of high quality at the important early stages of the research,

to Professor Dimitrios Tontis, Professor of Pathology at the Faculty of Veterinary Science of the University of Thessaly, for evaluating the biopsy samples of the original research

to Dr Evangelia Sofou, PhD student, for performing some of the ultrasound examinations, providing diagnostic images, and keeping accurate records.

My heartfelt thanks also go to Dr Svetlina Aleksandrova, Dr Elisa Badulescu, Dr Stathis Bairamoglou, Dr Dimitrios Balomenos, Dr Manolis Chatzis, Dr Dimitrios Doukas, Dr Girorgos Giannoulopoulos and Dr Panagiotis Koutsouvelis for assisting with the performance of some of the ultrasound examinations of the skin, following the guidelines and training that I provided them, to the best of their abilities.

Finally, I would like to thank all those heroes in the background that support me without ever requesting something back:

To my teachers for budling bridges for me to climb higher.

To my students that continue to teach me.

To my family for their love and support that is unwavering under any circumstances.

ΣΑΣ ΕΥΧΑΡΙΣΤΩ

PART ONE - REVIEW OF THE LITERATURE

1. Introduction

The skin is the largest organ in the body. Through hemostasis, inflammation, proliferation, and remodelling, it has the ability to heal itself. Traditionally, diagnosing skin diseases takes the route of history taking, clinical examination, laboratory teststing and histopathological evaluation of biopsy samples.

Ultrasonography is a diagnostic imaging modality that employs high frequency sound waves. It is non-invasive, non-ionising, allows the evaluation of deeper structures and, with the development of very high frequency transducers, the evaluation of more superficial structures and organs, like the skin. Cutaneous ultrasonography allows identification of the skin layers. With the correct choice of frequencies to be employed, cutaneous ultrasonography allows, to a certain limit, the resolution of the skin structures based on depth. Ultrasonographic equipment can be portable, allowing quick examination of the skin during consultation to obtain information regarding its layered appearance, thickness, and integrity. However, it cannot identify neoplastic or inflammatory cells or their distribution patterns and thus it is limited to more general changes in echogenicity and echotexture.

In human dermatology, cutaneous ultrasonography has been used for the evaluation of normal skin appearance, thickness and hydration status, and also for the evaluation of pathologic conditions, such as presence of foreign bodies, inflammatory lesions, scleroderma, neoplasms, cysts, oedema, and wounds (Alexander and Miller 1979; Miyauchi and Miki 1983; Fornage and Deshayes 1986; Fornage et al. 1993; Fornage 1993; Gniadecka 1996; Gniadecka and Quistorff 1996; Milner et al. 1997; Warszawski et al. 1997; Cammarota et al. 1998; Foster et al 2000; Eisenbeiss et al. 2001; Mirpuri et al. 2001; Dyson et al. 2003; Scope and Halpern 2003; Kong et al. 2008).

In veterinary medicine there are only very a few studies on the ultrasonography of the skin focusing on the appearance of the normal skin, the measurement of skin thickness and the evaluation of wound healing (Diana et al. 2004; Mantis et al. 2005; Mantis et al. 2007; Diana

et al. 2008; Zanna et al 2012; Mantis et al. 2014, Balomenos et al 2023, Balomenos et al 2023). There are no studies that evaluate pathologic conditions as in humans.

2. The technology

Cutaneous ultrasonography uses frequencies of more than 7 MHz and usually more than 15MHz. These frequencies can be classified as intermediate (7-15 MHz) or high (more than 20MHz) (Scope and Halpern 2003). Although lower frequencies may provide a borderline adequate resolution for the evaluation of the skin, frequencies higher than 15 MHz allow identification of skin layers, and frequencies of 50MHz and above allow discrimination between epidermis and dermis (Wortsman 2012; Mantis et al. 2014).

3. Anatomy of normal canine skin

The skin thickness (thickness of epidermis and dermis) varies from 0.5mm to 5mm depending on the body area. The skin is normally thicker dorsally on the trunk and proximally on the legs. Furthermore, the normal skin thickness varies in different breeds and in dogs of different ages (Noli 2008; Miller et al. 2013). Hair follicles and hair shaft are also present in the haired skin with the hair shaft traversing obliquely the epidermis and dermis (Noli 2008).

3a Intermediate frequency ultrasonography

Using a 13MHz linear array transducer the canine skin appears ultrasonographically as three layers:

a. Epidermal entry echo: interface between the coupling gel and the skin, visible as a hyperechoic line. The echogenicity depends on the amount of air trapped between the scales of the stratum corneum (Poziniak et al. 1989)

b. Epidermis and dermis: they appear as a single layer immediately bellow and hypoechoic to the epidermal entry echo. No differentiation between the epidermis and dermis is possible. The echogenicity of this middle layer depends on the components of the dermis (Fornage and Deshayes 1986; Fornage et al. 1993; Szymanska et al. 2000)

c. Subcutaneous fat: bellow epidermis and dermis, having an inhomogeneous hypoechoic pattern with thick linear hyperechoic bands (Diana et al. 2004).

Hair follicles, hair shafts and sebaceous glands require a frequency higher than 20 MHz and they are not visible with a 13 MHz transducer (Diana et al. 2004).

3b High frequency ultrasonography

It uses frequencies above 20 MHz. Commercially available scanners provide frequencies up to 25MHz with a limited resolution of approximately 80μm axial and 200μm lateral (Schmid-Wendtner and Dill-Müller 2008). With frequencies up to 25MHz, skin structures up to 8mm deep can be investigated (Hoffmann et al. 1992). Higher frequency machines have been developed for experimental use, and they allow structures of up to 2mm depth to be evaluated (El Gammal et al. 1999; Kumagai et al. 2012). For equipment with frequencies from 50MHz and above, the term ultrasound biomicroscopy is used (UB) because it permits visualization of tissue in microscopic resolution.

Using a 50MHz transducer the following layers can be seen:

a. Epidermis: thin, non-uniform hyperechoic linear layer

b. Dermis: visible bellow the epidermis as linear hyperechogenicities, roughly parallel or at a slight angle to the skin surface, hypoechoic to the epidermis and muscle fascia and hyperechoic to subcutaneous fat. Superficial and deep dermis may be distinguished in some cases. The hair follicles are visible as oblique, tubular hypoechoic structures with faint echogenic linear areas at their margins

c. Subcutaneous fat: bellow the dermis, as hypoechoic to anechoic tissue.

4 The diseased canine skin

Very few studies on non-healthy canine skin are available. Anatomic area of the skin and hydration status were correlated with the ultrasonographic findings and ultrasonographic measurement of skin thickness using a 13 MHz linear array transducer (Diana et al 2008) and ultrasound was found to be useful to assess skin thickness (Zanna et al 2012). High-frequency ultrasonography has been employed to evaluate wound size and would healing, along with the assessment of the effect of an aliamide-containing topical gel (Mantis et al 2005; Mantis et al 2007), to evaluate the incisional wound healing in dogs after closure with staples or tissue glue (Balomenos et al 2023) and to compare absorbable and nonabsorbable sutures for intradermal skin closure in dogs (Balomenos 2023).

High-frequency ultrasonographic appearance of human lesional and non-lesional atopic dermatitis (AD) skin, and healthy skin have been compared, the impact of treatment on the

ultrasonographic appearance of AD skin has been reported, and the width of the subepidermal low echogenic band (SLEB) has been shown to be positively correlated with clinical severity of AD (Kyllönen et al 2004;Holm et al 2006; Holm et al 2007; Polańska et al 2013; Sorokina et al 2020; Czajkowska et al 2021). In human AD, the echogenicity of the dermis depends on the severity of the cellular infiltration (Polańska et al 2013) and it was found to be lower in the lesional AD skin, followed by non-lesional AD skin, compared to normal healthy skin in some (Polańska et al 2013; Polańska et al 2013), but not in other studies (Sorokina et al 2020). No studies on the high frequency ultrasonographic appearance of canine AD skin are available.

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REVIEW ARTICLE

Ultrasonographic examination of the canine skin: a review

J HELLENIC VET MED SOC 2016, 67(1): 17-20
ΠΕΚΕ 2016, 67(1): 17-20

Review article
Ανασκόπηση

Ultrasonographic examination of the canine skin: a review

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Υπερηχοτομογραφική Εξέταση του Δέρματος στο Σκύλο

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ABSTRACT. Real time B-mode ultrasonography is a non-invasive diagnostic imaging modality that does not use radiation and allows examination of various soft tissue structures. For many years it is used in human dermatology and in the last decade it has entered the canine dermatology arena. Based on the frequency employed, cutaneous ultrasonography may be classified as intermediate- (7-15 MHz) or high-frequency (20 MHz or higher). Using intermediate frequency, the ultrasonographic features of normal canine skin are consistent and three distinct visible layers can be seen. Using a 50 MHz transducer, the epidermis and hair follicles are also identified and accurate measurements of skin thickness can be obtained. The aim of this article is to review the available published knowledge regarding ultrasonographic examination of the canine skin.

Keywords: *Ultrasound, dog, skin, ultrasound biomicroscopy*

INTRODUCTION

The skin is the largest organ of the body. This organ has the capability to heal itself after wounding through sequential phases that include hemostasis, inflammation, proliferation and remodeling. Traditionally, the diagnosis of skin diseases is based on history taking, physical examination and various laboratory tests including histopathology of skin biopsies. Recent advances into ultrasonography have broadened the application of this diagnostic imaging modality to a large number of soft tissue structures, including the skin. Ultrasonography is non-invasive and non-ionizing in nature and it allows visualization of these structures. For these abilities it lends itself as a useful tool for the evaluation of the skin that can be used to monitor the healing process and as an ancillary aid for diagnosis of skin diseases.

In comparison to other diagnostic modalities, ultrasonography has some advantages in the evaluation of the skin. Compared to histopathology, it allows good

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Date of initial submission: 06.01.2015
Date of revised submission: 12.02.2015
Date of acceptance: 13.02.2015

discrimination of skin layers without the need for biopsy; however it does not allow visualization of inflammatory or neoplastic cells or a detailed characterization of their distribution pattern. Cutaneous ultrasonography is totally non-invasive as it does not require even the injection of contrast media into the skin. Clipping of the skin is necessary except when glabrous or alopecic areas of the skin are to be examined. The possibility to use various frequencies during the examination, allows the choice of optimal penetration and resolution depending on the depth of the tissue in question. In addition, ultrasonography uses portable equipment with the ability to perform the examination in the consultation room and quickly obtain valuable information regarding skin appearance, thickness and layer integrity. In contrast, magnetic resonance imaging and computed tomography have limited resolution for the discrimination between epidermis and dermis and low discrimination for cutaneous lesions that measure less than 3 mm (Wortsman and Wortman 2010; Wortsman and Jemec 2010). Dermoscopy is rarely used in veterinary medicine. It enables the clinician to perform direct microscopic examination of diagnostic features of the skin not seen by naked eye. In humans it is mainly used in pigmented lesions especially for the early detection of melanoma and has been proved more accurate than naked eye in suspicious skin lesions (Vestergaard et al. 2008). The aim of this article is to review the available published knowledge regarding ultrasonographic examination of the canine skin.

THE HISTORY

Cutaneous ultrasonography has been extensively used in human dermatology to study normal skin echogenicity, thickness and hydration status, as well as various pathologic conditions, including cutaneous or subcutaneous neoplasms, cysts, inflammatory lesions, post-radiation reactions, scleroderma, edema, wounds, and presence of foreign bodies (Alexander and Miller 1979; Miyauchi and Miki 1983; Fornage and Deshayes 1986; Fornage et al. 1993; Fornage 1993; Gniadecka 1996; Gniadecka and Quistorff 1996; Warszawski et al. 1997; Milner et al. 1997; Cammarota et al. 1998; Foster et al 2000; Eisenbeiss et al. 2001; Mirpuri et al. 2001; Dyson et al. 2003; Scope and Halpern 2003; Kong et al. 2008). Contrary to the situation in human medicine, in veterinary medicine only few studies on cutaneous ultrasonography of the canine skin are available (Diana et al. 2004; Mantis et al. 2005; Mantis et al. 2007; Diana et al. 2008; Zanna et al 2012; Mantis et al. 2014). The studies in veterinary medicine aimed at evaluating the application of high frequency ultrasound for the evaluation and accurate measurement of the normal skin thickness in the dog (Diana et al. 2004, Zanna et al. 2012), the evaluation of wound healing in the dog (Mantis et al. 2005), the effect of an aliamide-containing topical gel on the reduction of wound volume (Mantis et al. 2007), and at evaluating the normal ultrasonographic appearance of the skin using ultrasound biomicroscopy (Mantis et al. 2014). These studies do not evaluate pathologic conditions, as it is the case with human studies.

TECHNICAL CONSIDERATIONS

Based on the frequency employed, cutaneous ultrasonography may be classified as intermediate- (7-15 MHz) or high-frequency (20 MHz or higher). (Scope and Halpern 2003) Although there are reports using lower frequency transducers, frequencies in excess of 15 MHz are generally recommended to get a better resolution and to identify the skin layers with higher clarity (Wortsman 2012). Ideally, frequencies of at least 50 MHz should be employed if the discrimination between epidermis and dermis is required (Mantis et al. 2014). Sedation is not normally required but it may be needed occasionally for an uncooperative dog. The skin should be shaved to improve contact between the transducer and the skin surface, thus providing a better image with reduced artifacts. Minimal pressure should be applied and transducer is placed perpendicularly to the skin.

NORMAL ANATOMY

The average thickness of the normal canine skin has been reported to range from 0.5 to 5 mm depending on body site, being higher dorsally than ventrally on the trunk and proximally than distally on the legs (Miller et al. 2013; Noli 2008). The skin of the forehead, dorsal neck, dorsal thorax, rump and tail base area is the thickest, whereas, skin of the ear pinnae, axillary, inguinal and perianal area is the thinnest (Miller et al. 2013). Thickness of the skin, excluding subcutis, is the sum of epidermal and dermal thickness. The former, if horny layer is not taken into account, ranges from 0.1 to 0.5 mm in haired skin but is substantially higher in footpads and nasal planum (up to 1.5 mm) (Noli 2008; Miller et al. 2013). Dermis, on the other hand, is the main determinant of normal canine haired skin thickness, which greatly depends on it's fiber, ground substance and water content (Miller et al. 2013). Finally, it is reported that the thickness of the normal skin varies according to the breed and the age of the dog (Noli 2008; Miller et al. 2013). Besides epidermis and dermis, hair follicles and hair shafts are present in normal canine haired skin. Hair shafts reside within their follicles and transverse, usually obliquely, the epidermis and the dermis

(Noli 2008). Their diameter may range from 0.01 up to 0.14 mm depending on the type of hair (primary, intermediate or secondary) (Noli 2008). Epitricheal sweat glands and sebaceous glands are associated with the hair follicles and their ducts open into their lumen.

INTERMEDIATE FREQUENCY ULTRASONOGRAPHY

Using a 13 MHz linear array transducer, the ultrasonographic features of canine skin have a consistent appearance with 3 distinct layers visible (Figure 1). Initially, the interface between the coupling gel and the skin is visible as a hyperechoic line termed "epidermal entry echo". The echogenicity of the epidermal entry echo depends on the amount of air trapped between the keratotic scales of the stratum corneum (Poziniak et al. 1989).

Immediately bellow this line, a hypoechoic, in comparison to the epidermal entry echo, layer is visible. This layer is thicker and corresponds to the epidermis and dermis. The axial resolution provided by a 13 MHz transducer, does not allow differentiation between the epidermis and dermis (Diana et al. 2004). This middle layer has variable achogenicity that probably depends on the various components of the dermis (e.g. collagenous fibers, dermal ground substance, water, and hair follicle adnexae like sebaceous glands) (Fornage and Deshayes 1986; Fornage et al. 1993; Szymanska et al. 2000).

