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Dermoscopic features of calcinosis cutis in 12 dogs: an observational study

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Dermoscopic features of calcinosis cutis in 12 dogs: an observational study

Abstract

Background: Dermoscopy is a non-invasive technique used to preliminary assess skin lesions in humans and, more recently, in animals. Calcinosis cutis (CC) is an uncommon condition in dogs characterized by cutaneous calcification typically secondary to iatrogenic hyperglucocorticism or endogenous hyperadrenocorticism.
Objectives: The aim of this study was to describe and characterize the dermoscopic features of cutaneous calcinosis in dogs affected by hypercortisolism, either iatrogenic or spontaneous and assess the inter-observer agreement of the dermoscopic parameters.
Materials and Methods: Twelve lesions from 12 client-owned dogs, histologically diagnosed as CC associated to spontaneous or iatrogenic hypercortisolism, were included in this study. Dermoscopic images of the lesions at 10x magnification were independently evaluated on a computer screen by three (ECVD) board-certified veterinary specialists and one ECVD resident.
Results: At 10x magnification, all lesions exhibited single or multiple structureless bright white areas and erythema. In eight of the twelve lesions, these white areas surrounded prominent plugs of yellowish to brown material emerging from follicular ostia and/or epidermal ulcerations. The main vascular pattern was dominated by a single vessel type (monomorphic pattern), consisting mainly of short, dilated, linear curved vessels (7/12), with short linear vessels observed in four lesions. Histopathology confirmed that the white structures observed dermoscopically corresponded to calcium deposits within the dermis. A specific association was not observed between dermoscopic features and the distribution or localization of mineralization described in the histological evaluations.
Conclusions: Dermoscopy represents a useful technique to assess calcinosis cutis in dogs.

Keywords: dermoscopy, dermoscopic features, dogs, calcinosis cutis, iatrogenic hyperglucocorticism, endogenous hyperadrenocorticism

Introduction

Dermoscopy has gained increasing importance as a non-invasive diagnostic technique for the evaluation of neoplastic and non-neoplastic dermatoses in humans and, more recently, in animals. It serves as a valuable adjunct to clinical examination, improving diagnostic accuracy and guiding further investigations.[1] In veterinary dermatology, dermoscopic features have been described in animals with a range of infectious diseases (e.g., feline and canine dermatophytosis),[2,3] parasitic infestations (e.g., canine demodicosis and sarcoptic mange),[4,5,6] and congenital disorders (e.g., acral congenital superficial dermal lymphatic malformation in two cats).[7] In addition, specific dermoscopic criteria have recently been established for the identification of sebaceous tumors, infundibular keratinizing acanthomas (IKAs), and follicular cysts in dogs.[8,9] To increase the reproducibility of dermoscopic examination, standardized criteria have been developed in human medicine for both tumors[1] and non-neoplastic dermatoses (inflammatory, infectious and infiltrative skin diseases).[10]

Calcinosis cutis (CC) is an uncommon condition characterized by the deposition of inorganic, insoluble mineral salts within the dermis, subcutaneous tissue, and, more rarely, the epidermis. These deposits typically involve collagen and elastin fibers in the dermis. CC may develop secondary to a variety of underlying conditions and is traditionally categorized into four main types: metastatic, iatrogenic, dystrophic, and idiopathic. Dystrophic calcification typically arises as a consequence of localized tissue damage. It is most commonly observed in dogs with either iatrogenic or endogenous hypercortisolism, but it may also be associated with systemic diseases such as leptospirosis or with inflammatory dermatoses, including follicular cysts and foreign body granulomas.[11] Clinically, CC presents with firm, often confluent papules and plaques, most commonly located on the flexural surfaces of the groin, the back of the neck, and the axillae. Ulceration and crusting may develop as gritty mineral salts mixed with keratin are extruded through the epidermis from older lesions.[12] In cases of dystrophic calcinosis, these lesions are frequently associated with other cutaneous manifestations of hypercortisolism, such as alopecia, skin atrophy, and secondary infections (pyoderma), along with systemic signs including polyuria, polydipsia, and polyphagia.[11,12]

Although the lesions associated with cutaneous calcinosis are usually distinctive on clinical ground, some cases can be diagnostically challenging. Hence, in this study we aimed to assess whether dermoscopic criteria can further aid in their characterization. Specifically, the objective of this analysis was to describe the dermoscopic features of 12 cutaneous lesions confirmed as calcinosis cutis by histopathological examination and to evaluate interobserver agreement in identifying these dermoscopic characteristics, using histopathological diagnosis as the gold standard.

