Clinical and Histologic Description of Lykoi Cat Hair Coat and Skin

リコイ猫の被毛と皮膚に関する臨床的および組織学的記述

Michelle L. LeRoy1,2*, David A. Senter1,2, Dae Young Kim3, Barbara Gandolfi2, John R. Middleton2, Karen E. Trainor4, Delia M. Bouhan2, Leslie A. Lyons2

1) Veterinary Allergy and Dermatology Clinic, LLC,
2) Department of Veterinary Medicine and Surgery, University of Missouri, College of Veterinary Medicine,
3) Department of Veterinary Pathobiology, University of Missouri, College of Veterinary Medicine,
4) Innovative Vet Path, LLC

Received April 9, 2016 and accepted June 7, 2016

Abstract: Hair and skin abnormalities of domesticated animals are readily identified and are biomedical models for ectodermal dysplasias. The hair coat of the Lykoi cat, a new cat breed, is a dramatic phenotype and has not been clinically or histologically described. Dermatoscopic examination was performed and skin biopsies were collected from seven Lykoi cats and seven dermatologically normal domestic shorthair (DSH) cats. All skin structures were examined on longitudinal and transverse sections. Immunohistochemistry for CD3 and Cytokeratin 8/18 was performed for comparison with DSH cats. Dermatoscopic images were compared. Lykoi had a significant reduction in average numbers of follicles per hair follicle group as compared to DSH cats, 14.7 ± 2.9 and 23.4 ± 5.4, respectively. Median (range) numbers of hairs per hair follicle group were 1.3 (0.4–5.7) and 18.8 (10.6–26.6), respectively. Mean (± SD) hair follicle depth was 0.95 mm ± 0.15 and 1.14 mm ± 0.21 for Lykoi and DSH cats, respectively. Mean (± SD) primary hair shaft diameters were 39 µm ± 0.029 and 47 µm ± 0.011 for Lykoi and DSH cats, respectively. Mean (± SD) total sebaceous gland area per hair follicle group was 19,937.7 pixels² ± 10,254.9 and 9,833.7 pixels² ± 5,784.5 for Lykoi and DSH cats, respectively. Unlike DSH, Lykoi had mild to severe perifollicular to mural lymphocytic infiltration in 77% of observed hair follicle groups, and follicles were often miniaturized, dilated, and dysplastic. The Lykoi has a unique feline phenotype that may serve as a novel dermatological biomedical model.

Key words: ectodermal dysplasia, feline, Felis silvestris catus, hair coat, hair follicle

要 約: イエネコの被毛や皮膚の異常は同定が容易であり、外観形成不全の疾患も出るに近くなっており、新種の猫であるリコイ猫の被毛は印象的な形態を示しているが、臨床および組織学的記述はこれまでのところ存在していない。本研究では7頭のリコイ猫および7頭の皮膚科学的に健常な短毛種猫を対象に、ダーモスコープを用いた評価ならびに皮膚生検が行われた。全ての検体について、垂直方向および水平方向の組織切片が作成されて解析に供された。またCD3とサイトテラチン8/18に関する免疫組織化学染色が行われ、短毛種猫と比較解析が行われた。さらにダーモスコーピー画像についても比較解析が行われた。毛単位あたりの毛包数の平均値を比較したところ、リコイ猫（14.7 ± 2.9）では短毛種猫（23.4 ± 5.4）よりも低値を示した。毛単位あたりの毛包数の中央値（範囲）は、リコイ猫で1.3（0.4–5.7）であったのに対し、短毛種猫では18.8（10.6–26.6）であった。毛包の深さの平均値（±標準偏差）は、リコイ猫で0.95 ± 0.15 mmであったのに対し、短毛種猫では1.14 ± 0.21 mmであった。一次毛の直径の平均（±標準偏差）は、リコイ猫で39 ± 0.029 µmであった。

* Correspondence to: Michelle LeRoy, (Veterinary Allergy and Dermatology Clinic, LLC), 11950 W. 110th St., Suite A, Overland Park, KS 66210, USA TEL: +1-913-381-3937 FAX +1-913-906-9277 E-mail: mleroy1026@gmail.com
Introduction

The hair coat of cats and all mammals is a selective advantage for thermal regulation, camouflage, protection from predators, and a barrier to diseases. In cats, coat color and hair quality are often selected as one of the main traits for developing a new breed. The rexoid coat of the domestic cat was first described as a wavy coat, absent of guard hairs by Jude in 1953, with additional descriptions by Searle & Jude in 1956. Eleven hairless or rexoid hair coat mutations have since been documented in the domestic cat and developed into breeds. These breeds include Cornish Rex, German Rex, Devon Rex, Sphynx, Selkirk Rex, Peterbald, American Wirehair, Ural Rex, LaPerm, Tennessee Rex and Lykoi. Although the former six have been scientifically documented as to their mode of inheritance and general phenotypic presentation, the latter five have had no scientific documentation.

