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Transverse sectioning in the evaluation of skin biopsy specimens from alopecic dogs

R. Bond[1,*], A. Hendricks*, J. Patterson-Kane†, K. Stevens* and H. W. Brooks Brownlie*

*Department of Clinical Sciences and Services, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts, AL9 7TA, UK
†Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow, UK

[1]Corresponding author email: rbond@rvc.ac.uk

Objectives: **Transverse sectioning of skin biopsy specimens has revolutionised assessment of human alopecia by demonstration of every hair in each specimen, allowing quantitative evaluation of follicular activity. Since only vertical sectioning is performed routinely in veterinary laboratories, we aimed to determine whether transverse sectioning was a valuable technique in assessment of canine alopecia.**

Methods: **Paired vertical and transverse sections of biopsy specimens from 31 alopecic dogs were examined independently in triplicate in random order and blinded to previous diagnosis using a standard check-list proforma. Assessments of key features (follicular activity [anagen/telogen], infundibular hyperkeratosis, sebaceous gland abnormalities, pigment clumping, dermal inflammation) by each sectioning method were compared.**

Results: **In the 31 cases, (atrophic [n = 13], dysplastic [n = 12], inflammatory diseases [n = 6]), follicular inactivity scores (median, [lower-upper quartile]) in transverse sections significantly exceeded those in vertical sections (transverse 4 [3-5], vertical 3 [2-4]). Agreement between the two sectioning planes was moderate for infundibular hyperkeratosis (kappa = 0.5210) and dermal inflammation (0.4351), fair for sebaceous gland abnormalities (0.3966) and pigment clumping (0.2197), but slight for follicular activity (0.1041). Vertical sectioning demonstrated diagnostically important epidermal pathology (n = 2) and dermal thinning (n = 3) whereas transverse sectioning enhanced assessment of hair growth phase (n = 11), follicular structure and architecture (n = 11), and focal luminal or mural folliculitides (n = 3).**

Clinical significance: **Transverse sectioning confers significant benefits and complements traditional vertical sectioning in the histological assessment of canine hair follicle diseases, particularly when subtle abnormalities comprise distorted compound follicle architecture, hair cycle arrest or when relatively few adnexal structures are affected.**

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Janet Patterson-Kane's current address is Morris Animal Foundation, 720 South Colorado Blvd., Suite 174A, Denver, Colorado 80246, USA.
H. W. Brooks Brownlie's current address is Department of Pathology, Bristol Veterinary School, Langford House, Langford, Bristol, BS40 5DU, UK.
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INTRODUCTION

The histopathological examination of skin biopsy specimens is an important tool in the diagnosis of alopecia in dogs when associated with atrophic, dysplastic and mural hair follicle diseases (Gross *et al*. 2005). In veterinary diagnostic laboratories, it is normal practice for skin biopsy specimens to be sectioned vertically, thus demonstrating changes in the full thickness of the skin from epidermis to subcutaneous fat (Dunstan 1990). This is not without limitations; only a few hair follicles are present in each section and slight deviations from the optimal plane of sectioning result in hair follicle segments at different levels that can be difficult to orient. By contrast, in human medicine, the use of transverse sectioning of skin biopsies, sometimes known as the "Headington technique" (Headington 1984, Flotte 2008) has revolutionised the assessment of alopecia, since transverse sections demonstrate every hair or fibrous tract remnant in the specimen (Frishberg *et al*. 1996, Elston *et al*. 2005). Examination of sections taken at different levels of the block allows anagen – telogen ratios and other abnormalities to be quantified, and the same hair follicle studied at different levels. Such follicular counts are valuable in the diagnosis and treatment of non-cicatricial alopecia (Whiting 2008). Furthermore, a recent consensus statement from a group of experts in the field of cicatricial alopecia of humans acknowledged that the combined transverse and vertical sectioning of biopsy specimens was optimal but suggested that in cases where only a single biopsy was available, sections should be transverse (Olsen *et al*. 2003).

Transverse sectioning is rarely utilised in routine veterinary dermatopathological practice, although this method was recommended for the definition of subtle alopecic disorders or dermatoses limited to focal areas of the adnexae by Robert W. Dunstan in 1990 (Dunstan 1990). One textbook of hair loss disorders in domestic animals dismisses the technique as being of "no benefit" because there is "no normal standard" and "no need to increase the hair follicle yield"(Mecklenburg 2009). Recent and contemporary scholarly accounts of canine hair follicle structure and function in health and disease describe features from only traditional vertical sectioning (Muntener *et al*. 2011, 2012, Welle & Wiener 2016). However, these authors commented on the need for serial vertical sectioning to overcome frequent difficulty in establishing the phase of hair growth (Muntener *et al*. 2011), which is likely impractical in commercial diagnostic laboratories.

