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JOURNAL: Journal of Small Animal Practice

PUBLISHER: Wiley

PUBLICATION DATE: 7 December 2016

DOI: https://doi.org/10.1111/jsap.12605

Clinical and pathological features of hair coat abnormalities in curly-coated retrievers from UK and Sweden.

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Structured summary

Objectives: To gain information on hair loss amongst curly-coated retrievers by questionnaire and to define the clinical and pathological features of hair coat abnormalities in affected dogs in UK and Sweden.

Methods: Questionnaires were completed by members of the curly-coated retriever clubs. Fourteen dogs (six in the UK, 8 in Sweden) were clinically examined and skin / hair samples collected for microscopy and histopathology. Blood was collected for haematological, biochemical and endocrine assays.

Results: Of 90 dogs surveyed, 39 had current or previous episodes of symmetrical, non-pruritic alopecia and or frizzy coat changes, usually affecting caudal thighs, axillae, dorsum and neck before 18 months of age; 23 dogs had a waxing / waning course. Examined dogs generally matched the pattern described in questionnaires. Hair shaft anomalies comprised occasional distorted anagen bulbs (10 dogs) and transverse fractures (8 dogs). Vertical histopathological sections showed infundibular hyperkeratosis (28/30 sections) and low-grade pigment clumping (17/30). Subtle telogenisation of hair follicles was unequivocally confirmed by transverse histomorphometric analyses.

Clinical significance: The follicular dysplasia of CCR reported here is similar to that of Irish water spaniels and Chesapeake Bay retrievers but distinct from that of Portuguese water dogs. The genetic basis requires further assessment.

Keywords: Alopecia; curly-coated retriever; hair follicle; transverse section; follicular dysplasia

Introduction

The curly-coated retriever (CCR), originally bred in England for hunting upland birds and waterfowl, has a tight, curly coat that is considered highly protective when working. There are reports of the breed being shown in 1860, and the breed standard was established in 1913 (Nicholls, 2015). Popularity reportedly declined during the World War years but increased again between 1950 and 1970, when dogs were exported to Scandinavia, Australia and USA. Only relatively small numbers of these dogs reside in the UK currently, with Kennel Club registrations between 2005 and 2014 ranging from 62 to 187 dogs per annum (median 81.5). Registrations with the Swedish Kennel Club between 2004 and 2012 ranged from 26 to 99 dogs per annum (median 55).

In a health survey of 81 CCR published by the Kennel Club in conjunction with the British Small Animal Veterinary Association's Scientific Committee in 2006, five were reported to have 'alopecia' without any further detail. The curly-coated Retriever Club is aware of a hair coat disorder within the breed which is referred to as "patterned baldness", "coat patterning" or the "curly-coat problem"; the club has observed that very few veterinary surgeons know about this presentation and that it is often misdiagnosed as hypothyroidism (Nicholls, 2015).

In standard texts of canine dermatology and dermatopathology, a hair loss disorder of CCR has been reported in single paragraphs grouped with 'follicular dysplasia' of the Irish water spaniel (IWS) and Portugese water dog (PWD), due to their similar hair coats and breeding background, although specific references for CCRs are not cited (Gross et al 2005, Miller et al 2013a). In their first report, Gross *et al* (1992) commented that there was insufficient data for histopathological characterisation of the disease in CCRs and, according to Cerundolo *et al* (2009), scientific reports on this disorder have not yet been published.

The purposes of the present study were, firstly, to establish the prevalence and pattern of hair loss amongst CCR in the UK and Sweden by review of questionnaires sent to members of the Curly-coated Retriever Club in both countries, and secondly to more fully define the clinical and pathological features of hair coat abnormalities in CCRs by examination of affected dogs.

Methods

Questionnaires

Questionnaires were distributed to members of the UK and Swedish Curly-coated Retriever Clubs via the clubs' websites and returned to the authors. Owners were asked to record the signalment, coat colour, reproductive history and or age/ date of neutering, country of origin, and general health of their dogs. In cases with hair coat abnormalities in the preceding 12 months, owners were asked to report current or previous episodes of hair loss or coat texture changes and their evolution, pruritus, eruptions or skin lesions and pigmentation changes. Owners were provided with a schematic diagram to record the distribution of coat abnormalities. Details of previous veterinary investigations and treatments, and their outcome, were also requested.