A third deeper layer is also visible and has an inhomogeneous hypoechoic pattern with thick linear hyperechoic bands. This layer corresponds to subcutaneous fat. (Diana et al. 2004) Hair follicles, hair shaft and sebaceous glands are not visible using a 13 MHz frequency and a frequency above 20 MHz needs to be used in order to visualize these structures (Diana et al. 2004). It has been shown that scanning the canine skin with a 13 MHz transducer allows the evaluation of the hydration status of the skin (Diana et al. 2008). Also, various studies (Diana et al. 2004; Zanna et al. 2012) have shown that there is high correlation between measurements of skin thickness with a 13 MHz transducer and histologic measurements, indicating that this frequency is adequate for measuring thickness of the canine skin.

HIGH FREQUENCY ULTRASONOGRAPHY

High frequency ultrasonography refers to the use of frequencies higher than 20 MHz. Today, commercially available scanners can provide frequencies up to 25 MHz; however they have a rather limited resolution of approximately 80 μm for axial and of 200 μm for lateral resolution (Schmid-Wendtner and Dill-Müller 2008). With such frequencies, skin structures up to 8 mm deep can be investigated (Hoffmann et al. 1992). In order to improve the resolution of the ultrasonographic images of the skin, higher frequency ultrasound machines have been developed for experimental use (El Gammal et al. 1999; Kumagai et al. 2012). They allow visualization of structures to a depth of 2mm bellow the skin surface.

For equipment that employs ultrasound frequencies between 50 and 100 MHz, the term ultrasound biomicroscopy (UB) is used. UB allows visualization of tissues at microscopic resolution. The technique is similar to real-time B-mode ultrasound examination but the main difference is that UB employs a moving transducer without covering membrane and uses a water bath technique.(El-Zawahry 2007).

Using a 50 MHz transducer, the epidermis is identified as a thin, slightly non-uniform, hyperechoic linear layer. Linear echogenicities, roughly parallel or at a slight angle to the skin surface, are visible within the dermis, while the hypoechoic to anechoic subcutaneous fat is visible bellow the dermis (Figure 2). The margin between epidermis and dermis is visible. Dermis appears

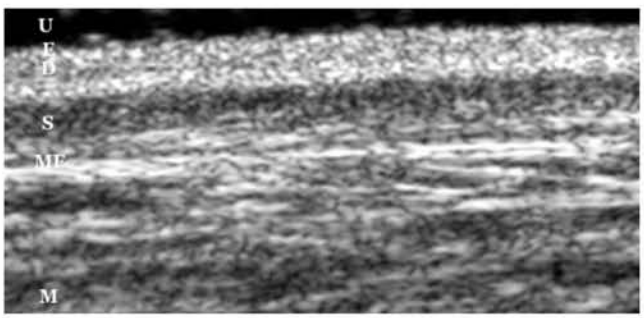

Figure 1: Ultrasonographic image of the skin of a normal dog from the center of the flank using 13 MHz frequency. The epidermal entry echo (E), epidermis and dermis (D) and subcutaneous fat (S) are visible.
U= ultrasound gel; MF= muscle fascia; M= muscle

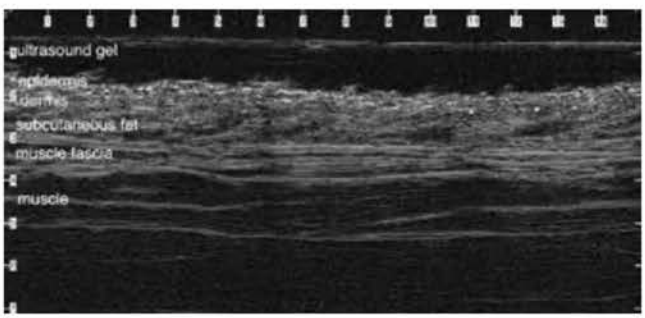

Figure 2: Ultrasonographic images of the skin of a normal dog corresponding to the dorsal thoracic area, over the upper palpable portion of the seventh rib using 50 MHz frequency. The epidermis, dermis and subcutaneous fat are clearly visible. The asterisks indicate hair follicles. The numbers indicate millimeters.

hypoechoic to the epidermis and muscle fascia and hyperechoic to the subcutaneous layer. The superficial and deep dermis can be distinguished in some instances. The subcutaneous fat appears as an anechoic layer with sparse echogenicities. The hair follicles are visible as oblique, tubular hypoechogenicities with faint echogenic linear areas at their margins. The ultrasonographic measurements performed with a 50 MHz transducer were almost identical to the histological measurements done in snap frozen skin biopsies (Mantis et al. 2014).

In conclusion, ultrasonography is applicable for the examination of the skin and it is safe and easy to use. The use of UB for the evaluation of canine skin has the potential to examine it at microscopic resolution and creates expectations for future clinical applications in canine dermatology. There are a limited number of studies describing ultrasonography of canine skin and more work, especially in various pathologic conditions, is needed before it can become a regular part of the daily clinical work-up.

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PART II-OUR STUDY

2. Research Study #1: High-frequency ultrasound biomicroscopy of the normal canine haired skin

Veterinary Dermatology

Vet Dermatol 2014; **25**: 176–e45 DOI: 10.1111/vde.12120

High-frequency ultrasound biomicroscopy of the normal canine haired skin

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Background – The ultrasonographic appearance of the normal canine haired skin examined using high-frequency ultrasonography has not been described.

Hyposthesis/Objectives – To describe the echogenicity of normal canine haired skin using high-frequency (50 MHz) ultrasonography and to compare ultrasonographic with histological measurements of skin thickness using snap-frozen tissue biopsy samples.

Animals – Ten normal healthy beagle dogs.

Methods – Ultrasonographic examination was performed on eight cutaneous sites by use of a 50 MHz polyvinylidene difluoride transducer. The skin echogenicity was evaluated, and the mean of 10 skin thickness measurements was calculated. Ultrasonography results were compared with histological findings of skin cryosections stained with haematoxylin and eosin, as well as with histometric measurements of skin thickness. Differences in the ultrasonographic and histological measurements among biopsy sites, age and sex of the animals were also examined.

Results – The skin layers and hair follicles could be identified with high-frequency ultrasound biomicroscopy in all eight examination sites of all 10 dogs. There was a highly significant, positive association between the ultrasonographic and histological measurements ($P < 0.001$) of skin thickness. For both ultrasonographic and histological skin thickness measurements, there were no statistically significant differences between sex, age or among the different examination sites.

Conclusions and clinical importance – Cutaneous ultrasound biomicroscopy using a 50 MHz transducer is a useful tool for the following applications: (i) to identify the skin layers (including the epidermis, dermis and subcutaneous fat); (ii) to demonstrate the hair follicles in various areas of the haired skin; and (iii) to measure the thickness of normal canine skin accurately.

Introduction

Cutaneous ultrasonography has been extensively used in human dermatology to study normal skin echogenicity, thickness and hydration status, as well as the echogenicity patterns of various pathological conditions, including cutaneous or subcutaneous neoplasms, cysts, inflammatory lesions, postradiation reactions, scleroderma, oedema, wounds and the presence of foreign bodies.[1 16] Depending on the tissue-penetration properties of the ultrasound beam, cutaneous ultrasonography has been classified into intermediate (7.5–10 MHz) and high-frequency ultrasonography (20 MHz or higher).[15]

Only a few studies on the ultrasonographic examination of canine skin have been published.[17 21] In three studies, the thickness and hydration of normal canine skin were examined;[17 19] in two other studies, high-frequency ultrasonography was employed to study wound healing.[20,21] The echogenicity[18,19] and thickness[17,18] of normal canine skin were evaluated using a transducer frequency of 13 MHz that resulted in an inability to visualize hair follicles. Also, the ultrasonographic measurements of normal canine skin thickness were found to be correlated significantly with, but were substantially higher than those obtained after histological examination; this difference was attributed to the inevitable shrinkage of the biopsy specimens during formalin fixation.[17,19] In previous studies, ultrasonographic examination included only one[18] or four different sites[17,19] of haired skin.

The aim of the present study was to describe the echogenicity of normal canine haired skin using high-frequency (50 MHz) ultrasonography and to compare the ultrasonographic with the histological measurements of skin thickness using snap-frozen tissue biopsy samples.

Accepted 20 December 2013
Sources of Funding: This study was self-funded.
Conflict of Interest: No conflicts of interest have been declared.

Materials and methods

Dogs

Ten clinically healthy, purpose-bred beagle dogs housed at the Department of Surgery, Faculty of Veterinary Science of the University of Thessaly were used; the experimental protocol was approved by the Greek National Animal Ethics Committee, and every effort was made to avoid or keep pain or discomfort of the animals to a minimum. There were seven males (three entire and four neutered) and three entire females, with an age ranging from 4 to 9 years (median, 5.75 years) and and body weight ranging from 12.5 to 20.5 kg (median, 16 kg). All dogs received regular treatment against ecto- and endoparasites. They were fed a commercial canine dry food at a quantity adjusted to their age and body weight throughout the study and were housed in runs with free access outdoors.

The inclusion criteria were as follows: (i) for female dogs, not being pregnant or lactating; (ii) ideal body condition (body condition score, 3/5);[22] (ii) no clinical evidence of dehydration; (iv) no systemic or cutaneous abnormalities identified during physical examination, except possible post-traumatic scars in areas of the skin that were not examined in this study; (v) no administration of any kind of medication during the previous 3 weeks; (vi) results of complete blood count, serum biochemistry and urinalysis were within reference range; and (vii) negative serology for canine leishmaniosis, monocytic ehrlichiosis, *Anaplasma phagocytophilum* infection, Lyme disease and heartworm disease.

Ultrasound biomicroscopy, skin biopsy and histological examination

Ultrasound biomicroscopy and skin biopsy were performed between 09.00 and 14.00 h, with the dogs in lateral recumbency; in humans, the time of the day and body posture can influence normal skin hydration and thickness.[12] Following premedication with a mixture of intramuscular dexmedetomidine (300 μg/m2 body surace area i.m.; Dexdomitor; Pfizer Hellas, Athens, Greece) and morphine (0.5 mg/kg i.m.; Morphine; Demo SA, Athens, Greece), anaesthesia was induced by intravenous administration of thiopental (Pentothal; Abbott Hellas, Athens, Greece) in a bolus injection of 5 mg/kg body weight and was maintained by inhalation of a mixture of isoflurane (Aerrane; Baxter, Thetford, UK) in oxygen.

A total of eight cutaneous sites, located on either the right or the left side of the body, were examined. These sites were as follows: (i) the dorsal thoracic area, over the upper palpable portion of the seventh rib; (ii) the middle thoracic area, over the middle of the seventh rib; (iii) the lower thoracic area over the joint between the seventh rib and the sternum; (iv) the upper lumbar area, over the fourth lumbar vertebra and 3 cm lateral to the spine; (v) the centre of the flank; (vi) the ventral abdominal area at the level of the umbilicus and 3 cm lateral to the mid-line; (vii) the lateral forelimb over the middle of the humerus; and (viii) the lateral thigh over the middle of the femur. Selection of the right or the left side of the body was based on the absence of scars in the respective examination sites. The hair over the examination sites was clipped, and a 4 cm straight line, parallel to the long axis of the body, was drawn with a marker pen over each clipped examination site.

A portable ultrasound scanner (Episcan I-200; Longport Inc., Chadds Ford, PA, USA) with a 50 MHz transducer was used for all ultrasonographic examinations. The scanner was fitted with a polyvinylidene difluoride transducer incorporated into a probe filled with distilled water, and scanning was performed using a digital stepping motor. The ultrasonic beam was propagated through an aperture covered with a disposable rubber membrane. Ultrasound images were obtained in sagittal orientation, with the aperture in absolute alignment with and just below the 4 cm line (the margin of the aperture was held parallel and in contact with the line), and with the centre of the aperture located 2 mm rostrally from the mid-point of the line, because the ultrasound beam is propagated 2 mm caudally from the centre of the aperture. The axial resolution in the conditions in which the equipment was used was expected to be ~50 μm. The focal zone of the transducer was set at 2 mm depth, with beam width at −6 dB (~25 μm), and each displayed pixel was 29 μm wide. The acquired images were 14.9 mm wide and 7.68 mm deep. They were saved and stored in the associated hard drive and were visualized using a grey-scale mirror palette from the same software with which the ultrasound machine operates (Episcan I-200, version 4.0-UL; Longport Inc.). The grey-scale mirror palette imaging mode uses all signal intensities, but it applies the same level of grey to all signals of the same size regardless of whether they are positive or negative, so that it rectifies the image.

Immediately after the ultrasound biomicroscopy, the scanned areas were biopsied, using 8 mm disposable biopsy punches (Kruuse, Langeskov, Denmark), without any additional preparation of the skin. Biopsies were obtained slightly below the middle of the 4 cm line, at exactly the same site that had previously been scanned, and were immediately bisected, under magnification, in a sagittal plane. One half of the biopsy sample was transferred into an embedding mould (Peel-A-Way^R Molds; Polysciences Inc., Warrington, PA, USA) that was filled with optimal cutting compound (Tissue-Tek OCT compound; Miles Scientific, Fergus Falls, MN, USA), immersed in isopentane, cooled to its freezing point in liquid nitrogen and stored at −80°C until used. Five-micometre-thick cryosections were stained with haematoxylin and eosin.

The echogenicity and the thickness (epidermis and dermis) of the skin were evaluated in the middle 8 mm portion of the 14.9-mm-wide ultrasound images, and they were compared with the same attributes of the 8-mm-wide histological sections. In particular, the thickness of the skin was measured in both the ultrasound image (ultrasound scanner software) and the histological section (NIKON DS Fi1-L2 digital camera; NIKON Eclipse E200 microscope, Nikon corporation, Tokyo, Japan) of each examination site using 10 evenly distributed lines that were drawn perpendicular to the surface of the skin until reaching the subcutaneous fat; the average thickness was recorded and used for statistical analysis.

Statistical analysis

A linear mixed-effects model with dog identity as a random effect was used to determine whether a significant association existed between the ultrasonographic and histological measurements of skin thickness. A likelihood ratio test compared the mixed-effects model with a simple linear regression model to determine whether the observations were correlated or not. A linear mixed-effects model with dog identity as a random effect was also used to evaluate the statistical significance of the difference in the thickness of the skin between different examination sites, between male and female dogs and between dogs of different ages (age was treated as an ordinal variable). All analyses were performed using Stata version 10 (StataCorp LP, College Station, TX, USA).

Results

The skin could be visualized with high-frequency ultrasound biomicroscopy in all eight examination sites of all 10 dogs. The epidermis appeared as a thin, relatively nonuniform, hyperechoic linear layer. Linear echogenicities, roughly parallel or at a slight angle to the skin surface, were identified in the dermis, while the hypoechoic to anechoic subcutaneous fat was visible bellow the dermis (Figure 1). The epidermis, dermis and subcutaneous fat corresponded to the same skin layers identified in the histological sections (Figure 2). In addition, muscle fascia and muscle were visible in some images (Figures 1 and 3).

A relatively thick, mildly nonuniform, hyperechoic linear layer, which probably reflects the epidermis, was clearly visible in all images. The thickness of this layer varied and probably depended on the angle of the ultrasound beam, the pressure applied and the examination site. The margin between epidermis and dermis was also visible (Figures 1–3).

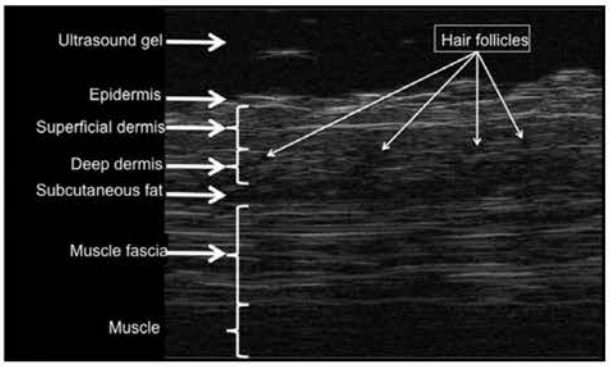

Figure 1. Close-up view of an ultrasonographic image of the centre of the flank skin of dog no. 9. The layers of the skin (epidermis, dermis and subcutaneous fat), hair follicles, muscle fascia and muscle are clearly visible. The superficial and deep dermis can be distinguished marginally by the length and thickness of the linear echoes they contain.

