



Dermoscopic findings and comparison of usefulness of longitudinal vs transversal sections in the histological diagnosis of alopecia X.

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Abstract:	<p>Background – A combination of dermoscopic and histological findings may provide useful information for the diagnosis of hair follicle diseases. However, there are no studies on dermoscopic-histopathologic correlations in dogs affected by alopecia X, and comparison of longitudinal versus transversal sectioning of skin biopsy specimens in the assessment of this hair loss disorder has not been thoroughly investigated. Hypothesis/objective – To correlate dermoscopic and histological features using both longitudinal and transversal sectioning of skin biopsy samples to gain additional information for the diagnosis of alopecia X. Animals – Nineteen Pomeranian dogs affected by alopecia X and 5 healthy Pomeranians as controls. Materials and methods – Dermoscopic-histological correlation was performed within the diseased group whereas histological comparisons against controls. The demographic and clinical characteristics were also related to the histological findings. Results – The dermoscopic findings revealed scattered, thinned, short hairs mixed with amorphous keratoseborrheic-like material (follicular plugging), perifollicular and intrafollicular scaling, and hyperpigmentation varying from pinpoint black spots to a diffuse texture. Dermoscopy correlated with histology for selected qualitative and quantitative findings. The usefulness of transversal sections was demonstrated in accurately determining the hair follicular density and counts, growth arrest phases, and in identifying mineralization of hair follicle basement membrane when compared to the longitudinal. Conversely, no correlations between histological findings and demographic and clinical characteristics were detected. Conclusions and clinical relevance – These data provide evidence of the usefulness of dermoscopy as an accessory diagnostic tool and of transversal sections of skin biopsies as complementary to the diagnosis of alopecia X.</p>

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Conclusions and clinical relevance – These data provide evidence of the usefulness of dermoscopy as an accessory diagnostic tool and of transversal sections of skin biopsies as complementary to the diagnosis of alopecia X.

Keywords

Dermoscopy, transversal sections, alopecia X

Introduction

In humans, trichoscopy, dermoscopic imaging of the scalp and hairs, has been successfully applied in practical dermatology as a non-invasive, adjunctive tool for diagnosing common hair loss disorders.¹⁻³ The technique reveals morphologic characteristics that are not readily visible to the naked eye, including cutaneous blood vessels, perifollicular and interfollicular features, and changes to hair shaft thickness and shape. In addition, trichoscopy may aid in the selection of a biopsy site when pathologic examination of a scalp disorder is warranted.^{4,5} For a complete dermoscopic-histopathological correlation, cutaneous transversal sectioning assessing morphological aspects of the same plane of the image obtained at dermoscopy needs to be utilized.⁶ Several studies have shown evidence that transversal sections better reflect the stage of the hair growth cycle, allow

pathologists to determine the severity of alopecia, and provide compelling statistical confirmation of a reduced anagen: telogen ratio in affected skin that may not be apparent in longitudinal sections.⁷⁻¹²

In veterinary dermatopathology, few studies correlating dermoscopic findings to transversal skin sections are available.¹³⁻¹⁵ Only recently, transversal sectioning has been demonstrated to confer significant benefits and to complement longitudinal sectioning in the histological evaluation of several canine hair follicle (HF) disorders.¹⁶ For example, in the study by Bond and co-workers, transversal and longitudinal sections were compared in different canine alopecic conditions such as atrophic, dysplastic, and inflammatory diseases, with one case of alopecia X also included. The authors concluded that longitudinal sections were more informative for epidermal changes and dermal thickness evaluation, whereas transversal sections were more useful for hair growth phase determination, follicular morphology, and follicular inflammation assessment, thus indicating that these techniques are both advantageous and complementary.¹⁶

As a hair cycle arrest disorder, alopecia X is characterized by symmetrical, nonpruritic, and noninflammatory alopecia that spares head and distal extremities with a predisposition in Pomeranians.^{17,18} Histologically, kenogen and telogen HFs predominate, whereas anagen follicles are sparse, thus suggesting impaired anagen induction and promotion. Atrophy of dermal collagen may be observed in cases of maximal severity.¹⁹

Against this background, the aims of this study were to: (i) describe the dermoscopic features and their histopathological correlations in dogs with alopecia X; (ii) assess the benefits of transversal sections when compared to longitudinal sections in the histological diagnosis of alopecia X; (iii) correlate the demographic and clinical characteristics as age, sex, season of biopsy, and duration of alopecia X with the histological findings. This was aimed to demonstrate the potential usefulness of additional techniques and morphological clues for the accurate diagnosis of alopecia X.

Material and methods

Study population

Client-owned Pomeranian dogs affected by non-inflammatory alopecia of any age and sex were enrolled in the study. Dogs were initially included in the study based on the following criteria: i) predisposed breed, ii) ideal body condition score (i.e., 5/9), iii) no dehydration on physical examination, iv) if female, not pregnant or lactating, v) absence of concomitant systemic signs, vi) no other gross lesions on dermatological examination besides truncal progressive hair loss and/or wooly coat quality, with or without cutaneous hyperpigmentation, vi) flea prevention for at least 3 months prior to sampling, including all other dogs and cats of the household, vii) no systemic or topical treatments associated with alopecia, such as glucocorticoids or diethylstilbestrol. Steroids whether administered, were withdrawn at least one month before enrolment.

