Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #8 09 October 2024

CASE I:

Signalment:
8-month-old, intact female, Pembroke Welsh Corgi, *Canis lupus familiaris*, canine.

History:
This patient presented with non-pruritic, erythematous, ulcerated, crusty skin lesions affecting the nose, muzzle, periocular tissue OU, ear pinna AU, and multiple nail beds of all four feet. Lesions started a month ago.

Gross Pathology:

Three punch biopsies of haired skin from multiple anatomic locations (forehead, muzzle, digit 4 of right hindfoot) were submitted for evaluation, with no overt gross findings.

Laboratory Results:
Aerobic bacterial culture – trace numbers of *Bacillus cereus, Staphylococcus equorum, Rummeliibacillus sp.*, and *Cellulolosimicrobium cellulans*

Fungal culture – absence of growth

Microscopic Description:
Haired skin (forehead, muzzle, digit 4 of right hindfoot): Multiple sections of haired skin are examined in which similar features are observed in all samples. Extending from the mid to deep dermis, and sharply demarcated from adjacent normal tissue in some

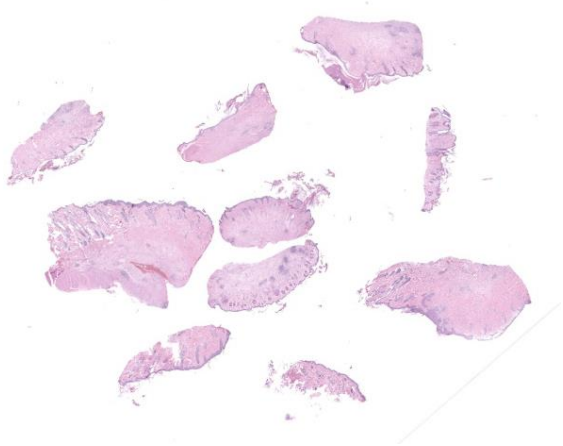

Figure 1-1. Haired skin, dog. Multiple sections of haired skin are submitted for examination. (HE, 4X)

sections, collagen fibers are regionally disorganized and pale to hypereosinophilic with variable loss of distinct bundle architecture. Medium to small caliber dermal vessels are distorted, characterized by pale, hyalinized eosinophilic tunics with loss of distinct separation of layers. Affecting other vessels, are circumferential perivascular cuffs of lymphocytes and plasma cells with fewer neutrophils and macrophages, which extend into the adjacent dermal stroma. Remaining pericytes and/or smooth muscle nuclei are frequently enlarged and prominent. Occasional aggregates of lymphocytes and histocytes are embedded in the dermis. Occasional clusters of lymphocytes and macrophages are also observed in the skeletal muscle layer, with separation, attenuation, and atrophy of affected fibers. Dermal nerve fibers have shrunken ax

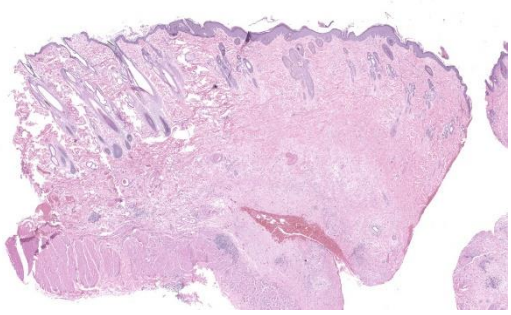

Figure 1-2. Haired skin, dog. In the right half of the section, beneath the hyperplastic epithelium, follicles are atrophic, and dermal collagen is poorly staining. There are cellular infiltrates surrounding deep dermal vessels and infiltrating the panniculus carnosus. (HE, 21X)

ons in decreased quantity, and rare lymphocytes and macrophages are embedded within affected fibers. Superficial to the affected dermis, multiple hair follicles are atrophied with a prominent fibrous root sheath. The overlying epidermis is hyperplastic, covered by segments of compact orthokeratotic to parakeratotic keratin that sometimes distend follicular ostia. Occasional serocellular crusts are also present.