The dermis was clearly visible in all samples, having a granular echotexture that, in some samples, appeared to become more linear in the deeper parts (Figure 1). The dermis was hypoechoic to the epidermis and muscle fascia and hyperechoic to the subcutaneous layer. The superficial dermis had a granular appearance more loosely arranged, with thin and irregularly distributed small echoes that probably arose from the collagen fibres. The deep dermis contained thicker linear echoes orientated more in parallel to the skin surface; however, the distinction between superficial and deep dermis was not always clearly visible.

Below the dermis, the subcutaneous fat was visualized as an anechoic layer with sparse echogenicities (Figures 1–3). The muscle fascia, when visualized, appeared as linear, relatively thick echogenic lines orientated roughly parallel to the skin surface. The muscle below, when visible, appeared hypoechoic to the dermis and epidermis, had equal to slightly higher echogenicity to the subcutaneous fat, and was interrupted in some areas by thin hyperechoic interfaces that probably represented the intermuscular fascia.

The hair follicles, especially their isthmus and inferior segment, were regularly identified (Figures 1 and 2). Their appearance varied depending on the orientation of their axis in relationship to the orientation of the ultrasound beam. When the hair follicles were viewed along their long axis, they appeared as oblique, roughly tubular hypoechogenicities with faint echogenic linear areas at the margins, while when viewed in an oblique fashion they appeared as oblong hypoechogenicities, mildly irregular in outline (Figure 2). The fat around the root of the anagen hair follicles appeared as a 'cloudy' hypoechogenicity with indistinct margination (Figure 2).

The thickness of the skin (epidermis and dermis) at each examination site is shown in Table 1. When controlling for the effect of individual dogs, there was a highly significant, positive association between the ultrasonographic and histological measurements ($P < 0.001$; Table 2); a one-unit increase in the latter resulted in a one-unit increase in the former. The likelihood ratio test comparing the linear mixed-effects model with a simple linear regression model showed that the model that accounted for clustering differed significantly from the model that assumed observations were independent ($P = 0.001$), confirming that the data were correlated; thus, the measurements of the eight samples taken from the same dog were more similar to each other than to samples taken from any of the other dogs. For both ultrasonographic and histological skin thickness measurements, there were no statistically significant differences between sexes, ages or among the different examination sites (Table 3).

Discussion

Ultrasound biomicroscopy is a safe, quick and easy-to-use tool that provides important diagnostic information for various skin diseases in humans and may find similar applications in veterinary medicine in the future.[23] The ultrasonographic appearance and thickness of normal canine skin have been reported previously.[17,19] In the present study, we have used a 50 MHz polyvinylidene difluoride transducer incorporated into a probe filled with

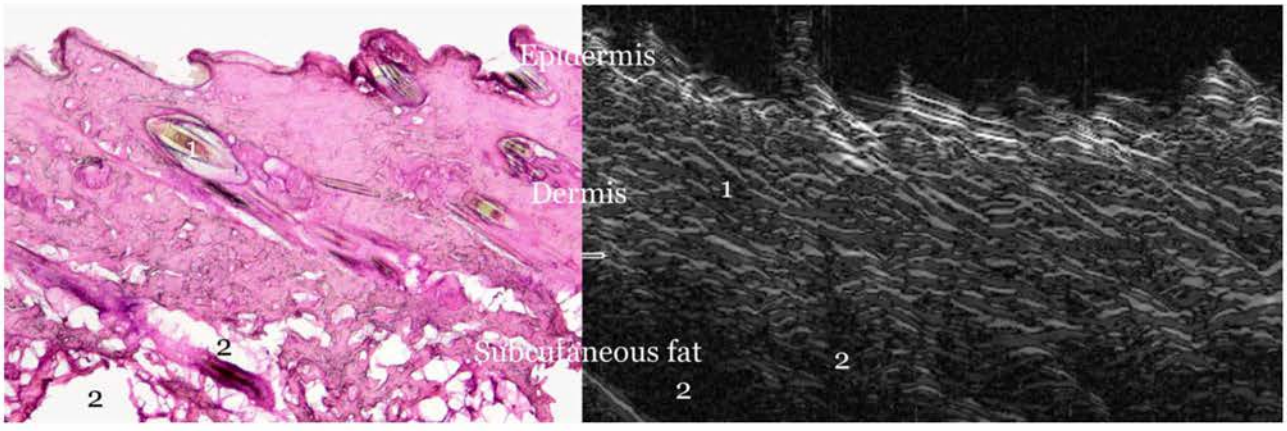

Figure 2. Haematoxylin- and eosin-stained histological and ultrasonographic images from the same examination site, i.e. the middle thoracic area (second examination site) of dog no. 1. The original ultrasonographic image has been cropped to include only the middle 8 mm portion of the original 14.9-mm-wide image and to have the same depth as the histological image. Numbers in the figure are as follows: 1, hair follicles; and 2, subcutaneous fat.

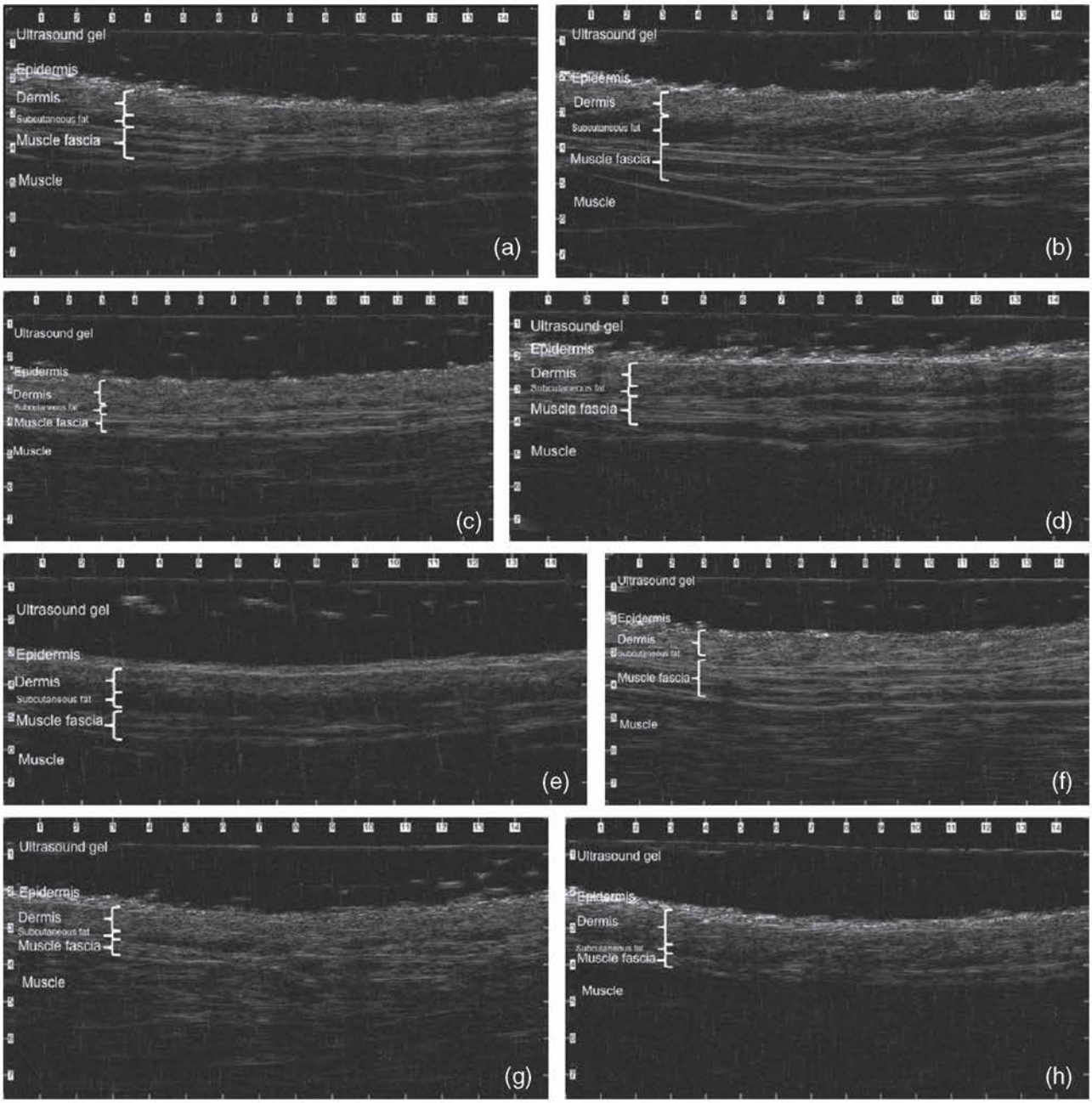

Figure 3. Ultrasonographic images of the skin of dog no. 1 corresponding to the dorsal thoracic area, over the upper palpable portion of the seventh rib (a), the middle thoracic area, over the middle of the seventh rib (b), the lower thoracic area over the joint between the seventh rib and the sternum (c), the upper lumbar area, over the fourth lumbar vertebra and 3 cm lateral to the spine (d), the centre of the flank (e), the ventral abdominal area at the level of the umbilicus and 3 cm lateral to the mid-line (f), the lateral forelimb over the middle of the humerus (g) and the lateral thigh over the middle of the femur (h). The epidermis, dermis and subcutaneous fat are clearly visible.

distilled water, and scanning was done using a digital stepping motor that allows image tissue resolution down to 40 μm. This is a much higher transducer frequency than the 13 MHz of the linear array transducers that have been employed previously,[17,19] thus allowing a more detailed imaging of the skin.

A mildly nonuniform hyperechoic layer, probably corresponding to the epidermis, was visible in all images. Although this layer was clearly visible, its lower margin at the epidermo-dermal interface was not distinct enough to allow accurate measurement of the epidermal thickness. This is probably related to the axial resolution of the 50 MHz transducer, and a higher frequency transducer would be expected to allow accurate measurement of the thickness of canine epidermis. This layer was followed by a second one, which was thicker and hypoechoic compared with the epidermal layer, had a granular appearance and was compatible with the dermis. The dermis was clearly visible and hyperechoic to the surrounding tissue and, in some samples, a distinction between the superficial and deep parts of the dermis was possible. The echogenicity of the dermis probably reflects its constituents, namely dermal ground substance, collagen and elastic fibres;[18] the different appearance of the linear echogenicities between the superficial and the deep dermis probably reflect differences in collagen

Table 1. Mean and SD of skin (epidermis and dermis) thickness measured using high-frequency ultrasound biomicroscopy images and snap-frozen skin biopsy histological sections for eight sites of the skin from 10 clinically healthy laboratory beagle dogs

Examination site*	1	2	3	4	5	6	7	8
Ultrasonographic measurement								
Mean (mm)	1.351	1.238	1.329	1.365	1.309	1.267	1.274	1.412
SD	0.172	0.158	0.198	0.193	0.153	0.107	0.146	0.159
Histological measurement								
Mean (mm)	1.354	1.251	1.343	1.365	1.319	1.276	1.279	1.433
SD	0.176	0.160	0.193	0.143	0.163	0.130	0.144	0.142

*Sites are as follows: site 1, dorsal thoracic area; site 2, middle thoracic area; site 3, lower thoracic area; site 4, upper lumbar area; site 5, centre of the flank; site 6, ventral abdominal area; site 7, lateral forelimb; and site 8, lateral thigh.

Table 2. Linear mixed-effects model with dog identity as a random effect to determine the association between ultrasonographic and histological measurements of the skin thickness (epidermis and dermis) for eight sites of the skin from 10 clinically healthy laboratory beagle dogs

	Coefficient	*P*-value	95% Confidence interval
Ultrasound	1.001	<0.001	0.947–1.054
Constant	−0.007	0.844	−0.080–0.065

fibres, which are normally thicker and less numerous in the deep dermis.[24] A third layer below the dermis was hypoechoic to the epidermis and dermis and represects subcutaneous fat. Also, in some samples, the muscle fascia and the superficial areas of the muscles were visible underneath the subcutaneous fat layer. The hair follicles could be identified in various orientations, and their location corresponded to that seen in the histological samples. To the authors' knowledge, this is the first time that the ultrasonographic appearance of the skin layers and hair follicles has been described for normal canine skin.

There was a highly significant association between ultrasonographic and histological measurements of skin thickness, with almost identical results from both modalities. This is different compared with previous studies, where the ultrasonographic measurements of skin thickness were higher than the histological ones. This is probably due to the use of snap-frozen rather than formalin-fixed skin biopsies, thus avoiding the shrinkage of the histology sample that always occurs during formalin fixation. In addition, 10 measurements were averaged in this study to calculate the skin thickness, which is markedly greater than the average of three measurements performed previously.[17,19] This was done to account for the variability that may result from different levels of skin compressibility during ultrasonography and also to minimize possible errors due to the subjective evaluation of the border between the dermis and subcutaneous fat and the expected variability between examiners regarding the exact location of placement of the callipers during thickness measurements for both modalities.

Table 3. Comparison of the mean ultrasonographic and histological measurements of skin thickness (epidermis and dermis) of 10 clinically healthy laboratory beagle dogs, between different sexes and ages and among eight examination sites

Variable	Histology	*P*-value	Ultrasound	*P*-value
Sex				
Male (n = 7)	1.35 ± 0.17	0.241	1.36 ± 0.17	0.201
Female (n = 3)	1.28 ± 0.14		1.28 ± 0.14	
Age				
4 years (n = 2)	1.33 ± 0.14	0.364	1.34 ± 0.14	0.472
6 years (n = 4)	1.30 ± 0.17		1.29 ± 0.17	
8 years (n = 1)	1.26 ± 0.09		1.25 ± 0.08	
9 years (n = 3)	1.38 ± 0.18		1.41 ± 0.17	
Examination site*				
1 (n = 10)	1.35 ± 0.18	0.084	1.36 ± 0.17	0.058
2 (n = 10)	1.25 ± 0.16		1.25 ± 0.16	
3 (n = 10)	1.34 ± 0.19		1.34 ± 0.21	
4 (n = 10)	1.36 ± 0.14		1.38 ± 0.15	
5 (n = 10)	1.32 ± 0.16		1.33 ± 0.16	
6 (n = 10)	1.28 ± 0.13		1.29 ± 0.10	
7 (n = 10)	1.28 ± 0.14		1.29 ± 0.16	
8 (n = 10)	1.43 ± 0.14		1.42 ± 0.17	

*Sites are as follows: site 1, dorsal thoracic area; site 2, middle thoracic area; site 3, lower thoracic area; site 4, upper lumbar area; site 5, centre of the flank; site 6, ventral abdominal area; site 7, lateral forelimb; and site 8, lateral thigh.

Canine skin thickness has been reported to decrease dorsally to ventrally in the trunk and proximally to distally in the limbs, with the thickest skin located on the head, dorsum of the neck, back and sacrum.[24,25] The findings of this study did not indicate a significant difference in skin thickness between the eight examination sites. A mild, nonsignificant decrease of the average thickness from dorsally to ventrally was found in the abdomen but not in the thoracic area, where the skin appeared to be thinner in the middle (examination site 2) by both ultrasonographic and histological measurements. This may be due to the measurement taking place over a rib and the stretching of the skin that may be greater in the middle.

A weak correlation was detected in beagle dogs between ultrasonographically measured skin thickness and sex and between skin thickness in dorsal neck or frontal regions and age in a previous study.[17] However, in the same study, no similar correlations were found in shar-pei dogs.[17] The present study was conducted in one breed, and the failure to find significant differences in skin thickness between male and female dogs and between different ages might have occurred because of the small number of individuals that were used. Also, with regard to age, the results should be evaluated cautiously because neither very young nor very old dogs were included in the study population. Further studies with a larger population of animals and standardization of the sex and neutering status in homogeneously aged groups are required to confirm these findings. Although only purpose-bred beagle dogs were studied, the authors consider that high-frequency cutaneous ultrasonography could be applied to privately owned dogs of various breeds with the aim of imaging normal skin and acquiring

baseline values of skin thickness in various areas of the body in an accurate and noninvasive way.

In conclusion, this study showed that cutaneous high-frequency ultrasound biomicroscopy using a 50 MHz transducer was a useful tool to identify the skin layers (including the epidermis, dermis and subcutaneous fat), to demonstrate the hair follicles and to measure accurately the thickness of normal canine haired skin.