Regarding laboratory findings, complete hematology and clinical chemistry including normal plasma total thyroxine concentration were performed, and dogs with alterations suggestive of endocrinopathy were excluded. Based on presentation, age, spaying status, absence of specific clinical signs nor microscopical findings, hyperestrogenism was also excluded.

To be enrolled all affected dogs underwent Wood's lamp analysis, hair plucking, forced combing, adhesive tape strips, and skin scrapings with negative results. Dermoscopic examination and biopsy collection for dermatopathology were performed as part of the diagnostic work-up in all cases. Duration of [disease](#) in dogs with alopecia X from its onset was classified as <3 months, 3-6 months, 6-12 months, or >12 months, and season of biopsy procedure was also recorded and classified as spring (March-May), summer (June-August), autumn (September-November), or winter (December-February) as previously reported in the study of Müntener et al.¹⁹. Informed owner consent was obtained to participate into the study and all procedures were performed in accordance with ethical guidelines (XXX).

Dermoscopic examination

A handheld light dermoscope (FotoFinder Handyscope®, FotoFinder Systems GmbH, Bad Birnbach, Germany) with polarized and non-polarized lights used independently of one another, customised to be attached to an iPhone® (Apple, Cupertino, CA, USA), and providing up to x20 magnification, was used on the area that was previously circled with a marker and intended for skin biopsy. For each patient, images at x20 magnification with an integrated scale bar of 5 mm were acquired and then stored through a dedicated iPhone app (Handyscope 3® app-version 3.0.6, FotoFinder Systems GmbH). Images were also taken using a videodermoscope (Fotofinder® TeachScreen Systems software GmbH; Bad Birnbach, Germany). This device is equipped with a software which allows the measurement of structures visualized in magnified images and provides results in real scale. [A real scale is used to consistently measure and monitor lesion dimensions and morphology over time. It is incorporated into the device, and it automatically generates an accurate distance measurement on the area of interest.](#)²⁰ Images of the skin were taken at a 20-fold magnification, which allows high quality enlargement of 1 cm² of skin area to the size of a computer screen, and at 70-fold magnification, which magnifies, in a similar manner, an area of 9 mm².²¹ All images were acquired and examined by a [dermatology](#) specialist. Qualitative dermoscopic findings such as perifollicular or intrafollicular scaling, pigment network, and vascular structures, scored as 0 (absent), 1 (mild), 2 (moderate), or 3 (severe) were evaluated and correlated to those detected histologically in longitudinal view. Hair follicle openings (HFOs) grouped in triplets and no-triplets (less or more than three grouped HFOs) were examined and counted on dermoscopy and correlated to the number of [HF complexes organized in triplets and non-triplets](#) detected histologically in transversal view. [To describe follicular arrangement, definitions were applied according with Meyer W.²² In detail: the HF complex indicates the typical arrangement of a central primary hair follicle and two lateral primary follicles. Each of these central and lateral groupings represents a compound follicle made of a primary follicle, secondary hair follicles, sebaceous and apocrine glands.](#)

Histological examination

Local anaesthesia (lidocaine hydrochloride; 20 mg/ml, Zoetis Srl, Italy) was applied on two diametrically opposed alopecic areas that were previously circled with a marker for dermoscopic purposes, and with the direction of hair growth also

indicated. Two punch biopsy samples were then taken: one of 4-6 mm from the maximal alopecic area of the left flank and intended for longitudinal sectioning, and a second of 4-6 mm from the diametrically opposite maximal alopecic area of the right flank and intended for [transversal](#) sectioning. Both specimens were immediately placed in neutral-buffered 10% formalin, and, after fixation, one was sectioned longitudinally and the other transversely, and then both were stained with haematoxylin and eosin for histological examination.

As control cases, normal skin samples from the flank region in the same area of the biopsies from alopecic dogs were obtained from deceased [healthy Pomeranians](#) for conditions unrelated to dermatological diseases and submitted for necropsy immediately postmortem prior to autopsy. Biopsies were fixed in formalin, routinely processed, and stained with haematoxylin and eosin. Morphological features were assessed independently by two pathologists. Inter-observer discrepancies were resolved by a third consensus reading.

For the longitudinal section examination, [HF complexes](#) were counted in each biopsy, and HFs were assigned to a specific [growth](#) cycle stage in accordance with Müntener *et al.*,¹⁹ namely early or late anagen, telogen, kenogen, or undetermined; dysmorphic follicles and flame follicles were also counted. The term dysmorphic was favoured and applied to misshapen HF as these were not of congenital origin as the term dysplasia may suggest. [Flame follicles were identified as those HFs characterized by excessive amount of trichilemmal keratin \(bright eosinophilic and amorphous isthmic keratin\) that generally irregularly interdigitates with the outer root sheath of the HF.](#)

The percentage of each follicle type was calculated. Thickness of the dermis was digitally measured for all cases at the maximum depth point, at regular intervals along the biopsy specimens (5 measurements [per case](#)) and expressed in μm . Additional changes affecting the epidermis, the dermis, and adnexal structures were recorded and scored. The following scores were applied to the assessment of epidermal hyperkeratosis, parakeratosis, hyperpigmentation, hyperplasia, atrophy, exocytosis of inflammatory cells, and spongiosis: absent (score 0), mild (score 1), moderate (score 2), and severe (score 3). Other lesions of dermis (fibrosis, collagen degeneration, mucinosis, elastosis, mineralization, pigmentary incontinence, edema, angiogenesis, inflammation) and adnexal structures (atrophy, hyperplasia, dysplasia, inflammation of sebaceous glands) were recorded as absent (score 0) or present (score 1).

