The Veterinary Journal 196 (2013) 510–514

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Morphometry of skin changes in Newfoundland dogs following coat clipping

Gila Zur [a,*], Keren Regal [a], Emmanuel Loeb [b]

[a] Veterinary Teaching Hospital, The Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel
[b] Pharmaseed Ltd., Hamazmera 9, POB 2119, Ness Ziona 74047, Israel

ARTICLE INFO

Article history:
Accepted 6 December 2012

Keywords:
Dogs
Hair clipping
Newfoundland dogs
Sebaceous glands
Sweat glands

ABSTRACT

Dog breeds are unique in their coat conformation and quality. Newfoundland dogs have a long and fine hair coat, and clipping may induce changes in newly grown hair. This study examined structural changes in the skin of Newfoundland dogs following clipping. Dogs included in the study had visible coat changes following clipping that appeared as loss of gloss, increased scaling and textural changes. The control groups consisted of two groups of dogs that had never been clipped: Newfoundland dogs served as within-breed controls, and long-haired dogs of other breeds served as between-breed controls. All dogs were healthy with no history of dermatological problems. Two skin biopsies were taken from each dog and evaluated for predetermined parameters.

A total of 41 samples were examined: 11 from clipped Newfoundland dogs, 16 from unclipped ones, and 14 from dogs of other breeds. By histopathology, the clipped dogs had a thicker cornified layer ($P = 0.006$) and smaller sebocytes ($P = 0.022$) than the unclipped ones. Newfoundlands had larger and more epitrichial sweat glands than other breeds ($P = 0.0002$, $P = 0.036$, respectively), and those were not affected by clipping. These results suggest that hyperkeratosis and decreased sebocyte size may explain the observed coat changes following clipping in Newfoundland dogs.

Introduction

Breed variation in dogs is very pronounced and one of the most prominent is hair coat appearance. Hair is usually classified as short, intermediate or normal and long, and is further subdivided into fine and coarse (Scott et al., 2001b). Hair regrowth following clipping may be impaired in some breeds, especially those with a long, fine hair coat, causing concern to owners and requiring frequent veterinary care (Scott et al., 2001a). Owners are also concerned about changes in the quality of hair coat that occur after clipping or spaying.

Several studies have been conducted to address problems associated with hair regrowth, in particular evaluating hormonal influence on hair cycle. Histopathology of the skin of dogs that experienced delay in hair regrowth post-clipping showed hair follicle arrest, which is also reported in endocrine alopecia (Frank, 2005; Gross et al., 2005). Other factors found to influence hair regrowth and replacement were body site, environmental changes, age, sex hormones and breed variation (Al-Bagdadi et al., 1977, 1979; Butler and Wright, 1981; Hale, 1982; Gunaratnam and Wilkinson, 1983; Dunstan et al., 2001). Reichler et al. (2008) reported coat changes in 20% of a cohort of spayed bitches, but could not identify the pathological mechanism underlying these changes.

Other changes in hair quality following clipping have yet to be addressed. The predominant changes in coat quality reported by owners are loss of gloss, increased scaling and texture changes. The aim of the present study was to investigate possible changes in skin structure in Newfoundland dogs with coat quality changes following hair clipping and to compare them with dogs that had never been clipped.

Materials and methods

Study animals and samples collection

The work was designed as a case-control study and the study group included skin samples from Newfoundland dogs that had a history of clipping of the entire body or large parts of it, and whose owners had noticed changes in hair quality demonstrated by loss of gloss, increased scaling and texture changes following clipping. This group is referred to as clipped Newfoundlands (C-NF). The hot and humid environment in which the dogs lived was the only reason for clipping. It is important to note that the owners of the clipped Newfoundlands did not seek veterinary care for the problem as their dogs did not have medical problems other than the skin and coat changes.