Acknowledgements

The authors would like to thank Longport Inc. for loaning the high-resolution ultrasound scanner used in this study and Paul Wilson for the technical assistance and advice provided.

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Résumé

Contexte – L'apparence échographique de peau velue normale de chien n'a jamais été décrite.

Hypothèses/Objectifs – Décrire l'échogénicité de peau velue normale de chien à l'aide d'ultrasons à haute fréquence (50 MHz) et de comparer les valeurs échographiques et histologiques de l'épaisseur de la peau sur des échantillons de biopsies congelées.

Sujets – Dix chiens beagles sains.

Méthodes – L'examen échographique a été réalisé sur huit sites cutanés à l'aide d'un transducteur en difluorure de polyvinylidène. L'échogénicité de la peau a été évaluée et la moyenne des mesures de 10 épaisseurs cutanée a été calculée. Les résultats échographiques ont été comparés avec les données histologiques de cryosections de peau colorées à l'hémalun-éosine ainsi que les mesures histométriques d'épaisseur cutanée. Les différences de mesures échographiques et histologiques parmi les sites de biopsies, l'âge et le genre des animaux ont également été observées.

Résultats – Les couches cutanées et les follicules pileux ont pu être identifiés par biomicroscopie ultrasonore à haute fréquence pour les huit sites examinés des 10 chiens. Il y avait une association positive hautement significative entre les données échographiques et histologiques ($P < 0.001$) de l'épaisseur de la peau. Pour les données d'épaisseur cutanée échographique et histologique, il n'y avait aucune différence statistique significative entre les genres ou l'âge ou parmi les différents sites d'examen.

Conclusions et importance clinique – La biomicroscopie cutanée par ultrason à l'aide d'un transducteur de 50 MHz est un outil utile pour les applications suivantes: (i) identifier les couches de la peau (y compris

l'épiderme, le derme et la graisse sous-cutanée); (ii) mettre en évidence les follicules pileux dans les différentes zones de la peau velue; et (iii) mesurer l'épaisseur de peau normale de chien avec précision.

Resumen

Introducción – la morfología ultrasonográfica de la piel canina normal mediante ultrasonografía de alta frecuencia no ha sido descrita.

Hipótesis/Objetivos – describir la ecogenicidad de la piel canina normal utilizando ultrasonografía de alta frecuencia (50 MHz) y comparar las medidas con ultrasonografía con las medidas histológicas de grosor de la piel utilizando biopsias rápidamente congeladas.

Animales – diez perros Beagle normales.

Métodos – el examen ultrasonográfico se desarrolló en ocho zonas de la piel mediante un sensor de polivinilideno difluorado. Se evaluó la ecogenicidad de la piel y se calculó la media de 10 mediciones de grosor. Los resultados de la ultrasonografía se compararon con los hallazgos histológicos de secciones de la piel congelada teñidas con hematoxilina y eosina, así como con medidas histométricas del grosor de la piel. Se examinaron las diferencias en las medidas ultrasonográficas e histológicas entre los distintos lugares de biopsia, edad y sexo de los animales.

Resultados – los estratos de la piel y los pelos pudieron identificarse con un biomicroscopio de ultrasonido de alta frecuencia en las ocho zonas de examen en los diez perros. Hubo una asociación positiva significativa entre las medidas por ultrasonografía e histología ($P < 0{,}001$) del grosor de la piel. Tanto para el grosor determinado por ultrasonografía como mediante histología no se observaron diferencias significativas en función del sexo, edad o zonas examinadas.

Conclusiones e importancia clínica – la biomicroscopía cutánea de ultrasonidos con un sensor de 50 MHz es una herramienta útil para las siguientes aplicaciones: (i) identificar los estratos de la piel (incluidos la epidermis, dermis y tejido adiposo subcutáneo); (ii) demostrar los folículos pilosos en varias zonas de la piel; y (iii) medir el grosor de la piel normal con precisión.

Zusammenfassung

Hintergrund – Bisher wurde das ultrasonografische Erscheinungsbild von normaler behaarter Hundehaut mittels hochfrequenter Ultrasonografie noch nicht beschrieben.

Hypothese/Ziele – Eine Beschreibung der Echogenität von normaler behaarter Hundehaut mittels hochfrequenter (50MHz) Ultrasonografie und ein Vergleich der ultrasonografischen mit histologischen Messungen der Hautdicke in Gewebsproben, die mit flüssigem Stickstoff schockgefroren waren.

Tiere – Zehn gesunde Beagle.

Methoden – Eine ultrasonografische Untersuchung wurde an acht Hautstellen mittels 50 MHz Polyvinylidendifluorid Schallkopf durchgeführt. Die Echogenität der Haut wurde evaluiert, und der Durchschnitt von 10 Hautdickenmessungen kalkuliert. Die ultrasonografischen Ergebnisse wurden mit den histologischen Befunden der Kryoschnitte der Haut, die mit Hämatoxylin und Eosin gefärbt worden waren, sowie mit histometrischen Messungen der Hautdicke verglichen. Unterschiede zwischen den ultrasonografischen und histologischen Messungen der Biospiestellen, des Alters und Geschlechts der Tiere wurde ebenfalls untersucht.

Ergebnisse – Die Hautschichten und die Haarfollikel konnten mittels hochfrequentem Ultraschall Biomikroskop an allen acht untersuchten Hautstellen aller 10 Hunde identifiziert werden. Es bestand ein hochsignifikanter, positiver Zusammenhang zwischen den ultrasonografischen und histologischen Messungen ($P<0{,}001$) der Hautdicke. Für sowohl die ultrasonografischen als auch die histologischen Messungen der Hautdicke bestand kein statistisch signifikanter Unterschied bezüglich Geschlecht, Alter und unterschiedlichen Hautstellen.

Schlussfolgerungen und klinische Bedeutung – Die Ultraschall Biomikroskopie der Haut mittels 50 MHz Schallkopf ist ein wichtiges Utensil für die folgenden Anwendungen: (i) Identifizierung der Hautschichten (inklusive Epidermis, Dermis und Subkutis); (ii) Darstellung der Haarfollikel an verschiedenen behaarten Hautstellen; und (iii) genaue Messung der Dicke der normalen Hundehaut.

要約

背景 – 高振動数の超音波を用いて検査した正常なイヌの被毛部皮膚の超音波での外観は述べられていない。**仮説/目的**–正常なイヌの被毛部皮膚のエコー源性を高振動数(50 MHz)超音波を用いて述べること、およびスナップ凍結組織生検材料を用いて、超音波による所見と皮膚の厚みの組織学的な計測を比較すること。

供与動物 – 10頭の健常ビーグル犬

方法 – 超音波検査は50 MHzの二フッ化ポリビニリデントランスデューサーを使用して8ヶ所の皮膚部位で行った。皮膚のエコー源性を評価し、10ヶ所の皮膚の厚さの測定値の平均を計算した。超音波での結果を皮膚の厚さの組織計測とヘマトキシリン・エオジン染色した皮膚凍結組織の組織学的な所見とを比較した。動物の生検部位、年齢ならびに性別間の超音波および組織学的な計測の差も検討した。

結果 – 皮膚の層および毛包は10頭全てのイヌの検査部位8ヶ所全てにおいて、高振動数超音波生体顕微鏡検査法で特定することができた。それらは非常に有意で、皮膚の厚みにおける超音波での測定と組織学的な測定($P < 0.001$)の間に明らかな相関がみられた。超音波と組織学的な皮膚の厚みの測定の両方において、性別、年齢あるいは異なった検査部位の間に統計学的な有意差は認められなかった。

結論および臨床的な重要性 – 50 MHzトランスデューサーを用いた皮膚の超音波生体顕微鏡検査法は以下の利用に対して有益なツールとなる: (i)皮膚の層の確認のため(上皮、真皮、皮下脂肪を含む); (ii)有毛部皮膚の様々な部位にて毛包を証明するため; (iii)正常なイヌの皮膚の厚さを正確に測定するため。

摘要

背景 — 没有资料描述正常犬有被毛皮肤高频探头的超声影像。

假设/目的 — 描述在高频探头（50 MHz）下正常犬有毛发皮肤回声反射性，并比较超声和组织学测量下急速冷冻的或组织样本的皮肤厚度。

动物 — 10只健康的比格犬。

方法 — 用50 MHz的聚偏氟乙烯传感器超声检查8个皮肤损伤位点。评估皮肤的回声反射性，计算10块皮肤厚度平均值。对比超声结果和经苏木精和伊红染色的皮肤冰冻切片的组织学结果。超声和组织学测量的同时，也调查了动物的活组织取样部位、年龄和性别。

结果 — 10只犬中有8只用高频探头可检查到皮肤表层和毛囊。皮肤厚度的超声检查结果与组织学测量值明显正向相关（$P < 0.001$）。在性别、年龄和不同的检查位点上用超声和组织学测量皮肤厚度都没有显著差异。

总结与临床意义 — 用50 MHz探头作为受损皮肤超声活组织镜，对于以下应用是一项很有用的工具：（1）确定皮肤表层（包括表皮、真皮和皮下脂肪）；（2）检查各部位有毛皮肤的毛囊；（3）准确测量正常犬的皮肤厚度。

3. Research Study #2: High-frequency ultrasound biomicroscopy findings of the skin of dogs with atopic dermatitis

Received: 14 July 2022 | Accepted: 4 March 2023

DOI: 10.1111/vde.13169

Veterinary Dermatology

ORIGINAL ARTICLE

High-frequency ultrasound biomicroscopy findings of the skin of dogs with atopic dermatitis

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Abstract

Background: The high-frequency ultrasonographic appearance of skin of dogs with atopic dermatitis (cAD) has not been described.

Objectives: To compare high-frequency ultrasonographic findings among lesional, macroscopically nonlesional skin of dogs with cAD, and the macroscopically nonlesional skin of healthy dogs. Additionally, to determine whether there is any correlation between the ultrasonographic findings in lesional skin and local Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04) or its domains (erythema, lichenification, excoriations/alopecia). As a secondary aim, six cAD dogs were re-evaluated after management intervention.

Animals: Twenty dogs with cAD (six were re-examined after treatment) and six healthy dogs.

Materials and Methods: In all dogs, ultrasonographic examination was performed on the same 10 skin sites, using a 50 MHz transducer. Wrinkling of skin surface, presence/width of subepidermal low echogenic band, hypoechogenicity of dermis and thickness of the skin were evaluated and scored/measured blindly.

Results: Dermal hypoechogenicity was more common and severe in lesional compared to macroscopically nonlesional skin of dogs with cAD. In lesional skin, presence/severity of wrinkling of skin surface and of dermal hypoechogenicity were positively correlated with presence/severity of lichenification, while severity of dermal hypoechogenicity was positively correlated with local CADESI-04. A positive correlation between the change in skin thickness and the change in the severity of erythema during treatment was noted.

Conclusions and Clinical Relevance: High-frequency ultrasound biomicroscopy may be useful for the evaluation of skin of dogs with cAD and for evaluating the progression of skin lesions during treatment.

KEYWORDS
4th iteration (CADESI-04), atopy, biomicroscopy, canine atopic dermatitis extent and severity index, cutaneous

INTRODUCTION

Canine atopic dermatitis (cAD) is one of the commonest skin diseases of dogs.[1–3] The cardinal clinical sign is pruritus,[4] typically involving the face, pinnae, distal limbs, paws and ventral trunk.[5] Lesions associated with cAD include alopecia, erythema, excoriations and lichenification.[6] It is currently recommended[7] to assess the extent and severity of these lesions using the Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04)[8] and/or the Canine Atopic Dermatitis Lesional Index (CADLI).[9] Despite being validated, CADESI-4 and CADLI are subjective scales and there is a continuing effort to develop more objective methods, such as dermatoscopy to measure specific features such as erythema.[10]

Few studies on the ultrasonographic appearance of canine skin are available.[11–16] Skin thickness and hydration have been evaluated,[11,12,15] and high-frequency ultrasonography has been employed to study wound healing,[13,14] and to measure the thickness of normal skin.[16] No studies on the high-frequency ultrasonographic appearance of the skin in cAD are available. By contrast, the high-frequency ultrasonographic appearance of human lesional AD skin (AD-L), nonlesional AD skin (AD-NL) and healthy skin have been compared, the impact of treatment on ultrasonographic appearance of AD skin has been reported, and the width of the subepidermal low echogenic band (SLEB) has been shown to be positively correlated with the clinical severity of AD.[17–23]

The primary aims of this study were to (i) compare the high-frequency ultrasonographic appearance among cAD-L, cAD-NL skin and the macroscopically nonlesional skin of healthy dogs (H-NL) and (ii) investigate the correlation between findings in cAD-L skin and local CADESI-04 or its domains (erythema, lichenification, excoriations and alopecia). A secondary aim was to determine whether the high-frequency ultrasonographic appearance of cAD-L skin changes in parallel with local CADESI-4 or its domains during diagnostic and/or therapeutic interventions, not including administration of anti-inflammatory drugs.

MATERIALS AND METHODS

The study protocol was approved by the Ethical Committee for Animal Use in Scientific Research of the Institution of the last author (50/23-1-18). Owners of all dogs signed an informed consent for participation in the study.

Initial examination of dogs

Twenty dogs with cAD and six healthy dogs were included in this prospective blinded study.

Privately-owned dogs diagnosed with cAD *sensu lato*[24] and fulfilling at least six of eight of the first set of diagnostic criteria proposed by Favrot and coworkers (2010)[25,26] comprised the AD group. Dogs with cAD in remission [i.e. CADESI-04 < 10^8 and/or pruritus Visual Analog Scale (PVAS) ≤ 1.9/10],[27] treated with topical and/or oral glucocorticoids during the previous month or with injectable long-acting glucocorticoids during the previous two months or with clinical signs of hypercortisolism were excluded. The severity of pruritus was assessed by the owner using PVAS. Overall skin lesions, as well as lesions at skin sites where high-frequency ultrasonographic examination was performed, were scored by a board certified veterinary dermatologist, a supervised dermatology PhD student or supervised dermatology residents, using CADESI-04 and local CADESI-04 (to assess a specific area), respectively. Any skin site with local CADESI-04 ≥ 1 was considered cAD-L, while if local CADESI-04 = 0 it was considered cAD-NL.

Privately-owned dogs, with an age > 6 months, no evidence of dehydration and no cutaneous or systemic diseases, comprised the healthy group.

High-frequency ultrasonographic examination

A portable ultrasound scanner (Episcan-I-200, v4.0-UL; Longport International Ltd) with a 50 MHz transducer was used for the ultrasonographic examinations, as described previously.[16] The scanner was fitted with a polyvinylidene difluoride transducer incorporated into a probe filled with distilled water, and scanning was performed using a digital stepping motor. The ultrasonic beam was propagated through an aperture covered with a disposable rubber membrane. Ultrasound images were obtained in sagittal orientation. The transducer had a spherical focus of 8 mm and the axial resolution in the conditions in which the equipment was used was expected to be ~50 μm. The focal zone of the transducer was set at 2 mm below the normal plane of the transducer membrane, with beam width at 6 dB (~25 μm), and each displayed pixel was 29 μm wide. The scan rate was one frame per s. The acoustic centre frequency was 50.5 MHz (±10%) with pulse duration 18 ns. The maximum power from the transducer was 1 μW. The spatial-peak temporal average intensity was 8.9 W cm^{-2} (±23%) with a spatial-peak pulse-average intensity of 360 W/cm^2 (±22%). The dynamic range was 66 dB. The acquired images were 14.9 mm wide and 7.68 mm deep. The images were saved and stored in the associated hard drive and visualised using a greyscale mirror palette, which rectifies the signal, from the same software with which the ultrasound machine operates (EPISCAN-I-200, software v4.0-UL; Longport Inc.). After clipping, 10 areas of the skin were examined from the dogs of both groups: (i) concave aspects of left and right ear pinnae (positions 1 and 2, respectively), which present lesions in more than half of dogs with AD[25,28]; (ii) symmetrical areas on the convex aspects of ear pinnae (positions 3 and 4, respectively), which are typically nonlesional in dogs with AD; (iii) left and right axillae (positions 5 and 6, respectively), which present skin lesions in approximately half of dogs with AD[25,28,29]; (iv) area of nonlesional skin closest to the left and right axillae and located on an imaginary line connecting the centre of each axilla to the middle of the seventh rib for dogs with AD or an area on the middle of the above imaginary line for healthy dogs (positions 7 and 8, respectively); and (v) left and right flanks (positions 9 and 10, respectively), which present skin lesions in approximately one quarter of dogs with AD.[25]

All scans were performed by two investigators trained by a veterinary radiologist. In order to blind the latter as to which group the dog was a member of and the area of the skin under investigation, an online random number generator (http://stattrek.com/statistics/random-number-generator.aspx) was used to assign a code number to each dog and to each of the 10 scanned areas of each dog, and these random numbers were used to code the saved images.