For the [transversal](#) examination, sections were chosen at the isthmus level after serial sectioning and as previously described.¹⁵ For morphometrical assessment, sections were examined using a light microscope (Nikon Eclipse 80i, XX) connected to a computer via a digital system (Nikon Digital sight DS-U1). Representative images were acquired at low magnification (10x) using NIS-Elements to perform the analysis on the following parameters: i) the entire transversal surface occupied by [HF complexes](#) was expressed in mm^2 , ii) the number of [HF complexes](#) organized in triplets and [those organized in](#) non-triplets (less or more than three [compounds HF](#)) was counted [in one standard field](#) and the total number of [HF complexes](#) per mm^2 of skin surface was also recorded, and, iii) the total area occupied by [HF complexes](#) per field was calculated and expressed as a percentage. Each follicle was assigned to a specific cycle stage or attributed to the undetermined category, and dysmorphic and flame follicles were also counted. Finally, follicle basement membrane mineralization was recorded for primary and secondary follicles when present.

Samples were obtained after signed informed consent of the owners and the use of animal tissue in the current study was approved by the Ethics Committee in charge for animal welfare of XX (with the protocol number OPBA_60_2022). Sensitive information regarding owners and animals were collected, managed, and preserved according to XX laws.

Statistical methods

To explore any agreement between dermoscopic and histological data, and within the histological results between longitudinal and transversal evaluations, the Spearman's rank correlation coefficient was used. Comparison between longitudinal and transversal section evaluations in affected dogs was performed using non-parametric Wilcoxon paired test. The comparisons between groups of affected Pomeranians vs healthy Pomeranians were performed using non-parametric Mann-Whitney test.

Season and duration distributions were compared in dogs with alopecia X using non-parametric Kruskal-Wallis test. $P < 0.05$ was considered significant. Statistical analysis was performed with commercial software (SAS 9.4, SAS Institute Inc., Cary, NC, USA, and XLStat, Addinsoft 2022, New York, USA).

Results

Dogs

The study included 24 Pomeranian dogs, 19 affected by alopecia X and 5 healthy controls. The clinical profile and demographic characteristics of cases and controls are summarized in Table 1.

Dermoscopic features

In dogs with alopecia X, scattered hair shafts, as thin hairs with no distinction between cortex and medulla and mixed with amorphous keratoseborrheic-like material (yellowish- brown follicular keratotic plugs) were detected. HFOs were evenly arranged, mainly in repetitive triplets, although non-triplets were also detected. Scaling, as greyish-white scales, relatively large, moderately adherent to the skin surface and varying from moderate to severe in their distribution, were observed along the perifollicular and intrafollicular skin surface. Pigmentation varied from interfollicular pinpoint black spots to a more diffuse pattern. Vascular structures were not visualized. Findings are reported in Figure 1.

Histological parameters in longitudinal view and agreement with dermoscopy

Basket-weave hyperkeratosis was observed in all the 19 dogs with Alopecia X with the highest score of 3 in 68% of cases (Table S1, the value of median score is 3) and more rarely in association with compact hyperkeratosis (3/19 cases). Parakeratosis was never observed. Variable degree of pigmentation was recorded in 14/19 cases with melanin distributed in the basal and suprabasal layers, and in the stratum corneum. Epidermal hyperplasia was a rare finding (1/19) whereas epidermal atrophy was seen in 9/19 cases; leukocyte epidermal exocytosis and spongiosis were

extremely rare and generally mild and focal. In the dermis no lesions were seen. Sebaceous glands were mildly atrophic in 3 dogs and mildly hyperplastic in 7 dogs. An average of 7 grouped HF complexes (range: 4-10) were counted in each biopsy, whereas an average of 24 HFs (range: 10-44) for each field were recorded. There was a moderate positive agreement between dermoscopy and histology for scaling ($r=0.616$, $p=0.005$) and a fairly strong agreement between dermoscopy and histology for hyperpigmentation ($r=0.854$, $p<0.0001$).

Histological parameters in transversal view and agreement with dermoscopy

HF complexes were arranged mainly in repetitive triplets, although non-triplets were also seen (Figure 2). For the transversal view, an average of 30 HF complexes (range: 9-72) was counted in each biopsy, whereas an average of 165 HFs (range: 43-308) for each field was recorded. There was a fairly strong positive correlation between the number of HFOs (grouped in triplets and non-triplets) counted dermoscopically and the number of triplets and non-triplets counted histologically with transversal view ($r=0.920$, $p<0.0001$).

Histological longitudinal-transversal section correlations

Pomeranian dogs affected by Alopecia X: comparison between histological examination performed in longitudinal and transversal view

A moderate agreement between longitudinal and transversal sections was observed for the percentage of telogen and kenogen HFs (Table 2, $r>0.500$). Regarding the comparison between the longitudinal and transversal sections, the follicular counts of telogen and kenogen as reported for longitudinal and transversal sections were not significantly different, whereas the percentage of undetermined, dysmorphic and flame follicles was significantly higher for the longitudinal sections (Table 3).