Recruitment of dogs, ethical approval and consent

The use of animals in this study was approved by the Royal Veterinary College's Ethics and Welfare committee, Uppsala Djurförsöksetiska Nämnd (The Ethical Committee on Animal Experiments, Uppsala, Sweden) and Jordbruksverket (Swedish Board of Agriculture). Cases for examination were recruited from the questionnaire returns by identifying dogs with broadly symmetrical, non-pruritic alopecia / hypotrichosis and, or, hair coat changes (texture, crimp) without skin inflammation and whose owners expressed an interest in participating in the study. Written informed consent was obtained from each owner and their local veterinary surgeon prior to enrollment. Each dog was physically examined by one or two authors (KV and, or, RB) and the nature and extent of any skin disease was recorded prior to conducting further investigations as described below.

Parasitology, microbiology and hair microscopy

In order to exclude infections and infestations as a cause of the hair loss, skin scrapings and hair plucks mounted in liquid paraffin were examined using a light microscope. Hair samples were incubated on Sabouraud's dextrose agar (Oxoid CM0041, Oxoid Ltd., Basingstoke, UK) containing

cycloheximide and chloramphenicol (Dermasel selective supplement SR0075E, Oxoid Ltd.) at 26ºC for four weeks in case of dermatophyte infection.

Hairs plucked from affected and unaffected areas were also collected in sterile universal containers, mounted in liquid paraffin and examined using a light microscope for evidence of structural anomalies. Hairs from normal, frizzy and alopecic areas on the dorsal skin were bundled together using cigarette papers then mounted in molten wax so that hair bundles could be sectioned transversely using routine histological methods. Image capture and analysis software were used to generate images of sectioned hairs (QCapturePro v6.0, QImaging, Surry, Canada) so that the hair shaft diameters of each hair in the specimens could be measured (Image J 1.48v; http://imagej.nih.gov/ij). Sectioning of the hair generated either circular forms or elliptical shapes of varying eccentricity, depending on the shape of the hair and how far the plane of section deviated from the perpendicular to the long axis of the hair; accordingly, the minor axes (shortest distance through centre) of elliptical hair sections were measured. Since hair shaft diameter might vary according to anatomical site (Vogt et al 2008), only hair samples from the dorsal trunk were examined by this method.

Blood sampling

Blood samples were obtained from peripheral veins for routine haematology and blood biochemistry to evaluate the general health of subject dogs. Serum concentrations of total T4 and thyroid-stimulating hormone (TSH), and free T4 measured by equilibrium dialysis where appropriate, were done to establish each dog's thyroid status. An ACTH stimulation test was performed by collecting serum samples before, and 60 minutes after, the intravenous injection of 250 ug of tetracosactride acetate (Synacthen, Alliance Pharmaceuticals Ltd, Chippenham, U.K.) in order to assess levels of cortisol and 17-hydroxyprogesterone (17-OHP), as indicators of adrenal gland function.

Histopathology

Skin biopsy specimens were obtained from affected (alopecic or 'frizzy') skin sites following local anaesthesia using 6 or 8 mm biopsy punches, fixed in formalin and submitted for histopathological

examination; a single additional sample was obtained from clinically-normal areas on the dorsal trunk for comparative purposes if acceptable to the owners. 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. Since transverse sectioning of skin biopsy specimens is essential for the full definition of hair follicle abnormalities in histological sections (Headington 1984), 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. In one case which had been biopsied previously, transverse sections were prepared by melting the wax of archived, vertically-sectioned tissue blocks; skin specimens of adequate thickness were turned and sectioned transversely whilst the wax was still molten, before being re-embedded as previously described). All sections were processed routinely and stained with haematoxylin and eosin for histopathological examination.