All images were examined by the veterinary radiologist. The following qualitative, semiquantitative and quantitative attributes of each image were recorded, scored or measured in the middle 10 mm portion of the 14.9 mm wide images: (i) wrinkling of skin surface,[17] subjectively scored as 0 (absent), 1 (mild), 2 (moderate) or 3 (severe); (ii) presence of SLEB[17,19,20]; (iii) width of SLEB (if present), measured using 10 evenly distributed lines drawn perpendicular to the surface of the skin from the upper to the lower border of SLEB and calculating their mean value; (iv) hypoechogenicity of dermis,[20] subjectively scored as 0 (absent), 1 (mild), 2 (moderate) or 3 (severe); and (v) thickness of the skin, evaluated using 10 evenly distributed lines drawn perpendicular to the surface of the skin until reaching the subcutaneous fat (or ear pinnae cartilage) and calculating their average.

Treatment and re-examinations of dogs with cAD

All dogs with cAD which were not receiving anti-inflammatory drugs that were re-examined during the study period underwent a repeat scan as above. All remaining diagnostic and therapeutic interventions currently recommended for cAD[30,31] were allowed and were prescribed by a veterinary surgeon, either as single interventions or in various combinations.

Statistical analyses

Comparisons were made between (i) cAD-L and cAD-NL, (ii) cAD-L and H-NL and (iii) cAD-NL and H-NL skin. The prevalence of wrinkling of the skin surface, SLEB and dermal hypoechogenicity were compared by Pearson's chi-square (χ^2) r Fischer's exact test. The distribution of continuous or ordinal data (i.e. severity of wrinkling of skin surface, SLEB width, severity of dermal hypoechogenicity) was examined by Lilliefors modification of Kolmogorov–Smirnov test and subsequent comparisons were done by independent sample Student's *t*-test (normal distributions) or Wilcoxon–Mann–Whitney *U*-test (non-normal distributions).

The prevalence of ultrasonographic findings in cAD-L skin was compared between body sites with or without each domain of CADESI-04 by Pearson's χ^2 or Fischer's exact test. Correlations between continuous or ordinal ultrasonographic findings and local CADESI-04 or its domains were examined by Spearman's rank correlation coefficient. The same test was used to examine for possible correlations between differences in continuous or ordinal ultrasonographic findings (delta of the severity of wrinkling of skin surface, of SLEB width, of the severity of dermal hypoechogenicity and of skin thickness) and differences in local CADESI-04 and its domains before and after treatment.

Significance was set at $p<0.05$ and all analyses were performed using IBM SPSS STATISTICS 23.

RESULTS

The mean age ± standard deviation (SD) of the 20 dogs with cAD was 6.2 ± 2.8 years and their body weights ranged from 3.5 to 45 kg (median 12.2 kg). There were four males (two neutered) and 16 females (eight spayed). The six healthy dogs had an age of 3.7 ± 3.2 years. There were two males (one neutered) and four females (two spayed). Their weight was 18.3 ± 10 kg. Table 1 shows the breed, PVAS and CADESI-04 scores for the 26 dogs.

Six dogs with cAD (nos 12, 13, 15, 17, 18 and 20) were re-examined after a median period of 48 days, during which they received isoxazolines (6 of 6), itraconazole (6 of 6), systemic antibiotics (3 of 6), a commercial hydrolysed diet (2 of 6) and topical treatment with a chlorhexidine/miconazole-containing shampoo (1 of 6), resulting in significant improvement of PVAS ($p=0.016$) and not of CADESI-04 ($p=0.327$).

Lesional and macroscopically nonlesional skin of dogs with cAD and macroscopically nonlesional skin of healthy dogs

Suboptimal images (poor technique from excessive pressure or insufficient transducer contact, or artefacts created by gas bubbles in the distilled water or on the skin surface), and images from healthy dog skin areas with local CADESI-04 ≥ 1, were discarded. Thus, 83 cAD-L, 66 cAD-NL and 37 H-NL skin images were examined (see Table S1).

The comparisons of the ultrasonographic findings between cAD-L, cAD-NL and H-NL skin are presented in Table 2, the scores/measurements of the ultrasonographic abnormalities for each area of the skin are presented in Tables S2–S4, the comparisons of these scores/measurements between cAD-L, cAD-NL and H-NL skin are presented in Table 3, and the comparisons/correlations with local CADESI-04 and its domains for cAD-L skin are presented in Table 4. Wrinkling of the skin surface in cAD-L skin was significantly more common in areas with lichenification ($p=0.02$), and there was a significant ($p=0.015$) weak ($r=0.265$) positive correlation between the severity of wrinkling and the severity of lichenification. The width of SLEB (Figure 1) was significantly larger in cAD-NL compared to cAD-L skin ($p=0.043$). Prevalence and severity of dermal hypoechogenicity (Figure 2) were significantly higher ($p=0.048$ and $p=0.041$, respectively) in cAD-L compared to cAD-NL skin; also, in cAD-L skin, dermal hypoechogenicity was significantly more common in areas with lichenification ($p=0.003$) and its severity was positively and weakly correlated with local CADESI-04 scores ($p=0.002$; $r=0.329$) and with the severity of lichenification ($p=0.003$; $r=0.325$).

Changes over time in dogs with cAD

After discarding suboptimal images, 38 pairs of images obtained before and after intervention in six dogs

TABLE 1 Breed, pruritus Visual Analog Scale (PVAS) score and Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04) score for each dog of the atopic dermatitis (cAD) and the healthy group.

Group	Dog no.	Breed	PVAS	CADESI-04
cAD	1	WHWT	2.2	26
	2	WHWT	3.9	15
	3	WHWT	5.4	19
	4	WHWT	10	42
	5	CB	2.2	10
	6	CB	2.5	25
	7	CB	8.6	17
	8	French bulldog	2.5	12
	9	French bulldog	7.8	25
	10	Maltese terrier	5.6	13
	11	Maltese terrier	8.7	22
	12	Pitbull terrier	3.8	15
	13	Pitbull terrier	8.9	35
	14	Boxer	4.5	15
	15	Bull terrier	5.7	17
	16	Cane corso	5.9	33
	17	Cavalier King Charles spaniel	6	99
	18	Dogo Argentino	3.6	42
	19	English bulldog	7.9	22
	20	Poodle	2.6	21
	All dogs with cAD		5.4 ± 2.5	21.5 (10–99)
Healthy	1	CB	1	5
	2	CB	1	6
	3	CB	1.8	5
	4	Cocker spaniel	1.5	8
	5	Dalmatian	1.3	7
	6	Doberman	0.3	2
	All healthy dogs		1.2 ± 0.5	5.5 ± 2.1

Abbreviations: CB, cross-breed; WHWT, West Highland white terrier.

TABLE 2 Prevalence of wrinkling of skin surface, subepidermal low echogenic band (SLEB) and hypoechogenicity of dermis in the lesional (cAD-L) and macroscopically nonlesional (cAD-NL) skin of 20 dogs with atopic dermatitis (cAD), and in the macroscopically nonlesional skin of six healthy dogs (H-NL).

	cAD			Healthy		*p*-values		
	No of dogs (%)	cAD-L (*n*=83)	cAD-NL (*n*=66)	No of dogs (%)	H-LN (*n*=37)	cAD-L versus cAD-NL	cAD-L versus H-NL	cAD-NL versus H-NL
Wrinkling	10 (50%)	11 (13.3%)	7 (10.6%)	2 (33.3%)	4 (10.8%)	0.622	1	1
SLEB	9 (45%)	6 (7.2%)	4 (6.1%)	1 (16.7%)	1 (2.7%)	1	0.435	0.652
Hypoechogenicity	16 (80%)	27 (32.5%)	12 (18.2%)	4 (66.7%)	8 (21.6%)	0.048	0.225	0.672

Note: Significance of the comparisons between cAD-L and cAD-NL, cAD-L and H-NL and cAD-NL and H-NL are given by *p*-values.

with cAD were examined. The only significant correlation between changes in ultrasonographic findings (delta severity of wrinkling of skin surface, delta SLEB width, delta severity of dermal hypochogenicity and delta skin thickness) and changes in local CADESI-04 (delta CADESI-04) and its domains (delta erythema, delta lichenification and delta alopecia/excoriations), was a moderate ($r=0.446$) positive correlation between delta skin thickness and delta local erythema ($p=0.005$).

DISCUSSION

To the best of our knowledge, this is the first study describing high-frequency ultrasonographic findings in the skin of dogs with cAD and comparing cAD-L, cAD-NL and H-NL skin. Furthermore, this is the first study on cAD in any species, including humans, that has been designed in a such a way that the single evaluator of ultrasound images was blinded to the health status (cAD or healthy), the area of the skin that corresponded to

each image (positions 1–10) and the macroscopic appearance of the skin (L or NL). We consider that blinding of the evaluator is of major importance to avoid observer bias, because most of the attributes of the images (presence and severity of epidermal wrinkling, presence of SLEB, presence and severity of hypoechogenicity of dermis) are subjective and, thus, their interpretation is prone to errors.

It has been shown that when normal canine haired skin is examined with a 50 MHz transducer, it presents as a thin, relatively nonuniform area that corresponds to the epidermis, followed by a hypoechoic dermis.[16] In addition to H-NL, this general ultrasonographic appearance of canine skin also was evident in cAD-L and cAD-NL skin in the present study.

Wrinkling of the skin surface (also called "unevenness of epidermal upper and lower contour"), has been found in 68% of children with AD when multiple areas of AD-L skin were scanned, and in 59% of the same children when multiple areas of AD-NL skin were scanned.[21] Our results in dogs with cAD are somewhat similar because half of them presented wrinkling in cAD-L and/or cAD-NL skin (Table 2). However, the same ultrasonographic finding was present in 33% of healthy dogs and there was no difference in the prevalence or in the severity of wrinkling among cAD-L, cAD-NL and H-NL skin (Tables 2 and 3). By contrast, in humans it is considered that wrinkling is more severe in AD-L compared to AD-NL and H-NL skin.[17] This discrepancy may reflect the lack of bias in the present study, owing to the blinding of the evaluator as to the group of the dog and to the macroscopic appearance of the skin corresponding to each image. Therefore, wrinkling may be considered a nonspecific ultrasonographic finding, without obvious clinical significance, that may be present in cAD-L, cAD-NL and in H-NL skin. However, in the cAD-L skin, wrinkling was significantly more common in skin areas with lichenification, and there was a weak – and significant – positive correlation between the severity of wrinkling and the severity of lichenification. This finding seems logical because the rough surface of lichenified skin is expected to be seen on ultrasound images as wrinkling. Further studies, combining clinical, ultrasonographic and histopathological examination, are needed to clarify if wrinkling is the ultrasonographic counterpart of lichenification and of the underlying epidermal hyperplasia in cAD-L skin.

In human dermatology, SLEB is a non-AD-specific finding of high-frequency ultrasound biomicroscopy that also can be seen in other inflammatory (e.g. psoriasis), environmental (e.g. solar dermatitis) and neoplastic (e.g. mycosis fungoides) skin diseases, secondarily to superficial dermal infiltration by inflammatory or neoplastic cells, oedema, accumulation of glycosaminoglycans, altered structure of collagen and elastic fibres and/or elongation of rete ridges.[17,19,32] Specifically in AD-L skin, the width of SLEB is positively correlated, not only with the severity of the inflammatory infiltrate, but also with the accompanying epidermal changes (hyperkeratosis, parakeratosis, hyperplasia and spongiosis).[32] In different patient populations, the prevalence of SLEB in the AD-L and AD-NL skin varies from 94%

TABLE 3 Comparisons (*p*-values) of the severity of wrinkling of skin surface, of the width of subepidermal low echogenic band (SLEB) and of the severity of hypoechogenicity of dermis among the lesional (cAD-L) and macroscopically nonlesional (cAD-NL) skin of 20 dogs with atopic dermatitis (cAD) and the macroscopically nonlesional skin of six healthy dogs (H-NL).

	cAD-L versus cAD-NL	cAD-L versus H-NL	cAD-NL versus H-NL
Wrinkling	0.607	0.695	0.974
SLEB	0.043	0.251	0.427
Hypoechogenicity	0.041	0.18	0.704

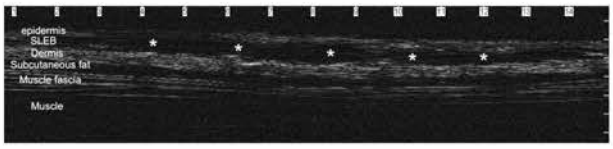

FIGURE 1 Subepidermal low echogenic band (asterisks) with a thickness of 0.838 mm in the ultrasonographic image obtained from the right flank (position 10) of Dog 7 with atopic dermatitis.

TABLE 4 Comparison and correlations (*p*- and *r*-values) between (a) the presence/severity of wrinkling of skin surface, the presence/width of subepidermal low echogenic band (SLEB) and the presence/severity of hypoechogenicity of the dermis and (b) local Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04) and the presence/severity of its domains (erythema, lichenification, excoriations/alopecia) in the lesional skin of 20 dogs with atopic dermatitis (cAD).

