Renal amyloid-A amyloidosis in cats: Characterization of proteinuria and biomarker discovery, and associations with kidney histology

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Abstract: BackgroundAmyloid A (AA) amyloidosis is a protein misfolding disease arising from serum amyloid A (SAA). Systemic AA amyloidosis recently was shown to have a high prevalence in shelter cats in Italy and was associated with azotemia and proteinuria.ObjectivesInvestigate urine protein profiles and diagnostic biomarkers in cats with renal AA amyloidosis.AnimalsTwenty-nine shelter cats.MethodsCase-control study. Cats with renal proteinuria that died or were euthanized between 2018 and 2021 with available necropsy kidney, liver and spleen samples, and with surplus urine collected within 30 days before death, were included. Histology was used to characterize renal damage and amyloid amount and distribution; immunohistochemistry was used to confirm AA amyloidosis. Urine protein-to-creatinine (UPC) and urine amyloid A-to-creatinine (UAAC) ratios were calculated, and sodium dodecyl sulfate-agarose gel electrophoresis (SDS-AGE) and liquid chromatography-mass spectrometry (LC-MS) of proteins were performed.ResultsTwenty-nine cats were included. Nineteen had AA amyloidosis with renal involvement. Cats with AA amyloidosis had a higher UPC (median, 3.9; range, 0.6-12.7 vs 1.5; 0.6-3.1; P = .03) and UAAC ratios (median, 7.18×10^3; range, 23×10^3-21.29×10^3 vs 1.26×10^3; 0.21×10^3-6.33×10^3; P = .04) than unaffected cats. The SDS-AGE identified mixed-type proteinuria in 89.4% of cats with AA amyloidosis and in 55.6% without AA amyloidosis (P = .57). The LC-MS identified 63 potential biomarkers associated with AA amyloidosis (P < .05). Among these, urine apolipoprotein C-III was higher in cats with AA amyloidosis (median, 1.38×10^7; range, 1.85×10^5-5.29×10^7 vs 1.76×10^6; 0.0×10^0-1.38×10^7; P = .01). In the kidney, AA-amyloidosis was associated with glomerulosclerosis (P = .02) and interstitial fibrosis (P = .05).Conclusions and Clinical ImportanceRenal AA amyloidosis is associated with kidney lesions, increased proteinuria and increased urine excretion of SAA in shelter cats. Additional studies are needed to characterize the role of lipid transport proteins in the urine of affected cats.

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ORIGINAL ARTICLE

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

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Abstract

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–histopathological 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/Objectives: The aim of this study was 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 five 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 also were related to the histological findings.

Results: The dermoscopic findings revealed scattered, thinned, short hairs mixed with amorphous keratoseborrhoeic-like material (follicular plugging), perifollicular and intrafollicular scaling, and hyperpigmentation varying from pinpoint black spots to a diffuse texture. Dermoscopic findings correlated with histological findings 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 mineralisation 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 dermoscopic evaluation as an accessory diagnostic tool and of transversal sections of skin biopsies as complementary to the diagnosis of alopecia X.

KEYWORDS
alopecia X, dermoscopic evaluation, transversal sections

Giordana Zanna and Francesca Abramo contributed equally to this work. They should be considered joint first author.

INTRODUCTION

In humans, trichoscopy—dermoscopic imaging of the scalp and hairs—has been successfully applied in dermatological practice as a noninvasive, adjunctive tool for diagnosing common hair loss disorders.[1–3] The technique reveals morphological 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 site for taking biopsy when pathological 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 dermoscopic evaluation needs to be utilised.[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, while 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 characterised 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, while 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; and (iii) correlate the demographic and clinical characteristics including age, sex, season of biopsy and duration of alopecia X, with the histological findings. This was aimed at demonstrating the potential usefulness of additional techniques and morphological clues for the accurate diagnosis of alopecia X.

MATERIALS AND METHODS

Study population

Client-owned Pomeranian dogs affected by noninflammatory 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. five of nine); (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 woolly coat quality, with or without cutaneous hyperpigmentation; (vii) flea prevention for at least three months before sampling, including all other dogs and cats of the household; and (viii) no systemic or topical treatments associated with alopecia, such as glucocorticoids or diethylstilbestrol. Steroids when administered were withdrawn at least one month before enrolment.