The area of images of the transverse sections at the lower isthmus level was obtained using image capture software (QCapturePro v6.0, QImaging, Surry, Canada V35 6KS) so that the density of compound follicles (CF) / mm^2 and the numbers of follicles / CF (HF/CF) could be recorded and compared in samples from unaffected, 'frizzy' and alopecic areas. The anagen : telogen (+catagen) (A:T) ratios were also compared, according to the methods of Headington (1984) and of Whiting (2008). Briefly, anagen hairs were identified by the presence of an intact inner root sheath (IRS) and an absence of apoptosis in the trichilemma (outer root sheath [ORS]) at the lower isthmus level, whereas catagen was recognised by apoptosis or shrinkage of the trichilemma. Telogen was identified by the presence of either club hairs (loss of IRS, irregular stellate configuration of keratotic remnants), telogen germinal units (irregular islands of basaloid cells with peripheral nuclear palisading and little or no centripetal keratinisation and no apoptosis) or, rarely, angiofibrotic tracts (follicular streams or stele; vascularised fibrous tracts). Catagen follicles were included in the telogen counts because catagen hairs inevitably become telogen hairs (Whiting 2008). The overall A:T ratio was calculated using combining counts for both primary and secondary hairs and expressed as either

A:T ratio per CF or per histological section. Since a preferential loss of primary hairs has been reported in certain dysplastic diseases of hair follicles (Gross et al 2005), the A:T ratios for primary hairs alone and secondary hairs alone per section were also calculated. The investigator (RB) was blinded to the origin of each section during the cutting process.

Statistical analyses

The density of CF / mm^2, HF/CF, A:T ratio / CF, A:T ratio / section, and hair shaft diameter from alopecic, frizzy and unaffected sites were compared using a linear mixed model to account for repeated measures from the same dog. Statistical significance was set at $P < 0.05$ (SPSS v20, IBM United Kingdom Ltd., Portsmouth, U.K.). Data for A:T ratio per section were log_{10}-transformed prior to analysis.

Results

Questionnaires

Completed questionnaires regarding 70 dogs from 155 U. K. members, and 20 dogs from 134 Swedish members were received. These comprised 70 black (56 UK) and 19 liver (14 U.K.) dogs (1 colour not recorded), aged 6 months - 13 years (median 4 years), of which 47 were female (19 neutered) and 43 were male (15 neutered). Two dogs were imported into the UK (from Finland, USA) and two were imported into Sweden (from Finland, Norway) with the remainder having been bred in their home countries. Thirty-nine (24 UK, 15 Sweden) had current or previous episodes of broadly symmetrical, non-pruritic alopecia / hypotrichosis without skin inflammation. Twelve of these 39 had coat abnormalities when acquired by the respondent (median age 5.5 months, range 2-12 months). In dogs that developed coat abnormalities after acquisition, the age at onset reportedly (22 / 27) ranged from 2-74 months (median 12 months). Thirty-five had been affected in the 12 months preceding the survey, with 32 currently affected. In the 12 months preceding the survey, 24 dogs (4 ME, 5 MN, 11 FE, 4 FN) had a waxing and waning course, five had become progressively worse (3 FE, 2 ME), three progressively better (2 MN, 1 FE), and three had remained unchanged (2 FN, 1 FE). Alopecia affected one or more regions, including dorsum (n=22), neck (n=21), caudal thighs

(n=21), axillae (n=18), sternum (n=11), tail (n=7), flanks (n=7) and skin overlying the scapula (n=4). Focal abnormalities of hair texture/quality ("frizzy", "dry") were reported by 18 owners. Increased skin pigmentation in alopecic areas was reported by only seven owners. Seven cases waxed and waned in association with oestrus; three dogs lost hair before oestrus with full re-growth afterwards (in one case, this was a single episode), whereas two dogs improved after oestrus without regaining a normal coat. Two owners reported worsening 8 weeks after oestrus with substantial or complete regrowth before next oestrus. One owner reported complete recovery after ovario-hysterectomy. One dog was reportedly more severely-affected after whelping, and another which had been affected for three years had a full recovery after whelping. Three dogs with perennial coat abnormalities were reportedly less-severely affected in the summer months, whereas three dogs were affected in the summer months with full recovery in the winter months. One dog affected for two years recovered for unknown reasons. No pattern to the waxing and waning was specified for the remaining seven dogs.

Other skin abnormalities reported in other dogs included pruritus (atopic dermatitis [n=1], cutaneous adverse food reaction [n=1], unspecified [n=3]), onychodystrophy (n=2), tail gland hyperplasia in entire male dogs (n=2), mammary neoplasia (n=2) and mast cell tumour (n=1). Notable disorders of other organ systems included epilepsy (n=4), and gastric dilatation-volvulus (n=2).