	Local CADESI-04		Erythema		Lichenification		Excoriations/alopecia	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Wrinkling								
Presence	—	—	—	0.44	—	0.02	—	0.352
Severity	0.105	0.345	0.01	0.931	**0.265**	0.015	**−0.16**	0.148
SLEB								
Presence	—	—	—	0.264	—	0.148	—	0.586
Width	−0.372	0.468	−0.338	0.512	**−0.123**	0.816	—	—
Hypoechogenicity								
Presence	—	—	—	1	—	0.003	—	0.191
Severity	0.329	0.002	0.129	0.243	**0.325**	0.003	**0.167**	0.131

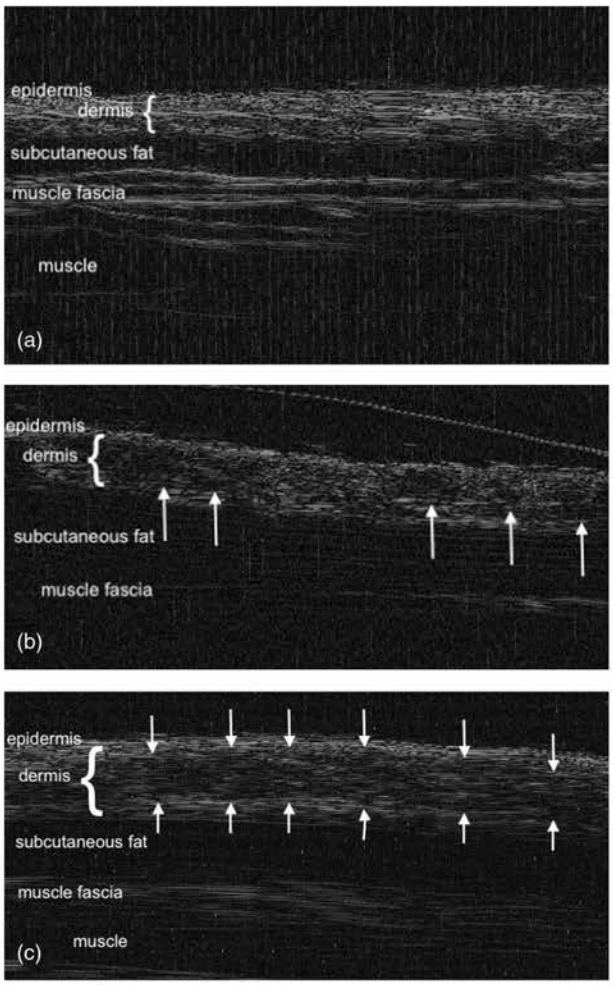

FIGURE 2 Images showing absence or presence of dermal hypoechogenicity of variable severity. (a) Absence of dermal hypoechogenicity in the ultrasonographic image obtained from the area of nonlesional skin closest to the left axillae (position 7) of Dog 20 with atopic dermatitis (open parenthesis shows the extent of the whole dermis). (b) Mild dermal hypoechogenicity in the ultrasonographic image obtained from the convex aspect of the right ear pinna (position 4) of Dog 16 with atopic dermatitis (arrows indicate the hypoechoic patches in the dermis). (c) Moderate dermal hypoechogenicity in the ultrasonographic image obtained from the concave aspects of the right ear pinna (position 2) of Dog 18 with atopic dermatitis (arrows indicate the upper and lower limits of the extensive hypoechoic area in the dermis).

to 100% and from 13% to 77%, respectively.[19–21,32,33] In the present study, the prevalence of SLEB was much lower compared to humans, because it was found in only 45% of dogs with cAD after scanning 10 areas of the skin of each dog (Table 2). A possible explanation could be that we used a transducer of higher frequency (50 MHz) than those used in most human studies (20 MHz), and this resulted in higher image resolution and an inability to clearly differentiate SLEB from an underlying hypoechogenic dermis. However, in a study of 22 children with AD, when a 75 MHz transducer was used, SLEB was present in 100% AD-L and in 77% AD-NL skin.[21] Therefore, the most reasonable explanation for this difference between human AD and canine cAD is the different structure of the skin between the two species, and especially, the increased number of larger and compound hair follicles in the haired canine skin that do not leave sufficient space for the superficial oedema and inflammatory infiltrate to create a hypoechogenic area large enough to be clearly identified as SLEB on high-frequency ultrasound biomicroscopy. In human dermatology there is debate regarding the specificity of SLEB as an indicator of skin disease, because in some studies it was absent in H-NL skin,[19,20,32] yet in other studies it was present.[17,18,21] Our results are in line with the latter studies, because the blinded examiner identified SLEB in a single image from a healthy dog (Table 2). Surprisingly, the width of SLEB was significantly higher in the four cAD-NL areas of the skin where SLEB was present compared to the six areas of cAD-L skin; a possible explanation may be that in these four areas of cAD-NL skin there was acute inflammation, accompanied by superficial oedema that resulted in both a wider SLEB and in a collapse of the capillaries that resulted in the absence of macroscopic erythema.

In human AD, the echogenicity of the dermis depends on the severity of inflammatory cell infiltration.[32] When measured with image analysis software, it was found to be lower in AD-L compared to AD-NL and H-NL skin, and in AD-NL compared to H-NL skin, in some[20,32] and not in all studies.[21] Furthermore, to the best of our knowledge, possible correlations between the severity of hypoechogenicity of AD-L skin and the severity of local lesions have not been investigated. In our study, where the presence and severity of hypoechogenicity of dermis were evaluated blindly and subjectively, it was found to be more common and more severe in cAD-L compared to cAD-NL skin, to be more common in cAD-L skin with lichenification, and to be weakly and significantly correlated with local CADESI-04 and local lichenification. These results can be explained by the more severe infiltration of inflammatory cells in the cAD-L compared to cAD-NL skin, in the cAD-L skin with more severe macroscopic lesions and in the chronically inflamed lichenified skin.[34–36] The lack of difference between cAD-L (and perhaps also cAD-NL) and H-NL skin may be a consequence of the small number of healthy dogs and/or the subjective evaluation of hypoechogenicity of dermis. Future studies including more healthy dogs and using image analysis software are needed to further clarify this.

In order to study the changes of high-frequency ultrasonographic findings during diagnostic and therapeutic interventions for cAD, we intentionally excluded dogs treated with anti-inflammatory drugs. Although these drugs are expected to reduce epidermal hyperplasia/lichenification, oedema and inflammatory cell infiltration and thus to reduce wrinkling of skin surface, width of SLEB, hypoechogenicity of dermis and thickness of the skin, they may have additional indirect effects on skin thickness, a parameter that can be measured accurately with high-frequency biomicroscopy.[16] Glucocorticoids can decrease skin thickness due to the inhibition of collagen synthesis and extracellular matrix production by fibroblasts, calcineurin inhibitors can increase skin thickness due to transforming growth factor beta (TGF-b)-mediated stimulation of collagen and ground substance synthesis, and, although it is unknown if oclacitinib can have similar effects, this is possible because TGF-b signalling is mediated by

Janus kinase-coupled receptors.[23,37–40] Despite the low number of dogs, a significant, moderately positive correlation was found between reduction of skin thickness and of surface erythema, which is the principal marker of acute inflammation. Further studies are needed to examine the changes of high-frequency ultrasonographic findings during treatment of cAD with anti-inflammatory drugs and to investigate if these changes can be used as an objective measure of treatment efficacy.

A limitation of our study is that skin biopsies were not obtained and, thus, the correlations between ultrasonographic and histological findings could not be examined. However, obtaining such a large number of biopsies (10 per dog) from dogs with cAD and healthy dogs would have raised ethical concerns, it would probably have met with owner's reluctance to participate, and it would have been meaningless if significant differences of the ultrasonographic findings between cAD-L, cAD-NL and H-NL skin had not been found. However, based on our results, future studies examining the correlations between ultrasonographic and histological findings in cAD-L and cAD-NL skin are warranted. Another limitation is that the ultrasonographic examination of the cAD skin necessitates specialised equipment and a highly experienced examiner.

In conclusion, blinded evaluation of high-frequency ultrasound biomicroscopy images from cAD-L, cAD-NL and H-NL skin showed that dermal hypoechogenicity was more common/severe in cAD-L compared to cAD-NL skin. Also, in cAD-L skin the presence/severity of wrinkling of skin surface and of dermal hypoechogenicity were positively correlated with the presence/severity of lichenification, while severity of dermal hypoechogenicity was positively correlated with local CADESI-04. Finally, a moderate positive correlation between the skin thickness and the severity of local erythema was seen in the dogs in which repeat ultrasounds were performed during diagnostic and therapeutic interventions for cAD.

AUTHOR CONTRIBUTIONS

Panagiotis Mantis involved in conceptualisation, investigation, data curation, methodology, project administration, writing original draft preparation, review and editing. **Evangelia Sofou** involved in investigation and data curation. **Svetlina Aleksandrova, Panagiotis Koutsouvelis, Stathis Mpairamoglou, Manolis Chatzis and Elisa Badulescu** involved in investigation. **David Church and David Lloyd** involved in supervision, conceptualisation, project administration, writing review and editing. **Manolis Saridomichelakis** involved in conceptualisation, data curation, project administration, methodology, investigation, project administration, writing review and editing.

ACKNOWLEDGEMENT

The authors would like to thank Longport Inc. for loaning the high-resolution ultrasound scanner used in this study and Paul Wilson for the technical assistance and advice provided.

FUNDING INFORMATION

This study was self-funded.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Mantis P, Sofou EI, Aleksandrova S, Badulescu E, Church D, Lloyd D, et al. High-frequency ultrasound biomicroscopy findings of the skin of dogs with atopic dermatitis. Vet Dermatol. 2023;00:1–10. https://doi.org/10.1111/vde.13169

摘要

背景: 特应性皮炎(cAD)患犬的皮肤高频超声表现尚未被报道。

目的: 比较cAD患犬的病变、肉眼可见的非病变皮肤和健康犬的肉眼可见的无病变皮肤的高频超声检查结果。此外,为了确定病变皮肤的超声检查结果与局部犬特应性皮炎程度和严重程度指数第4次迭代(CADESI-04)或其区域(皮肤发红、苔藓化、抓痕/脱毛)之间是否存在相关性,。作为次要目的,在管理干预后对6只cAD患犬进行了重新评估。

动物: 20只cAD患犬(6只在治疗后重新检查)和6只健康犬。

材料和方法: 在所有犬中,使用50MHz换能器对相同的10个皮肤部位进行超声检查。对皮肤表面的皱纹、表皮下低回声带的存在/宽度、真皮的低回声和苔藓化厚度进行评估和盲目评分/测量。

结果: 与cAD患犬肉眼可见的非病变皮肤相比，病变中的皮肤低回声更常见和更严重。在病变皮肤中，皮肤表面皱纹和真皮低回声的存在/严重程度与苔藓化的出现/严重程度呈正相关，而真皮低回声严重程度与局部CADESI-04呈正相关。治疗期间，皮肤厚度的变化与皮红严重程度的变化呈正相关。
结论和临床相关性: 高频超声生物显微镜可用于评估cAD犬的皮肤，并可用于评估治疗过程中皮肤病变的进展。

Résumé
Contexte: L'aspect échographique avec une sonde à haute fréquence de la peau des chiens atteints de dermatite atopique (cAD) n'a pas été décrit.
Objectifs: Comparer les observations échographiques réalisées avec une sonde à haute fréquence de la peau lésionnelle macroscopiquement non lésionnelle de chiens atteints de cAD et la peau macroscopiquement non lésionnelle de chiens sains. De plus, déterminer s'il existe une corrélation entre l'aspect échographiques de la peau lésionnelle et le score local d'étendue et de gravité de la dermatite atopique canine, 4e édition (CADESI-04) ou ses paramètres (érythème, lichénification, excoriations/alopécie). Comme objectif secondaire, six chiens cAD ont été réévalués après l'intervention de la direction.
Animaux: Vingt chiens atteints de DAC (six ont été réexaminés après traitement) et six chiens sains.
Matériels et méthodes: Chez tous les chiens, un examen échographique a été effectué sur les dix mêmes sites cutanés, à l'aide d'une sonde de 50 MHz. Le plissement de la surface de la peau, la présence/largeur d'une bande sous-épidermique faiblement échogène, l'hypoéchogénicité du derme et l'épaisseur de la peau ont été évalués et notés/mesurés à l'aveugle.
Résultats: L'hypoéchogénicité cutanée était plus fréquemment observée et plus prononcée pour la peau lésionnelle que pour la peau macroscopiquement non lésionnelle des chiens atteints de DAC. Dans la peau lésionnelle, la présence/sévérité des rides de la surface de la peau et de l'hypoéchogénicité dermique étaient positivement corrélées avec la présence/sévérité de la lichénification, tandis que la sévérité de l'hypoéchogénicité dermique était positivement corrélée avec le CADESI-04 local. Une corrélation positive entre la variation d'épaisseur de la peau et l'évolution de la sévérité de l'érythème pendant le traitement a été notée.
Conclusions et pertinence clinique: La biomicroscopie échographique à haute fréquence peut être utile pour évaluer la peau des chiens atteints de DAC et l'évolution des lésions cutanées durant le traitement.

Zusammenfassung
Hintergrund: Das Hochfrequenz Ultraschallerscheinungsbild der Haut von Hunden mit atopischer Dermatitis (cAD) wurde noch nicht beschrieben.
Ziele: Das Ziel dieser Studie war der Vergleich von Hochfrequenz Ultraschallerscheinungsbildern läsionaler, makroskopisch nicht-läsionaler Haut von Hunden mit cAD und makroskopisch nicht-läsionaler Haut gesunder Hunde. Zusätzlich sollte festgestellt werden, ob eine Korrelation zwischen den Ultraschallbefunden in läsionaler Haut und beim lokalen Canine Atopic Dermatitis Extent and Severity Index 4te Auflage (CADESI-04) oder seiner Domains (Erythem, Lichenifizierung, Exkoriationen/Alopezie) besteht. Als zweites Ziel wurden sechs cAD-Hunde nach einer Management Veränderung nochmals evaluiert.
Tiere: Zwanzig Hunde mit cAD (sechs wurden nach der Behandlung neuerlich evaluiert) und sechs gesunde Hunde.
Materialien und Methoden: Bei allen Hunden wurde eine Ultraschalluntersuchung mittels 50 MHz Schallkopf an den 10 gleichen Hautstellen durchgeführt. Es wurden eine Faltenbildung der Haut, das Auftreten/Weite des subepidermalen niedrig echogenen Bandes, Hypoechogenität der Dermis und die Dicke der Haut evaluiert und blind bewertet/gemessen.
Ergebnisse: Die dermale Hypoechogenität war häufiger und deutlicher bei läsionaler Haut im Vergleich zu makroskopisch nicht läsionaler Haut von Hunden mit cAD. In läsionaler Haut war das Auftreten/Schweregrad der Faltenbildung auf der Hautoberfläche und die dermale Hypoechogenität positiv korreliert mit dem Auftreten/Schweregrad der Lichenifizierung, während das Ausmaß der dermalen Hypoechogenität positiv korreliert war mit einem lokalen CADESI-04. Eine positive Korrelation zwischen der Veränderung der Hautdicke und der Veränderung vom Ausmaß des Erythems während der Behandlung wurde festgehalten.
Schlussfolgerungen und klinische Bedeutung: Hochfrequenz Ultraschall Biomikroskopie kann für die Evaluierung der Haut der Hunde mit cAD und für die Evaluierung des Fortschritts der Hautveränderungen während der Behandlung nützlich sein.

要約
背景: アトピー犬の皮膚に対する高周波超音波検査像はこれまで記述されていない。
目的:　本研究の目的は、犬アトピー性皮膚炎(cAD)の病変部、肉眼的に非病変部の皮膚、健常犬の肉眼的に非病変部の皮膚の高周波超音波所見を比較することであった。さらに、病変部皮膚の超音波所見および局所的な Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04) **またはその領域(紅斑、苔癬化、擦過傷/脱毛症)の間に相関があるかどうかを明らかにすることであった。二つ目の目的として、6頭のアトピー犬が管理介入後に再評価した。**
対象動物: cAD犬20頭(6頭は治療後に再検査)および健常犬6頭。
材料と方法:　すべての犬において、50MHzのトランスデューサを用い、同じ10箇所の皮膚に対して超音波検査を実施した。皮膚表面のしわ、表皮下低エコー帯の有無/幅、真皮の低エコー性、皮膚の厚さを評価し、盲目化でスコア/測定した。

結果: 真皮の低エコー源性は、cAD犬の病変部皮膚では、非病変部皮膚とマクロ的に比較して、より一般的で重度であった。病変部では、皮膚表面のしわの有無や真皮低エコー化は苔癬化の有無や程度と正の相関があり,真皮低エコー化の程度は局所CADESI-04と正の相関があった。また、治療中の皮膚厚の変化と紅斑の重症度の変化には正の相関が認められた。
結論と臨床的関連性 高周波超音波生体顕微鏡検査は、cADを有する犬の皮膚の評価や治療中の皮膚病変の進行の評価に有用であると考えられた。

Resumo
Contexto: A aparência da pele de cães com dermatite atópica (DAC) por ultrassonografia de alta-frequência ainda não foi descrita.
Objetivos: Comparar os achados de ultrassonografia de alta frequência na pele lesional e macroscopicamente alesional em cães com DAC, e na pele macroscopicamente alesional em cães saudáveis. Além disto, determinar se há alguma correlação entre os achados ultrassonográficos na pele lesional e o *Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04)* local ou os seus domínios (eritema, liquenificação, excoriações/alopecia). Como objetivo secundário, seis cães com DAC foram reavaliados após tratamento.
Animais: Vinte cães com DAC (seis foram re-examinados após o tratamento) e seis cães saudáveis.
Materiais e métodos: Em todos os cães, exame ultrassonográfico foi realizado nas mesmas 10 regiões cutâneas, utilizando um transdutor de 50 MHz. Enrugamento da superfície cutânea, presença/profundidade de bandas subepidérmicas de baixa ecogenicidade, hipoecogenicidade dérmica e espessura da pele foram avaliadas e classificadas cegamente.
Resultados: Hipoecogenicidade dérmica foi mais comum e mais grave na pele lesional comparada à pele macroscopicamente alesional de cães com DAC. Na pele lesional, presença/gravidade do enrugamento da superfície cutânea e da hipoecogenicidade dérmica foram positivamente correlacionadas com o CADESI-04 do local. Observou-se uma correlação positive entre as alterações na espessura cutânea e na alteração da gravidade do eritema durante o tratamento.
Conclusões e Relevância Clínica: Biomicroscopia ultrassonográfica de alta frequência pode ser útil para a avaliação da pele de cães com DAC e para avaliação da progressão das lesões cutâneas durante o tratamento.