Regarding laboratory findings, complete haematological and clinical chemical analysis including normal plasma total thyroxine concentration were performed, and dogs with alterations suggestive of endocrinopathy were excluded. Based on the presentation, age, spaying status and absence of specific clinical signs or microscopical findings, hyperoestrogenism also was 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 dermatopathological examination 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 when biopsy was collected also was recorded and classified as spring (March–May), summer (June–August), autumn (September–November) or winter (December–February) as reported previously in the study of Müntener et al.[19]

Informed owner consent was obtained to participate into the study, and all procedures were performed under good clinical practices in accordance with ethical guidance published in no. 289 of the national Gazzetta Ufficiale, 10 December 1996, pp. 47–53.

Dermoscopic examination

A handheld light dermoscope (Handyscope; FotoFinder Systems GmbH) with polarised and nonpolarised lights used independently of one another, customised to be attached to an iPhone® (Apple), and providing up to ×20 magnification, was used on the area that was previously circled with a marker and intended for removal of skin biopsy. For each patient, images at ×20 magnification with an integrated scale bar of 5 mm were acquired and then stored through a dedicated iPhone app (HANDYSCOPE 3 v3.0.6; FotoFinder Systems GmbH). Images also were taken

using a videodermoscope (Fotofinder TeachScreen Systems software GmbH). This device is equipped with software which allows the measurement of structures visualised 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 magnification, which allows high-quality enlargement of 1 cm^2 skin area to the size of a computer screen, and at ×70 magnification, which magnifies, in a similar manner, an area of 9 mm^2.[21]

All images were acquired and examined by a first-opinion dermatological specialist veterinary surgeon.

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 with those detected histologically in longitudinal view. Hair follicle openings (HFOs) grouped in triplets and nontriplets (fewer or more than three grouped HFOs) were examined and counted on dermoscopic evaluation and correlated to the number of HFs complexes organised in triplets and nontriplets detected histologically in transversal view. To describe follicular arrangement, definitions were applied according to 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) 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 4–6-mm-diameter punch biopsy samples were then taken: one from the maximal alopecic area of the left flank and intended for longitudinal sectioning, and a second 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 & 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 post-mortem before autopsy. Biopsies were fixed in formalin, routinely processed, and stained with H&E. Morphological features were assessed independently by two pathologists. Interobserver 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 also were counted. The term dysmorphic was favoured and applied to misshapen HFs as these were not of congenital origin, as the term dysplasia may suggest. Flame follicles were identified as those HFs characterised by an 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 (in μm) for all cases at the maximum depth point, at regular intervals along the biopsy specimens (five measurements per case). 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 (1), moderate (2) and severe (3). Other lesions of dermis (fibrosis, collagen degeneration, mucinosis, elastosis, mineralisation, pigmentary incontinence, oedema, angiogenesis and inflammation) and adnexal structures (atrophy, hyperplasia, dysplasia and inflammation of sebaceous glands) were recorded as absent (score 0) or present (1).

For the transversal examination, sections were chosen at the isthmus level after serial sectioning and as described previously.[15] For morphometrical assessment, sections were examined using a light microscope (Eclipse 80i; Nikon) connected to a computer via a digital system (Digital sight DS-U1; Nikon). Representative images were acquired at low magnification (×10) using NIS-Elements to perform the analysis using the following parameters: (i) the entire transversal surface occupied by HF complexes was expressed in mm^2; (ii) the number of HF complexes organised in triplets or nontriplets (fewer 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 also was 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 mineralisation 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 Organo Preposto al Benessere Animale (OPBA) of the University of Milano, Italy (with the protocol number OPBA_60_2022). Sensitive information regarding

owners and animals was collected, managed and preserved according to law.

Statistical methods

In order 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 the nonparametric Wilcoxon paired test. The comparisons between groups of affected Pomeranians and healthy Pomeranians were performed using the nonparametric Wilcoxon–Mann–Whitney *U*-test.

Season and duration distributions were compared in dogs with alopecia X using the nonparametric Kruskal–Wallis test; $p<0.05$ was considered significant. Statistical analysis was performed with commercial software [SAS 9.4 (SAS Institute Inc.) and XLStat (2022; Addinsoft)].

RESULTS

Dogs

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

TABLE 1 Characteristics of dogs with 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 when biopsy taken		
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.

Abbreviation: n.a., not available.

Dermoscopic features

In dogs with alopecia X, scattered hair shafts, as thin hairs with no distinction between cortex and medulla and mixed with amorphous keratoseborrhoeic-like material (yellowish-brown follicular keratotic plugs) were detected. HFOs were evenly arranged, mainly in repetitive triplets, although nontriplets also were 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 visualised. Findings are reported in Figure 1.