Dogs

Fourteen affected CCR (11 black, 3 liver) were clinically examined; six dogs lived in the UK and eight lived in Sweden. The age at presentation of the dogs (three male [two neutered, one entire] and eleven female [four neutered, seven entire]) ranged from 2 y 1 mo to 9 y 6 mo (median 5 y 2 mo). The age at onset of skin disease reportedly ranged from 4 mo to 6 y (median 13 mo); in nine cases, signs developed in the first 14 months of life, although in one case the owner could be no more accurate than 'young'. Initial signs reported comprised focal areas of, frequently symmetrical, alopecia without pruritus, inflammation or changes in skin pigmentation, often accompanied by poor coat condition with either a localised or more generalised distribution. In the male entire dog, the alopecia was reportedly slowly progressive, whereas in the two neutered male dogs, a waxing and

waning course was described without full hair regrowth. A waxing and waning course was described in six of the seven entire female dogs; in one case significant alopecia followed by full regrowth developed after whelping on two occasions, although alopecia remained on the sternum after a third litter. Two entire female dogs were permanently affected but showed clear deterioration after each oestrus, another waxed and waned but was noted to be worst in the autumn, and two dogs had varying levels of severity without any clear seasonal or oestrus-associated pattern. One neutered female dog had partial hair regrowth after oestrus as a young dog but a more static presentation subsequent to neutering, whereas the other three neutered female dogs had varying levels of severity without any clear seasonal pattern.

The most frequently affected anatomic sites were the dorsum, ventral neck (Fig 1), caudal thighs (n=10) (Fig 2) and sternum (n=9). In five Swedish dogs, alopecia extended to involve the entire neck. Other areas affected included the flanks (n=5), axillae (n=4), lateral thighs (n=2), abdomen and medial thighs, pre-auricular area and tail (one dog). Frizzy coat changes, sometimes associated with a subtle lightening of coat colour, were evident on the dorsum (six black and one liver-coloured dog) but extended to the caudal thighs in one dog, and formed a saddle-shaped pattern in the liver-coloured dog (which breeders refer to as ‘saddleback’)(Fig 3).

Parasitology, microbiology and hair microscopy

Ectoparasites were not identified inskin scrapings or hair plucks and dermatophytes were not isolated from any of the dogs.

Primary hairs from liver-coloured dogs were brownish in colour and the cortices and medullae could be clearly distinguished using the scanning (x4) objective of the microscope, whereas primary hairs from black-coloured dogs had a uniform black appearance. Secondary hairs from dogs of both coat colours were brownish in colour. A typically subtle variation in shaft diameter along the length of the hair was noted in samples from all dogs and in hairs from both healthy and affected sites; an uneven diameter can be expected when twisted or curled oval structures are viewed in two dimensions (Price 1990). Distorted and misshapen anagen hair bulbs were occasionally observed in specimens from 10

of the 14 dogs (Figs 4a-d). In primary hairs from two unaffected sites and three affected sites, one or more areas of symmetrical bulging of the outline of the primary hair was observed (Fig. 5a). Transverse breaks in the continuity of the hair fibre were observed in nine specimens (five unaffected sites, four affected) from eight dogs. In seven cases, transverse fracture defect across the shaft occurred without the brush-like splaying out of individual cortical cells and their fragments as seen in trichorrhexis nodosa (Price 1990)(Figs. 5b-d); these were most often seen distally in secondary hairs although splits in the hair bulb region were observed in two cases (Fig. 5c). In two cases, a notch in one side of the primary hair was noted (Fig. 5a).

The diameter (mean ± se) of hair shafts from alopecic (44.6 ± 4.9 um), frizzy (50.0 ± 2.7) and normal (55.3 ± 3.1) areas on the dorsal trunk did not vary significantly (P=0.205).

Blood sampling

Other than one dog with mild neutrophilia (14.2 x 10^9/L, reference interval: 3.0-11.5), and another with a mild elevation of alkaline phosphatase (517 U/L, reference interval: 19-285), routine haematology and blood biochemistry showed no significant abnormalities.

All dogs were considered euthyroid based on serum concentrations of total (or free) T4 and endogenous thyroid-stimulating hormone (TSH); three dogs with mild elevations of TSH had normal values for free T4 when measured by equilibrium dialysis (Table 1). Serum concentrations of cortisol before and after ACTH stimulation were within normal ranges with the exception of one pre-stimulation cortisol value (Table 1), and taken together with haematological, biochemical, and clinical features, did not support diagnoses of hyperadrenocorticism. Concentrations of 17-OHP were normal in nine dogs but mildly elevated following ACTH simulation in one entire female and one neutered female, and elevated before and after stimulation in two entire female dogs sampled during oestrus. One entire female had a ‘borderline’ 17-OHP value post-ACTH stimulation.