Comparison between cases with alopecia X and healthy controls for the transversal view

In affected Pomeranians, the percentages of kenogen and dysmorphic telogen HF were significantly higher while the percentage of anagen HF was significantly lower in Pomeranians with Alopecia X compared to normal Pomeranian skin. A positive trend towards significance was observed for flame and mineralized follicles ($p=0.06$ and $p=0.09$) in affected compared to healthy dogs. Of note, mineralized primary and secondary HFs were only detected in the diseased dogs. Findings are illustrated in Figure 3 and results in Table S2.

Dermal thickness assessment and comparison between diseased dogs and controls

In the 19 Pomeranians affected by alopecia X the median thickness of the dermis calculated in longitudinal view was 749 μm (range: 545-1368) against 899 μm (range 821-969) in the healthy Pomeranians. No significant difference was detected between the diseased dogs and controls.

Demographic and clinical characteristics in dogs with alopecia X and comparison with histological findings

No effect of age or sex was detected on follicular counts in transversal or longitudinal sections for the 19 dogs affected by alopecia X. Among the 19 dogs with alopecia X, the disease onset was recorded in winter for 15 dogs and spring for 4. In longitudinal view, the season effect was observed for flame follicles ($p=0.045$) with percentages greater in spring than in winter. No significant effect was detected for disease duration in this group.

Discussion

In this study, dermoscopic findings in dogs affected by alopecia X and the correlation between dermoscopic features and histopathology using transversal sections are evaluated for the first time. HFOs were easily assessed dermoscopically, and histological findings correlated positively with dermoscopic calculations. The key observational dermoscopic feature for alopecia X was the presence of scattered and thin short hair shafts, lacking demarcation between cortex and medulla, and admixed with keratoseborrheic-like material occluding the HFO.

In humans, several hair shaft structural abnormalities are evaluated by trichoscopy, with clinicians often adopting a classification established on a structure-based approach that encompasses a group of congenital and acquired alterations.²³

Trichoscopic observations are broadly grouped as hair signs, vascular, pigment and interfollicular patterns. Abnormalities may include decreased hair density, amorphous hair residues, broken or coiled hairs, tapered or up-right regrowing hairs and/or “yellow dots” as trichoscopic findings corresponding to dilated follicular infundibula filled with keratotic material.²⁴

To visualize distinctive morphological features of the cutaneous vasculature, it is important to perform dermoscopy without inducing a firm direct pressure (diascopy) that might result in their blanching although in the pigmented skin, the heavy pigment also obfuscates the vascular patterns.²⁵

In this study, the type of HF findings observed dermoscopically were supposedly related to the hair cycle arrest and/or, at least for some of them, to the singular shape of flame/dysmorphic HFs. However, it cannot be excluded that this finding could be detected also in other hair cycle arrest disorders and in the future, we expect to isolate also from other alopecic conditions all those features that are significantly associated with a particular diagnosis.

The severity of scaling detected dermoscopically (varying from moderate to severe) was related to the dryness of the skin that may be detected in dogs with alopecia X, whereas the different degree of pigmentation was considered to result from the progressive solar exposure in the balding areas as previously reported,² also overlapping the vascular pattern.

Secondly, in this study and in line with observations in other reports,¹⁶ the usefulness of transversal sectioning in accurately determining HF density compared to longitudinal sectioning was confirmed by the higher number of follicle units and hair follicles counted in the transversal sections compared to the longitudinal ones and allowed us to partially overcome some of the difficulties in establishing HF growth phase by histopathology.

Despite a trend in recent years, the paucity of studies in veterinary dermatopathology directly comparing longitudinal and transversal section morphology may be because historically the transversal technique has been considered as providing little benefit.

Indeed, it is complex to carry out because it requires training and expertise in orientation, trimming and embedding, as well as multiple serial [sectioning need to](#) be performed to obtain all the representative information of the different structures and depths. Thus, longitudinal orientation of biopsies has continued to represent the standard in the investigation of most diseases, including hair growth disorders.

[In general, orientation of follicles in tissue samples is critical to assess hair growth cycle stages and this information is deemed necessary for the diagnosis of specific alopecic conditions. Although longitudinal sections are satisfactory for the study of many dermatological diseases, their utility in the evaluation of HF status and type and severity of alopecia alone may have some limitations. Indeed, because HF grows at an angle in relation to the epidermis, the result is that it can be often missed, cut tangentially, or incompletely in the longitudinal section.](#)²⁶

[This is more relevant in canine skin where compound HFs are present and thus HF density determination and cell cycle phase identification in all HFs may be underscored by the tangential sectioning of most HFs. Therefore, the assessment of the number, HF type \(primary versus secondary\) and the identification of the specific cell cycle phase of most HFs in any skin biopsy loss \(number reduction\) may bear some limitations. On the contrary, although transversal sectioning may offer a quantitative approach to alopecic conditions because allows for the assessment of HF at different levels, provide with the number and the relative size of the HFs in a specific/standard area and include most bulbs in lower sections thus increasing sensitivity of histology in the diagnosis, it does not allow an adequate qualitative approach based on the repetitive criteria that can be obtained in longitudinally oriented section.](#)