Resumen
Introducción: No se ha descrito la apariencia ultrasonográfica de alta frecuencia de la piel de perros con dermatitis atópica (cAD).
Objetivos: Comparar los hallazgos ultrasonográficos de alta frecuencia entre la piel lesionada y macroscópicamente no lesionada de perros con cAD y la piel macroscópicamente no lesionada de perros sanos. Además, determinar si existe alguna correlación entre los hallazgos ultrasonográficos en la piel lesionada y el índice de extensión y gravedad de la dermatitis atópica canina local, 4.ª revisión (CADESI-04) o sus dominios (eritema, liquenificación, excoriaciones/alopecia). Como objetivo secundario, seis perros con cAD fueron reevaluados después del manejo de la enfermedad.
Animales: Veinte perros con cAD (seis fueron reexaminados después del tratamiento) y seis perros sanos.
Materiales y Métodos: En todos los perros, se realizó un examen ultrasonográfico en los mismos 10 sitios de la piel, utilizando un transductor de 50 MHz. Se evaluaron y midieron a ciegas las arrugas de la superficie de la piel, la presencia/anchura de la banda subepidérmica de baja ecogenicidad, la hipoecogenicidad de la dermis y el grosor de la piel.
Resultados: La hipoecogenicidad dérmica fue más frecuente y grave en la piel lesionada en comparación con la piel macroscópicamente no lesionada de los perros con cAD. En la piel lesionada, la presencia/gravedad de las arrugas de la superficie de la piel y de la hipoecogenicidad dérmica se correlacionaron positivamente con la presencia/gravedad de la liquenificación, mientras que la gravedad de la hipoecogenicidad dérmica se correlacionó positivamente con el CADESI-04 local. Se observó una correlación positiva entre el cambio en el grosor de la piel y el cambio en la gravedad del eritema durante el tratamiento.
Conclusiones y relevancia clínica: La biomicroscopía ultrasónica de alta frecuencia puede ser útil para la evaluación de la piel de perros con cAD y para evaluar la progresión de las lesiones cutáneas durante el tratamiento.

Supporting information

Table S1. Number of images that were discarded and number of images that were examined for each of the 10 scanned areas of the skin of 20 dogs with atopic dermatitis (AD) and of six healthy dogs

	Local CADESI-4	Erythema	Lichenification	Excoriations/alopecia
Presence of wrinkling	-	0.44	0.02	0.352
Severity of wrinkling	0.345	0.931	0.015	0.148
Presence of SLEB	-	0.264	0.148	0.586
Width of SLEB	0.468	0.512	0.816	-
Presence of Hypoechogenicity	-	1	0.003	0.191
Severity of Hypoechogenicity	0.002	0.243	0.003	0.131

Table S2. Presence and score of wrinkling of skin surface for each of the 10 scanned areas of the skin of 20 dogs with atopic dermatitis (AD) and of six healthy dogs

Position	**Dogs with AD**				**Healthy dogs**			
	Presence	**Mild**	**Moderate**	**Severe**	**Presence**	**Mild**	**Moderate**	**Severe**
1	3/15	2	1		1/3	1		
2	1/16	1			0/2			
3	1/12	1			0/5			
4	1/14	1			1/6	1		
5	0/15				0/3			
6	3/15	3			0/3			
7	3/16	3			1/5	1		
8	2/15	2			1/5	1		
9	2/16	2			0/3	0		
10	2/15	2			0/2			
Total	**18/149**	**17/18**	**1/18**	**0/18**	**4/37**	**4/4**	**0/4**	**0/4**

Table S3. Presence and width of subepidermal low echogenic band for each of the 10 scanned areas of the skin of 20 dogs with atopic dermatitis (AD) and of six healthy dogs

Position	**Dogs with AD**		**Healthy dogs**	
	Presence	**Thickness (mm)**	**Presence**	**Thickness (mm)**
1	2/15	0.403/0.422	0/3	
2	0/16		0/2	
3	1/12	0.778	0/5	
4	0/14		0/6	
5	0/15		0/3	
6	0/15		0/3	
7	3/16	0.372/0.511/0.62	1/5	0.534
8	1/15	0.529	0/5	
9	1/16	0.464	0/3	
10	2/15	0.504/0.838	0/2	
Total	**10/149**	**0.544 ± 0.157**	**1/37**	**0.534**

Table S4. Presence and score of hypoechogenicity of dermis for each of the 10 scanned areas of the skin of 20 dogs with atopic dermatitis (AD) and of six healthy dogs

Position	**Dogs with AD**				**Healthy dogs**			
	Presence	**Mild**	**Moderate**	**Severe**	**Presence**	**Mild**	**Moderate**	**Severe**
1	6/15	3	3		0/3			
2	3/16		3		0/2			
3	1/12	1			0/5			
4	3/14	3			2/6	1	1	
5	4/15	2	2		1/3	1		
6	4/15	3	1		0/3			
7	7/16	5	2		2/5	1	1	
8	4/15	2	2		3/5	3		
9	4/16	1	3		0/3			
10	3/15	3			0/2			
Total	**39/149**	**23/39**	**16/39**	**0/39**	**8/37**	**8/8**	**2/8**	**0/8**

CONCLUSIONS

This is the first study to describe the appearance of normal canine haired skin on ultrasound biomicroscopy and the differences in its appearance among various anatomic locations. It is also the first study to record the ultrasound biomicroscopy findings in an inflammatory skin disease of dogs, namely canine atopic dermatitis, and their changes during treatment.

In conclusion:

1. Cutaneous ultrasound biomicroscopy is applicable for the examination of normal and diseased canine skin. It is safe and easy to perform and allows the examination of canine skin at microscopic resolution.
2. Cutaneous ultrasound biomicroscopy using a 50 MHz transducer allows identification of the skin layers including epidermis, dermis, and subcutaneous fat.
3. Cutaneous ultrasound biomicroscopy allows accurate measurement of the thickness of normal canine skin.
4. Cutaneous ultrasound biomicroscopy can demonstrate the hair follicles in various areas of haired skin.
5. High frequency ultrasound biomicroscopy showed that dermal hypoechogenicity is more common and more severe in the lesional skin of dogs with atopic dermatitis compared to their non-lesional skin, and that the presence and the severity of wrinkling of the skin surface and of dermal hypoechogenicity are positively correlated with local lesional score.
6. High frequency ultrasound biomicroscopy identified a positive correlation between the change in skin thickness and the severity of local erythema during diagnostic and therapeutic interventions for canine atopic dermatitis that did not include the administration of anti-inflammatory drugs.

PROPOSALS FOR FUTURE STUDIES

The results of this research are highly suggestive that cutaneous ultrasound biomicroscopy may have numerous clinical applications in canine dermatology. Future studies are required to expand the scope of this technique, to enter the daily clinical practice when evaluating dogs with skin diseases.

Areas of future development may include:

1. Evaluation of ultrasound biomicroscopy findings in diseases like cutaneous and subcutaneous neoplasia, cysts, post-radiation reactions, and various inflammatory skin diseases.
2. Use of ultrasound biomicroscopy to monitor the response to therapy of the above diseases
3. Doppler ultrasonographic examination of normal and diseased canine skin.
4. Ultrasonographic examination of normal and lesional canine skin with frequencies higher than 50MHz.

SUMMARY

ULTRASOUND BIOMICROSCOPY OF CANINE SKIN

Traditionally, the diagnosis of skin diseases is based on history, clinical examination, laboratory test results and cutaneous histopathology. The continuous advancement in ultrasound has enabled this diagnostic imaging modality to be introduced in the evaluation of normal and diseased skin. The fact that ultrasonography is non-ionising and non-invasive makes it a useful tool aiding the diagnosis and monitoring of skin diseases.

Based on the frequency employed, cutaneous ultrasonography can be classified as intermediate (7-15 MHz) or high-frequency (20 MHz or higher). Generally, frequencies more than 15 MHz are recommended to get a better resolution and to identify skin layers with higher clarity. Sedation is not normally required but it may be needed, occasionally, for an uncooperative dog. The skin should be shaved to improve contact between the transducer and the skin surface providing a better image and avoiding the creation of artifacts. The transducer is placed perpendicularly to the skin and minimal pressure should be applied. In some instances, skin saving may be avoided, and diagnostic images may be acquired with the use of copious amounts of ultrasound gel and steady pressure with the transducer.

Cutaneous ultrasonography has been used in human dermatology to study normal skin echogenicity, thickness, and hydration status, as well as various pathologic conditions, including cutaneous or subcutaneous neoplasms, cysts, inflammatory lesions, post-radiation reactions, scleroderma, oedema, wounds, and presence of foreign bodies. Contrary to human medicine, there are only a few studies on the ultrasonography of canine skin, focusing on the evaluation of high frequency ultrasonography for the accurate measurement of skin thickness and for the evaluation of wound healing. The ultrasonographic and histologic measurements of normal canine skin thickness have been found to be significantly correlated, but the former were found to be substantially higher than the latter. This finding may be explained by the inevitable shrinkage of skin biopsy specimens during formalin fixation. Furthermore, in the previous studies, ultrasonographic examination included only one or four different sites of haired skin.

The aim of our first study was to describe the echogenicity of normal canine haired skin using high-frequency (50 MHz) ultrasonography and to compare the ultrasonographic and the histologic measurement of skin thickness using snap-frozen biopsy samples. The study was performed on eight cutaneous sites of 10 clinically healthy, purpose-bred, Beagle dogs. The skin echogenicity was evaluated, and the mean of 10 measurements of skin thickness per site was calculated. Ultrasonography results were compared with histological appearance of skin cryosections stained with haematoxylin and eosin, as well as with the histometric measurement of skin thickness. Differences in the ultrasonographic and histologic measurements among biopsy sites, age and sex of the animals were examined.

For the ultrasound examination, a portable ultrasound scanner (Episcan I-200; Longport Inc., Chadds Ford, PA, USA) with a 50 MHz transducer was used. The scanner was fitted with a polyvinylidene difluoride transducer incorporated into a probe filled with distilled water, and scanning was performed using a digital stepping motor. The axial resolution was expected to be ~50 μm. The focal zone of the transducer was set at 2 mm depth and the acquired images were 14.9 mm wide and 7.68 mm deep. Immediately after ultrasound biomicroscopy, the scanned areas were biopsied, using 8 mm disposable biopsy punches, without any additional preparation of the skin. One half of the biopsy sample was transferred into an embedding mould (Peel-A-WayR Molds; Polysciences Inc., Warrington, PA, USA) that was filled with optimal cutting compound (Tissue-Tek OCT compound; Miles Scientific, Fergus Falls, MN, USA), immersed in isopentane, cooled to its freezing point in liquid nitrogen and stored at -80°C until used. Five-micrometre-thick cryosections were stained with haematoxylin and eosin.

The epidermis was identified as a thin, relatively nonuniform, hyperechoic linear layer. The dermis was hypoechoic to the epidermis and muscle fascia, and hyperechoic to the subcutaneous layer. The superficial dermis had a granular appearance more loosely arranged, with thin and irregularly distributed small echoes that probably arose from the collagen fibres. The deep dermis contained thicker linear echoes orientated more in parallel to the skin surface. The subcutaneous fat was visualized as an anechoic layer with sparse echogenicities. The muscle fascia, when visualized, appeared as linear, relatively thick echogenic lines orientated roughly parallel to the skin surface. The muscle below, when visible, appeared hypoechoic to

the dermis and epidermis, and had equal to slightly higher echogenicity to the subcutaneous fat. The epidermis, dermis and subcutaneous fat corresponded to the same skin layers identified in the histologic sections. The hair follicles were regularly identified, and their appearance varied depending on the orientation of their axis in relationship to the ultrasound beam. When viewed along their long axis, they appeared as oblique, roughly tubular hypoechogenicities with faint echogenic linear areas at their margins, while when viewed in an oblique orientation they appeared as oblong hypoechogenicities, mildly irregular in outline. The fat around the root of the anagen hair follicles appeared as a 'cloudy' hypoechogenicity with indistinct margination. Furthermore, there was a highly significant, positive association between ultrasonographic and histologic measurements of skin thickness. There were no significant differences between sex, age or among the different examination sites, for both ultrasonographic and histological skin thickness measurements.

In conclusion, this study showed that cutaneous high-frequency ultrasound biomicroscopy using a 50 MHz transducer was a useful tool to identify the skin layers (epidermis, dermis, and subcutaneous fat), to demonstrate the hair follicles and to measure the thickness of normal canine haired skin.

In the second study, we attempted, for the first time, to apply ultrasound biomicroscopy for the examination of the skin of dogs with atopic dermatitis. The aims were a) to compare high-frequency ultrasonographic findings among lesional skin of dogs with atopic dermatitis, their macroscopically non-lesional skin, and the macroscopically non-lesional skin of healthy dogs; b) to examine for possible correlations between ultrasonographic findings in lesional skin and local Canine Atopic Dermatitis Extent and Severity Index-4 (CADESI-4) or its domains (erythema, lichenification, and excoriations/alopecia), and c) to examine if ultrasonographic findings change in parallel with local CADESI-4 or its domains during treatment of atopic dermatitis. Twenty privately-owned dogs with atopic dermatitis *sensu lato* and fulfilling at least 6 out of 8 of the first set of diagnostic criteria proposed by Favrot and co-workers (2010) comprised the atopic dermatitis group (six of them before and after treatment). Six healthy, privately-owned dogs, with an age of more than 6 months, no evidence of dehydration, and no cutaneous or systemic diseases were included in the healthy group. The same portable

ultrasound machine and probe, as in the previous study, was used to scan 10 skin sites in all dogs. All scans were evaluated by the PhD student who was blinded to the group the animal and to the part of the body the scanned image was from. Wrinkling of skin surface, presence and width of subepidermal low echogenic band, hypoechogenicity of dermis, and thickness of the skin were evaluated and scored or measured blindly.

This study confirmed the findings from the previous study that a thin, relatively non-uniform area that corresponds to the epidermis, followed by relatively hypoechoic dermis can be identified using a 50MHz transducer. In addition to the normal non-lesional skin, this general ultrasonographic appearance was also evident in the lesional and the non-lesional skin of dogs with atopic dermatitis.

Based on the results of this study we concluded that: (i) dermal hypoechogenicity was more common and more severe in the lesional compared to non-lesional skin of dogs with atopic dermatitis; (ii) in the lesional skin of dogs with atopic dermatitis the presence and the severity of wrinkling of skin surface and of dermal hypoechogenicity were positively correlated with the presence and with the severity of lichenification, whereas the severity of dermal hypoechogenicity was positively correlated with local CADESI-4; and (iii) there is a positive correlation between the changes in skin thickness, as measured by ultrasound biomicroscopy, and the changes in the severity of local erythema during diagnostic and therapeutic interventions for atopic dermatitis that did not include the administration of anti-inflammatory drugs. These results showed that high-frequency ultrasound biomicroscopy has the potential to be useful for the evaluation of the skin of dogs with atopic dermatitis and of its changes during treatment.

ΠΕΡΙΛΗΨΗ

ΥΠΕΡΗΧΟΤΟΜΟΓΡΑΦΙΚΗ ΒΙΟΜΙΚΡΟΣΚΟΠΙΑ ΤΟΥ ΔΕΡΜΑΤΟΣ ΤΟΥ ΣΚΥΛΟΥ

Παραδοσιακά, η διάγνωση των δερματοπαθειών στηρίζεται στο ιστορικό, στα ευρήματα της κλινικής εξέτασης και των εργαστηριακών εξετάσεων και, όταν χρειάζεται, στην ιστοπαθολογική εξέταση των βιοψιών από το δέρμα. Οι συνεχείς τεχνολογικές εξελίξεις στο χώρο της υπερηχοτομογραφίας έχουν επιτρέψει τη χρήση αυτής της απεικονιστικής μεθόδου για την εξέταση του φυσιολογικού δέρματος αλλά και διάφορων δερματοπαθειών. Το γεγονός ότι η υπερηχοτομογραφία δεν χρησιμοποιεί ιονίζουσα ακτινοβολία και δεν είναι επεμβατική, την κάνει δυνητικά χρήσιμο εργαλείο για τη διάγνωση και την παρακολούθηση της θεραπείας διάφορων δερματοπαθειών.