Histopathology

Skin biopsy specimens were obtained from clinically normal skin (n=6 from 6 dogs) and affected areas (frizzy, n=4; alopecic, n=26 from 14 dogs). In sections from normal skin, three showed follicular keratosis without dilation of the infundibula, and three sections (from black dogs) showed pigment clumping in inferior portions of the follicles. In vertical sections from affected areas, there was infundibular hyperkeratosis (Fig 6) in 28 out of 30 sections (3 frizzy, 25 alopecic), 21 (2 frizzy, 19 alopecic) of which showed dilation extending into secondary follicles ("witches feet") (Fig 7). Pigment clumping in the hair shaft or follicular epithelium (Figs 7 and 8) was accompanied by pigmentary incontinence around follicles in 15 transverse and 17 vertical sections; these features were more prominent in specimens from black dogs.

Although anagen hairs were abundant in 14 vertical sections (3 frizzy, 11 alopecic), greater proportions of telogen hairs were recognised in transverse sections, especially in secondary follicles; abundant anagen hairs were evident in normal skin and in four of four transverse sections from frizzy areas and only four of 26 alopecic areas (Fig 9). An eccentric location of the hair shaft within the follicular epithelium was observed in one of six transverse sections from unaffected areas, and 11 of 30 sections from affected areas (Fig 10), but in none of 35 sections previously obtained from six healthy beagle dogs in the course of a separate study (Bond & Brooks 2013).

Compound follicle density (mean CF/mm2 ±se) in healthy (3·55 ±0·32), frizzy (2·95 ±0·43) and alopecic (3·44 ±0·22) areas were comparable (P=0·47) and did not vary between anatomical sites (P=0·14). The number of hair follicles per compound follicle (mean ±se) in frizzy areas (17·2 ±1·1) exceeded (P=0·01) those of unaffected (14·6 ±0·88) and alopecic (14·39 ±00·078) areas. Mean A:T ratios for primary and secondary hairs combined in alopecic areas were lower (P<0·001) than those of unaffected areas when calculated either per CF or per section (Table 2). Mean A:T ratios per section in alopecic areas were also lower (P<0·001) than in healthy areas for primary hairs alone, and secondary hairs alone. Mean A:T ratios per section in frizzy areas for secondary hairs alone, and primary plus secondary hairs, exceeded (P<0·01) those of alopecic areas; a similar relationship between ratios for secondary hairs alone and when combined with primary hairs can be expected

given that secondary hairs typically account for more than 93% of hairs per CF. There was no evidence of a relationship between A:T ratios per section for primary and secondary hairs (P=0·533).

Discussion

"Dysplasia" is a somewhat ambiguous term that is defined in both a veterinary (Studdert et al 2012) and medical (Anderson et al.) dictionary as "an abnormality of development; in pathology a developmental abnormality leading to alteration in size, shape and organisation of adult cells". "Canine follicular dysplasia" consists of a group of skin diseases with varied clinical and microscopical features, loosely characterised by abnormal growth and development of hair, leading to varying degrees of hair loss and altered quality of the hair coat (Gross et al 2005). By convention, the term is used mostly in relation to breed-specific disorders of young animals (not congenital) with a suspected or proven hereditary basis. Some authors classify these diseases according to whether they are coat-colour linked or not, and cyclical or non-cyclical in their course. Whilst a permanent or progressive alteration in adnexal structure may occur, it is recognised that some types are associated with intermittent expression (Laffort-Dassot et al 2002, Gross et al 2005). Common histological features include infundibular hyperkeratosis, varying degrees of pigment clumping or incontinence and follicular distortion, and atrophic hair follicles or telogenisation without size reduction and with an otherwise normal epidermis and dermis (Gross et al 2005).

The clinical and pathological features reported and observed in this study fit well with the definition of follicular dysplasia proposed by Gross et al (2005). The hair loss and frizzy coat changes, often with a waxing and waning course, in young CCR that are otherwise healthy, is consistent with follicular dysplasia. Other potential causes of alopecia, such as infection or infestation of the hair follicles by microbes or parasites, were excluded by their absence in samples examined microscopically, cultures and histopathological specimens.