Previous reports of the use of transverse sections in veterinary dermatology include studies of the hair cycle, particularly in species where hair growth is of commercial relevance, such as sheep and goats (Nixon 1993, Parry *et al*. 1995). The first report of transverse morphometry in canine hair follicle disease was in relation to experimentally induced hypothyroidism (Credille *et al*. 2001). In a subsequent study of the morphology of hair follicles in young growing dogs by the same group, the authors concluded that transverse sectioning was "critical" for accurate assessment of canine hair follicle morphology (Credille *et al*. 2002). Transverse sectioning has since been used to assess hair regrowth in clipped Siberian huskies (Diaz *et al*. 2006); to study sebaceous gland morphometry in healthy canine skin and in cases of sebaceous adenitis (Bond & Brooks 2013); to define histopathological features of follicular dysplasia in curly-coated retrievers (CCR) (Bond *et al*. 2016); and to evaluate the depth of topically applied drug permeation in an *in vitro* model of canine skin (Frosini *et al*. 2017). Studies in other veterinary species include assessment of anagen:telogen ratios in horses with pituitary *pars intermedia* dysfunction (Innera *et al*. 2013), and a histological description of the skin of Sphynx cats (Genovese *et al*. 2014). However, reports from routine diagnostic practice are lacking. Current veterinary dermato-pathological practice would appear to be at significant variance from, and potentially inferior to, the "standard of care" for the histopathological investigation of alopecia in humans.

We investigated whether this relatively simple modification to histological processing would enhance the routine diagnostic histopathological evaluation of the pilosebaceous units of dogs with alopecic disorders thereby maximising the diagnostic benefit from those skin biopsies.

MATERIALS AND METHODS

The study was approved by the Chair of the Royal Veterinary College's Ethics and Welfare committee. The samples were submitted by clinicians in referral dermatology or first opinion prac-

Table 1. Outline of the data capture form used in the blinded histological assessment of paired vertical and transverse sections in 31 dogs with alopecia

Skin compartment	Criterion	Section orientation	Assessment
Epidermis	Hyperkeratosis/ parakeratosis	V	Absent, present
	Living epidermis	V	Normal, abnormal (with description)
	Degree of pigmentation	V	None/slight, mild, marked
	Pigment clumping	V	None/slight, mild, marked
Dermis	Inflammation	V&T	Absent, present (pattern, cell types)
Sebaceous glands	Presence	V&T	Absent, present
	Pathology	V&T	Normal, abnormal (with description)
Hair follicle infundibulum	Hyperkeratosis/ parakeratosis	V&T	Absent, present
	Living epidermis	V&T	Normal, abnormal (with description)
	Degree of pigmentation	V&T	None/slight, mild, marked
	Pigment clumping	V&T	None/slight, mild, marked
Hair follicle isthmus/inferior	Follicular inactivity scale	V&T	1, 2, 3, 4, 5* (with description)
	Degree of pigmentation	V&T	None/slight, mild, marked
	Pigment clumping	V&T	None/slight, mild, marked
Overall	Morphological diagnosis	V&T	Comment

V, vertical section; T, transverse section
*Follicular inactivity scale: 1, anagen only; 2, anagen:telogen 75%:25%;3, anagen:telogen 50%:50%; 4, anagen:telogen 25%:75%; 5, telogen only

tice in the UK and France as part of their routine approach to the diagnosis of alopecia in dogs. Written informed consent was obtained from the dog owners before skin biopsy sampling in each case. Skin biopsy specimens were taken from appropriate skin lesions in 31 alopecic dogs following local anaesthesia using 6 or 8 mm biopsy punches, fixed in formalin and submitted for histopathological examination. These dogs were clinically suspected of having a range of atrophic, dysplastic and inflammatory diseases of the hair follicles and or adnexal glands.

After fixation, samples were bisected vertically and one half section was embedded in the traditional vertical orientation to generate histological sections which included all layers of the epidermis, dermis and subcutis. The other half of the sample was placed cut-surface down on a cutting board and sectioned transversely (two or three cuts depending on the thickness of the skin); the resultant semi-circular sections were then arranged in one paraffin wax block. This meant that the resulting glass slide from these sections represented multiple transverse views of the skin at different levels. All sections (4 um) were processed routinely and stained with haematoxylin and eosin for histological examination, and the results reported to the submitting veterinary surgeon. The sections generated were further reviewed independently in triplicate in random order and blinded to previous diagnosis using a standard check-list proforma (Table 1). Slides were examined independently in triplicate by (1) two authors who contemporaneously agreed the features in each case (RB and HBB), (2) by another specialist veterinary dermatologist (AH) and by (3) a collaborating veterinary dermatopathologist (JPK). Initially, the vertical sections were examined. Subsequently transverse sections were examined in random order and without reference to submission paperwork or the previous report. The process included a graded assessment of the anagen:telogen ratio in each slide by use of a "follicular inactivity" scale of 1 (anagen predominant) to 5 (telogen predominant) (Table 1); the stages of the hair cycle were defined according to the methods of Headington (Headington 1984) and of Whiting (Whiting 2008), as previously described (Bond *et al*. 2016).

Statistical analyses

The kappa statistic (IBM SPSS Statistics software, IBM, New York) was used to assess the agreement between the histological findings for the criteria assessed in the paired vertical and transverse sections (Table 1), where 1 is perfect agreement, 0 is exactly what would be expected by chance, and negative values indicate agreement by less than chance (Viera & Garrett 2005). The Wilcoxon signed rank test (Unistat v3.0 statistical software package, Unistat Ltd.) was used to compare follicular inactivity scores in the paired transverse and vertical sections, with $P < 0.05$ for significance.