Histological parameters in longitudinal view and agreement with dermoscopic features

Basket-weave hyperkeratosis was observed in all 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 (three of 19 cases). Parakeratosis was never observed. Variable degree of pigmentation was recorded in 14 of 19 cases with melanin distributed in the basal and suprabasal layers, and in the stratum corneum. Epidermal hyperplasia was a rare finding (one of 19), while epidermal atrophy was seen in nine of 19 cases; leucocyte 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 three dogs and mildly hyperplastic in seven.

An average of seven 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 dermoscopic and histological results for scaling ($r=0.616$, $p=0.005$) and a fairly strong agreement between dermoscopic and histological results for hyperpigmentation ($r=0.854$, $p<0.0001$).

Histological parameters in transversal view and agreement with dermoscopic features

HF complexes were arranged mainly in repetitive triplets, although nontriplets also were 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 nontriplets) counted dermoscopically, and the number

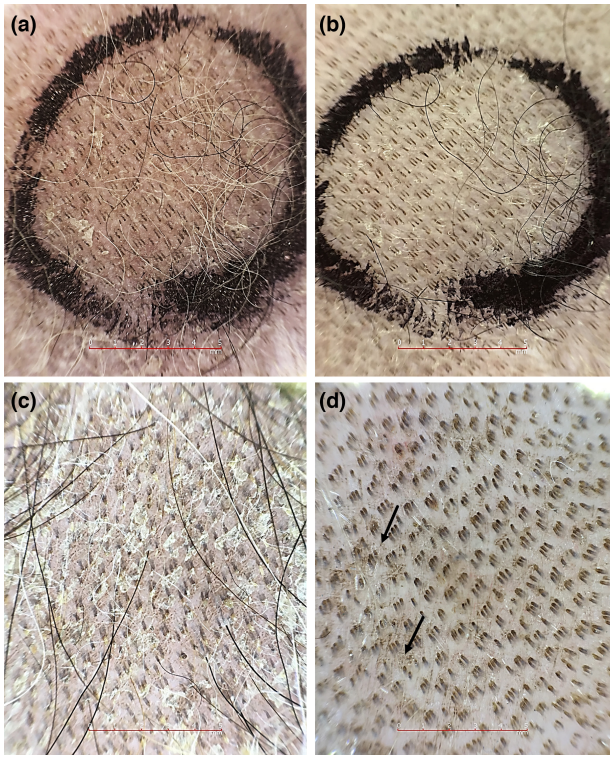

FIGURE 1 Handheld dermoscopic examination: representative dermoscopic features in a dog affected by alopecia X. (a,c) By nonpolarised dermoscopic examination. Whitish scales are observed on the skin surface. (b,d) By polarised dermoscopic examination. Thin and short hairs are observed. Hair follicle openings (HFOs) are arranged in triplets and nontriplets depending upon whether the follicular unit comprised three or another number of compound follicles; pigmentation appears as pinpoint black spots [black arrows in (d)]. ×20. Bar, 5 mm.

of triplets and nontriplets 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 (r>0.500; Table 2). 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, while 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 HFs were significantly higher while the percentage of anagen HF was significantly lower in Pomeranians with alopecia X compared with normal Pomeranian skin. A positive trend towards significance was observed for flame and mineralised follicles (p=0.06 and p=0.09, respectively) in affected compared with healthy dogs. Of note, mineralised primary and secondary HFs were detected only 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 µm) versus 899 µm (range 821–969 µm) 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 and histopathological features 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 keratoseborrhoeic-like material occluding the HFO.

In humans, several hair shaft structural abnormalities are evaluated by trichoscopic examination, 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 upright regrowing hairs and/or 'yellow dots', as trichoscopic findings corresponding to dilated follicular infundibula filled with keratotic material.[24]

In order to visualise distinctive morphological features of the cutaneous vasculature, it is important to