Contributor's Morphologic Diagnosis:
Haired skin (forehead, muzzle, digit 4 of right hindfoot): Vasculitis with ischemic dermatopathy, regionally extensive, severe

Contributor's Comment:
Based on the clinical history, signalment, and histopathologic findings, a diagnosis of ischemic dermatopathy was warranted in this case. Ischemic dermatopathies (ID) refer to a group of cutaneous diseases that involve inflammation of the skin, vessels, and sometimes muscle or subcutaneous tissue, derived from a variety of causes. These conditions have been reported in humans[7] and dogs.[1,4,6,7,9,10] There are 5 main subtypes reported in dogs which include: 1) familial dermatomyositis (DM), 2) juvenile onset ischemic dermatopathy (dermatomyositis-like disease), 3) localized post-rabies vaccine panniculitis, 4) generalized vaccine-induced ischemic dermatopathy, and 5) generalized idiopathic ischemic dermatopathy.[4,6,10] Although the pathogenesis is incompletely elucidated for these diseases, the generally accepted premise is that of a cell-poor vasculitis, leading to decreased oxygen perfusion of the tissue, and subsequent ischemic change.[1,4,9]

Across the various subgroups of ischemic dermatopathies, clinical, gross, and histopathologic features are often similar. Clinically overall, anatomic locations most susceptible for mechanical trauma (i.e., bony protuberances), or distal extremities (i.e., phalanges) are preferentially affected.[4] Gross lesions include alopecia, scab formation, variation in pigmentation (hyper- or hypo-), and thinning of the skin.[4,8] Histopathologic lesions consist of follicular atrophy, hyperkeratosis, smudgy and indistinct collagen fibers, interface dermatitis and/or clefting at the epidermal basement membrane.[6,8] Diagnosing a specific subtype can sometimes be challenging, which requires a thorough clinical history and signalment, coupled with gross and histopathologic features. The use of electromyography or muscle physiology studies have also been useful in some cases.[1,3]

Familial Dermatomyositis (FDM) and juvenile onset ischemic dermatopathy (dermatomyositis-like or DM-like) have several identical clinicopathological features. They both occur in young dogs. Most commonly, lesions originate on the muzzle, periorbital and perioral tissues, dorsum of the distal phalanges and sometimes footpads,[4,8] but additionally the tips and folds of the pinnae, tip of the tail, and claw folds can also be affected.[4] Muscle changes and clinical abnormalities

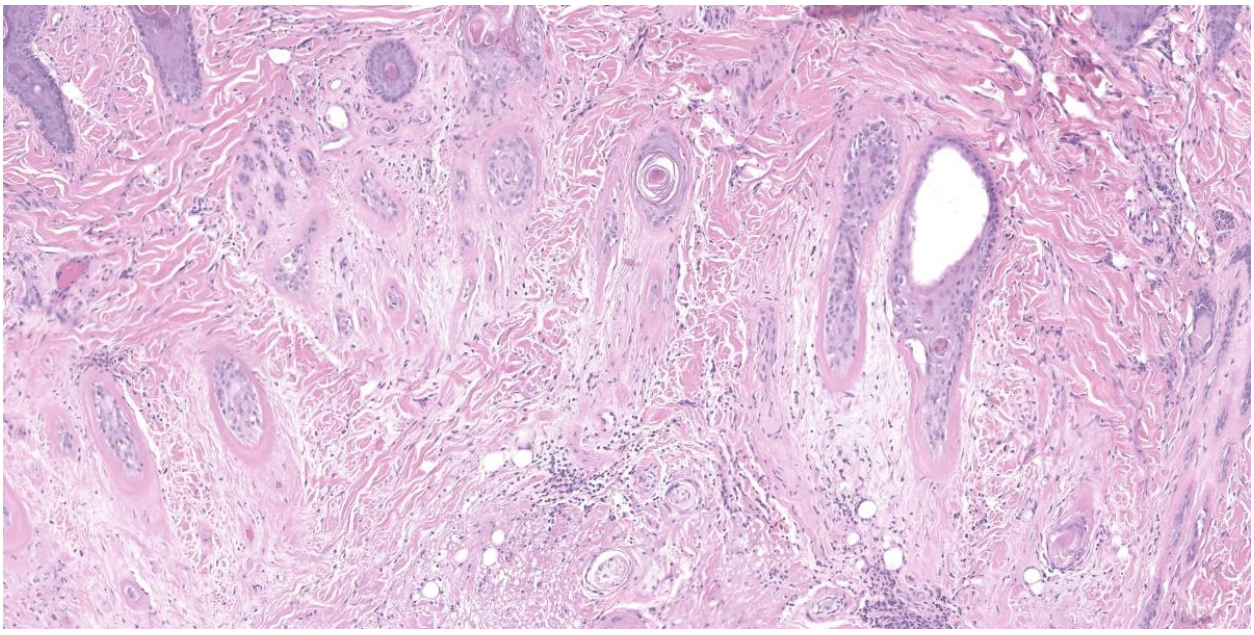

Figure 1-3. Haired skin, dog. Hair follicles are atrophic and surrounded by smudgy, diminished collagen fibers.