RESULTS

Among the 31 cases studied, 18 breeds were represented, comprising 18 males (10 neutered), and 11 females (8 neutered), aged between 12 months and 14 years (median 6 years). There were 13 examples of atrophic diseases of the adnexa (Table 2), 12 examples of dysplastic diseases of the adnexa (Table 3) and 6 cases of inflammatory skin disease (Table 4). As expected, there was substantial overlap in the principal pathological features observed in the 90 paired vertical and transverse sections, but there were also numerous examples where one or other of the two sectioning planes yielded superior visualisation of key features.

Table 2. Comparison of the principal histopathological features in paired vertical and transverse sections in 13 dogs with atrophic diseases of the adnexa, from a series of 31 dogs with alopecia

Final diagnosis	Number of cases	Features in vertical sections	Number	Features in transverse sections	Number	Conclusion
Hyperadrenocorticoidism*	7	Telogen predominant	4	**Telogen predominant**	7	Thin dermis (3/7) noted only in V. Telogen best noted in Tx (3 cases, including preferentially in secondary follicles [1 case]).
		Small follicles	4	Small follicles	3	
		Infundib hyperK (3 mild)	7	Infundib hyperK	7	
		Thin dermis	3	–	–	
Acquired pattern alopecia (pattern baldness)	2	(**Ep hyperpigmentation**)	1	–	–	Miniaturised secondary follicles in anagen observed in Tx only in one case.
		(Infundib hyperK)	2	Infundib hyperK	1	
		Small secondary follicles	1	Small secondary follicles	2	
		–	1	**Miniature anag follicles**	1	
		Perifollicular fibrosis		Perifollicular fibrosis	1	
				Pig clump inferior	2	
Postclipping alopecia	2	Telogen predominant	2	Telogen predominant	2	Typical features of Postclipping alopecia best demonstrated in Tx.
		–	–	**1ary follicles haired telogen**	2	
		(Infundib hyperK)	1	(Infundib hyperK)	1	
		–		**Excess tricholemmal corn**	2	
Alopecia X	1	**(Ep hyperpigmentation)**	1	–	–	Comparable features in V and Tx, except for the mild epidermal changes in V only.
		Telogen predominant	1	Telogen predominant	1	
		Infundib hyperK	1	Infundib hyperK	1	
		Excess tricholemmal corn	1	Excess tricholemmal corn	1	
Hypothyroidism	1	Telogen predominant	1	Telogen predominant	1	Comparable features in V and Tx.
		Infundib hyperK	1	Infundib hyperK	1	
		Excess tricholemmal corn	1	Excess tricholemmal corn	1	

Features in (parenthesis) indicate a mild feature. Bold text indicates features that were prominent in one sectioning plane but not the other
A:T, anagen:telogen; anag, anagen; CF, compound follicle; corn, cornification; Ep, epidermal; hyperK, hyperkeratosis; incont, incontinence; infundib, infundibulum; predom, predominant; pig, pigment; Tx, transverse section; V, vertical section; 1ary, primary
*Comprising three naturally-occurring cases and four associated with corticoid administration in hypoadrenocorticism. One case had concurrent hypothyroidism

Generally, the degree of telogenisation was under-estimated by vertical sectioning (Fig 1B), notably in 7 of 12 cases of dysplastic diseases of the adnexa (Tables 2 and 3). Among the six examples of inflammatory diseases, there were three cases where key pathological processes that affected a small proportion of adnexal structures were seen only in the transverse sections and not in the paired vertical sections (Table 4).

Atrophic diseases of the adnexa

Vertical sectioning allowed observation of dermal thinning in three out of seven dogs with hyperadrenocorticoidism (naturally occurring [n = 2], corticoid supplementation in hypoadrenocorticoidism [n = 1]) that could not be appreciated in transverse sectioning (Table 2). By contrast in three cases of hyperadrenocorticoidism, transverse sectioning highlighted a clear predominance of follicles in the telogen phase where vertical sections showed a mixture of anagen and telogen follicles, presumably in sections taken from partially alopecic skin. A histological diagnosis of postclipping alopecia was strongly supported by the presence of a haired telogen phase in primary follicles accompanied by excessive tricholemmal cornification (Gross *et al*. 2005), noted only in the transverse sections in biopsies from two plush-coated dogs (Fig. 2). In one of two cases of acquired pattern alopecia (Yager & Wilcock 1994), the key diagnostic feature of miniaturised anagen follicles was observed only in the transverse sections.

Dysplastic diseases of the adnexa

The histopathological features observed in the paired vertical and transverse sections in five examples of seasonal flank alopecia were broadly comparable with no advantage from either method (Table 3). Among seven examples of breed-associated follicular dysplasia (5 CCR, 1 Doberman, 1 dachshund), vertical sectioning showed epidermal hyperkeratosis in specimens from three dogs (1 CCR, 1 Doberman and 1 dachshund) (Table 3). However, transverse sectioning was advantageous in demonstrating a greater predominance of telogen follicles than that observed in vertical sections in all seven cases of follicular dysplasia; in two cases this appeared to preferentially affect secondary follicles. In addition, transverse sectioning illustrated a distortion of the normal architecture of individual follicles within the compound follicles in two CCR (Fig 3B).