Recently, clinical descriptions of Sphynx and Cornish Rex phenotypes and analyses of skin and follicle presentation have been published. Despite the relative paucity of hairs seen on the majority of the Sphynx cat’s body, Sphynx were found to have follicular dysplasia and abnormal hair shaft keratinization without a decrease in overall numbers of hair follicles. The curly coated Cornish Rex was found to also have abnormal hair follicle formation with resultant thin, wavy hairs covering the body. No published data are available regarding skin and hair follicle abnormalities of the other hairless and rexoid breeds and what impact these have on the hair coat.

The Lykoi cat, or “Werewolf Cat”, is a newly recognized cat breed and was first recorded in 2011 by the breed founders. Two unrelated litters of kittens were presented to the founders with a unique appearance. The cats appeared to have an absent undercoat, and the majority of guard hairs were black with some all-white hairs, producing a roan coat color. When two unrelated cats from these litters were bred, the first intentionally bred Lykoi was produced. The mother of one of the litters was a black domestic shorthair cat, and, therefore, other black domestic shorthairs have been used to outcross the breed to prevent genetic inbreeding and maintain the dramatic phenotype. Several other unrelated cats with the Lykoi phenotype have since been discovered in the United States. Currently categorized as an “Advanced New Breed” by The International Cat Association, Lykoi breeders aim to achieve championship status. At the time of sample collection for this study, approximately 20 Lykoi cats were present in the worldwide breeding program. At the time of writing, the breed has expanded to 63 standard Lykoi cats.

Several genes have been recognized that affect hair morphology in distinct feline breeds. Variant in keratin 71 gene (KRT71) has been associated with abnormal hair follicle and hair shaft development. KRT71 variants are responsible for the unique hair coat of the Sphynx, Devon Rex and Selkirk Rex cats. A second locus involved in hair follicle development, lysophosphatidic acid receptor 6 (LPAR6), is associated with the Cornish and German Rex coat phenotype.

Lykoi do not have any of the known cat rexoid variants (unpublished data, L.A. Lyons and B. Gandolfi). The identified genes and causal variants are genes that cause ectodermal dysplasia in humans and demonstrate that these novelty phenotypes can be valuable dermatological biomedical models. Current genetic research aims to genetically characterize the Lykoi cat’s unique hair coat.

The goals of this study were to: (i) provide a clinical description of the Lykoi cat hair coat; (ii) provide a histological description of the Lykoi cat skin, hair follicle, hair shafts, hair follicle density, and other pilosebaceous components; (iii) examine Lykoi cat skin
dermatoscopically; and (iv) compare Lykoi histologic findings to control domestic shorthair (DSH) cats.

Materials and Methods

A prospective, descriptive study was undertaken to achieve the aforementioned goals. Seven client-owned Lykoi cats were presented to local veterinary clinics (Overland Park, KS and Vonore, TN, USA) for examination and biopsy collection. All procedures were performed with informed owner consent under the University of Missouri institutional animal care and use protocol 8701. Seven control DSH cats were examined and biopsied opportunistically by shelter veterinarians at animal shelters while anesthetized for routine castration. Measures taken to minimize pain or discomfort during procedures are described below.

Examination of the hair coat, skin, vibrissae, claws, and teeth of seven Lykoi cats and seven DSH cats with normal coats was performed prior to biopsy. Trichograms were performed to determine if hair shaft abnormalities were evident. Following examination, full thickness skin biopsies were collected with the cats sedated. Sedation or general anesthesia was achieved with intravenous dexmedetomidine hydrochloride (Dexdomitor; Orion Pharma, Espoo, Finland) (one Lykoi cat), masked sedation with isoflurane gas (six Lykoi cats), or the anesthetic protocol set by the respective shelter medicine programs during the time of castration (seven control cats). Sedative and anesthetic procedures were supervised by a veterinarian and/or trained veterinary technician. The skin was lightly clipped with a #40 clipper blade and local anesthesia was performed by injecting 2% lidocaine hydrochloride (Sparhawk Laboratories, Inc., Lenexa, KS, USA) at the biopsy site. Site selection was based on the observed density of the Lykoi hair coat on different body sites to allow for comparison of sites. One biopsy sample was collected each from the dorsal thorax between the scapulae (interscapular) and the left lateral antebrachium proximal to the level of the elbow from all cats. A 6 mm punch biopsy (Acu-Punch®, Acuderm, Inc.) was used to collect samples. Tissue samples were fixed in 10% buffered formalin.

After processing, the samples were embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin (H&E). Both longitudinal and transverse sectioning was performed. All slides were examined using a Nikon Eclipse E400 light microscope (Nikon Instruments Inc.; Melville, NY, USA). Digital images were taken with a Canon Vivia HF S30 camera (Canon Inc.; Ōta, Tokyo, Japan).