Similarly, neither the general physical examinations, intermittent occurrence of alopecia, frequent occurrence in neutered dogs nor the results of blood investigations support the endocrine skin diseases of hypothyroidism, hyperadrenocorticism and functional gonadal neoplasia as a cause of the

alopecia. The measurement of 17-OHP before and after ACTH stimulation is promoted as a test for 'sex hormone alopecia', although the effect of this hormone on the hair growth cycle is not well-defined (Frank et al 2003). Elevated concentrations have been reported in Pomeranians and poodles with 'alopecia X' (Frank et al 2003) and in dogs with various forms of hyperadrenocorticism (Chapman et al 2003). The significance of the infrequent elevations in the CCR studied here is uncertain, and contrasts with the routine elevations in IWS and Chesapeake Bay retrievers (CBR) with follicular dysplasia reported by Cerundolo et al (2000, 2005). The increased levels of 17-OHP in samples from dogs in oestrus is in accordance with previous reports (Bromel et al 2010).

The histological features observed in traditional vertical sections were essentially similar to those reported in IWS with follicular dysplasia. Infundibular hyperkeratosis, reduced follicular activity and pigment clumping within hair shafts and follicular epithelium are unifying features of the diseases of IWS and the CCR reported here. By contrast, the increased sebaceous lobulation and presence of 'flame follicles' reported in IWS were not observed in the present study. Flame follicles, wherein large spikes of trichilemmal keratin project and protrude radially and irregularly through the outer root sheath towards the vitreous layer (Gross et al 2005), must be differentiated from prominent trichilemmal keratin seen in some telogen club hairs. The distinction between 'normal' and 'excessive' trichilemmal keratin appears to be subjective with the definition being applied inconsistently in the literature; for example, the illustrations of 'flame follicles' reported by Cerundulo et al (2000) do not appear to match the aforementioned definition. However, the lack of excessive trichilemmal keratinisation serves to clearly distinguish the histopathology of follicular dysplasia of CCRs from that of Siberian Huskies (Post et al 1988, Gross et al 2005).

The skin biopsy specimens from the CCR in this study did not show the marked accumulations of melanin within follicular epithelium that typify colour dilution alopecia and black hair follicular dysplasia (Miller 1990, Gross et al 2005). The follicular dysplasia of PWD also appears to be histologically distinct from that of CCR and IWS; the unique features of vacuolar alteration of the inner root sheath and dissolution of the hair matrices seen in PWD (Miller and Scott 1995) were not

present. It is therefore questionable whether the diseases of these three breeds should be grouped together in textbooks.

In 2005, Cerundolo et al reported adult-onset hair loss in ten CBR with similar clinical signs and histopathological features to those seen in the present study. Notable differences between those CBR and the CCR reported here include neck involvement in CCR but not CBR, absence of frizzy coat changes in CBR, and a younger age at onset and more frequent waxing and waning course in CCR retrievers. Both breeds share histological features of infundibular hyperkeratosis and pigment clumping but in the absence of trichography and hair follicle morphometrics in the CBR study, the role of hair fracture and or telogenisation in the pathogenesis of alopecia cannot be fully determined.

Whilst the term 'pattern baldness' has been used by breeders to describe this presentation in CCR, our observations indicate that this is not technically correct; pattern baldness (aka acquired pattern alopecia) refers to a syndrome seen in small dogs with fine hair coats, notably dachshunds, that present at a young age with progressive thinning of hair on the pinnae, and / or caudal thighs and ventrum associated with miniaturised but otherwise cycling hairs (Gross et al 2005).