Materials and methods

Study population

Cases were included if a well-circumscribed papule or plaque, firm to hard on palpation, had been evaluated by dermoscopy, prior to surgical excision, and subsequently confirmed as CC by histopathological examination. Informed consent was obtained from all owners before any clinical, surgical, or diagnostic procedures,

in accordance with ethical guidelines and good clinical practice (published in No. 289 of the Italian *Gazzetta Ufficiale*, 10 December 1996, pp. 47–53).

Dermoscopic examination

Lesions were examined using polarized and non-polarized light with a hybrid dermoscope (Heine Delta 20 Plus; Intermed SRL, San Giuliano Milanese, (MI), Italy). A layer of transparent ultrasound gel (GIMA SpA, Gessate, (MI), Italy) was applied to each lesion prior to dermoscopic examination to minimize pressure on blood vessels. The dermoscope was connected to a digital camera (Nikon D3100; Europe BV, Campi di Bisenzio, (FI), Italy), and all images were captured in JPEG format. Morphological analysis was performed according to the descriptive and standardized terminology proposed by Errichetti *et al* on behalf of the *International Dermoscopy Society*,[10] which has recently been applied in studies on the dermoscopic evaluation of cutaneous lesions in dogs.[8,9] In particular, five basic parameters have been identified, with a range of subitems, including (I) vessels (morphology and distribution); (II) scales (colour and distribution); (III) follicular findings; (IV) 'other structures' (colour and morphology); and (V) 'specific clues'.[10]
Dermoscopic images were displayed on a computer screen to two evaluators experienced in dermoscopy, a board-certified veterinary dermatologist (FS) and a dermatologist (NP), who independently assessed all images. Following the individual assessments, the two observers systematically discussed each case, paying particular attention to discrepancies between their evaluations. A consensus diagnosis was finally reached and was considered the gold standard for dermoscopic assessment. The scores assigned during the evaluation were then used for correlation analysis to assess associations between individual dermoscopic and histological parameters.
To assess interobserver agreement, dermoscopic images were also independently evaluated by two additional board-certified veterinary dermatologists (DD, SC) and a (ECVD) resident (PB) with less experience in dermoscopy.

Histologic examination

After dermoscopic examination and image acquisition, lesions were surgically excised and immediately fixed in 10% buffered formalin for 48–72 hours. Samples were trimmed and routinely processed, embedded in paraffin wax, cut into 4 µm sections, stained with haematoxylin and eosin, and assessed by two ECVP board-certified pathologists. Microscopical lesions consistent with mineralization were assessed and sites of mineralization were recorded for each specimen. To demonstrate transepidermal or transfollicular elimination of mineralized material, von Kossa Stain was performed utilizing a commercial kit (Bio Optica, Italy), following the manufacturer's instructions. Positive mineralized material was identified as black extracellular deposits.

Statistical analysis

The association between histological diagnosis and each dermoscopic feature considered was investigated with the Pearson chi-square test. The significance threshold of *p-value* was set at 0.05. Analysis of the agreement was made through Cohen's Kappa coefficient: the agreement between the three evaluators was considered modest for kappa values between 0.21 and 0.40, moderate for values between 0.41 and 0.60, and good for values between 0.61 and 0.80. For values

greater than 0.8 there was a near-perfect degree of agreement between the evaluators. All analyses were performed with the software *STATA 18.0*.

Results

A total of 12 cutaneous lesions were included in the study. The lesions were obtained from eleven purebred dogs and one mixed-breed, with a mean age of 7 years (range: 4–11 years). The breeds included four French Bulldogs, one Border Collie, one English Bulldog, one American Staffordshire Terrier, one Labrador Retriever, one Dogue de Bordeaux, one Vizsla, one American Bully and one Chihuahua. Seven dogs were spayed females, two were neutered males and three were intact males. Six dogs had iatrogenic hypercortisolism, while six were diagnosed with spontaneous hypercortisolism. The duration of lesions ranged from one week to eight months. Lesions appeared as well-circumscribed, firm papules or plaques, located on the trunk (6/12), neck (5/12), and limbs (1/12).