The number of hair follicles and hair shafts per hair follicle group were counted on transverse sections for 10 hair follicle groups per cat at the level of the isthmus (5 from each biopsy site). The type of hair follicle (central primary, lateral primary, or secondary) and stage of growth (anagen, telogen, or hairless) was noted for 40–50 hairs per cat on transverse sections. Anagen hairs were characterized by the presence of an inner root sheath, and telogen hairs were characterized by the presence of trichilemmal keratin. Diameters of primary and secondary hairs were measured for each cat on transverse sections (5–15 primary and secondary hairs per cat). The presence or absence of a hair shaft medulla was noted on transverse sections for 20 hairs per cat. Anagen bulb diameters were measured for visible anagen bulbs in each longitudinal section (0–7 anagen bulbs per cat). The depths of hair follicles, regardless of hair follicle stage, were measured from longitudinal sections (5–13 follicles per cat). Anagen bulb depth was compared separately from average total hair follicle depth.

Additionally, the follicular density was calculated by counting all hair follicles within a 1 mm² area. The following equation was used: follicular density = number of hair follicles in histological section / area of histological section. To determine if there was a difference in hair follicle density at different body sites, the measured density of hair follicles at interscapular and lateral antebrachial sites was compared.

Two dimensional sebaceous gland areas were quantified on transverse sections using the frehand selection tool of the Image J software (National Institutes of Health; Bethesda, MD, USA) applied to digital images of six hair follicle groups per cat (three from each biopsy site) at the level of the isthmus. The software was calibrated using a digital image of a slide micrometer at each of the necessary objectives (4×, 10×, and 20×). The total area of each hair follicle group was also measured. Average sebaceous gland area per hair follicle group was compared for Lykoi and control cats.

Next, the total area of sebaceous glands per cat was compared to the total area of the hair follicle groups to determine a percentage area of sebaceous glands per hair follicle group area. The following ratio was used: total...
sebaceous gland area of hair follicle group / total hair follicle group area. The average sebaceous gland area per average hair follicle group area (as a percentage) for both biopsy sites was compared between populations.

Biopsy samples from the interscapular and lateral antebrachial regions of Lykoi cats were immunohistochemically stained for CD3. Additionally, a subset of domestic shorthair and Lykoi cat samples were stained with Cytokeratin 8/18 to help differentiate between follicular and glandular components of the pilosebaceous units. Briefly, the tissue slides were pretreated by a heat-induced antigen retrieval method using citrate buffer (0.01 M, pH 6.0). The primary antibodies were rabbit anti-CD3 polyclonal antibody (Dako, Carpinteria, CA) and rabbit anti-cytokeratin 8/18 monoclonal antibody (Dako, Carpinteria, CA). EnVision™+ system (Dako, Carpinteria, CA) was used for antigen detection and the immunoreactivity was visualized by using AEC Chromogen (Biocare Medical, Concord, CA). Haematoxylin was used for counterstain.

Ten hair follicle groups per Lykoi cat were examined on CD3 immunohistochemistry sections. A preset scale for quantifying lymphocyte numbers was developed by the authors, and the number of perifollicular to mural lymphocytes was quantified as none, mild (< 15 lymphocytes), moderate (15–40 lymphocytes), or severe (> 40 lymphocytes) for each examined hair follicle group.

Dermatoscopic examination of seven Lykoi cats and four control cats was performed. All cats’ hair coats were lightly clipped with a #40 clipper blade. A Firefly DE300 digital dermatoscope (FireflyGlobal; Belmont, MA, USA) was used to capture images, and the FireFlyPro software program (FireflyGlobal, Belmont, MA, USA) was used to view images and compare observed density of the hair coat. The hairs were not numerically assessed for density on the images.

Results

Clinical description of animals

Lykoi cats

Four Lykoi cats were male and three were female. Lykoi cats from different genetic backgrounds were used for sample collection when possible. All Lykoi cats were sexually intact and ages ranged from three months to two years with a median age of 9 months. An analysis of the developed pedigree as reported by the breeders suggested an autosomal recessive mode of inheritance (Fig. 1). All outcrosses of a Lykoi cat with an unrelated DSH produced cats with normal hair coats for the breed, including male offspring from both male and female Lykoi cats. Because multiple unrelated litters from different parts of the country were presented to the founders with this unique appearance, a multigenic or compound heterozygous inheritance trait cannot be excluded.