Of note, when the transversal orientation of biopsies has been used in veterinary medicine, the bisectonal technique, dividing a single skin biopsy sample for longitudinal and [transversal](#) views, has been demonstrated to increase the diagnostic accuracy of the dermatopathological analysis.¹⁶ Nevertheless, in the present study we preferred to correlate and compare two skin biopsies from two diametrically opposed alopecic areas, thus increasing the material available, facilitating the cutting of the [transversal](#) biopsies, and minimizing technical difficulties associated with reduction of the sample. Moreover, [this approach facilitated and improved the assessment](#) and the clinical significance of dermoscopic-histologic [correlations especially for the quantitative parameters.](#)

Dogs with alopecia X showed [lesser numbers of HF complexes](#) per section in longitudinal specimens compared to the higher number observed in the corresponding [transversal](#) biopsy section. Although this finding may be not surprising, it confirmed the utility of [transversal](#) section in the visualization of virtually all the HFs present in a given biopsy and provided a better assessment of the real HF density and counts. [Additionally](#), although Credille et al. demonstrated that hair follicles in transversal sections are mainly arranged in triplets,²⁷ in this study, [several groups of HF arranged in non-triplets \(two or more than three\)](#) were observed and counted.

Moreover, [transversal](#) sections allowed for better identification of HF arrest phases and were superior to longitudinal sectioning in estimating the percentage of telogen, dysmorphic, and kenogen HFs. Conversely, anagen and flame follicles were better assessed using longitudinal sections. As expected, and in line with previous studies, anagen HFs were observed (generally in the early phase) by detecting hair bulbs in the superficial panniculus, thus explaining why longitudinal sections are more reliable for determination of anagen growth cycle phase.^{19,28} In contrast, the finding of a

better estimation of flame follicles in longitudinal sections was unexpected; this was likely due to the increased possibility of identifying the irregular interlocking of trichilemmal keratin along the entire length of the HF wall, while only one area of the isthmus region was utilized to assess HFs in transversal sections.

Also of note was the identification of a subgroup of dogs with alopecia X with evidence of HF basement membrane mineralization. It is commonly accepted that dystrophic mineralization of the HF basement membrane can be seen in dogs with spontaneous or iatrogenic hyperadrenocorticism and that this may represent a senile change in poodles, although it has also been demonstrated as not always being associated with calcium deposition.^{29,30} In this study, the severity and type of distribution (mainly scattered) of this finding was better appreciated in transversal sections, and was considered as novel because, to the best of the author's knowledge, it has not been previously reported in canine alopecia X; follicle mineralization was detected in juvenile and adult dogs making an etiopathogenetic comparison with the same finding in poodle (related to old age) unlikely. However, further investigations are warranted to confirm this finding, and to determine whether it is linked to this alopecic condition.

Alopecia X has been reported to be a disease associated with maximal dermal atrophy.¹⁹ The finding of dermal atrophy was only partially confirmed by our study where for example, the depth of the dermis was reduced in the affected Pomeranians when compared with the healthy counterparts. However, this finding was not as severe as previously described.

Finally, this is the first report providing comparison of transversal sectioning between dogs with alopecia X and healthy dogs. The area occupied by HFs was similar in dogs with alopecia X and controls with affected dogs demonstrating a significant increase of HF kenogen arrest and decrease in anagen, thus confirming findings previously reported for alopecia X.¹⁹

Conversely, this study failed to identify any effect of specific demographic features on the phase of the hair growth in the 19 affected dogs. These findings are in contrast with previous studies that, for example, showed that neutered females had significantly fewer telogen follicles and significantly more kenogen and atrophic follicles than male dogs.¹⁹ However, in our study most of the affected dogs were intact mature-adult males, and this may have influenced the results. Interestingly, in most dogs, disease onset was recorded during the winter season but those developing the disorder during spring displayed more flame follicles. However, all these observations need further investigations and confirmation in a larger number of cases.

A limitation of this study was the impossibility of reporting the dermoscopic findings in healthy dogs, for ethical reasons, since clipping is necessary to observe the surface in normal areas and permission was not granted by the owners.

In conclusion, this study demonstrates the efficacy of dermoscopy as a useful accessory diagnostic tool in alopecia X. However, because the pathogenesis of this disorder remains unclear, in the future it would be desirable to compare the dermoscopic findings in alopecia X with those observed in other non-inflammatory hair disorders characterized by hair cycle arrest. Finally, transversal sections are recommended as complementary and adjunctive tools for the assessment of several parameters including follicular density and counts, basement membrane mineralization, and in general to further determine the severity of alopecia.

Table legends

Table 1. Characteristics of dogs with alopecia X (n=19) and healthy controls (n=5).

Table 2. Alopecia X in 19 dogs: correlation between histological examination performed in longitudinal and transversal views (Spearman rank correlation index).

Table 3. Alopecia X in 19 dogs: comparison between histological examination performed in longitudinal and transversal views (non-parametric Wilcoxon paired test).

Table S1. Histology in longitudinal view in dogs with alopecia X.

Table S2. Histology in transversal view: comparison of HFs between Pomeranians with alopecia X (n=19) and healthy Pomeranians (n=5) (non-parametric Mann-Whitney test)

Figure legends

Figure 1. Handheld dermoscope: representative dermoscopic features in a dog affected by alopecia X. (a,c) By non-polarized dermoscopy. Whitish scales are observed on the skin surface; (b,d) By polarized-dermoscopy. Thin and short hairs are observed. HFOs are arranged in triplets and non-triplets depending upon whether the follicular unit comprised three or another number of compound follicles; pigmentation appears as pinpoint black spots (black arrows in d). (20x scale bar 5mm).