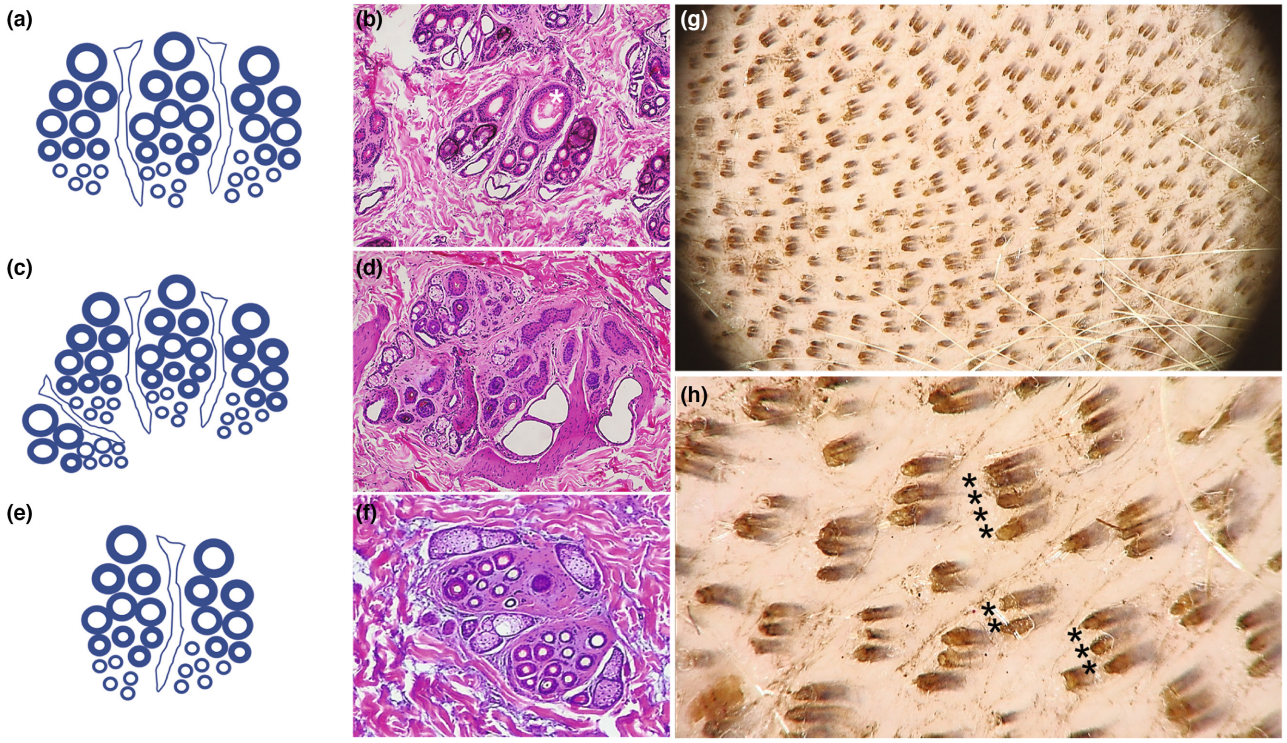

FIGURE 2 Sketches [from Credille et al., 2001, modified (a,c,e)] and related images of hair follicle (HF) triplets and nontriplet arrangement. (a, b) Classic triplet of three compound follicles each composed of a large primary HF and numerous secondary follicles; note mineralisation in (b) (marked with a white asterisk). (c,d) Nontriplet HF arrangement composed of four compound follicles; note two primary dysmorphic primary HFs in (d). (e,f) Nontriplet HF arrangement composed of two compound follicles. Haematoxylin & eosin, ×200. (g,h) Videodermoscopic images at ×20 (g) and ×70 (h): hair follicle openings (HFOs) in triplets and nontriplet arrangements are observed (black asterisks); scattered, short and thin hairs inside their HFOs and mixed with amorphous keratoseborrhoeic-like material (follicular plugging) are detected.

perform dermoscopic examination 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 all those features that are significantly associated with a particular diagnosis from other alopecic conditions also.

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, while the different degree of pigmentation was considered to result from the progressive solar exposure in the balding areas as reported previously,[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 with longitudinal sectioning was confirmed by the higher number or follicle units and hair follicles counted in the transversal sections compared with the longitudinal ones, and allowed us to partially overcome some of the difficulties in establishing HF growth phase by histopathological evaluation.