Dermatologic examination revealed a variably decreased density of hairs on the body with comedone formation along the dorsum and dorsal tail. Variability in hair coat thickness was observed between cats. Lykoi cats were more sparsely haired on the limbs, feet, face, and ears compared to the trunk and head. A common finding included complete alopecia of the rostral muzzle and periocular skin (Fig. 2). Visualized hairs appeared to be mostly primary guard hairs with fewer secondary hairs present. Pigmentation of the hairs included a mixture of white and black hairs in a roan pattern, and the pigment remained the same throughout the entirety of the hair shaft. Vibrissae were straight and appeared similar in length to control cats. All cats had darkly pigmented adherent debris on parts or the entirety of the claws, and some cats had mild amounts of brown debris on the feet and face. The claws were otherwise considered normal. No abnormalities in dentition were detected. No significant abnormalities in hair shaft structure were noted on trichography.

Control cats

All control cats were DSH cats. Three were male, four were female, and all animals were sexually intact. Ages ranged from three months to three years with a median age of 12 months. Longitudinal and transverse sections of biopsy samples were available for all seven cats. No dermatological abnormalities were observed. No abnormalities in hair follicles, hair shafts, or periadnexal structures were observed from control cat samples.

Histologic description of skin

Epidermis

Lykoi cats often had mild to moderate surface orthokeratotic hyperkeratosis. The epidermis was considered of normal thickness for the anatomical body sites examined for Lykoi and DSH cats and ranged from two to four cell layers thick.

Hair follicles

Lykoi cats had a compound hair follicle structure similar to DSH cats. Numerous dilated primary and secondary hair follicles (Fig. 3c, 3f) were noted on both longitudinal and transverse sections from Lykoi cats; some Lykois had a higher proportion of dilated follicles than other Lykoi cats. Occasionally, dilated follicles were filled with excessive keratin.

Fig. 1. Lykoi cat pedigree. Black circles and squares are Lykoi females and males, respectively. White circles and squares are black domestic shorthair females and males, respectively. Half-filled symbols indicate obligate carriers of the Lykoi coat. Black arrows in the lower left corner of individual cats are the Lykoi probands identified from different litters and different areas of the USA.

Fig. 2. Typical appearance of the Lykoi cat. Note periocular, rostral muzzle, and partial limb alopecia with a mixture of black and white hairs on the trunk, tail, and head. Photo courtesy of Brittney Gobble.
Lykoi follicular infundibula were dilated and contained excess keratin consistent with the comedones seen on physical examination. Hair follicle depth from Lykoi samples was overall more shallow relative to DSH cats (Fig. 3b). While some of these follicles were telogenized, some anagen bulbs were seen in the more superficial dermis compared to DSH cats (Fig. 4). Not all Lykoi anagen follicles were miniaturized. Some anagen follicles adjacent to the superficially located anagen and telogen follicles reached the deep dermis and/or subcutis as seen in normal DSH cats. Hair follicle epithelium was often thin and follicles were sometimes curved (Fig. 5c). Dermal papillae appeared normal in organization but were often “kinked” in a different orientation compared to the rest of the hair follicle (Fig. 5d). Occasional hair follicles and hair shafts were seen in a different orientation than surrounding normally oriented follicles (Fig. 5a). Variable amounts of lymphocytic inflammation were consistently appreciated around and within Lykoi hair follicle walls (see below: Lymphocytic mural...

No obvious abnormalities of the inner and outer root sheath structures were observed.

**Hair shafts**

Hair shaft numbers were sparse in Lykoi cat hair follicle groups compared to DSH cats (see below: Quantitative assessment of hair follicles). Many Lykoi follicles (both primary and secondary) in all growth stages (including anagen) were devoid of hair shafts all together, whereas controls exhibited only hairless telogen follicles. Hair follicle groups commonly had only one or two hair shafts present within secondary hair follicles. Subjectively, Lykoi hair shafts appeared smaller compared to DSH cats. A mixture of medullated and nonmedullated hair shafts was seen for both primary and secondary hairs. Many hair shafts appeared normal, but occasional dysplastic hair shafts were noted (Fig. 5b).

**Sebaceous glands, epitrichial (apocrine) glands, and arrector pili muscles**

Lykoi cats often subjectively appeared to have an increased proportion of sebaceous glands at the level of the isthmus compared to DSH cats (Fig. 3e). Sebaceous glands had a normal appearance. Some epitrichial glands were mildly dilated in the deeper dermis. Epitrichial glands were immunoreactive for Cytokeratin 8/18 (Fig. 6). Arrector pili muscles appeared large next to the Lykoi cat’s miniaturized hair follicles (Fig. 5c), especially on interscapular sections where arrector pili muscles are expected to be larger than other body sites. No abnormalities were seen in the observed arrector pili muscles.

**Dermis and subcutis**

All findings in the dermis and subcutis were considered normal. Mild infiltration of mast cells in the superficial dermis of Lykoi and DSH cats was seen.