Dermoscopic findings

The dermoscopic features of the lesions and interobserver agreement are summarized in Table 1.

The main findings included bright white structureless areas (12/12; 100%) (Figure 1 a-j; Figure 2a; Figure 3a), yellow to brown prominent keratin plugs emerging from follicular infundibula, and ulcerations (8/12; 66,7%) (Figure 1a, c,d,e,f,g,h,i; Figure 2a; Figure 3a). Regarding vascular structures, they were detected in 7out 12 lesions (58.3%), most of which displayed a monomorphic pattern predominantly consisting of short, linear curved vessels (Figure 1d,e,g,l,j and 2a). A polymorphic arrangement, characterized by the coexistence of linear and linear curved vessels (Figure 3a), was observed in three lesions. In all cases, vessels were distributed in a random pattern. Scales and crusts were present in the majority of lesions (7/12; 58,3%), appearing either as thin white scales or as yellow to brownish crusts.

Interobserver agreement was good for yellow to brown keratin plugs (κ = 0.79) and for scales and crusts (κ = 0.66), and moderate for vessel morphology (κ = 0.48 for linear and κ = 0.58 for linear curved vessels) as well as for a polymorphic vessel arrangement (κ = 0.50). A modest level of agreement was observed for a monomorphic vessel arrangement (κ = 0.39). All assessors reported the presence of white structureless areas and erythema in all evaluated images. For these two parameters, since there was no variability in our sample, the concordance coefficient calculation was not appropriate.

No significant association was observed between the aforementioned dermoscopic features and either the type of hypercortisolism (spontaneous or iatrogenic) or the duration of the lesions.

Histopatological findings

Histological evaluation confirmed the presence of dermal mineralization in all examined cases (Figure 2b,c and Figure 3b). Dermal mineralization was mild, ranging from focal to multifocal, in 3 out of 12 lesions, whereas it was moderate to severe with multifocal or multifocal-to-diffuse distribution in 5/12 and 4/12 cases, respectively. Osseous metaplasia was identified in two lesions. Mineralization of the outer root sheath (Figure 2b,c) was observed in 4/12 lesions, while transfollicular and

transepidermal elimination of calcium (Figure 3b) were noted in 6/12 and 1/12 lesions respectively. Mild fibrosis was present in 7 of the 12 lesions, whereas moderate fibrosis was found in 4 cases. A granulomatous inflammatory infiltrate characterized by macrophages and, in 3 cases, multinucleated giant cells, was observed in 9 of the 12 lesions.
No specific association was observed between dermoscopic features and the distribution or localization of mineralization described in the histological evaluations.

Discussion

To the best of the authors' knowledge, this study is the first to describe the dermoscopic features of CC in dogs. Although calcinosis plaques have distinctive features, ulceration and secondary infection can obscure their appearance, making differentiation from granulomatous lesions of other etiologies, such as deep bacterial or fungal infections, challenging for general practitioners. [12] In our analysis, we identified recurrent dermoscopic features, most notably bright white structureless areas and yellow-brown prominent follicular plugs, together with short linear curved vessels, which may aid in distinguishing this condition from other granulomatous lesions of different etiologies.

In dermoscopy, the white color has been associated with several histological findings in dogs, including subsurface keratin, as observed in IKAs and follicular cysts, as well as with fibrosis.[9] In CC, however, white color is more likely related to calcium deposits themselves, given their bright shade and consistent with the gross and histological aspects of this condition.

