

SEM Study of Hair Cuticle in Some *Canidae* Breeds

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Abstract

The hair fiber study is very actual due to its implication especially in forensic medical expertise. It was studied the usual and SEM microscopic morphology of primary hair fibers collected from mature male dogs of five well-known breeds: Husky, Bichon Maltese, Golden retriever, Labrador and German Shepherd. Descriptive observations and quantitative data obtained by micrometry do not lead to significant differences to permit identification of one breed by simple hair examination. But, comparing the hair fiber quantitative parameters obtained in our study with those ones known in specialty literature concerning the human hair, we can conclude that there are differences among medullary index values and as index could be an identification criterion.

Keywords: *hair cuticle, Scanning Electron Microscopy (SEM), Canidae.*

INTRODUCTION

Trichology is a domain of biological sciences, which studies the structure, functions and pathology of hair follicles and their products –hair fibers. The complex structure, formed at least by ten distinct cellular types, gives to pilous follicles as very dynamic organs a cyclic function to provide mammal hair continuous turnover (Hausman, 1980). The hair is a cylindrical, flexible formation, composed by dead keratinocytes, resistant to post-mortem chemical decomposition, thus keeping for long time their structural particularities. This quality made both human and animal hair study to have a great importance in forensic medical expertise (Kshirsagar *et al.*, 2009), in archaeological studies and in mammal species identification (Paliu *et al.*, 1979; Cadar, 2009; Cadar and Szentkuti, 2013; Cadar *et al.*, 2014). In all these domains, hair can be biochemical analysed to testing the presence of some chemical contaminant substances, drugs or some nutritional deficiency. The presence of some tissue portions from hair root or blood allow for DNA and as well as mDNA analysis (sex determination). But, the

most frequent examination is the microscopic one, both in optic microscope and SEM one, to put into evidence the hair morphology: hair fiber thickness, medullar thickness and structure, cuticle type, melanin pigment distribution etc.

In this study was followed the hair fiber microscopic morphology examination and if the hair from some dog breeds has structural particularities to make it different from those ones known for human hair. Also, was followed if quantitative and qualitative parameters offered by hair fiber microscopic examination allow dog breed identification. At same time, was taken into consideration the hypothesis that hair fiber microscopic morphology, as genetically determined character, could serve in genealogy analysis of *Canis familiaris domesticus* subspecies (Lorenz, 2000).

MATERIALS AND METHODS

Hair fibers were collected from 5 breeds of mature male dogs (3 Husky, 3 Bichon Maltese, 2 Golden retriever, 3 Labrador and 3 German Shepherd) in very good health conditions. There were collected

only primary hair fibers from shoulder blade region. For optic microscopic examination, the hair fibers were washed with distillate water, degreased with absolute ethylic alcohol and mounted in anhydrous glycerine between port-object slide and cover slide. For hair fibers collected from German Shepherd breed was necessary depigmentation with a preparation of cosmetic use based on hydrogen perchloride, ethyl alcohol, phosphoric acid, oxiquinoleine sulphate, tetra-sodium EDTA and disodium pyrophosphate. Optic microscopic examination was done with Novex-B (Holland) microscope and GrabBeeX+deluxe Video Home-2007 soft for video camera (resolution 720x480 PAL mode 720x576) (Cellular Biology Dept., Faculty of Animal Science and Biotechnologies, UASVM Cluj-Napoca, Romania). For hair cuticle cells' examination, after degreasing with alcohol, the hair fibers were fixed on port-object of Vega©Tescan SEM (Biophysics Dept., Faculty of Veterinary Medicine, UASVM Cluj-Napoca, Romania), were examined under different increasing degrees and photographed.

For quantitative parameters, such as hair fiber diameter, medullar diameter, height and width of cuticle cells, was used the micrometry technique. Was calculated the medullary index (ratio between medullar average diameter and hair fiber average diameter) and the cuticular index (ration between average height of cuticular cells and hair fiber average diameter).

RESULTS AND DISCUSSION

In Husky breed dogs, on electron micrographs obtained in SEM, the free portion of cuticle cells appears of polygonal irregular shape, wider (average value 52 μm) than high (average value 18 μm), thus the hair fiber circumference can comprise 2-3 associated cells. For the hair fiber average diameter of 60 μm was calculated the cuticular index of 0.30. The cells' outline is lightly sinuous, almost linear, like that of cells from a simple squamous epithelium as vascular endothelia, fact that we frame this cuticle into squamous type (*Fig. 1*).