can be subtle but are often detected with histopathologic evaluation and/or electromyographic studies.[3] When present, atrophy can be most apparent affecting the temporal and masseter muscles.[4] Megaesophagus, growth retardation, and infertility are also reported findings.[4] FDM was first described in Collies,[5] with later reports in Shetland sheepdogs, Portuguese water dogs, and Belgian Tervuren dogs[3,4,6,10] whereas DM-like disease occurs sporadically in breeds without a scientifically proved genetic or familial component.[2,10]

Localized post-rabies panniculitis typically occurs 1 to 3 months after vaccine administration,[6] and initially, there is focal alopecia at the affected site that can progress to hyperpigmentation.[6] The neck and shoulder region in the vicinity of the scapulae, are primarily affected.[4]. Deposition of rabies viral antigens into the wall of vessels is suspected as the inciting stimulus.[4] Breeds predisposed to this condition include Toy and Miniature Poodles and Bichon Frises,[4,6] but there are reports also involving Yorkshire terriers and Chihuahuas.[8] The most characteristic histopathologic findings are lymphocytic perivascular inflammation and vasculitis,[11] coupled with other previously described features of ID. This condition can become more widespread and severe in some adult dogs, with the development of fever, lethargy, depression, and/or elevation in liver enzymes (generalized vaccine-induced ischemic dermatopathy).[4,6]

Generalized vaccine or idiopathic ischemic dermatopathy are identical clinicopathologically, and the presence of alopecia over a previous rabies vaccination site can assist in differentiating the two subtypes.[9] Generalized idiopathic ischemic dermatopathy is a diagnosis of exclusion.

For this case, the top three subtype differentials included: dermatomyositis-like disease, generalized idiopathic ischemic dermatopathy, and generalized vaccine-induced dermatopathy.

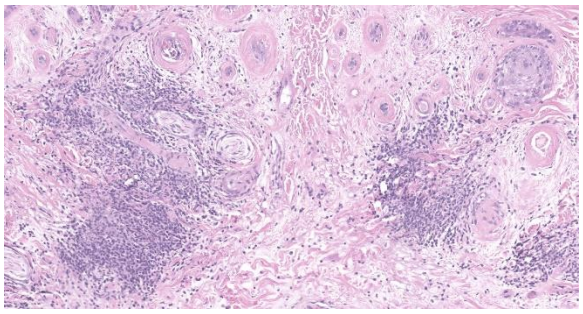

Figure 1-4. Haired skin, dog: In the deep dermis, vessel walls are thickened and vessels are surrounded by low to moderate numbers of lymphocytes, histiocytes and plasma cells. Vessels walls lack detail and are brightly eosinophilic (vasculitis). (HE, 158X)

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JPC Diagnosis:
Haired skin, dermis: Vasculitis, lymphohistiocytic, multifocal, moderate with regionally extensive adnexal atrophy, rare thrombi, and cell poor interface dermatitis.

JPC Comment:
This week's moderator was Dr. Charles Bradley, Associate Professor of Anatomic Pathology at the University of Pennsylvania. A perennial visitor to the WSC, Dr. Bradley emphasized cutaneous patterns of skin disease with participants. The first case is a good example of an interface dermatitis, albeit a cell-poor one. Case features are best observed first from low magnification with disruption of dermal collagen, loss of adnexal structures, and vasocentric lesions being helpful pickups that point at the underlying pathogenesis. Key histologic features on high magnification include disruption of the dermal-epidermal basement membrane, rare thrombi, and mild vasculitis. Although not strictly necessary for this case, PAS highlighted the basement membrane nicely and Movat's pentachrome highlighted select vessels.