Inflammatory diseases

A range of inflammatory diseases was represented in the examined specimens, comprising two cases with superficial pyoderma, and single examples of ischaemic dermatopathy/dermatomyositis in a Shetland sheepdog, exfoliative cutaneous lupus erythematosus (ECLE) in a German short-haired pointer, sebaceous adenitis and mural folliculitis (Table 4). Transverse sectioning was inappropriate for the evaluation of ECLE since the pivotal features of epidermal hyperkeratosis, basal cell vacuolation and lymphocyte exocytosis were evident only in vertical sections. Sebaceous adenitis was diagnosed histologically in both vertical and transverse sections, although infundibular hyperkeratosis and unaffected sebaceous glands were recorded only in transverse sections. In the ischaemic dermatopathy/dermatomyositis case, the central ischaemic features of "faded follicles," rarefaction of collagen fibres, and panniculus myofibre degeneration/regeneration and necrosis (Gross *et al*. 2005) were recognised in

Table 3. Comparison of the principal histopathological features in paired vertical and transverse sections in 12 dogs with dysplastic diseases of the adnexa, from a series of 31 dogs with alopecia

Final diagnosis	Number of cases	Features in vertical sections	Number	Features in transverse sections	Number	Conclusion
Seasonal flank alopecia	5	**Ep pigmentation**	5	–	–	Both sectioning methods comparable in assessment of key features
		Infundib hyperK	4	Infundib hyperK	5	
		Telogen predom	4	Telogen predom	5	
		Pig clump infundib	2	Pig clump infundib	2	
		Pig clump inferior	4	Pig clump inferior	3	
		–		**Excess tricholemmal corn**	2	
Follicular dysplasia curly coated retriever	5	**Ep pigmentation**	5	–	–	Enhanced assessment of telogen in transverse section confirmed in wider study (Bond *et al*. 2016)
		Epidermal hyperkeratosis	1	–	–	
		Infundib hyperK (mild)	5	(Infundib hyperK)	5	
		Pig clump infundib	1	Pig clump infundib	1	
		Pig clump inferior	5	Pig clump inferior	5	
				Telogen predominance	5	
				Distorted CF architecture	2	
Follicular dysplasia Doberman	1	**(Ep hyperkeratosis)**	1	–	–	Telogen dominance and pigment incont move evident in Transverse.
		(Infundib hyperK)	1	Infundib hyperK	1	
		Pig clump inferior	1	Pig clump inferior	1	
				Pig incont inferior	1	
				Telogen predominant	1	
Follicular dysplasia dachshund	1	**(Ep hyperkeratosis)**	1	–	–	Telogen dominance and pigment abnormalities move evident in Transverse.
		(Infundib hyperK)	1	Infundib hyperK	1	
		A:T equal proportion	1	**(Pig clump inferior)**	1	
				(Pig incont inferior)	1	
				Telogen predominant	1	

Features in (parenthesis) indicate a mild feature. Bold text indicates features that were prominent in one sectioning plane but not the other
A:T, anagen:telogen; CF, compound follicle; corn, cornification; Ep, epidermal; hyperK, hyperkeratosis; incont, incontinence; infundib, infundibulum; predom, predominant; pig, pigment

Table 4. Comparison of the principal histopathological features in paired vertical and transverse sections in 6 dogs with inflammatory diseases, from a series of 31 dogs with alopecia

Final diagnosis	Number of cases	Features in vertical sections	Number	Features in transverse sections	Number	Conclusion
Ischaemic dermatopathy/ dermatomyositis*	1	**Ep hyperker, paraker**	1	–	–	Epidermal changes only in V.
		Hydropic degen basal cells	1	–	–	Faded follicles and telogen predominance
		Pigmentary incontinence	1	–	–	
		Interstitial dermatitis	1	Interstitial dermatitis	1	more evident in Tx.
		Faded	1	Faded follicles	1	
		Rarefaction of collagen	1	Rarefaction of collagen	1	
		Myofibre degen/regen/nec	1	Myofibre degen/regen/nec	1	
		–	–	**Telogen predominance**	1	
Exfoliative cutaneous lupus erythematosus	1	**Ep hyperker**	1	–	–	Key features observed only in V.
		Basal cell vacuolation	1	–	–	
		Lymphocyte exocytosis	1	–	–	
		Perivascular dermatitis	1	Perivascular dermatitis	1	
		–	1-	[Telogen predominant]	1	
		[Telogen predominant]		**[SG atrophy]**	1	
		–				
Mural folliculitis	1	Telogen predominant	1	Telogen predominant	1	Key features observed only in Tx.
		Infundibular hyperK	1	Infundibular hyperK	1	
				Mural folliculitis	1	
				Sparse follicles, with replacement by fibrosis	1	
Pyoderma-neutrophilic folliculitis	1	Neutro derm, pv and pf	1	Neutro derm, pv and pf	1	Key features observed only in Tx.
		Rare mural inflammation	1	Rare mural inflammation	1	
				Luminal follic/furunc	1	
Pyoderma-Perifolliculitis and hidradenitis	1	**Ep pigment**	1	–	1	Epidermal changes only in vertical. Small secondary follicles and apocrine adenitis only in Tx.
		(Ep lymphocyte exocytosis)	1	–	1	
		Lympho-plasmacytic, perivasc D & perifolliculitis	1 1	Lympho-plasmacytic, pv D & perifolliculitis	1 1	
		Pig clump inferior	–	Pig clump inferior		
		-	–	**Small secondary follicles**		
		-		**Hidradenitis (apocrine adenitis)**		
Sebaceous adenitis	1	**(Ep pigment)**	1	–	–	Unaffected SG and infundib hyperK only observed in Tx.
		PV dermatitis	1	PV dermatitis	1	
		SG absent or inflamed	1	SG absent or inflamed	1	
		–	–	**Unaffected SG**	1	
				Infundib hyperK	1	