Cortical layer has average thickness of 10 μm , homogenous structure, lacked of pigment granules, but appear small clearly oblong spaces, named cortical *fusi*. The hair fiber medullar layer is continuous, homogenous, multiserial, and with average diameter of 40 μm . Reported to hair diameter, results a medullary index of 0.60, so the

medullar diameter represents more than 1/3 of hair fiber diameter.

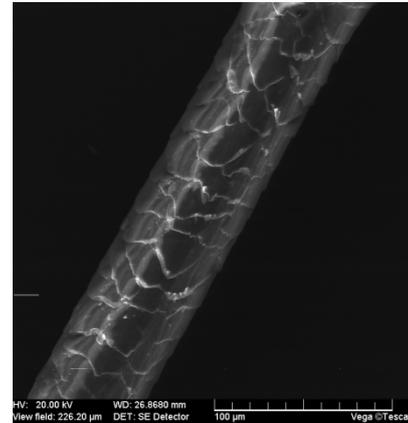


Fig. 1. Electron micrograph of hair fiber from Husky breed

The hair fibers of Bichon Maltese breed dogs have average diameter of 45 μm . Cells enough similar, with approximate rectangular oblong shape, form the hair cuticle, thus only 2 cells can be comprised in hair fiber circumference. Cell average height of 13.8 μm gives cuticular index value of 0.30. By his cell shape and sinuous outline, the cuticle frames also in squamous type. Hair fiber cortical layer appears clear, with rare melanin pigment granules, instead of cuticle cells, which appear intense-pigmented. Medullar layer is homogenous, continuous, multiserial and very-pigmented, having average diameter of 25 μm and medullary index value of 0.55.

In dogs of Golden retriever breed, hair fiber has average diameter of 45 μm , and the cuticle is also of squamous type, with very similar cells as shape and sizes to those ones of Bichon Maltese breed excepting the cell height that is more reduced (0.60 μm) resulting a smaller value of cuticular index (0.23). Examination in optical microscope makes evident a moderate-pigmented cuticle and a clear homogenous and non-pigmented cortical layer. Medullar layer is continuous and richly pigmented, his cells' outline being distinct as well cells are set into multiserial columns (*Fig. 2*). Medullar average thickness is 25 μm and results a medullary index value of 0.55.

The dogs of Labrador breed had an average hair fiber thickness of 77 μm , with cuticle formed by very unequal cells as shape and sizes, and the cell underline have numerous and profound sinuosity. For cuticle cell average height of 10.7 μm ,

it was obtained a cuticular index value of 0.13. The ensemble aspect of cuticle is also of squamous type. Examination in optical microscope makes evident moderate cuticle pigmentation and cuticle cells are prominent to the hair fiber surface. Cortical layer is clear, homogenous, with rare melanin pigment granules. Medullar layer is continuous, homogenous in his central zone, but at periphery are observed intense-pigmented cellular columns. For the average diameter of medullar layer of 47 μm there is a medullary index of 0.61.

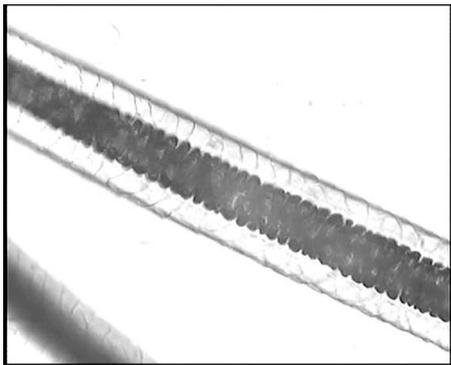


Fig. 2. Aspect of hair fiber from Golden retriever breed. Microphotograph 200x.

Among the five studied dog breeds, the German Shepherd breed presented the thickest hair fibers with average diameter of 110 μm . SEM examination made evident a cuticle of squamous type, with cells generally rectangular, very oblong, but height of only 12.8 μm and with cuticular index of 0.12. In optical microscope, hair fibers of this dog breed appear completely opaque because of melanin pigment presence in cuticle, cortical and medullar layers (*Fig. 3*).

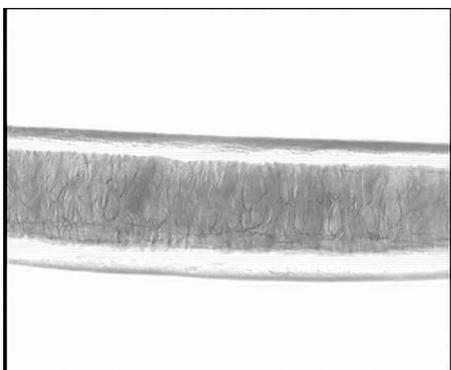


Fig. 3. Aspect of depigmented hair fiber from German Shepherd breed. Microphotograph 200x.