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 to provide 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 needing to be performed to obtain all of 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. Longitudinal sections are satisfactory for the study of many dermatological diseases, yet 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 often can be missed, or 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. However, although transversal sectioning may offer a

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

	Transversal						
	Telogen (%)	Kenogen (%)	Anagen (%)	Undetermined (%)	Dysmorphic telogen (%)	Dysmorphic kenogen (%)	Total dysmorphic (%)	Flame (%)
Longitudinal								
Telogen (%)	0.62 (p=0.004)							
Kenogen (%)		0.53 (p=0.018)						
Early anagen (%)			0.42 (p=0.072)					
Late anagen (%)			0.42 (p=0.072)					
Undetermined (%)				0.01 (p=0.966)				
Dysmorphic (%)					0.18 (p=0.457)	−0.25 (p=0.301)	0.13 (p=0580)	
Flame (%)								0.19 (p=0.427)

quantitative approach to alopecic conditions because it allows for the assessment of HF at different levels, provides the number and the relative size of the HFs in a specific/standard area and includes most bulbs in lower sections, thus increasing the histological sensitivity in the diagnosis, it does not allow an adequate qualitative approach based on the repetitive criteria that can be obtained in longitudinally orientated section.

It is of note that when the transversal orientation of biopsies has been used in veterinary medicine, the bisectional 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 minimising technical difficulties associated with reduction in the sample. Moreover, this approach facilitated and improved the assessment and the clinical significance of dermoscopic–histological correlations, especially for the quantitative parameters.

Dogs with alopecia X showed lower numbers of HF complexes per section in longitudinal specimens compared with the higher numbers observed in the corresponding transversal biopsy section.

Although this finding may be not surprising, it confirmed the utility of transversal section in the visualisation of virtually all 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 HFs arranged in nontriplets (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] By contrast, the finding of a better estimation of flame follicles in longitudinal sections was unexpected; this was likely to have been a result of 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 utilised 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 mineralisation. It is commonly accepted that dystrophic mineralisation 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 also has been demonstrated as not always being associated

TABLE 3 Alopecia X in 19 dogs: comparison between histological examination performed in longitudinal and transversal views (nonparametric 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 (%)[a]	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

Note: Data expressed as median and interquartile range in brackets.
[a]Per anagen in longitudinal view, early and late anagen have been summed.

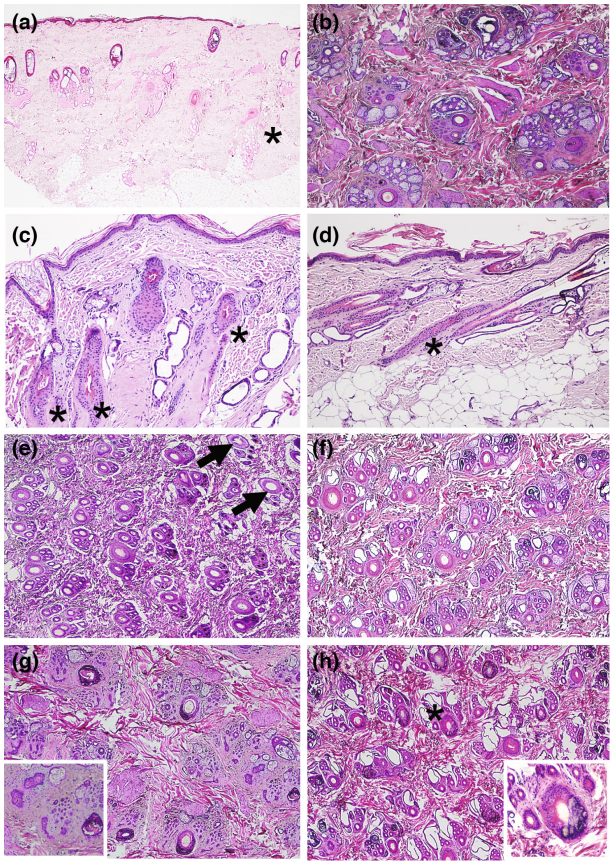

FIGURE 3 (a,b) Longitudinal and transversal sections from a dog with alopecia X. Nine compound follicles were counted in the longitudinal section (a) and 12 compound follicles in the transversal section (b). No flame follicles are visible in the transversal section. Haematoxylin & eosin, ×4 (a), ×10 (b). (c,d) Longitudinal sections from a dog with alopecia X. Flame follicles extend from the isthmus region downwards. H&E, ×10. (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, ×10. (g) Dog with alopecia X: most hair follicles are in kenogen with some characterised by dysmorphic morphology; mineralised hair follicles also are present (inset). (h) Dog with alopecia X: most hair follicles are in telogen; 'flame' and mineralised hair follicles (inset) can be seen. H&E, ×20. All flame follicles in (a,c,d,h) have been marked with an asterisk.

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, and to the best of the authors' knowledge, it has not been reported previously in canine alopecia X; follicle mineralisation was detected in juvenile and adult dogs making an aetiopathogenetic 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 described previously.