White structureless areas were observed in all lesions analyzed in this study. These findings parallel those described in humans, where dermoscopy of calcinosis cutis typically reveals homogeneous white areas corresponding to dermal mineralization. Notably, in people these white areas provide a useful clue for distinguishing calcinosis cutis from clinical mimickers, particularly keratotic lesions such as warts, corns, callosities, or lichen planus-like keratosis, which instead usually display dull white areas related to compact keratin[13]. Similarly, milia-like calcinosis cutis in children, especially in association with Down syndrome, also presents with discrete white papules showing dermoscopic homogeneous bright white areas, underscoring the cross-species reproducibility of this feature.[13]

Surface keratin and keratin plugs were frequent additional findings that corresponded histologically to keratin accumulation admixed with mineral transepidermal/transfollicular extrusion. This process has been emphasized in human studies as a hallmark of calcinosis cutis, where dermoscopy highlights the pathophysiological mechanism of trans-epidermal or trans-follicular elimination of calcium deposits.[13] Yellow to brown follicular plugs and perifollicular scales are also common dermoscopic features in canine demodicosis, particularly in short-haired dogs;[4] however, in this condition these features were not associated with white structureless areas or linear curved vessels, as observed in CC in the present study.. This observation suggests that the concomitant presence of yellow/brown follicular plugs and white structureless areas represents a key dermoscopic criterion for differentiating the two conditions.

Scales and crusts were observed in most lesions. In our cohort, thin white scales corresponded to compact orthokeratotic hyperkeratosis, while thick yellow to brown crusts reflected keratin admixed with serum, inflammatory cells, and calcium salts. Their perifollicular or diffuse arrangement further supports their inclusion as a possible useful dermoscopic clue in CC, helping to differentiate it from other keratotic or cystic lesions. Vascular structures characterized by short, dilated, linear, and linear curved vessels were observed in more than half of the lesions.. These findings are reasonably consistent, as observed in humans, with chronic inflammatory changes and superficial vascular congestion.[10] In human dermatology, vascular patterns represent a fundamental component of dermoscopic evaluation, as they provide information that is often not appreciable to the naked eye and may significantly narrow the differential diagnosis. It has been demonstrated that different diseases in people induce characteristic changes in vessel morphology, distribution, and density, making vascular features valuable diagnostic clues. The currently available evidence suggests a comparable relevance in veterinary dermoscopy; however, further species-specific studies correlating dermoscopic and dermatopathological findings are required to define disease-oriented vascular patterns.

All evaluators reported the presence of white unstructured areas and erythema in all images evaluated, and good inter-observer agreement was obtained for yellow-to-brown keratin plugs as well as for scales and crusts. These results emphasize the reproducibility of the aforementioned dermoscopic findings, reflecting the significant agreement among observers.

Conclusions

The study underlines that dermoscopy may be considered a possible useful, non-invasive and reproducible tool to assist the non-invasive recognition of CC and its distinction from clinically similar lesions such demodicosis, deep pyoderma or granulomatous conditions. The characteristic combination of bright white structureless areas, and features of prominent mineral elimination (yellow/brown keratin plugs) appears distinctive of CC and may serve as practical diagnostic clue. The strong agreement between observers supports the applicability of dermoscopy for the diagnosis of CC across different levels of expertise.

The limitations of the present pilot study include the small sample size and, with regard to the technique, the inability to accurately assess the depth of mineralization or to reliably differentiate between mineral deposition and osseous metaplasia (osteoma cutis), both of which remain dependent on histopathological evaluation.

Therefore, further studies are needed to confirm our preliminary data.

Caption/legends

Table 1. List of dermoscopic features observed in the 12 lesions of calcinosis cutis, with their presence expressed as percentages in lesions and agreement among the four observers.

Dermoscopic features	**DCC**	*Cohen's Kappa (95% CI)*
White structureless areas	12/12 (100%)	*
Yellow/brown plugs	8/12 (66.7%)	0.79 (0.44-100)
Linear vessels	4/12 (33,3%)	0.48 (0.36-0.61)
Linear curved vessels	7/12 (58,3%)	0.58 (0.44-100)
Vascular pattern polymorphic	3/12 (25,0%)	0.50 (0.46-0.59)
Vascular pattern monomorphic	6/12 (50.0%)	0.39 (0.33-0.47)
Erythema	12/12 (100%)	*
Scales/crusts	7/12 (58,3%)	0.67 (0.51- 073)