Figure 2. Sketches [from Credille et al., 2001, modified (a,c,e)] and related images of HF triplets and non-triplet arrangement. (a,b) Classic triplet of three compound follicles each composed of a large primary HF and numerous secondary follicles; of note, in b, mineralization (marked with a white asterisk). (c-d) Non-triplet HF arrangement composed of four compound follicles; of note in d, two primary dysmorphic primary HFs. (e-f) Non-triplet HF arrangement composed of two compound follicles. H&E (200x). (g-h) Videodermoscopy: images at 20x (g) and 70x (h): HFOs in triplets and non-triplet arrangements are observed (black asterisks); scattered, short, and thin hairs inside their HFOs and mixed with amorphous keratoseborrheic-like material (follicular plugging) are detected.

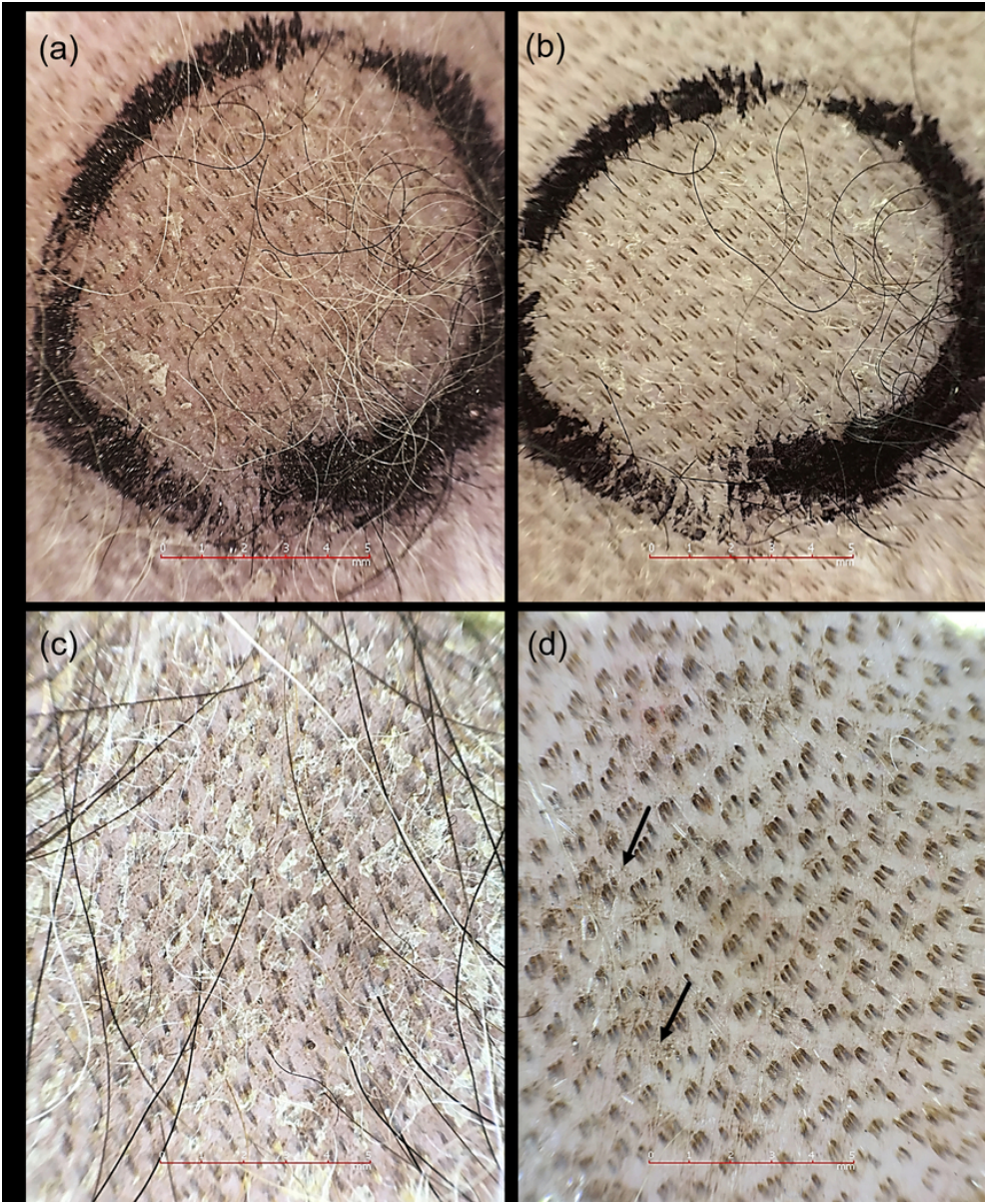
Figure 3. (a-b) Longitudinal and transversal sections from a dog with alopecia X. Nine compound follicles were counted in the longitudinal section (a) and 12 compound follicles in the transversal section (b). No flame follicles are visible in the transversal section (H&E;4x-10x, respectively). (c-d) Longitudinal sections from a dog with Alopecia X. Flame follicles extend from the isthmus region downward (H&E; 10x) e) Healthy control: telogen and anagen hair follicles (arrows on the upper right). f) Dog with alopecia X. Most hair follicles are in telogen (H&E; 10x). g) Dog with alopecia X: most hair follicles are in kenogen with some characterized by dysmorphic morphology; mineralized hair follicles are also present (inset). h) Dog with alopecia X: most hair follicles are in telogen; 'flame' and mineralized hair follicles (inset) can be seen (H&E; 20x). All flame follicles in figures a, c, d, h have been marked with an asterisk.