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 in HF kenogen arrest and decrease in anagen, thus confirming findings reported previously for alopecia X.[19]

Notwithstanding this, the present study failed to identify any effect of specific demographic features on the phase of the hair growth in the 19 affected dogs. These findings contrast with those in previous studies which, 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 yet 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, because 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 dermoscopic evaluation 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 noninflammatory hair disorders characterised 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 mineralisation and in general to further determine the severity of alopecia.

AUTHOR CONTRIBUTIONS

Giordana Zanna: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; visualization; writing – review and editing; supervision; project administration. **Francesca Abramo:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; supervision; project administration. **Barbara Contiero:** Methodology; software; formal analysis; data curation; validation; visualization; writing – review and editing. **Eric Zini:** Methodology;

validation; software; formal analysis; data curation; conceptualization; visualization; writing – review and editing. **Francesco Albanese:** Methodology; investigation; visualization. **Elena Borio:** Investigation; visualization; methodology. **Francesco Godizzi:** Methodology; visualization; investigation. **Fabiano Necci:** Validation; methodology; investigation. **Luca Luciani:** Methodology; validation; investigation. **Paola Roccabianca:** Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; visualization; writing – review and editing; supervision.

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CONFLICT OF INTEREST STATEMENT
All authors have no conflict of interest to declare.

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

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摘要

背景: 联合皮肤镜和组织学检查结果,可能为毛囊疾病的诊断提供有用的信息。然而,目前还没有关于X脱毛患犬的皮肤镜-组织病理学关联的研究,在评估这种脱毛症时,皮肤活检标本的纵向与横向切片的比较也没有得到彻底的研究。

假设/目的: 使用皮肤活检样本的纵向和横向切片,将皮肤镜和组织学特征关联起来,以获得诊断X脱毛的额外信息。

动物: 19只X脱毛博美患犬,以及5只健康博美犬作为对照。

材料和方法: 在患病组内关联皮肤镜-组织学结果,而与对照组进行组织学比较。皮肤形态和临床特征也与组织学发现进行关联。

结果: 皮肤镜检查结果显示,被毛分散、稀疏、变短,混合有无定形角化脂溢样物质(毛囊堵塞)、毛囊周围和毛囊内脱屑,色素沉着从点状黑点到弥漫性纹理不等。在选定的定性和定量检查中,皮肤镜检查结果与组织学检查结果相关。与纵向切片相比,横向切片在准确确定毛囊密度和数量、生长停滞期以及识别毛囊基底膜矿化方面的实用性得到了证明。相反,组织学发现与皮肤形态和临床特征之间没有相关性。

结论和临床相关性: 这些数据证明了皮肤镜评估作为辅助诊断工具的实用性,以及皮肤活检的横截面可以作为X脱毛诊断的补充。

Résumé

Contexte: La combinaison des résultats dermatoscopiques et histologiques peut fournir des informations utiles pour le diagnostic des maladies du follicule pileux. Cependant, il n'existe pas d'études sur la corrélation entre la dermatoscopie et l'histopathologie chez les chiens atteints d'alopécie X, et la comparaison entre la coupe longitudinale et la coupe transversale des échantillons de biopsie cutanée dans l'évaluation de ce trouble alopécique n'a pas fait l'objet d'une étude approfondie.

Hypothèse/Objectifs: Corréler les caractéristiques dermatoscopiques et histologiques en utilisant des coupes longitudinales et transversales d'échantillons de biopsie cutanée afin d'obtenir des informations supplémentaires pour le diagnostic de l'alopécie X.

Animaux: Dix-neuf poméraniens atteints d'alopécie X et cinq poméraniens sains comme témoins.

Matériels et méthodes: Une corrélation dermatoscopique-histologique est réalisée au sein du groupe malade, tandis que des comparaisons histologiques sont effectuées par rapport aux témoins. Les caractéristiques démographiques et cliniques ont également été mises en relation avec les résultats histologiques.

Résultats: Les résultats dermatoscopiques révélent des poils courts, clairsemés et amincis, associés à une matière amorphe de type kératoséborrhéique (bouchon folliculaire), une desquamation périfolliculaire et intrafolliculaire, et une hyperpigmentation allant de macules noires ponctuelles à une pigmentation diffuse. Les résultats dermatoscopiques sont corrélés aux résultats histologiques pour certains résultats qualitatifs et quantitatifs.