***** Present in all the lesions and observed by all the evaluators

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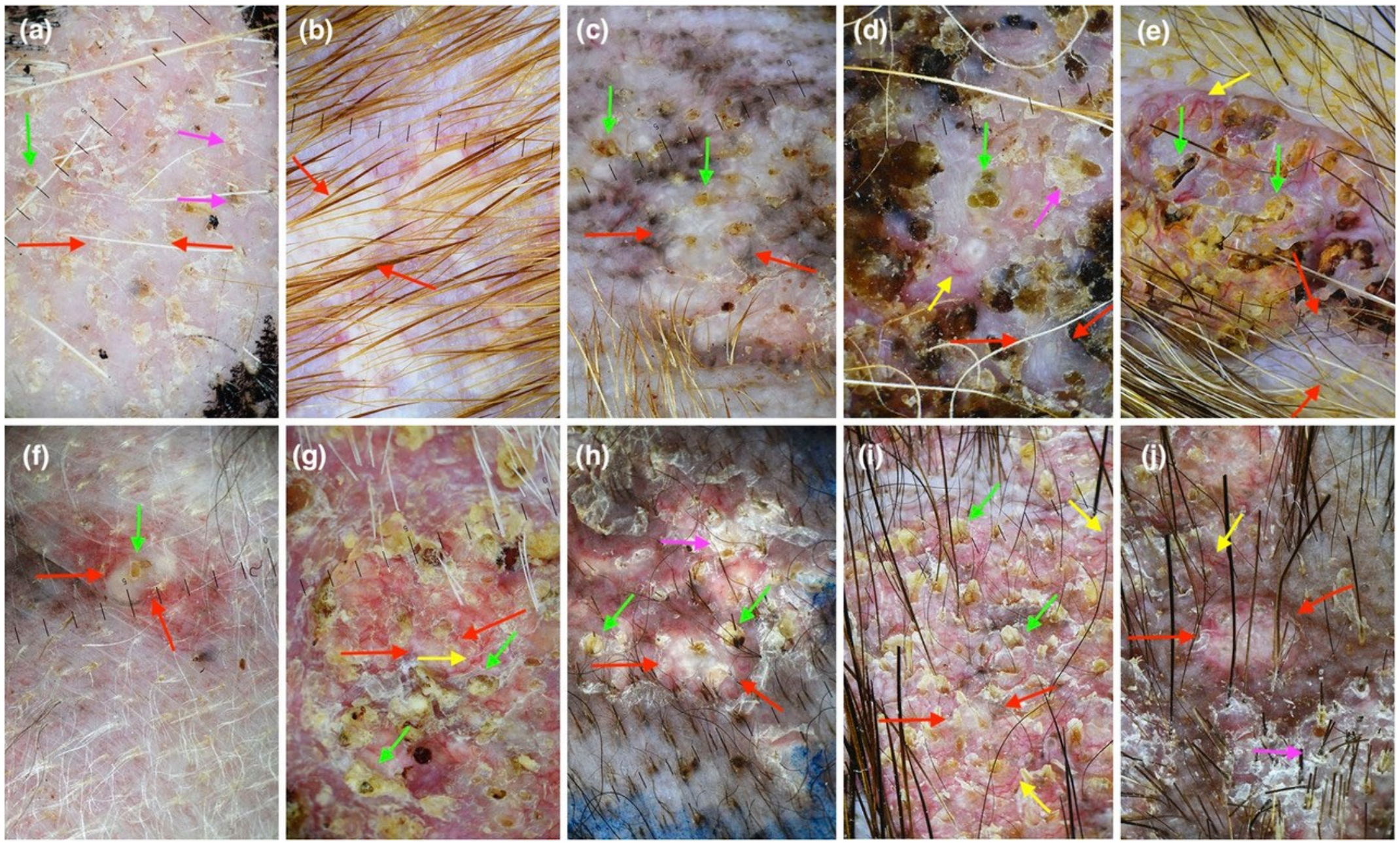
Figures legend:

Figure 1. Dermoscopic features in dogs with calcinosis cutis. Red arrows indicate structureless white areas; green arrows point to prominent plugs of yellowish/brown material emerging from follicular ostia and/or epidermal ulcerations; yellow arrows highlight short vessels; pink arrows point to scales.