Features in (parenthesis) indicate a mild feature. [] indicates not all sections. Bold text indicates features that were prominent in one sectioning plane but not the other

CF, compound follicle; D, dermatitis; degen, degeneration; Ep, epidermal; follic/furunc, folliculitis/furunculosis; hyperK, hyperkeratosis; incont, incontinence; infundib, infundibulum; nec, necrosis; neutro, neutrophilic; paraker, parakeratosis; pf, perifollicular; predom, predominant; pig, pigment; pv, perivascular; SG, sebaceous glands; regen, regeneration; Tx, transverse section; V, vertical section

*Classified here due to diffuse, mild inflammatory response in the dermis but follicular atrophy was also prominent

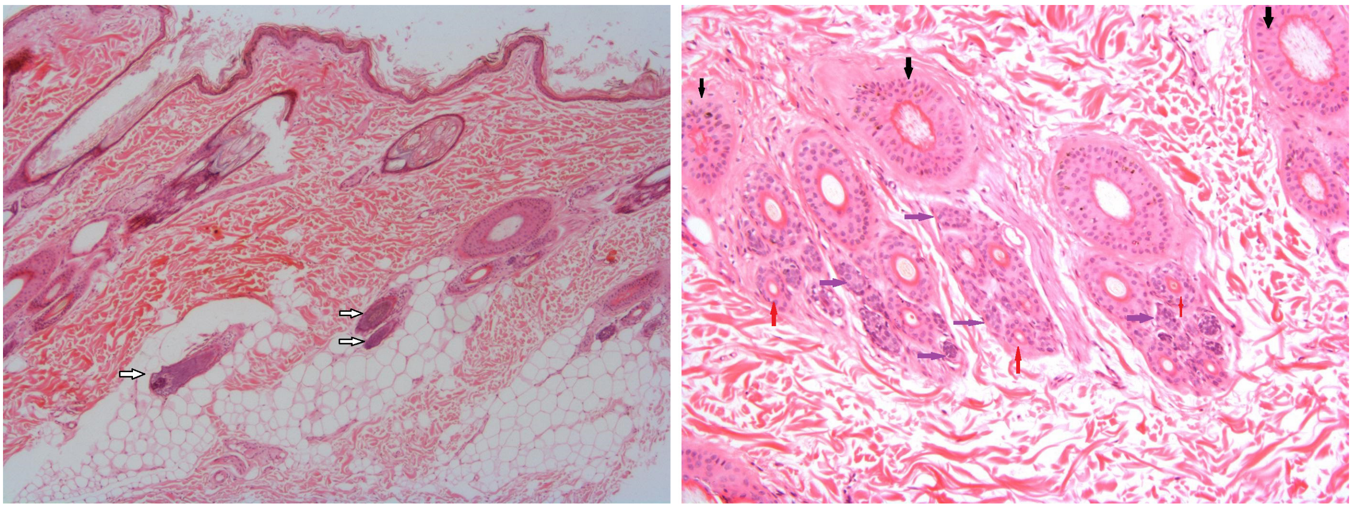

FIG 1. (A) Biopsy specimen from a dog with a final diagnosis of pattern baldness; vertical section. There is infundibular hyperkeratosis with some infundibula containing small diameter hair fibres. Numerous anagen bulbs (arrows) are present in the subcutaneous fat creating the impression of predominant anagen follicular activity. (B) Biopsy specimen from a dog with a final diagnosis of pattern baldness; paired transverse section. This section at the deeper level of the follicular isthmus shows four complete compound follicles. Although several follicles are in the anagen phase, some of these are small (red arrows), the telogen phase of hair growth is predominant as manifest by telogen primary hairs (black arrows) and abundant telogen germinal units (purple arrows)

both vertical and transverse sections. The follicular changes were more evident in transverse sections (where the contrast between affected and unaffected follicles was most apparent, along with a telogen predominance), whereas additional epidermal features of epidermal keratosis, hydropic degeneration of basal keratinocytes and intra-epidermal pigmentary incontinence were confined to vertical sections.

In two cases of superficial pyoderma, perivascular and perifollicular dermatitis was readily appreciated in both vertical and transverse sections (Table 4). Transverse sectioning, but not vertical sectioning, showed luminal folliculitis/furunculosis in one case, and hidradenitis (apocrine adenitis), regarded an as extension of follicular pyoderma (Gross *et al.* 2005), in another. In a case of mural folliculitis, lymphocytic inflammation targeting the follicular isthmus, and a loss of hair follicles with replacement by fibrosis, was observed only in transverse sections; by contrast, the interpretation from the paired vertical sections was of an atrophic dermatosis (Fig 4 B, C) .