L'utilité des coupes transversales est démontrée pour déterminer avec précision la densité et le nombre de follicules pileux, les phases d'arrêt de croissance et pour identifier la minéralisation de la membrane basale des follicules pileux par rapport aux coupes longitudinales. En revanche, aucune corrélation entre les résultats histologiques et les caractéristiques démographiques et cliniques n'est détectée.

Conclusions et pertinence clinique: Ces données démontrent l'utilité de l'évaluation dermatoscopique en tant qu'outil de diagnostic complémentaire et de l'analyse histopathologique des biopsies cutanées des coupes transverses en tant que complément au diagnostic de l'alopécie X.

Zusammenhang

Hintergrund: Eine Kombination der dermoskopischen und histologischen Befunde könnten eine nützliche Information zur Diagnose von Haarfollikelerkrankungen liefern. Es gibt jedoch keine Studien über dermoskopisch-histopathologische Korrelationen bei Hunden, die an einer Alopezia X erkrankt sind. Ebenso wenig wurden bisher Vergleiche von longitudinalen versus transversalen Hautschnitten der Hautbiopsie Proben bei der Erfassung dieser Erkrankung mit Haarausfall untersucht.

Hypothese/Ziele: Das Ziel dieser Studie war die Feststellung einer Korrelation der dermoskopischen und histologischen Merkmale mittels sowohl Longitudinal- wie auch Transversalschnitten der Hautbiopsien, um zusätzliche Informationen für eine Diagnose der Alopezia X zu erhalten.

Tiere: Neunzehn Pomeranier mit einer Alopezia X und fünf gesunde Pomeranier als Kontrollen.

Materialien und Methoden: Eine dermoskopisch-histologische Korrelation wurde in der erkrankten Gruppe durchgeführt, während gegenüber den Kontrollen histologische Vergleiche gemacht wurden. Die demografischen und klinischen Charakteristika standen mit den histologischen Ergebnissen im Zusammenhang.

Ergebnisse: Die dermoskopischen Befunde zeigten verstreute, ausgedünnte, kurze Haare vermischt mit amorphem keratoseborrhoeisch-ähnlichem Material (follikuläre Pfropfen), perifollikuläre und intrafollikuläre Schuppenbildung, und eine Hyperpigmentierung, die zwischen völlig schwarzen Punkten bis zu diffuserer Textur variierten. Die dermoskopischen Befunde korrelierten mit den histologischen Befunden, was die ausgewählten qualitativen und quantitativen Ergebnisse betraf. Die Nützlichkeit von Transversalschnitten konnte im Vergleich zu Longitudinalschnitten durch eine genauere Bestimmung der Haarfollikeldichte und -Anzahl, der Ruhephasen des Wachstums und der Feststellung einer Mineralisation der Basalmembran der Haarfollikel gezeigt werden. Im Gegensatz dazu konnte zwischen den histologischen Befunden und den demografischen und klinischen Charakteristika keine Korrelation gefunden werden.

Schlussfolgerungen und klinische Bedeutung: Diese Daten liefern Evidenz für die Nützlichkeit einer dermoskopischen Evaluierung als diagnostisches Hilfswerkzeug sowie für die Nützlichkeit der Transversalschnitte der Hautbiopsien, um die Diagnose einer Alopezia X zu erleichtern.

要約

背景: ダーモスコピーおよび組織学的所見の組み合わせは、毛包疾患の診断に有用な情報を提供する可能性がある。しかしながら、脱毛症Xに罹患した犬におけるダーモスコピーと病理組織学的相関に関する研究はなく、この脱毛症の評価における皮膚生検標本の縦切片と横切片の比較は十分に検討されていない。
仮説/目的: 本研究の目的は、脱毛症Xの診断のための追加情報を得るために、皮膚生検標本の縦切片と横切片の両方を用いて、ダーモスコピーと組織学的特徴を相関させることであった。
対象動物: 脱毛症Xに罹患したポメラニアン19頭とおよび対照として健常ポメラニアン5頭。
材料と方法: 皮膚組織学的相関は罹患群内で、組織学的比較は対照群に対して行った。人口統計学的および臨床的特徴も組織学的所見と関連させた。
結果: ダーモスコピー所見では、無定形の角化脂漏様物質(毛包栓塞)と混在する散在した細く短い毛、毛包周囲および毛包内の鱗屑、ピンポイントの黒色斑からびまん性の質感まで様々な色素沈着が認められた。ダーモスコピーの所見は、選択された質的・量的所見について組織学的所見と相関していた。横断面切片の有用性は、縦断面切片と比較して、毛包密度と数、成長停止段階、毛包基底膜の石灰化を正確に判定できることが示された。逆に、組織学的所見と人口統計学的および臨床的特徴との間には相関は認められなかった。
結論と臨床的意義: これらのデータは、ダーモスコピーの補助的診断ツールとしての有用性、および皮膚生検の横断切片が脱毛症Xの診断を補完するものであることを証明するものであった。