The presence of abundant anagen hairs in vertical sections from affected areas raised questions about the patho-mechanism of the alopecia in these dogs and prompted the histomorphometric analyses of transverse sections. These data provided compelling statistical confirmation of the subjective impression of increased telogenisation in transverse sections from alopecic areas, and further substantiates the pivotal role of the transverse sectioning method in the assessment of the hair and adnexa in skin biopsies from dogs (Credille et al 2002, Bond et al 2014). A standard textbook states that hair loss in CCR is due to hair fracture (Miller et al 2013a), and whilst a variety of structural anomalies likely to result in broken hairs was seen in eight dogs, these were not prominent features in our study population. Our data indicates that telogenisation must also be regarded as a contributing factor for alopecia in CCRs, as an increase in the percentage of follicles in telogen leads to increased hair shedding (Trueb 2008). By contrast, as expected due to the lack of a scarring process observed in routine sections, and the waxing and waning course of the disease in many dogs, transversehistomorphometry confirmed that the alopecia was not associated with reduced hair follicle

density; the values for compound follicle density in this study are mid-range for the values of 1.0 to 6.0 groups / mm^2 proposed in a standard text of veterinary dermatology (Miller et al 2013b). To our knowledge, the eccentric location of hair shafts observed in transverse sections has not been reported previously. Although this feature likely fits within the range of structural anomalies seen in follicular dysplasias, a more widespread use of transverse sectioning is needed to determine whether this feature is unique to CCR follicular dysplasia or occurs in other breeds, particularly those with curled hairs.

Whilst this study has defined the clinical and pathological features of follicular dysplasia, effective therapy has not been identified and in view of the presumed genetic basis, is not likely to be readily available. Further studies are now required to define the molecular and genetic basis of this disorder in CCRs so that tests can be developed to guide selection of dogs that are most suitable for breeding.

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Table 1. Results of hormonal assays in 14 curly coated retrievers with hair coat abnormalities.

Case number	Gender	Total T4	TSH	Free T4	Cortisol pre	Cortisol post	17OHP pre	17OHP post
1	MN	29.8	0.08	ND	NA	NA	<1.0	1.7
2	MN	23.1	0.13	ND	29	241	<1.0	2.7
3	FN	17.6	**0.66**	17.4	67	282	<1.0	1.1
4	FN	22.5	**1.00**	9.8	**277**	471	<1.0	<1.0
5	FN	**12.3**	0.23	ND	<27	171	<1.0	1.0
6	F	21.9	0.22	ND	53	235	<1.0	5.3
SW1	F	24.7	0.14	ND	41	244	<1.0	9.5
SW2	F	51.6	0.07	ND	31	183	***8.2****	***19.4****
SW3	F	22	**0.75**	12.0	102	213	1.9	***10.2***
SW4	F	47.9	0.04	ND	48.7	189	***7.5****	***12.2****
SW5	M	31	<0.1	ND	<27	185	<1.0	5.3
SW6	F	26.4	0.11	ND	42	270	2.4	6.6
SW7	FN	28.3	0.07	ND	49	189	<1.0	**12.9**
SW8	F	18.7	0.27	ND	66	279	<1.0	7.4

M, male; F, female; N, neutered; 17OHP, 17 hydroxyprogesterone; ND, not done; NA, not available.

Total T4 normal range 13-52 nmol/L; TSH normal range <0.41 ng/mL; free T4 normal range 7.0-40 pmol/L.

Cortisol normal range pre-ACTH <250 nmol/L, post-ACTH <500 nmol/L.

17OHP normal range pre-ACTH <3.0 nmol/L, post-ACTH 8.0 -10.0 'borderline', >10.0 'possible sex hormone excess'.

*dog in oestrus at time of sampling.

Values in **bold** are outwith the normal range.

Table 2. Anagen : telogen (+catagen) ratios (mean ± se) for primary and or secondary hairs assessed by examination of transverse sections at the lower isthmus level in skin biopsy specimens obtained from healthy, alopecic and frizzy sites in 14 curly-coated retrievers. Ratios are calculated and expressed either per compound follicle or per histological section.

Method	Healthy	Frizzy	Alopecia
Primary + secondary / CF	1.45 ± 0.23^{a}	1.15 ± 0.29	0.67 ± 0.21^{c}
Primary hairs only / section	1.88 ± 0.31^{bd}	0.94 ± 0.35	0.59 ± 0.16
Secondary hairs only / section	1.07 ± 0.21^{a}	1.12 ± 0.26^{b}	0.57 ± 0.17
Primary + secondary / section	1.11 ± 0.19^{a}	1.16 ± 0.26^{b}	0.54 ± 0.15

Comparison with alopecic sites within method: [a] $P<0.001$; [b] $P<0.01$
Comparison with frizzy sites within method: [c] $P=0.051$; [d] $P=0.057$
CF, compound follicle.