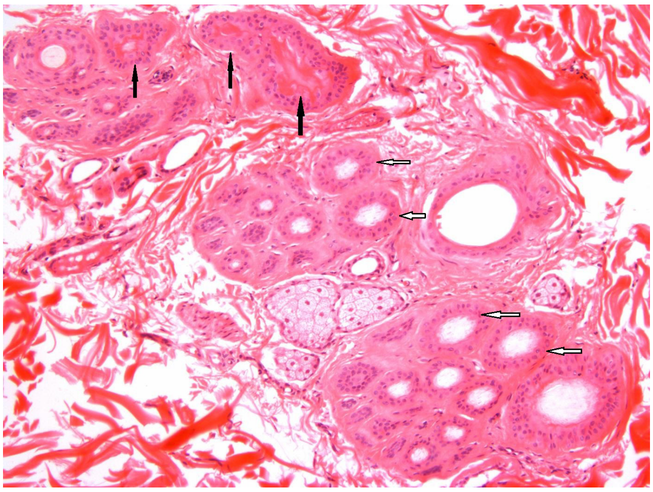

FIG 2. Biopsy specimen from a Pyrenean mountain dog with a final diagnosis of postclipping alopecia; transverse section. This section shows a complete follicular unit at the level of the isthmus. The primary and two secondary follicles of the top compound follicle show excess tricholemmal cornification (black arrows). Otherwise, haired telogen is prominent throughout the follicular unit (white arrows)

Quantitative comparisons of vertical and transverse sections

Follicular inactivity scores (median, [lower-upper quartile]) in transverse sections significantly exceeded those in vertical sections (transverse 4 [3-5], vertical 3 [2-4]; $P < 0.001$). These differences were reflected by a "slight" agreement between follicular inactivity scores from transverse and vertical sections when assessed using the kappa statistic (0.104). Agreement between the two sectioning planes was moderate for hyperkeratosis of follicular infundibulum (0.521) and dermal inflammation (0.435), fair for sebaceous gland pathology (0.397) and the degree of pigmentation (0.340) and pigment clumping (0.2197) in follicular isthmi/inferior portions and slight for changes in the living epidermis of the follicular infundibula (0.190). Pigmentary changes in the follicular infundibulae were not assessed since they were rarely present.

DISCUSSION

The results of this study confirm previous reports of significant benefits conferred by transverse sectioning in the histological assessment of hair follicles and their disorders, and indicate that use of this sectioning method will frequently complement traditional vertical sectioning when evaluating canine follicle diseases.

Traditional vertical sectioning is clearly advantageous whenever there are important pathological changes in the inter-follicular epidermis and in its adjacent dermis. The inclusion in our

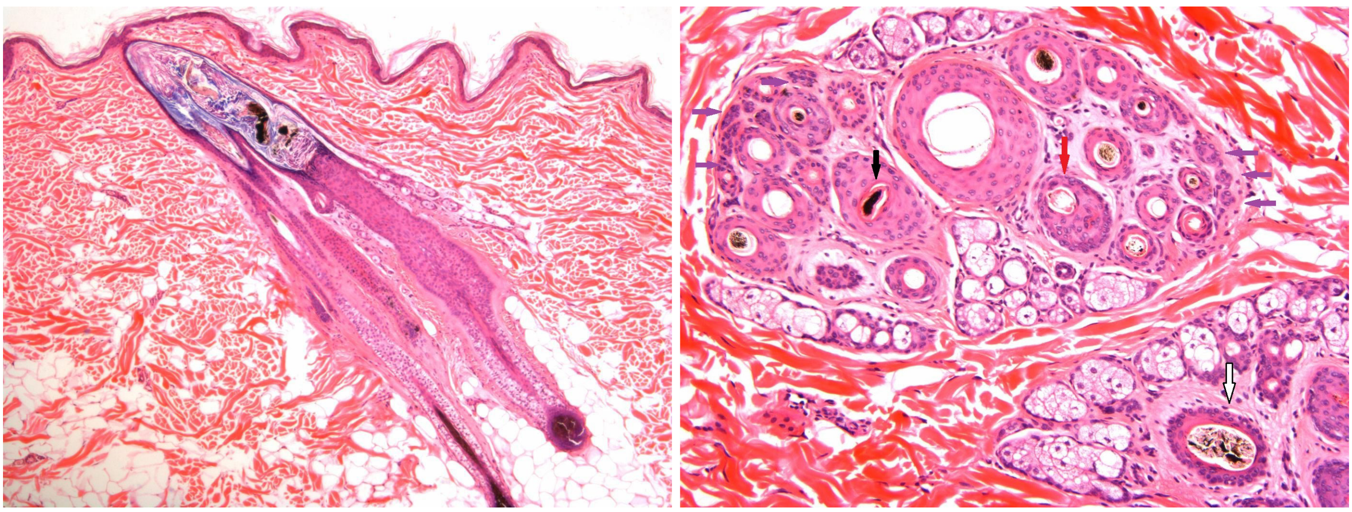

FIG 3. (A) Biopsy specimen from a curly coated retriever with a final diagnosis of follicular dysplasia; vertical section. Infundibular orthokeratotic hyperkeratosis and pigment clumping in a compound follicle with two large anagen hair follicles and four smaller secondary follicles likely in telogen. (B) Biopsy specimen from a curly coated retriever with a final diagnosis of follicular dysplasia; paired transverse section. This section shows a complete follicular unit and part of an adjacent compound follicle at the level of the isthmus. One hair shaft is distorted (black arrow), another fragmented (white arrow) and one hair fibre has an eccentric location within the follicular epithelium (red arrow). There are abundant telogen germinal units at the periphery of the follicular unit (purple arrows)