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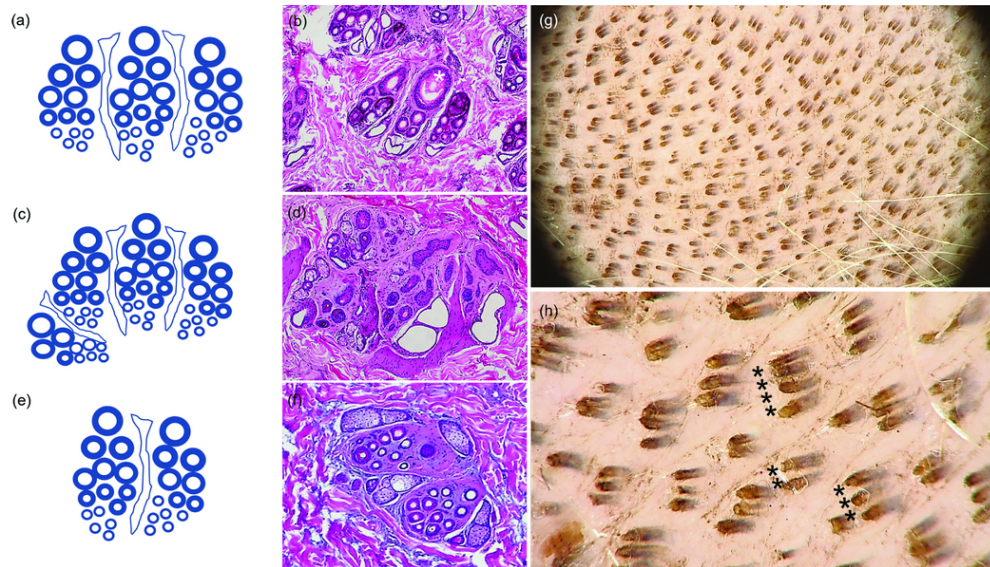
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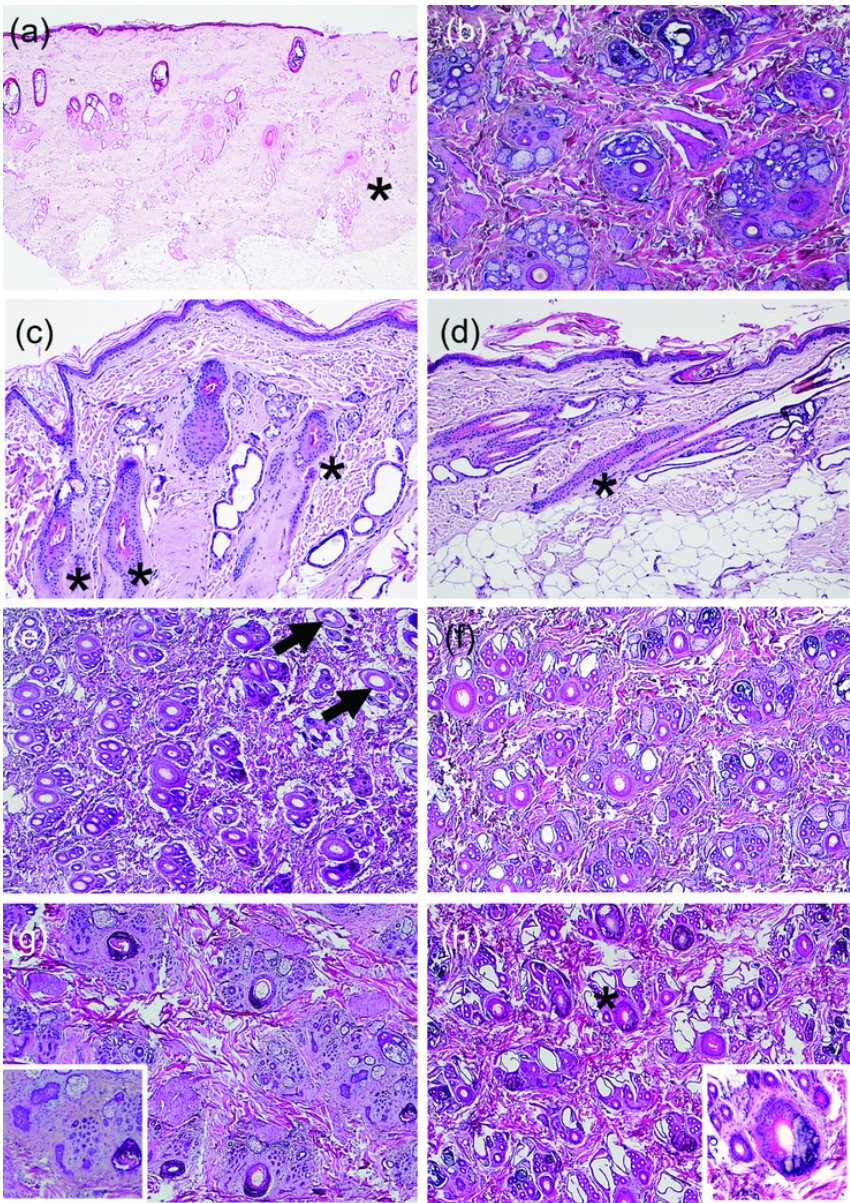
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145x176mm (150 x 150 DPI)



178x100mm (150 x 150 DPI)



124x176mm (150 x 150 DPI)

Tables

Table 1. Characteristics of Pomeranian dogs affected by alopecia X (n=19) and healthy controls (n=5).