Figure 2. Calcinosis cutis: (a) Dermoscopic image and corresponding histopathology at x4 (b) and x10 magnification (c). Multiple structureless bright white areas surrounding prominent plugs of yellowish to brown material emerging from follicular ostia, along with short, dilated, linear curved vessels, are visible in the dermoscopic image (a). Histological examination of the area observed with the dermoscope shows deposition of black material consistent with mineralization of collagen fibers and of the external root sheath of a hair follicle (b, c). von Kossa stain, x4 and x10.

Figure 3. Calcinosis cutis: (a) Dermoscopic image and corresponding histopathology at x4 magnification (b). Prominent plugs of yellowish to brown material emerging from epidermal ulcerations surrounded by white unstructured areas and short, dilated linear and linear curved vessels are visible in the dermoscopic image (a). Histological examination of the same area shows the presence of extruded material from a hair follicle infundibulum consistent with mineralized material as demonstrated by positivity to von Kossa stain. von Kossa (b) x4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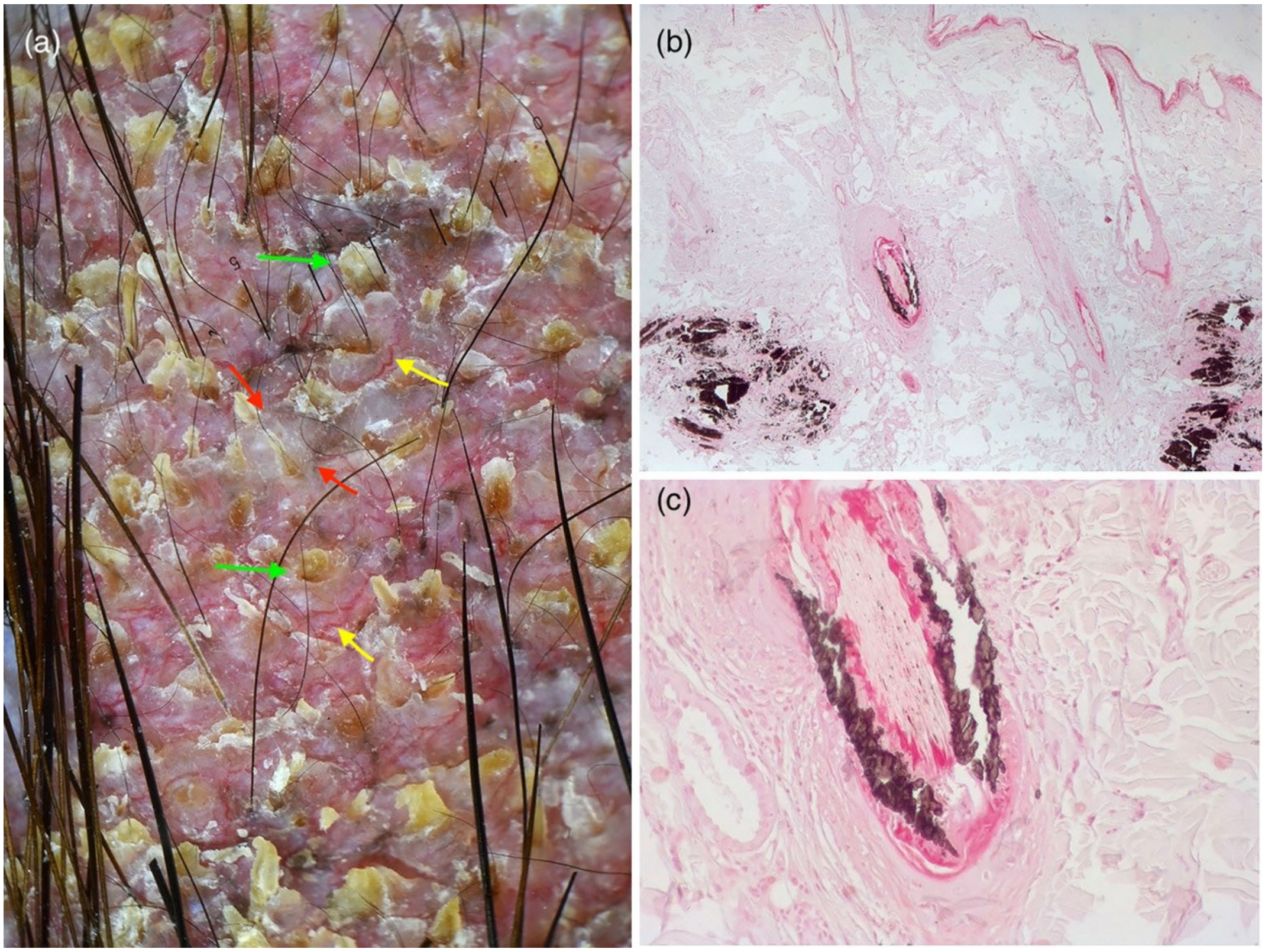
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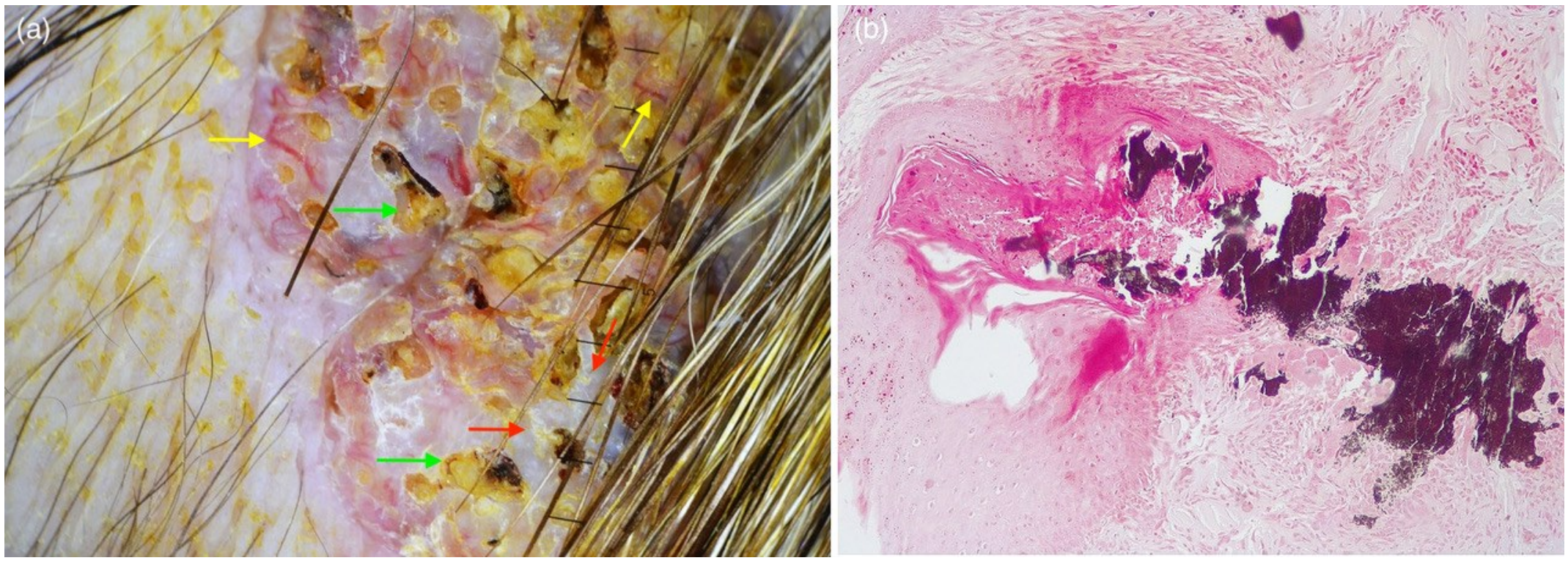
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Response to reviewers