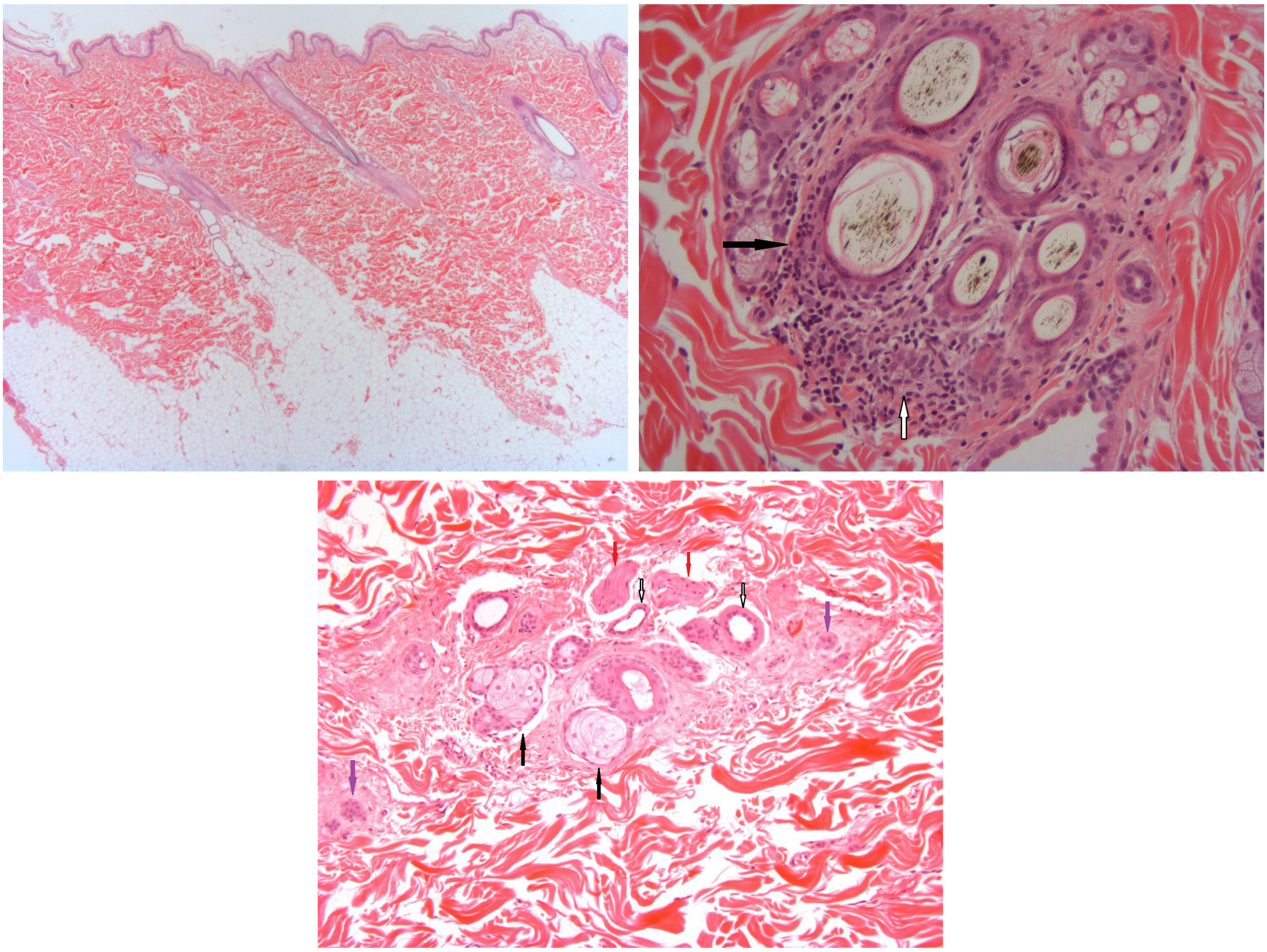

FIG 4. (A) Biopsy specimen from a Labrador retriever with a final diagnosis of mural folliculitis; vertical section. This section creates an impression of an atrophic process, with infundibular orthokeratotic hyperkeratosis, and few hair follicles with no evidence of anagen activity. Inflammation is not evident in this specimen. (b) Biopsy specimen from a Labrador retriever with a final diagnosis of mural folliculitis; paired transverse section. At the level of the isthmus, lympho-plasmacytic cells infiltrate the epithelium but not the lumen of a primary follicle (black arrow) and efface an adjacent telogen germinal unit (white arrow). (C) Biopsy specimen from a Labrador retriever with a final diagnosis of mural folliculitis; transverse section. The remnants of a follicular unit at the level of the isthmus comprise sebaceous glands (black arrows), sweat glands (white arrows), arrector pilae muscle (red arrows) and small islands of follicular epithelium embedded in a loose fibrous stroma (purple arrows)

series of an example of ECLE with basal cell vacuolation and lymphocyte exocytosis of interfollicular epidermis highlights the importance of routine vertical sectioning. Similarly, the presence of a thin dermis noted only in vertical sections in three dogs with atrophic skin diseases associated with disorders of the adrenal cortex further emphasises the value of this method. Such dermal thinning, in association with marked follicular and sebaceous gland atrophy and thinning of surface and infundibular epithelium, is considered highly suggestive of (but not definitive for) hyperadrenocorticoidism/hyperglucocorticoidism (Yager & Wilcock 1994), enabling the dermatopathologist to direct the attending clinician to evaluate the patient's hypothalamic-pituitary-adrenal axis or recent drug history. By contrast, the comparable features noted in vertical and transverse sections in all five cases of seasonal flank alopecia suggest that either method is appropriate for this disorder, although review of a larger number of cases is required to confirm this.