**Quantitative assessment of hair follicles**

Mean ± SD hair follicles per hair follicle unit differed between Lykoi (14.7 ± 2.9) and DSH (23.4 ± 5.4) cats (p<0.01). Similarly, the median (range) number of hair shafts per hair follicle group differed between Lykoi (1.3, range: 0.4–5.7) and DSH (18.8, range: 10.6–26.6) cats.
186 Histology of Lykoi Cat Skin

Finally, the mean follicle density (follicles/mm²) differed between Lykoi (interscapular: 54.5 ± 13.5; lateral antebrachium: 49.7 ± 11.0) and DSH (interscapular: 84.3 ± 12.6; lateral antebrachium: 132.9 ± 24.0) cats at both interscapular and lateral antebrachial biopsy sites (p<0.01). The follicle density did not differ between the interscapular and antebrachial regions within Lykoi cats despite observed body region hair shaft density differences (p=0.49).

Hair cycle stage

Hair cycle stage (anagen, telogen, hairless) was compared between Lykoi and DSH cats. Mean ± SD percentage of total anagen hair follicles for Lykoi cats (25 ± 14%) was significantly lower than DSH cats (45 ± 22%) (p=0.03). Mean ± SD percentage telogen follicles did not significantly differ between Lykoi (21 ± 13%) and DSH (21 ± 11%) cats (p=0.46). Lykoi cats had a significantly higher percentage of hairless follicles (55 ± 22%) compared to DSH (34 ± 13%) cats (p=0.03).

Anagen bulb diameter

Mean ± SD anagen bulb diameters were 87 µm ± 22 and 84 µm ± 14 for Lykoi cats and DSH cats, respectively (p=0.41).

Hair follicle depth

Mean ± SD anagen bulb depth was not significantly different between Lykoi (1.38 mm ± 0.27) and DSH (1.25 mm ± 0.13) cats (p=0.17). However, average total hair follicle depth (regardless of hair cycle stage) was significantly lower for Lykoi (0.95 ± 0.15 mm) cats compared to DSH (1.14 ± 0.21) cats (p=0.04).

Hair shaft diameter

Mean (± SD) primary hair shaft diameter for Lykoi cats and DSH cats was 39 µm (± 29) and 47 µm (±11), respectively (p=0.04). Mean (± SD) secondary hair shaft diameter for Lykoi cats and DSH cats was 13 µm (± 2) and 16 µm (± 2), respectively (p=0.02).

Medullated hair shafts

The mean percentage (± SD) medullated hair shafts for Lykoi cats was 67% (± 25%) and for DSH cats was 41% (± 26%) (p=0.04). Conversely, the mean percentage (± SD) nonmedullated hair shafts for Lykoi cats was 33% (± 25%) and for DSH cats was 59% (± 26%) (p=0.04).

Sebaceous gland area

Two dimensional measurement was used to determine average sebaceous gland area per follicle group. Mean (± SD) interscapular sebaceous gland volume per hair follicle group for Lykoi cats was 19,937.7 pixels³ (± 10,254.9) compared to 9,833.7 pixels³ (± 5,784.5) for DSH cats (p=0.02). Mean (± SD) lateral antebrachial

(p<0.01). Finally, the mean follicle density (follicles/mm²) differed between Lykoi (interscapular: 54.5 ± 13.5; lateral antebrachium: 49.7 ± 11.0) and DSH (interscapular: 84.3 ± 12.6; lateral antebrachium: 132.9 ± 24.0) cats at both interscapular and lateral antebrachial biopsy sites (p<0.01). The follicle density did not differ between the interscapular and antebrachial regions within Lykoi cats despite observed body region hair shaft density differences (p=0.49).

Hair cycle stage

Hair cycle stage (anagen, telogen, hairless) was compared between Lykoi and DSH cats. Mean ± SD percentage of total anagen hair follicles for Lykoi cats (25 ± 14%) was significantly lower than DSH cats (45 ± 22%) (p=0.03). Mean ± SD percentage telogen follicles did not significantly differ between Lykoi (21 ± 13%) and DSH (21 ± 11%) cats (p=0.46). Lykoi cats had a significantly higher percentage of hairless follicles (55 ± 22%) compared to DSH (34 ± 13%) cats (p=0.03).

Anagen bulb diameter

Mean ± SD anagen bulb diameters were 87 µm ± 22 and 84 µm ± 14 for Lykoi cats and DSH cats, respectively (p=0.41).

Hair follicle depth

Mean ± SD anagen bulb depth was not significantly different between Lykoi (1.38 mm ± 0.27) and DSH (1.25 mm ± 0.13) cats (p=0.17). However, average total hair follicle depth (regardless of hair cycle stage) was significantly lower for Lykoi (0.95 ± 0.15 mm) cats compared to DSH (1.14 ± 0.21) cats (p=0.04).

Hair shaft diameter

Mean (± SD) primary hair shaft diameter for Lykoi cats and DSH cats was 39 µm (± 29) and 47 µm (±11), respectively (p=0.04). Mean (± SD) secondary hair shaft diameter for Lykoi cats and DSH cats was 13 µm (± 2) and 16 µm (± 2), respectively (p=0.02).