Resumo

Contexto: Uma combinação de achados dermoscópicos e histológicos podem proporcionar informações úteis para o diagnóstico de doenças dos folículos pilosos. No entanto, não existem estudos sobre as correlações dermatoscópicas-histopatológicas em cães afetados pela alopecia X, e a comparação do corte longitudinal versus transversal de amostras de biópsia de pele na avaliação deste distúrbio de queda de pelos não foi completamente investigada.
Hipótese/Objetivos: Correlacionar características dermatoscópicas e histológicas usando cortes longitudinais e transversais de amostras de biópsia de pele para obter informações adicionais para o diagnóstico de alopecia X.
Animais: Dezenove cães da Lulu da Pomerânia afetados pela alopecia X e cinco lulus da Pomerânia saudáveis como controles.
Materiais e Métodos: A correlação dermatoscopia-histologia foi realizada dentro do grupo doente, enquanto as comparações histológicas foram realizadas com os controles. As características demográficas e clínicas também foram relacionadas aos achados histológicos.
Resultados: Os achados dermatoscópicos revelaram pelos curtos e finos esparsos, misturados com material amorfo tipo ceratoseborreico (tampão folicular), descamação perifolicular e intrafolicular e hiperpigmentação variando de manchas pretas pontuais a uma textura difusa. Os achados dermatoscópicos correlacionaram-se com os achados histológicos em determinados achados qualitativos e quantitativos. A utilidade dos cortes transversais foi demonstrada pela acurácia na determinação da densidade e contagem de folículos pilosos, fases de sequestro folicular e na identificação da mineralização da membrana basal do folículo piloso quando comparado ao longitudinal. Por outro lado, não foram detectadas correlações entre os achados histológicos e as características demográficas e clínicas.
Conclusões e Relevância Clínica: Estes dados evidenciam a utilidade da avaliação dermatoscópica como ferramenta diagnóstica acessória e dos cortes transversais de biópsias cutâneas como complementares no diagnóstico da alopecia X.

Abstracto

Introducción: una combinación de hallazgos dermatoscópicos e histológicos puede proporcionar información útil para el diagnóstico de enfermedades de los folículos pilosos. Sin embargo, no existen estudios sobre las correlaciones dermatoscópicas-histopatológicas en perros afectados por alopecia X, y no se ha investigado a fondo la comparación del corte longitudinal versus transversal de muestras de biopsia de piel en la evaluación de este trastorno de pérdida de pelo.
Hipótesis/Objetivos: Correlacionar características dermatoscópicas e histológicas mediante cortes longitudinales y transversales de muestras de biopsia de piel para obtener información adicional para el diagnóstico de alopecia X.
Animales: Diecinueve perros Pomerania afectados por alopecia X y cinco Pomerania sanos como controles.
Materiales y métodos: se realizó correlación dermatoscópica-histológica dentro del grupo enfermo, mientras que se realizaron comparaciones histológicas con controles. Las características demográficas y clínicas también fueron relacionadas con los hallazgos histológicos.
Resultados: Los hallazgos dermatoscópicos revelaron pelos cortos, adelgazados y dispersos mezclados con material amorfo de tipo queratosborreico (taponamiento folicular), descamación perifolicular e intrafolicular e hiperpigmentación que variaba desde puntos negros puntuales hasta una textura difusa. Los hallazgos dermatoscópicos se correlacionaron con los hallazgos histológicos para hallazgos cualitativos y cuantitativos seleccionados. La utilidad

de las secciones transversales se demostró para determinar con precisión la densidad y el recuento de folículos pilosos, las fases de detención del crecimiento y para identificar la mineralización de la membrana basal del folículo piloso en comparación con las longitudinales. Por el contrario, no se detectaron correlaciones entre los hallazgos histológicos y las características demográficas y clínicas.

Conclusiones y relevancia clínica: Estos datos aportan evidencia de la utilidad de la evaluación dermatoscópica como herramienta diagnóstica accesoria y de los cortes transversales de biopsias de piel como complementaria al diagnóstico de alopecia X.