It is recognised that, in some atrophic or dysplastic hair follicle diseases, changes in aspects such as hair follicle size and density can be subtle (Dunstan 1990, Gross *et al.* 2005). Accordingly one standard textbook recommends that multiple biopsy samples should be obtained from areas of complete alopecia, along with a control specimen from as grossly normal an area as possible (Gross *et al.* 2005). The interpretation of the stage of the hair growth cycle (or degree of "telogenisation") is also challenging, particularly when single vertical sections are used (Muntener *et al.* 2011). We underestimated the degree of telogenisation within the vertical sections in this slide collection, as indicated by the significantly higher follicular inactivity scores in transverse sections and the low agreement between sectioning methods as assessed by the kappa statistic. This might reflect the relative difficulty in appreciating the presence of significant numbers of small telogen germinal units in vertical sections, a feature readily observed in transverse sections (Whiting 2008). The presence of abundant anagen hairs in vertical sections can raise questions about the patho-mechanism of alopecia when the dermatopathologist attempts to correlate clinical reports with their own observations (Bond *et al.* 2016). Transverse sectioning allows the pathologist to determine the severity of the alopecia (Dunstan 1990); *e.g.*, in a larger study of follicular dysplasia in CCR, transverse sectioning provided compelling statistical confirmation of a reduced anagen:telogen ratio in affected skin, that was not apparent in vertical sections (Bond *et al.* 2016).

Our observation of several cases with disparity between the phase of hair growth in primary and secondary hair follicles within the same compound follicle or follicular unit is in accordance with those of Credille *et al.* (2001). These authors reported that untreated beagle dogs rendered hypothyroid by radioactive iodine had a greater number of secondary hairs in telogen when compared with treated hypothyroid dogs, whereas proportions of primary hairs in anagen and telogen did not vary significantly between these groups. This aspect of the canine hair growth cycle appears to have received little attention. Preferential loss of primary hairs has been reported in certain dysplastic diseases of hair follicles (Gross *et al.* 2005) and marked seasonal variations in the relative activity of primary and secondary hair follicles are reported in other species such as the Angora goat (Nixon 1993). Similarly, the number of active secondary hair follicles within the compound follicle varies markedly during seasonal moulting in ferrets (Nixon 1993). Transverse sectioning is advantageous because observations of this nature can be readily made in a single section at the level of the isthmus (Dunstan 1990, Credille *et al.* 2001, Whiting 2008) whereas multiple serial sections would be required with traditional vertical sectioning (Muntener *et al.* 2011). The marked dog breed variation in the normal proportions of hairs in the anagen or telogen phase (Credille *et al.* 2001, 2002) represents a challenge to the dermatopathologist in determining whether any given anagen: telogen ratio is normal for that breed, particularly when anagen follicles are evident in a section from an alopecic dog. These observations support the recommendation that inclusion of skin biopsy specimens from apparently unaffected skin is warranted (Gross *et al.* 2005), whenever possible, in the histological assessment of alopecia.

This study also highlights the value of transverse sectioning in the diagnosis of diseases where key features may involve only limited numbers of the adnexa. In human medicine, transverse skin sections have facilitated the diagnosis of HIV-associated eosinophilic folliculitis (Piantanida *et al.* 1998), and Fox-Fordyce disease (characterised by a keratotic plug at the follicular orifice and spongiosis of the infundibular epithelium at the level of entry of the apocrine duct) (Stashower *et al.* 2000). Transverse sections were also of particular value in creating a wider definition of the features of chronic cutaneous lupus erythematosus (Chung & Goldberg 2017). In the present study, the key histological features in three dogs with folliculitides were observed only in the transverse sections, likely reflecting the observation of a much larger number of hair follicles by this method. In a previous study of healthy beagle skin, transverse sectioning lead to more than threefold increase in the number of compound hair follicles observed when compared with paired vertical sections (Bond & Brooks 2013). Additionally, transverse sections illustrate every follicle within the compound follicle, typically 10-18 depending on the breed (Credille *et al.* 2001, 2002, Bond *et al.* 2016). By contrast, only about one third of the hair follicles are likely evident in a vertical section; taken together, transverse sectioning can be expected to generate a 10-fold increase in the number of observed follicles in the same biopsy specimen. Increasing the yield of adnexal structures by transverse sectioning has also been found to be advantageous in the assessment of canine sebaceous adenitis (Bond & Brooks 2013).

The results of this study indicate that the use of both traditional vertical sectioning and transverse sectioning should be regarded as complimentary in the evaluation of alopecic skin diseases in dogs, supporting the recommendations of Dunstan made almost 30 years ago (Dunstan 1990). Transverse sectioning of a portion of the biopsy has the potential to change the histopathological diagnosis; it is especially valuable in evaluating the phase of hair growth in the specimen; the number, size and arrangement of follicles within compound follicles and follicular units. It is also of value when key histopathological features such as inflammatory processes affect relatively small numbers of adnexa within the specimen. Veterinary dermatopathologists should routinely utilise this additional sectioning method when evaluating biopsy specimens from alopecic dogs.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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