Medullated hair shafts

The mean percentage (± SD) medullated hair shafts for Lykoi cats was 67% (± 25%) and for DSH cats was 41% (± 26%) (p=0.04). Conversely, the mean percentage (± SD) nonmedullated hair shafts for Lykoi cats was 33% (± 25%) and for DSH cats was 59% (± 26%) (p=0.04).

Sebaceous gland area

Two dimensional measurement was used to determine average sebaceous gland area per follicle group. Mean (± SD) interscapular sebaceous gland volume per hair follicle group for Lykoi cats was 19,937.7 pixels³ (± 10,254.9) compared to 9,833.7 pixels³ (± 5,784.5) for DSH cats (p=0.02). Mean (± SD) lateral antebrachial

(p<0.01). Finally, the mean follicle density (follicles/mm²) differed between Lykoi (interscapular: 54.5 ± 13.5; lateral antebrachium: 49.7 ± 11.0) and DSH (interscapular: 84.3 ± 12.6; lateral antebrachium: 132.9 ± 24.0) cats at both interscapular and lateral antebrachial biopsy sites (p<0.01). The follicle density did not differ between the interscapular and antebrachial regions within Lykoi cats despite observed body region hair shaft density differences (p=0.49).

Hair cycle stage

Hair cycle stage (anagen, telogen, hairless) was compared between Lykoi and DSH cats. Mean ± SD percentage of total anagen hair follicles for Lykoi cats (25 ± 14%) was significantly lower than DSH cats (45 ± 22%) (p=0.03). Mean ± SD percentage telogen follicles did not significantly differ between Lykoi (21 ± 13%) and DSH (21 ± 11%) cats (p=0.46). Lykoi cats had a significantly higher percentage of hairless follicles (55 ± 22%) compared to DSH (34 ± 13%) cats (p=0.03).

Anagen bulb diameter

Mean ± SD anagen bulb diameters were 87 µm ± 22 and 84 µm ± 14 for Lykoi cats and DSH cats, respectively (p=0.41).

Hair follicle depth

Mean ± SD anagen bulb depth was not significantly different between Lykoi (1.38 mm ± 0.27) and DSH (1.25 mm ± 0.13) cats (p=0.17). However, average total hair follicle depth (regardless of hair cycle stage) was significantly lower for Lykoi (0.95 ± 0.15 mm) cats compared to DSH (1.14 ± 0.21) cats (p=0.04).

Hair shaft diameter

Mean (± SD) primary hair shaft diameter for Lykoi cats and DSH cats was 39 µm (± 29) and 47 µm (±11), respectively (p=0.04). Mean (± SD) secondary hair shaft diameter for Lykoi cats and DSH cats was 13 µm (± 2) and 16 µm (± 2), respectively (p=0.02).

Medullated hair shafts

The mean percentage (± SD) medullated hair shafts for Lykoi cats was 67% (± 25%) and for DSH cats was 41% (± 26%) (p=0.04). Conversely, the mean percentage (± SD) nonmedullated hair shafts for Lykoi cats was 33% (± 25%) and for DSH cats was 59% (± 26%) (p=0.04).

Sebaceous gland area

Two dimensional measurement was used to determine average sebaceous gland area per follicle group. Mean (± SD) interscapular sebaceous gland volume per hair follicle group for Lykoi cats was 19,937.7 pixels³ (± 10,254.9) compared to 9,833.7 pixels³ (± 5,784.5) for DSH cats (p=0.02).

Sebaceous gland volume per hair follicle group for Lykoi cats was 21,092.2 pixels² (± 10,232.1) compared to 9,248.5 pixels² (± 6,031.0) for DSH cats (p=0.01).

When sebaceous gland area was compared to total hair follicle group area as a percentage of total area of the hair follicle group, results indicated that there was no significant difference between Lykoi (21.3 ± 6.0%) cats and DSH (16.5 ± 4.0%) cats (p=0.10). Mean sebaceous gland area per average hair follicle group area for Lykoi and DSH cats was not significantly different at the interscapular (p=0.26) or lateral antebrachial (p=0.14) biopsy sites.

Lymphocytic mural folliculitis

Perifollicular to mural T-cell lymphocytic infiltration was verified with CD3 immunohistochemistry (Fig. 7). Seventy-seven percent of follicle groups observed for the seven Lykoi cats (10 follicle groups per cat) had a mild to severe amount of lymphocytic perifollicular to mural infiltration (Table 1).