	Alopecia X	Healthy controls
Sex		
Intact female	2	2
Neutered female	2	0
Intact male	14	3
Castrated male	1	0
Age		
<1 year, puppy	1	0
1-2 years, adolescent	2	2
2-6 years, mature adult	13	2
>6 years, senior	3	1
Season of biopsy		
Spring (March-May)	4	n.a.
Summer (June-August)	0	n.a.
Autumn (September-November)	0	n.a.
Winter (December-February)	15	n.a.
Duration of alopecia		
<3 months	5	n.a.
3-6 months	7	n.a.
6-12 months	6	n.a.
>12 months	1	n.a.

n.a., not available.

Table 2. 19 Pomeranian dogs affected by Alopecia X: correlation between histological examination performed in longitudinal and transversal views (Spearman rank correlation index).

[illegible]

Table 3. 19 Pomeranian dogs affected by Alopecia X: comparison between histological examination performed in longitudinal and transversal views (non-parametric Wilcoxon paired test).

	Longitudinal	Transverse	P-value
Telogen (%)	30% (13-45%)	37% (14-60%)	0.104
Kenogen (%)	53% (35-65%)	50% (34-81%)	0.515
Anagen (%)*	0% (0%-0.08%)	0% (0%-0%)	0.046
Undetermined (%)	12% (8-22%)	6% (3-8%)	0.036
Dysmorphic (%)	13% (10-20%)	4% (2-6%)	<0.001
Flame (%)	9% (5-15%)	1% (0-2%)	0.001

Data expressed as median and interquartile range in brackets.

* Per anagen in longitudinal view, early and late anagen have been summed

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Table S1. Histology in longitudinal view in 19 Pomeranian dogs affected by alopecia X: descriptive.

Pomeranians	
Hair follicles	
Miniaturization (0/1)	1 (5%)
Fibrous sheath thickening (0/1)	12 (63%)
Fibrous sheath mineralization (0/1)	4 (21%)
Inflammation (0/1)	0 (0%)
Pigmentary dysplasia, inferior segment (0-3)	0(0-0)
Atrophy (0-3)	3(3-3)
Infundibular keratosis (0-3)	2(2-3)
Trichilemmal keratin excess (0-3)	2(1-2)
Epidermis	
Crusts (0/1)	1 (5%)
Basket weave hyperkeratosis (0-3)	3 (2-3)
Compact hyperkeratosis (0-3)	0 (0-0)
Parakeratosis (0-3)	0 (0-0)
Stratum corneum hyperpigmentation (0-3)	1 (0-2)
Suprabasal hyperpigmentation (0-3)	1 (0-1)
Basal keratinocyte hyperpigmentation (0-3)	1 (0-2)
Epidermal hyperplasia (0-3)	0 (0-0)
Epidermal dysplasia (0-3)	0 (0-0)
Epidermal atrophy (0-3)	0 (0-1)
Exocytosis/epidermal inflammation (0-3)	0 (0-0)
Epidermal spongiosis (0-3)	0 (0-0)
Dermis	
Fibrosis (0/1)	0 (0%)
Collagen degeneration (0/1)	0 (0%)
Mucinosi (0/1)	1 (5%)
Elastosis (0/1)	0 (0%)
Mineralization (0/1)	0 (0%)
Pigmentary incontinence (0/1)	1 (5%)
Edema (0/1)	3 (16%)
Angiogenesis (0/1)	3 (16%)
Inflammation (0/1)	3 (16%)
Sebaceous glands	
Atrophy (0/1)	3 (16%)
Hyperplasia (0/1)	7 (37%)
Dysplasia (0/1)	0 (0%)
Inflammation (0/1)	0 (0%)

0-3, score from absent (0) to severe (3); (0/1), absent (0) or present (1).

Data are expressed as median and interquartile range in brackets (quantitative data) and as absolute number of cases and percentage in brackets (qualitative data). n.a., not available.

Table S2. Histology in transversal view: comparison of HFs between Pomeranians with alopecia X (n=19) and healthy Pomeranians (n=5) (non-parametric Mann-Whitney test)

	Pomeranians		P-value
	Alopecia X	Healthy controls	
Telogen (%)	37% (14-60%)	19% (18-34%)	0.446
Kenogen (%)	50% (34-81%)	17% (11-30%)	0.005
Anagen (%)	0% (0-0%)	46% (21-48%)	<0.001
Not determined (%)	6% (3-8%)	11% (3-27%)	0.406
Dysmorphic telogen (%)	3% (1-6%)	0% (0-0%)	0.003
Dysmorphic kenogen (%)	0% (0-1%)	0% (0-0%)	0.206
Flame (%)	1% (0-2%)	0% (0-0%)	0.061
Mineralized primary hairs (%)	0.4% (0-3%)	0% (0-0%)	0.094
Mineralized secondary hairs (%)	0% (0-0.3%)	n.a.	n.a.

Data expressed as median and interquartile range in brackets. n.a., not available.

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