Dr. Bradley also covered ancillary changes in section with conference participants. These included vacuolation of basal keratinocytes (likely a mild processing artifact) and focal parakeratosis which was attributable to tissue response to injury. In the superficial dermis, there was also granulation tissue present suggestive of previous ulceration and re-epithelization of the epidermis, perhaps from a previous episode of diminished blood flow. Likewise, the mild myositis in this case is likely a bystander lesion rather than a primary dermatomyositis – in Dr. Bradley's experience, muscle changes are rare to absent in histologic section in dermatomyositis (DM). Finally, it is also important to rule out other processes in the skin with ischemic changes such as reactive histiocytosis.

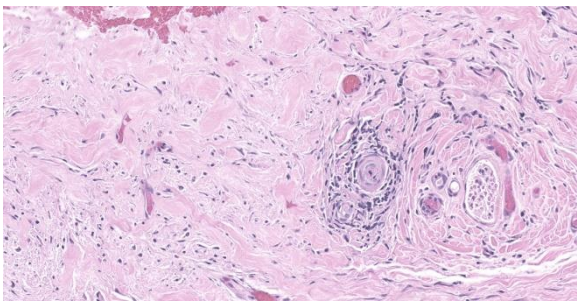

Figure 1-5. Haired skin, dog. Higher magnification of an affected vessel. Dermal collagen fibers are often markedly decreased in diameter, giving it a "smudgy" appearance (HE, 275X)

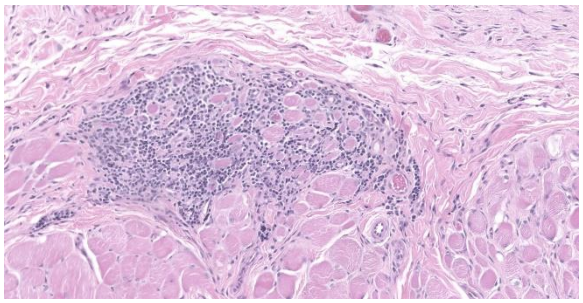

Figure 1-6. Haired skin, dog. The panniculus carnosus is occasionally infiltrated by inflammatory cells and in these areas, muscle fibers demonstrate evidence of degeneration and atrophy. (HE, 240X)

The clinical history of a young animal is helpful in this case and is suggestive of a vaccine-associated event. Histologically however, this is not distinguishable from DM. In addition, the location of the hyperpigmented alopecic skin ('poodle patch') may or may not be close to the site of vaccination, reflecting the systemic nature of this vasculitis. Deeper punch biopsies that sample the vaccine site may identify lymphohistiocytic aggregates within the subcutis that may contain vaccine adjuvant though. Dr. Bradley also touched on the role of anti-IL-31 therapy in dermatology cases, to include the use of oclacitinib and lokivetmab in modulating immune-related conditions. Apoquel, Cytopoint, and similar emerging therapies have been tried for a variety of canine skin conditions with reasonable success as an adjunct to more conventional drugs such as glucocorticoids.[8]

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CASE II:

Signalment:

5 month old, intact female, Shar Pei, *Canis familiaris*, Dog.

History:

Limited history available. Alopecia and dermatitis, has been on cephalexin. epresentative samples submitted from face and legs.

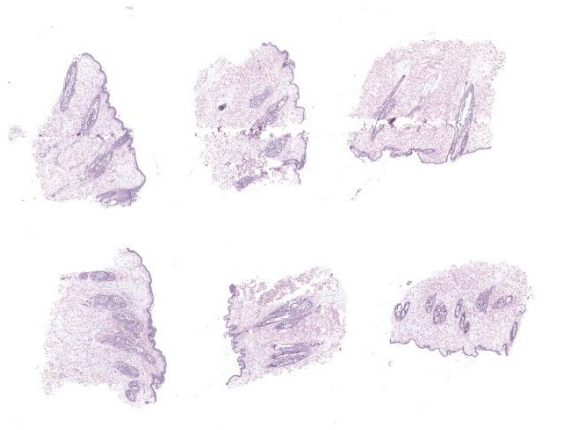

Figure 2-1. Haired skin, dog: Six punch biopsies are submitted for examination. (HE, 5X)

Gross Pathology:
3 haired skin punch biopsy specimens, 5-6mm diameter, ranging from 3-4mm deep

Microscopic Description:
Haired skin: All 3 punch biopsy samples are similar.