The within-breed control group included skin samples from pure bred Newfoundland dogs that had never been clipped, and is referred to as unclipped Newfoundlands (N-NF). The between-breed control group included skin samples from dogs of other breeds with fine long hair coat that had never been clipped, and is referred to as other breeds (OB). All dogs were healthy according to their records and the physical examination performed by the first author (GZ) and had no history of skin problems. The dogs did not receive any topical or systemic treatments for at least 1 month prior to obtaining the skin samples.

* Corresponding author. Tel.: +972 3 9688547.
E-mail address: zurgila@agri.huji.ac.il (G. Zur).

1090-0233/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2012.12.005

From each dog two samples were taken for histological evaluation: one from the chest and one from the back. The chest was chosen because Newfoundlands have a heavy coat and more dogs were clipped only at this site. The back was chosen because the coat and skin changes were easily demonstrated there and for the purpose of having samples from another site. The chest sample was taken in the medial line between the forelimbs and the back sample was taken 2–3 cm lateral to the spine at the line of the last rib. All samples were taken by one author (KR). The biopsies were taken during the months March and April 2007 and 2009; however, no attempt was made to take the samples from the clipped dogs at a particular time after clipping.

For skin biopsy, the area was gently shaved, smeared with local anesthetic cream containing lidocaine 2.5% and prilocaine 2.5% (Emla, AstraZeneca), and after 20–30 min the area was injected subcutaneously (SC) with a local anesthesia solution containing lignocaine–HCl 1% (Esracain, Rafa). The skin biopsies were obtained using a 6 mm biopsy punch and stored in 10% (v/v) neutral buffered formalin until processing for histological examination.

All owners signed an informed consent form and all procedures in the study were approved by the Ethical Committee for Clinical Trials of the Koret School of Veterinary Medicine (Approval Number: KSVM-VTH 07/2007).

Histology and tissue examination

Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). From each sample, 3 μm serial sections were put on a slide and the most intact ones were examined microscopically. The following parameters were evaluated: (1) the thicknesses of the epidermis and of the cornified layer and the ratio between them; (2) the number of follicular units in a section (a follicular unit was defined as a group of primary and secondary hair follicles with the sebaceous gland connected to them. These structures are associated also with the arrector pili muscle and are known as a pilosebaceous unit); (3) the number of hair follicles and sebaceous glands per follicular unit and the number of epitrichial sweat glands; (4) in some follicular units the largest sebaceous gland was measured (in μm) by choosing the longest longitudinal and horizontal lines; (5) sizes of sebocytes; numbers of sebocytes in a gland, number of reserve cells, and the ratio between them repeated for 3–4 sebaceous glands in every section.

The reserve cells are deeply basophilic basal cells located at the basement membrane zone which bordered the sebaceous lobules. These cells become sebocytes when progressively accumulating lipids, and then disintegrate to form sebum towards the center of the lobule. The measurements of the epitrichial sweat glands were made as described for sebaceous glands. The epitrichial sweat glands evaluated were chosen arbitrarily in the section. The measurements were done using a special in-lens calibrator (Olympus) in which 10 calibrated lines at a magnification ×100 is equal to 100 μm. Histological examinations and measurements were performed blindly by all the authors.

Statistical analysis

Chi-Square Fisher's exact test was applied for assessing the associations between two categorical variables, and included testing the associations between groups for genders. One-way ANOVA was used to compare the ages of the groups. The Kruskal–Wallis analysis of variance was used to compare quantitative variables between the groups. This included testing the associations between groups, between and within body sites for all the measurable histological variables in skin samples. The Mann–Whitney test was used to examine associations between the gender and histological variables.

In cases where statistical significances were found, analysis of variance was applied to detect which of the two variables (group or gender) had a greater effect on the results. Pearson's correlation coefficient was applied for all histological variables and ages. In cases where the differences between groups were statistically significant and the correlation was found high, analysis of covariance was applied for examining the effect of each variable on the results. All tests applied were two-tailed, and $P \leqslant 0.05$ was considered statistically significant.

For associations where statistical significances reached 0.05 or less, multiple pairwise comparisons using the Mann–Whitney test were done, and the significance was set at a value of 0.017. The Bonferroni correction was applied for the three paired comparisons: C-NF and N-NF, C-NF and OB, N-NF and OB.