Table 1. Lykoi perifollicular to mural lymphocytic infiltration

<table>
<thead>
<tr>
<th>Lykoi Cat</th>
<th>Sex</th>
<th>Age</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>1 year</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>F</td>
<td>1 year</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>8 months</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>F</td>
<td>2 years</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>F</td>
<td>3 months</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>M</td>
<td>9 months</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Number of Lykoi cat hair follicles (10 follicles per cat) with lymphocytic perifollicular to mural inflammation presented according to cat A–G. (None = 0, Mild ≤ 15 lymphocytes, Moderate = 15–40 lymphocytes, Severe ≥ 40 lymphocytes).

Dermatoscopic examination of animals

Lykoi cat hairs were subjectively reduced in density compared to DSH cats on dermatoscopic images (Fig. 8). Primary guard hairs with fewer secondary hairs were seen on evaluation of Lykoi cat images compared to DSH cat images. Both Lykoi and DSH cats occasionally had mild scale on the skin between the hairs.

Discussion

Cat breeds generally are developed by “novelty” selection. A novel trait, often an unusual hair coat, coloration or morphology, is recognized by the public and then cats with this novel trait are crossbred to develop a breed. During the production of the breed individuals, health concerns can sometimes be recognized that are associated with the traits. Examples
include lameness and incontinence in the Manx breed or osteochondrodysplasia in the Scottish Fold\(^1, 8, 29\). Many of these associated diseases prove useful as biomedical models for human disease. If proven the same gene as a human condition, therapies used in humans may help cats, and new therapies developed in cats may support human health. In addition, now that cat registries recognize the health concerns in some historically acceptable breeds, such as Manx and Scottish Fold\(^1, 8, 29\), cat registries are more diligent to promote new breeds only after they have shown that the new traits of interest do not affect the cat’s health. Thus, the clinical definitions of novel traits in cats can support their breed development and perhaps be used as biomedical models.

Currently, there are no published clinical data describing the hair coat or a histologic analysis of the skin, hair follicles, and adnexal structures of the Lykoi cat. Breeding advancements have resulted in the growth of Lykoi cat numbers and dispersal of the breed to new parts of the world. Increased numbers and recognition of the breed by cat fanciers will impact how frequently the Lykoi cat is encountered in clinical practice.

Lykoi cats have a unique distribution of alopecia on the rostral muzzle and periorcular skin as well as partial alopecia of the remainder of the body. This distribution of alopecia is undescribed in any other feline breed in current literature. Additionally, the hair coat consists of a combination of white and black hairs in a roan pattern. A “roan” coat pattern can be found in other species such as horses\(^24\) and cattle\(^26\), but is not documented in any other feline breeds.

Histologic analysis showed that Lykoi cats had a significantly decreased density of both hair follicles and hair shafts within hair follicle groups compared to control cats. These findings were further corroborated by dermatoscopic examination which showed a reduced density of hair shafts for Lykoi cats compared to DSH cats. This reduction in hair follicles and hair shafts resulted in visible alopecia compared to DSH cats. A reduced number of hair follicles are the result of either a reduction in hair placode formation during embryogenesis or from the complete loss of hair follicles due to defects in morphogenesis\(^17\). Many cases of congenital alopecia in dogs, Sphynx cats, horses, and cattle are a result of structural changes in hair follicles that result in abnormal, fragile hair shaft fibers or even complete lack of hair shaft formation\(^17\). Concurrent reduction in follicle number and hair shaft formation (due to hair follicle dysplasia) is not described for any other feline hair coat and is unique to the Lykoi cat. Specific causes for the reduced number of follicles require further characterization.

Miniaturization of hair follicles has not been clearly defined in veterinary medicine. In human medicine, miniaturized follicles in male pattern alopecia are defined by the conversion of terminal hairs to vellus hairs\(^40\). A vellus hair has a cross-sectional (transverse) diameter of 0.03 mm or less, and its diameter does not exceed the thickness of the investing root sheath\(^11, 32\). In veterinary medicine, conditions such as acquired pattern alopecia (pattern baldness) are characterized by moderate to occasional severe diminution (miniaturization) in size, moderately thinner and shorter follicles, and fine residual hair shafts with no follicular distortion or irregularity of contour or arrest of the hair growth cycle\(^9\). Lykoi cat primary and secondary hairs were statistically smaller in diameter than controls, and the average hair follicle depth was decreased for Lykoi cats consistent with the description of miniaturized follicles in canine pattern baldness\(^9\). Additionally, Lykoi cat follicular epithelium was often fine, especially for shorter follicles. Lykoi cats did, however, exhibit hair follicle dysplasia not seen in canine pattern baldness.

A recent study on the canine hair follicle cycle suggests that the most easily visible and important feature to determine hair cycle stage is the absolute position of the dermal papilla in the dermis or subcutaneous fat\(^9\). Though this information has not been applied to cats, feline hair cycle stages may behave similarly to those of the canine. The Lykoi cat exhibited occasional anagen bulbs and dermal papilla much higher in the dermis than control DSH cats. This finding along with the presence of other superficial dermal shallow follicles in other hair cycle stages further supports the conclusion that follicles are truly miniaturized.