Chemical depigmentation was necessary to make possible delimitation of cortical and medullar layers. In medullar structure appear intercellular clear spaces, thus can be observed the multiserial disposition of cellular columns. Average medullar thickness of 70 μm gives a medullary index value of 0.63.

From the study in optical microscope and SEM of guard hair fibers, collected from five domestic dog breeds, the resulted quantitative and qualitative data cannot be considered sufficient to establish certainly the hair fiber provenience. Hair fiber cuticle morphology, as basis element in species identification, does not presented inter-breed significant differences.

In recent articles, there were admitted only three cuticle types -coronar type (cells in crown shape, which completely surround the hair fiber), spinous type (cells in oblong petals' shape) and imbricate type (cells disposed as tiles on roof)- the last one being present in human hair fibers and those ones of numerous mammal species (*Fig. 4*).

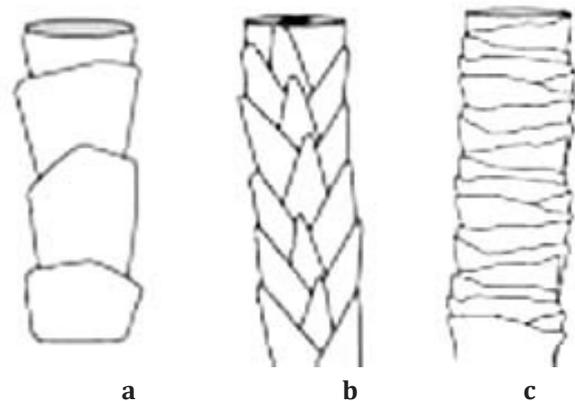


Fig. 4. Hair cuticle types in mammals: coronar type (a), spinous type (b) and imbricate type (c) (after Douglas and Koch, 2004).

We opted for a more ample typifying of hair fiber cuticle proposed by Paliu *et al.* (1979), which subdivides the imbricate type in 8 subtypes, observed in numerous domestic and wild mammals (rectangular, oval, interpenetrated, non-undulated, squamous, denticulate, crenellated and reticulated). Among these, we identified in hair fibers from the five studied dog breeds only the squamous one.

Our observations are more concluding as concerns the morphological differences of hair

Tab. 1. Quantitative and qualitative parameters of hair fiber in some *Canidae* breeds

Parameters	Dog breeds				
	Husky	Bichon Maltese	Golden retriever	Labrador	German Shepherd
Cuticle type	squamous	squamous	squamous	squamous	squamous
Width of cuticle cells (μm)	52	34.4	33	45	56.8
Height of cuticle cells (μm)	18	13.8	10.6	10.7	12.8
Hair fiber diameter (μm)	60	45	45	77	110
Medullar diameter (μm)	40	25	25	47	70
Medullary index	0.66	0.55	0.55	0.61	0.63
Cuticular index	0.30	0.30	0.23	0.13	0.12

fibers from studied dog breeds and those ones known for human hair fibers (Douglas and Koch, 2004). Thus, human hair fiber pigmentation is more intense in cortical zone surroundings the cuticle, while in dog hair fiber the melanin pigment is located much more in medullar layer. But, the most important difference consists in medullar structure and especially in medullar index value. In human hair fiber, medullar layer appears continuous and amorphous, while in hair fiber from dog the medullar layer is also continuous but structured. In human hair fiber, the medullar diameter is always under 1/3 of hair fiber rod diameter (Saferstein, cited by Kshirsagar *et al.*, 2009).

Micrometric data, effected on hair fibers from 12 mammal species and on human hair fibers from different body regions, had specified that medullary index in human hair is under 0.25 and in mammals over 0.44 (Kshirsagar *et al.*, 2009). In hair of our studied dog breeds, minimal medullary index was 0.55 in Bichon Maltese and Golden retriever breeds and over 0.60 in Husky, Labrador and German Shepherd breeds (*Tab.1*).

We consider that microscopic examination of hair fibers from a large number of domestic dog breeds could provide valid elements in identifying by hair of some breeds. For increasing the diagnostic value of hair cuticle morphology, we consider more efficient the SEM examination than hair fiber impress one in optical microscope (Bancroft and Stevens, (1996).

CONCLUSION

The micrometric data do not always offer significant differences among breeds, while medullar structure and especially value of medullary index will make the difference among studied dog breeds and could be used to make distinction from those ones known in human hair.

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