Με βάση την κάθε φορά χρησιμοποιούμενη συχνότητα του υπερήχου, η υπερηχοτομογραφία του δέρματος διακρίνεται στην ενδιάμεσης (7-15 MHz) και την υψηλής (≥20 MHz) συχνότητας. Γενικά, προτιμάται η χρήση συχνοτήτων μεγαλύτερων από 15 MHz, προκειμένου να υπάρχει ικανοποιητική ευκρίνεια της εικόνας και να είναι εφικτή η διαφοροποίηση μεταξύ των στοιβάδων του δέρματος. Συνήθως δε χρειάζεται χημική συγκράτηση (εκτός από τη, σχετικά σπάνια, περίπτωση ζώων που δε συνεργάζονται), αλλά καλό είναι να κουρεύεται το δέρμα της περιοχής που πρόκειται να εξεταστεί, προκειμένου να υπάρχει καλύτερη επαφή μεταξύ του ηχοβολέα και της επιδερμίδας και κατά συνέπεια καλύτερη απεικόνιση και αποφυγή τεχνουργημάτων. Εναλλακτικά, τα αποτελέσματα της εξέτασης μπορεί να είναι διαγνωστικά χωρίς να προηγηθεί κούρεμα, με τη χρήση μεγάλης ποσότητας υπερηχοτομογραφικής γέλης και τη διατήρηση σταθερής πίεσης του δέρματος από τον ηχοβολέα καθ’ όλη την εχέταση. Σε κάθε περίπτωση, ο τελευταίος τοποθετείται κάθετα στην επιφάνεια του δέρματος στην οποία και ασκεί ελάχιστη πίεση.

Η υπερηχοτομογραφική εξέταση έχει χρησιμοποιηθεί στον άνθρωπο για τη μελέτη της απεικόνισης του φυσιολογικού δέρματος, του πάχους και του βαθμού ενυδάτωσής του, καθώς και διάφορων δερματοπαθειών, όπως, για παράδειγμα, των νεοπλασμάτων του δέρματος και

του υποδόριου ιστού, των κύστεων, διάφορων δερματιτίδων, της δερματοπάθειας μετά από ακτινοθεραπεία, του σκληροδέρματος, του οιδήματος του δέρματος, των τραυμάτων και της παρουσίας ξένων σωμάτων. Αντίθετα, στην Κτηνιατρική δεν υπάρχουν παρά ελάχιστες σχετικές μελέτες, όπου η υπερηχοτομογραφία υψηλής συχνότητας χρησιμοποιήθηκε για τη μέτρηση του πάχους του δέρματος και την εκτίμηση της επούλωσης των τραυμάτων. Αν και οι υπερηχοτομογραφικές και ιστολογικές μετρήσεις του πάχους του δέρματος σχετίζονταν σημαντικά μεταξύ τους, οι πρώτες ήταν συστηματικά μεγαλύτερες από τις δεύτερες, πιθανά λόγω της συρρίκνωσης των βιοψιών του δέρματος κατά τη μονιμοποίησή τους στη φορμόλη. Επιπλέον, στις προηγούμενες μελέτες η υπερηχοτομογραφική εξέταση πραγματοποιήθηκε μόνο σε μια ή σε τέσσερεις διαφορετικές ανατομικές περιοχές του δέρματος.

Ο στόχος της πρώτης από τις μελέτες μας ήταν να περιγράψουμε την ηχογένεια του φυσιολογικού δέρματος του σκύλου με τη χρήση υπερηχοτομογράφου υψηλής συχνότητας (50 MHz) και να συγκρίνουμε το πάχος του δέρματος μεταξύ των μετρήσεων με την παραπάνω εξέταση και εκείνων σε ιστοτεμάχια που καταψύχθηκαν αμέσως μετά τη βιοψία, χωρίς να μονιμοποιηθούν σε φορμόλη. Χρησιμοποιήθηκαν 10 κλινικά υγιή πειραματόζωα, φυλής beagle και εξετάσθηκαν οκτώ ανατομικές περιοχές του δέρματος από κάθε σκύλο, στις οποίες εκτιμήθηκε η υπερηχοτομογραφική απεικόνιση του δέρματος και του υποδόριου ιστού και μετρήθηκε το πάχος του δέρματος (διάμεσος 10 μετρήσεων/ανατομική περιοχή της απόστασης από την επιδερμίδα μέχρι το όριο χορίου-υποδόριου ιστού). Τα ιστοτεμάχια βάφτηκαν με αιματοξυλίνη-εωσίνη και χρησιμοποιήθηκαν για να ερμηνευθεί η υπερηχοτομογραφική εικόνα του δέρματος και να συγκριθούν οι μετρήσεις του πάχους του. Τα αποτελέσματα ελέγχθηκαν για τυχόν διαφορές μεταξύ των ανατομικών περιοχών, μεταξύ των δύο φύλων ή μεταβολών που σχετίζονταν με την ηλικία των σκύλων.

Χρησιμοποιήθηκε φορητός υπερηχοτομογράφος (Episcan I-200; Longport Inc., Chadds Ford, PA, USA) με ηχοβολέα 50 MHz. Ο σαρωτής ήταν συνδεδεμένος με μετατροπέα από διφθορίδιο του πολυβινυλιδενίου που ήταν ενσωματωμένος σε μορφοτροπέα που γέμιζε με απεσταγμένο νερό, ενώ η σάρωση του δέρματος έγινε με ψηφιακό κινούμενο ενισχυτή. Η αξονική διακριτική ικανότητα ήταν της τάξης των 50 μm, η εστιακή ζώνη του μετατροπέα ορίστηκε σε βάθος 2 mm και οι εικόνες που ανακτήθηκαν είχαν πλάτος 14,9 mm και βάθος

7,68 mm. Αμέσως μετά την υπερηχοτομογραφική εξέταση, χωρίς να γίνει χειρουργική προετοιμασία του δέρματος, λαμβάνονταν βιοψίες, από τα ίδια ακριβώς σημεία, με διατρητή μίας χρήσης διαμέτρου 8 mm. Τα ιστοτεμάχια κόβονταν κάθετα ως προς την επιδερμίδα και μεταφέρονταν σε ειδικούς μεταλλικούς υποδοχείς (Peel-A-WayR Molds; Polysciences Inc., Warrington, PA, USA) που αρχικά γεμίζονταν με optimal cutting compound (Tissue-Tek OCT compound; Miles Scientific, Fergus Falls, MN, USA) και στη συνέχεια ψύχονταν σε ισοπεντάνιο, που είχε παγώσει στο σημείο τήξης του ύστερα από εμβάπτιση σε υγρό άζωτο. Τα παγωμένα ιστοτεμάχια διατηρούνταν στους -80°C μέχρι να χρησιμοποιηθούν για την παρασκευή τομών, πάχους 5 μm, σε κρυοτόμο που στη συνέχεια βάφονταν με αιματοξυλίνη-εωσίνη.

Η επιδερμίδα απεικονίστηκε ως λεπτή, σχετικά ανομοιογενής, υπερηχογενής γραμμοειδής στοιβάδα. Το χόριο του δέρματος απεικονιζόταν υποηχογενές σε σύγκριση με την επιδερμίδα και τις περιτονίες των μυών και υπερηχογενές σε σύγκριση με τον υποδόριο ιστό. Οι περισσότερο επιφανειακές στοιβάδες του χορίου εμφανίζονταν κοκκώδεις με λιγότερο πυκνές τοπικές εστίες μικρών ήχων, με λεπτές και ανώμαλα κατανεμημένες ακουστικές ανακλάσεις που πιθανότατα αναπαριστούσαν τις ίνες του κολλαγόνου. Αντίθετα, στα βαθύτερα στρώματα του χορίου, υπήρχαν παχύτερες γραμμικές ανακλάσεις με διάταξη περισσότερο παράλληλη προς την επιφάνεια του δέρματος. Το υποδόριο λίπος απεικονίζονταν ως ανηχογενής στοιβάδα με σποραδικές εστίες υπερηχογένειας. Οι μυϊκές περιτονίες, όταν απεικονίζονταν, ήταν γραμμικές και σχετικά παχιές ηχογενείς δομές με διάταξη σχετικά παράλληλη ως προς την επιφάνεια του δέρματος. Οι υποκείμενοι μύες, όταν απεικονίζονταν, ήταν υποηχογενείς σε σύγκριση με το χόριο και την επιδερμίδα και εξίσου ή περισσότερο ηχογενείς σε σύγκριση με τον υποδόριο ιστό. Η επιδερμίδα, το χόριο και ο υποδόριος ιστός, όπως απεικονίστηκαν με την υπερηχοτομογραφική εξέταση, αντιστοιχούσαν στις ίδιες στοιβάδες του δέρματος όπως αυτές φαίνονταν στα ιστοτεμάχια. Επιπλέον, με την υπερηχοτομογραφική εξέταση απεικονίστηκαν οι θύλακες των τριχών, η εικόνα των οποίων διέφερε ανάλογα με τον προσανατολισμό του επιμήκη άξονά τους σε σύγκριση με εκείνον του υπερήχου. Όταν απεικονίζονταν κατά τον επιμήκη άξονά τους, οι θύλακες εμφανίζονταν ως σχετικά σωληνοειδείς, υποηχογενείς κατασκευές με αχνές ηχογενείς γραμμικές περιοχές στα

όριά τους. Αντίθετα, όταν ο προσανατολισμός τους ήταν λοξός, εμφανίζονταν ως επιμήκεις υποηχογενείς δομές με σχετικά ανώμαλο περίγραμμα. Το υποδόριο λίπος γύρω από το κατώτερο τμήμα των αναγονικών θυλάκων απεικονίζονταν ως θολερή υποηχογενής δομή με ασαφή όρια. Τέλος, διαπιστώθηκε στατιστικά σημαντικός συσχετισμός μεταξύ της υπερηχοτομογραφικής και της ιστολογικής μέτρησης του πάχους του δέρματος, το οποίο δε διάφερε ανάμεσα στις οκτώ ανατομικές περιοχές ή μεταξύ των αρσενικών και θηλυκών σκύλων και δεν σχετιζόταν με την ηλικία τους.

Συμπερασματικά, η μελέτη αυτή έδειξε ότι η υψηλής συχνότητας (50 MHz) υπερηχοτομογραφική εξέταση, επιτρέπει τη διάκριση των στοιβάδων του δέρματος (επιδερμίδα, χόριο, υποδόριος ιστός), απεικονίζει τους θυλάκους των τριχών και επιτρέπει την αξιόπιστη μέτρηση του πάχους του δέρματος.

Στη δεύτερη μελέτη χρησιμοποιήσαμε, για πρώτη φορά, την υπερηχοτομογραφική εξέταση για να μελετήσουμε το δέρμα σκύλων με ατοπική δερματίτιδα. Οι στόχοι της μελέτης ήταν: α) η σύγκριση των ευρημάτων αφενός του δέρματος σκύλων με ατοπική δερματίτιδα που εμφάνιζε μακροσκοπικές αλλοιώσεις, και του δέρματος των ίδιων σκύλων σε περιοχές όπου δε εμφανίζει μακροσκοπικές αλλοιώσεις και αφετέρου του δέρματος των υγιών σκύλων, β) η διερεύνηση για τυχόν συσχετισμούς μεταξύ των ευρημάτων της υπερηχοτομογραφικής εξέτασης και της βαρύτητας των δερματικών αλλοιώσεων στην αντίστοιχη περιοχή του δέρματος, όπως αυτή βαθμολογήθηκε με την κλίμακα Canine Atopic Dermatitis Extent and Severity Index-4 (CADESI-4) ή συσχετισμών μεταξύ των ευρημάτων της υπερηχοτομογραφικής εξέτασης και των επιμέρους δερματικών αλλοιώσεων (ερύθημα, λειχηνοποίηση, δρυφάδες/αλωπεκία) που περιλαμβάνονται στην κλίμακα CADESI-4, και γ) η διερεύνηση τυχόν συσχετισμών μεταξύ των μεταβολών των ευρημάτων της υπερηχοτομογραφικής εξέτασης και των μεταβολών της κλίμακας CADESI-4 ή των επιμέρους δερματικών αλλοιώσεων στη διάρκεια της θεραπευτικής αντιμετώπισης της ατοπικής δερματίτιδας. Στην ομάδα των σκύλων με ατοπική δερματίτιδα συμπεριλήφθηκαν 20 σκύλοι ιδιοκτητών, που πληρούσαν τουλάχιστον 6 από τα 8 διαγνωστικά κριτήρια κατά Favrot και συν. (2010), ενώ οι έξι από τους 20 αυτούς σκύλους ελέγχθηκαν πριν και μετά τη θεραπεία. Στην ομάδα των υγιών σκύλων συμπεριλήφθηκαν έξι κλινικά υγιείς σκύλοι ιδιοκτητών, ηλικίας

άνω των 6 μηνών. Η υπερηχοτομογραφική εξέταση έγινε σε 10 ανατομικές περιοχές του δέρματος με τον ίδιο υπερηχοτομογράφο, όπως στην πρώτη μελέτη, και οι εικόνες εξετάστηκαν από τον υποψήφιο διδάκτορα, ο οποίος δεν γνώριζε την ανατομική περιοχή ή την ομάδα του σκύλου στην οποία αντιστοιχούσε η κάθε εικόνα. Βαθμολογήθηκαν ή μετρήθηκαν η ρυτίδωση της επιφάνειας του δέρματος, η παρουσία και το πλάτος της υποεπιδερμικής υποηχογενούς ζώνης, η υχοδομή του χορίου και το πάχος του δέρματος.

Στη μελέτη αυτή επιβεβαιώθηκαν τα ευρήματα της προηγούμενης έρευνας όσον αφορά την απεικόνιση του δέρματος (επιφανειακά η λεπτή, σχετικά ανομοιογενής επιδερμίδα και από κάτω το σχετικά υποηχογενές χόριο), όχι μόνο στο δέρμα των υγιών σκύλων αλλά και σε εκείνο των σκύλων με ατοπική δερματίτιδα, με ή χωρίς μακροσκοπικές αλλοιώσεις.

Τα σημαντικότερα ευρήματα της μελέτης είναι: α) το χόριο του δέρματος σκύλων με ατοπική δερματίτιδα που εμφανίζει μακροσκοπικές αλλοιώσεις απεικονίζεται με μειωμένη ηχογένεια συχνότερα και εντονότερα σε σύγκριση με το δέρμα των ίδιων ζώων σε περιοχές χωρίς μακροσκοπικές αλλοιώσεις, β) στο δέρμα των σκύλων με ατοπική δερματίτιδα και μακροσκοπικές αλλοιώσεις η παρουσία και ή ένταση της ρυτίδωσης της επιφάνειας του δέρματος και της υποηχογένειας του χορίου σχετίζονταν σημαντικά με την παρουσία και την ένταση της λειχηνοποίησης και επιπλέον, η μείωση της ηχογένειας του χορίου σχετιζόταν με το CADESI-4 στην ίδια περιοχή του δέρματος, και γ) υπήρχε θετικός συσχετισμός μεταξύ της μεταβολής του πάχους του δέρματος και της μεταβολής της έντασης του ερυθήματος ύστερα από την εφαρμογή διάφορων διαγνωστικών και θεραπευτικών μέτρων για την ατοπική δερματίτιδα, που δεν περιλάμβαναν τη χορήγηση φαρμάκων με αντιφλεγμονώδη δράση. Τα παραπάνω αποτελέσματα δείχνουν ότι η υπερηχοτομογραφία υψηλής συχνότητας είναι χρήσιμη στη μελέτη των αλλοιώσεων του δέρματος σκύλων με ατοπική δερματίτιδα και των μεταβολών τους κατά τη διάρκεια της θεραπείας.