Diffusely, hair follicle lumina are moderately distended and filled with multiple longitudinal, transverse, and tangential sections of arthropods. These arthropods are elongate, up to 40µm diameter and 200µm in length; have a thin, eosinophilic, chitinous exoskeleton; short, jointed appendages; a hemocoel; striated musculature; digestive tract; and male or female reproductive tracts. There is minimal perifollicular as well as perivascular inflammation consisting of low numbers of lymphocytes, plasma cells, and eosinophils.

Dermal collagen fibers are diffusely fragmented and widely separated by abundant clear space and wispy, fibrillar, beaded, basophilic to amphophilic mucin. There are low numbers of histiocytes and lymphocytes scattered within the mucinous matrix.

There is clumping of melanin pigment within the follicular bulb matrix epithelium and to a lesser extent within the non-matrical follicular epithelium and follicular lumina.

Contributor's Morphologic Diagnosis:
1. Haired skin: Follicular ectasia, moderate, diffuse, with mild lymphoplasmacytic and eosinophilic perifolliculitis and numerous follicular intraluminal *Demodex canis* mites

2. Haired skin: Dermal mucinosis, diffuse, marked

3. Haired skin, hair follicles: Matrical epithelial, follicular epithelial, and intraluminal melanin clumping

Contributor's Comment:
This case represents three entities: juvenile-onset demodicosis, dermal mucinosis (a feature of "normal" skin in Shar Pei dogs), and color dilution.

Canine demodectic mange, also termed follicular mange or red mange, is one of the most common skin diseases of dogs.[6-10] It is a noncontagious disease that occurs when commensal *Demodex* mites are allowed to proliferate resulting in overpopulation.[1,5,6,9] Canine demodicosis is most commonly caused by *D. canis*, but *D. injai* can also cause disease.[1,5,8,9] *D. canis* is approximately 300µm in length, while *D. injai*, the "long-bodied mite", is 334-368µm in length.[8] Predisposing factors to *Demodex* overpopulation in its host include multiple causes of host immunosuppression.[1,6,8,9] Canine demodicosis can be either localized or generalized.[1,5,8,9] Localized disease is mild and typically self-limiting with spontaneous resolution, whereas generalized disease may spontaneously resolve but can continue into adulthood if inadequately treated, and may be fatal.[5,8] Canine demodicosis can also be either juvenile-onset or adult-onset.[1,5,8] Disease is considered adult-onset if disease onset occurs at 4 years of age or older,[8] although differentiation between these may be difficult.[1] Generalized demodicosis is typically a juvenile-onset disease.[4,8] Juvenile-onset demodicosis is thought to be

due to a genetically mediated immunodeficiency resulting in decreased T-cell function.[4,6,8,9] A recent study into the molecular pathogenesis of canine demodicosis found evidence that disease is associated with *Demodex*-induced host immune tolerance.[6] Studies into this immune tolerance identified host cellular endoplasmic reticulum stress which in turn results in the accumulation of unfolded proteins (i.e., unfolded protein response) which regulates signaling pathways involved in Toll-like receptors (especially TLR2) and promotion of M2-phenotype immunosuppressive macrophages.[7] Disease relapse, recurrence, or persistence is uncommon.[1]

Excessive accumulation of dermal mucin is termed cutaneous mucinosis.[2,5,8,10] This condition is abnormal and rare in most dogs.[5,8] In shar pei dogs, however, cutaneous mucinosis is due to a genetic mutation and is considered a normal feature which leads to their distinctive thick, wrinkled skin.[5,8,9] The mucinous material in the shar pei dermis has been identified as hyaluronan (or hyaluronic acid [HA]).[2,10] A study found that HA is produced in shar pei dermal fibroblasts in greater quantities than in control cells.[10] Shar pei cutaneous mucinosis has further been linked to increased mRNA expression of the HAS2 isoform of hyaluronan synthase (HAS), resulting in increased transcription of HAS2 by dermal fibroblasts.[2,9,10] The authors of that study suggest using the term "hereditary cutaneous hyaluronosis (HCH)" for the shar pei specific version of cutaneous mucinosis.[2] Diffuse canine cutaneous mucinosis has also been associated with hypothyroidism; this is rarely reported but clinically and histologically striking.[5,8,9] Cutaneous mucinosis is termed "myxedema" when associated with hypothyroidism.[5,8,9] Increased focal areas of dermal mucin have been reported in association with numerous inflammatory and neoplastic processes, such as mast cell tumor, severe pyoderma, and eosinophilic diseases.[5,8,9] A specific example of a cause of focal mucinosis in dogs is infection with the trombidioid mite *Stralensia cynotis* which causes characteristic mucinosis of the perifollicular dermis and pseudoepitheliomatous hyperplasia of the follicular epithelium (see WSC Conference 2, Case 4, 2018-2019).[8,9]