We thank all reviewers for their observations that allowed us to improve this paper
We have made the requested corrections as follows:

Revewer 1

Page 2, Introduction
In the first paragraph of the introduction, it seems that some citations are missing (first 3 sentences).

Response: Citations have been added, the bibliography has been expanded, and references in the text have been updated.

Page 3, Materials and methods
Be consistent describing instruments, software and reagents in brackets (you put full address in some of it, while in others – there are only name of the company and country; I think that the best idea is to put the name of the company, city and country, without full address).

Response: We have harmonized the description as suggested.

Page 4, Results
Correct Figure citation, to be consistent with the figures – i.e. curved vessels are also in Fig.3, and these keratin plugs are not pointed out in Fig. 1a. Furthermore, be consistent with numerical results (always put number out of 12 and percentage, in each lesion – and percentages should be written in the similar manner – not 66,6 in one place, and 58,33 in other place; maybe 66.7 would be more appropriate, as it is also in the table?).

Response: The citations of the figures have been corrected and the numerical data in the results and in Table 1 have been harmonized as requested.

Discussion, page 5, lines 207-208
Are there any other lesions, that could be difficult to distinguish from DCC in dogs? Maybe some citations should be placed here.

Response: We have cited rare examples in which cutaneous calcinosis could be confused with other diseases and included the reference. *Lines 203-206*

Page 8, Figure legends
I suggest to remove from the figure legend 1 letters in brackets (c,d,e…. etc.) – it is unnecessary, as the arrows point the same structures in all figures. Furthermore, these letters in brackets are not in line with the figures (red arrows are also in figure a and b, but these are not specified in brackets…).
Red arrows on Figure 2b seem to point hair shafts (especially the bottom one), not white structureless areas.

Response: Letters have been removed from figure legend 1 as suggested

Page 7, References
All references should be written in the similar way, according to journal requirements.

Response: The references have been rectified according to journal guidelines

Reviewer 2

Line 1: Please remove "dystrophic" and correct throughout the manuscript. The disease name is calcinosis cutis (see reference 5 and 6).

Response: The name of disease has been correct throughout the manuscript

Line 23: What is a monomorphic vascular pattern?

Response: We have modified the sentence on line 23 to make the definition clearer. Information regarding the dermoscopic definition of blood vessels has been provided in publications describing the dermoscopic appearance of sebaceous proliferations, as well as in a subsequent article on IKA, both of which are cited in the text. *Lines 106-112* However, should the reviewer consider it necessary, we would be willing to include a section specifically dedicated to dermoscopic definitions in the "Materials and Methods" section.

Why are vascular profiles important to describe?

Response: We have attempted to explain the usefulness of describing vascular patterns in the discussion. *Lines 265-275*

Line 55: Please provide references.

Response: The reference has been added as requested.

Line 173: Show polymorphic arrangement in figure.

Response: Arrows (yellow) have been added to Figure 3 to indicate the linear and curved linear vessels that together form the polymorphic pattern.

Line 189: Please reference figures in the paragraph.

Response: *The reference figures has* added as requested.

*Line 223: Please provide reference. (*Notably, in people these white areas provide a useful clue for distinguishing calcinosis cutis from clinical mimickers, particularly keratotic lesions such as warts, corns, callosities, or lichen planus-like keratosis, which instead usually display dull white areas related to compact keratin)

Response: The reference has been added as requested.

Line 235: "… as typically described in DCC." Do you mean as observed in this study?

Response: Yes. The sentence was changed from: "as typically described in DCC" to "as observed in CC in the present study".

Line 245: "A monomorphic pattern of short, dilated linear curved vessels, randomly distributed, was the most common, while a polymorphic combination of linear and linear curved vessels was less frequent." This is redundant, already stated in the results.

Response: The sentence has been shortened and modified as follows: "*Vascular structures characterized by short, dilated, linear, and linear curved vessels were observed in more than half of the lesions*" and is followed by a justification of the importance of describing the vascular arrangement, as requested by the reviewer.

Line 247: "These findings are consistent with chronic inflammatory changes and superficial vascular congestion". Which findings? The monomorphic pattern or the polymorphic pattern? Important to make that sentence clear to the reader.

Response: The presence of short, dilated vessels represents a dermoscopic vascular feature suggestive of chronic inflammation and vascular congestion in people. The sentence has been reformulated to clarify the concept (lines 243–246).

Line 265: " … that assesses the specific association between dermoscopic features and the distribution and localization of mineralization described in the histological evaluations." This statement is unclear, that looks like what the study attempted to do? Please clarify.

Response: We have rephrased the sentence to make it clearer as follow: "*The limitations of the present pilot study include the small sample size and, with regard to the technique, the inability to accurately assess the depth of mineralization or to reliably differentiate between mineral deposition and osseous metaplasia (cutaneous osteoma), both of which remain dependent on histopathological evaluation"*.

"