Results

Study animals

The C-NF group comprised of 11 samples from seven dogs: eight samples from four dogs with whole body clipping, of which two were intact females, one was a neutered female and one a castrated male. Three more samples were from dogs that were clipped only on their ventral coat, consisting of two females and one male. The age range was 5–10 years with a median of 7 years (standard deviation (SD) = 1.72).

The N-NF group included 16 samples from 10 dogs, 12 from five intact females and one intact male; another sample was taken from one intact female in which only the chest was sampled and three more samples were taken from two intact females and one intact male for which only the back samples were included in this group (these dogs were clipped only on their chests and their chest samples were included in the C-NF group, as described above). The dogs were 2–8 years old with a median of 4 years (SD = 2.12).

The OB group included 14 samples from seven dogs, five males, one of which was castrated, and two intact females. One dog was a Saint Bernard, one a Border collie, and the rest mixed breeds with fine, long hair coats. The age range in this group was 1–8 years old with a median of 4 years (SD = 2.32).

The dogs in the C-NF group were significantly older than the other groups ($P = 0.003$) and there were more neutered dogs in this group than in the other groups ($P = 0.017$), but the number of neutered dogs was small. In the OB group there were more males than in the other groups ($P = 0.01$).

Tissue examination

A total of 41 biopsies were examined: 11, 16 and 14 from the C-NF, N-NF and OB groups, respectively. Overall, 21 samples were taken from the chest (seven samples from each group) and 20 samples were taken from the back (four from the C-NF group, nine from the N-NF group, and seven from the OB group). The morphometric data are presented in Table 1.

The cornified layer was significantly thicker in the C-NF group than in the other groups ($P = 0.011$) (Figs. 1–3), and especially thicker than in the N-NF group ($P = 0.006$ in the pairwise comparisons). The C-NF group had a basket-weave diffuse, orthokeratotic hyperkeratosis (Fig. 1). The epidermis was also thicker (regular mild hyperplasia) in the C-NF group than in the other groups and in both Newfoundland groups than in the OB ($P = 0.011$) (Figs. 1–3). In pairwise comparisons the two Newfoundland groups were similar ($P = 0.195$), but a very statistically significant difference was found between the C-NF and the OB groups ($P = 0.0002$) and to a lesser extent between the N-NF and the OB groups ($P = 0.004$).

The ratio cornified layer/epidermis was significantly higher in the C-NF and OB groups than in the N-NF group ($P = 0.011$). In multiple pairwise comparisons, only the difference between the N-NF and OB groups remained statistically significant ($P = 0.002$).

The number of follicular units in the sections was similar among the groups. The number of hair follicles per follicular unit was similar in the two Newfoundland groups, with a median of 5, but lower in the OB group with a median of 4 ($P = 0.0434$). In multiple pairwise comparisons, however, no significant differences were found between groups.

The number of sebaceous glands in a follicular unit was significantly higher in the C-NF group ($P = 0.0399$, median 6 vs. 3 and 3.5 in the N-NF and OB groups, respectively). In multiple pairwise comparisons, the significance of the differences between the C-NF and OB groups was $P = 0.021$, between the C-NF and the N-NF groups was $P = 0.030$, while the two unclipped groups were similar ($P = 0.79$). The size of sebaceous glands and the number of sebocytes per gland were not statistically different between groups (Table 1) ($P = 0.930$ and 0.083 respectively). The C-NF group had the smallest sebocytes and the OB group the largest (medians 35 μm and 45.5 μm respectively ($P = 0.0222$; multiple pairwise comparisons $P = 0.003$). The number of reserve cells in the sebaceous glands was smallest in the C-NF group ($P = 0.07$), with medians of 14, 25 and 35.5 in the C-NF, N-NF and OB groups, respectively.

The ratios between reserve cells and sebocytes were similar between the groups. Newfoundland dogs had more sweat glands per