Based on H&E staining alone, dilated hair follicles could not be easily distinguished from dilated epidermal glands. Multiple immunohistochemical stains were considered to conclusively identify each structure correctly. Cytokeratins 8 and 18 are located in most secretory glandular cells in humans\(^1\). Cytokeratin 8/18 has also been used in feline tissue to detect simple glandular epithelium\(^10, 12\). Lykoi and DSH cat biopsy specimens were stained with Cytokeratin 8/18 to differentiate dilated hair follicles from epithelial
glands. Lykoi and DSH cat normal epithrichial glands were immunoreactive on Cytokeratin 8/18 immunohistochemistry, but the observed dilated hair follicles, normal hair follicles, and sebaceous glands and ducts were not. The presence of rare hair shafts and excessive keratin within the dilated follicles helped further support that the dilated structures were in fact dilated hair follicles.

A high percentage of hair follicle groups were affected by mild to severe lymphocytic perifollicular to mural folliculitis in all Lykoi cats. Previous studies suggest that lymphocytes in the periadnexal regions and infiltrative lymphocytic mural folliculitis do not exist in normal cats. The Lykoi cats sampled are presumed to be considered “normal” for their breed, although the breed standard is new and under development. One study showed that cats with inflammatory dermatosis of various etiologies were more likely to have infiltrative lymphocytic mural folliculitis with allergic dermatoses than nonallergic dermatoses. Lykoi cats were not described by the owners as having any consistent dermatologic abnormalities or allergic symptoms. The significance of lymphocytic mural folliculitis in Lykoi cats is unclear at this time.

Further characteristics of Lykoi cats include adherent brown debris on the claws, feet, and face which has been described in other breeds such as the Sphynx cat. Possible explanations include an increased sebaceous gland volume and sebum production. Measurement comparisons showed Lykoi cats did have an increased average sebaceous gland area per hair follicle group compared to DSH cats. Chemical analysis of sebum and comparison to control cats was beyond the scope of this study and may be warranted for further characterization of differences in sebum content.

Dermal inflammation consisting of mild infiltration of mast cells was seen for both Lykoi and DSH cats. A recent study quantified superficial dermal mast cells in felines with both normal skin and inflammatory skin disease (allergic and nonallergic) and found that median mast cell counts did not significantly differ between groups. The presence of occasional mast cells in the superficial dermis of Lykoi cats and DSH cats is therefore likely a normal finding.

Intact Lykoi cats reportedly undergo molting of the hair coat at least once in life shortly after birth (2–4 weeks) as well as during times of hormonal change such as sexual maturity. Molting caused by hormonal fluctuation is a unique characteristic of Lykoi cats that warrants further investigation.

One limitation of the current study includes the small number of relatively young Lykoi cats. Since the Lykoi cat is a newly recognized breed, the total number of Lykoi cats in the breeding pool was limited to just over 20 cats in the world at the time of sample collection. Additionally, all cats were under the age of four years. However, based on the statistically significant differences documented above, the study had a sufficient power to differentiate Lykoi cats from DSH cats for most of the measured outcomes.

In summary, Lykoi cats exhibit a novel variant of the feline coat phenotype. The data presented here provides a thorough phenotypic characterization of Lykoi cat skin and supports the cat as a useful biomedical model for dermatological diseases and disorders. Decreased follicular density, follicles per hair follicle group, and hair shaft density compared to DSH control cats were features of the Lykoi cat. Lykoi cats also had a reduced percentage of anagen follicles, increased percentage of hairless follicles, decreased average hair shaft diameter, and decreased average hair follicle depth compared to DSH controls. Abnormally miniaturized, dilated, and dysplastic hair follicles were commonly observed. Lykoi cats had an increase in average sebaceous gland area per hair follicle group compared to DSH cats. Lymphocytic mural folliculitis was a consistent finding for all the Lykoi cats examined in this study. Further studies are indicated to elucidate the impact of this inflammation on the breeds’ relative alopecia. Studies examining hair follicle morphogenesis and larger numbers of Lykoi cats during different seasons, hormonal changes, and ages are also indicated.

Acknowledgments

The authors would like to thank the Lykoi cat breeders, Dr. Johnny Gobble, Brittnay Gobble, and Wendy Barnes. We would also like to thank the Great Plains SPCA (Independence, MO), Wayside Waifs (Kansas City, MO), and Central Missouri Humane Society (Columbia, MO) animal shelters for their efforts to make this study possible. We would also like to thank Brittnay Gobble for her professional Lykoi cat photographs.

This study was funded by University of Missouri
College of Veterinary Medicine Clinician Scientist Research Grant and the Gilbreath McLorn Endowment.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

References