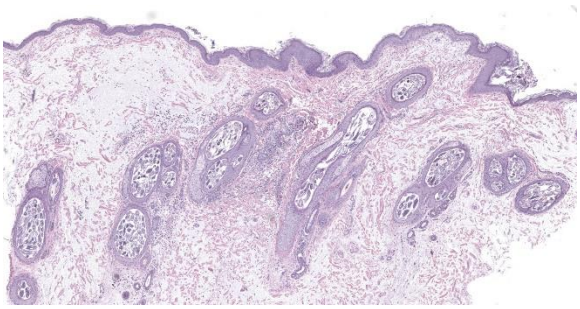

Figure 2-2. Haired skin, dog. Hair follicles are markedly expanded by numerous mites. Collagen fibers are diffusely and widely separated by abundant mucin. The overlying epidermis is mildly hyperplastic. (HE, 44X)

Color dilution has been reported in many species as well as in many breeds of dogs.[3,8,9] The condition in dogs is inherited as an autosomal recessive trait and is due to abnormalities in melanin transfer and storage.[3,5,8,9] In affected dogs, the coat color is pale, manifesting as blue, fawn, etc. This dilute color appearance is due to clumping of large melanin granules within hair follicles and characteristically within the epidermis.[5,8,9] There is preliminary evidence that the genetic cause of color dilution in dogs is an autosomal recessive mutation in the melanophilin gene (MLPH).[3,9] In dogs, the condition of color dilution may be associated with alopecia (i.e., color dilution alopecia), but is not always associated with alopecia.[5,8,9] Color dilution alopecia should only be diagnosed if histologic findings of both color dilution (clumped melanin) and follicular dysplasia/distortion are present.[5,9]

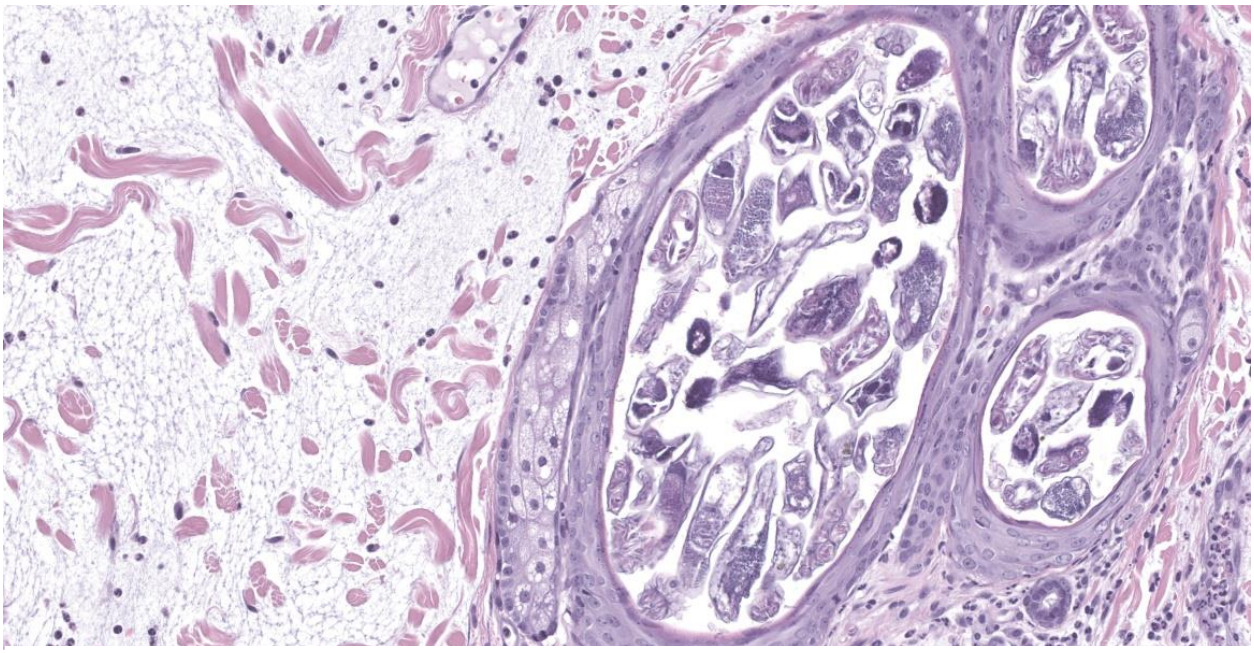

Figure 2-3. Haired skin, dog. Higher magnification of mite-laden hair follicles and adjacent mucinous dermis. Mites have chitinous exoskeletons, jointed appendages, striated muscles, a hemocoel, and nervous and reproductive tracts. (HE, 248X)

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JPC Diagnosis:
1. Haired skin: Follicular ectasia, moderate, diffuse, with mild lymphoplasmacytic and eosinophilic perifolliculitis and numerous follicular intraluminal adult *Demodex* mites.
2. Haired skin, dermis: Mucinosis, diffuse, moderate.

JPC Comment:
Case 2 has several entities for participants to consider. *Demodex* mites and dermal mucinosis are abundantly present in these sections. These findings together are interesting and should prompt consideration of immunosuppression such as Cushing's disease or severe hypothyroidism (myxedema) though the breed of this dog (Shar Pei) is an important detail as the contributor notes. Though these look similar histologically, the clinical presentation sorts these two camps out quickly. Other considerations for mite presence include long-term immunomodulatory drugs for control of allergic skin disease. In section, there are multiple examples of jointed appendages (figure #) and skeletal muscle that help to distinguish that hair follicle lumina are distended with many *Demodex* that the contributor nicely describes. Although not needed to distinguish the myxomatous dermis in this case, an Alcian blue pH 2.5 does highlight mucin nicely. One feature not present in this case is epidermal hyperplasia which is a secondary change due to the animal scratching (owing to mural folliculitis, rupture, and periadnexal mite fragments). Conference participants compared *Demodex* species across dogs and cats with *D. cati* being similarly follicularly-focused like *D. canis* while *D. gatoi* is found in the stratum corneum and is directly transferable between cats. The profound sebaceous hyperplasia induced by *D. injai* residing within sebaceous

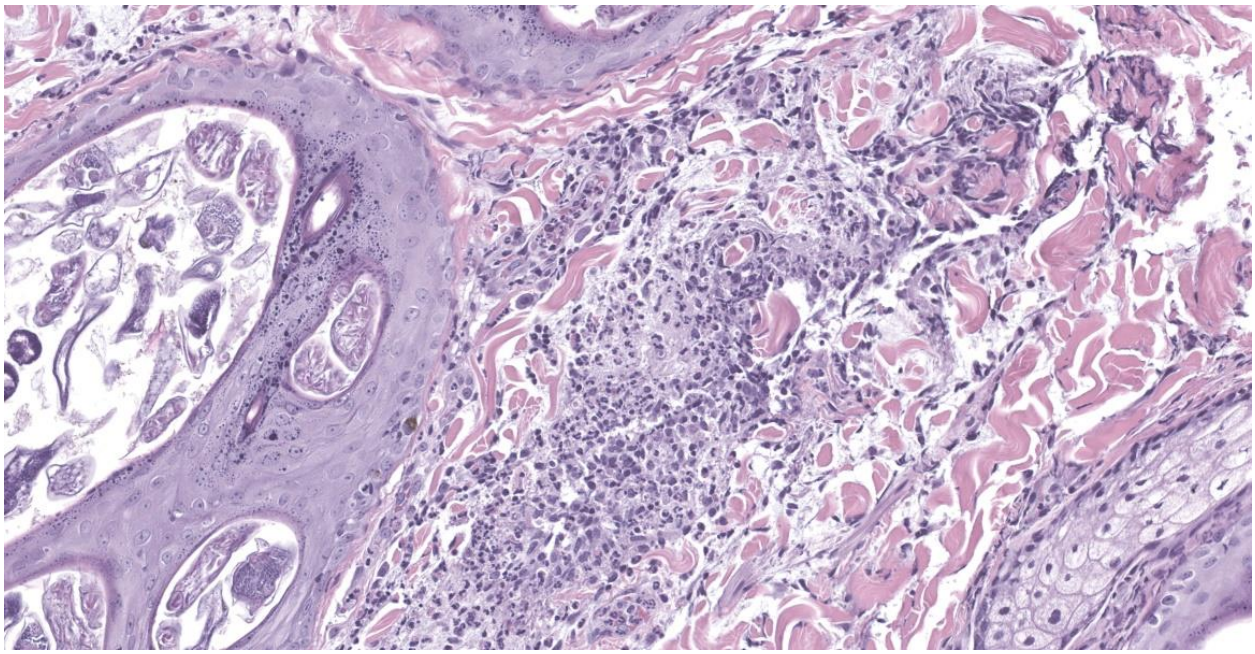

Figure 2-4. Haired skin, dog. Adjacent to ectatic follicles, there are variable amounts of mixed inflammation, including numerous lymphocytes, histiocytes, plasma cells, and eosinophils. (HE, 248X)

and ducts while lacking other epidermal changes explains the markedly greasy phenotype noted clinically.

The color dilution noted by the contributor prompted an interesting discussion among the group. Notably, many of the approved Shar Pei breed standard coats include dilute variants. As such, this could be a normal observation. We did not observe melanin clumping within epidermal melanocytes or the hair shaft in large quantity, though the hair bulb clumping is evident and worth describing. Though not in these section, distortion and fracture of the hair shaft is a helpful corroborating feature for color dilution alopecia.

Finally, Dr. Bradley touched on the role of inflammation in demodicosis. Although *Demodex* may be an incidental finding during routine skin biopsy, this case is clearly in excess of a single mite within a single follicle. Nonetheless, conference participants noted the overall muted inflammatory response in this case. Demodex is thought to play a role in the development of human rosacea in a two-fold manner. Foremost, proliferation of the mite activates TLR2 on inflammatory cells which upregulates production of cathelicidin (LL-37 peptide) which has both antimicrobial and proangiogenic effects.[4] Activation of endothelial cells by LL-37 increases in conjunction with UVB damage from sunlight, which then promotes increased production of VEGF which has both proangiogenic and immunosuppressive effects on immune cells.[4] In excess, VEGF binding causes loss of lymphocyte function and essential T cell exhaustion.[4] Secondly, Demodex also express a surface glycan Thomsen-Nouveau Antigen (Tn Ag) which is recognized by the dendritic cell galactose-type lectin receptor. Once bound to this receptor, Tn Ag induces production of IL-10 and recruitment of regulatory T-cells that further tamp down inflammation and allow *Demodex* to evade a committed host response and proliferate further.[4] These factors together hint at the interplay of

VEGF, LL-37, and IL-10 in a complex feedback loop for which the flushed face, papules, and telangiectasia are only a hint of the sinister scheme occurring at the molecular level.

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CASE III:

Signalment:

Horse (*Equus caballus*), Iceland pony, adult, male neutered.

History:

The horse has had penile lesions with multifocal mucosal thickenings and swelling for years.

Gross Pathology:

The penis was severely reddened and oedematous, the mucosa exhibited multifocal nodular thickenings and superficial yellow deposits. At the apex of the caecum, the mesentery of the large intestine and in the omentum there were multiple solid beige nodules about 1 to 3 cm in diameter.

Microscopic Description:

Penis/prepuce: The subepithelial connective tissue (submucosa) is severely expanded by a multifocal to coalescent, variable densely cellular inflammatory infiltrate consisting of high numbers of macrophages and lymphocytes, fewer numbers of plasma cells, and multinucleated giant cells (predominantly Langhans type). Lymphocytes often accompany the mucosa or surround deeper blood

Figure 3-1. Penis and prepuce, pony. The penis was reddened and edematous, the mucosa exhibited multifocal nodular thickenings and superficial yellow deposits (left and center). Similar changes were seen in the prepuce (right). (*Photo courtesy of:* Department of Veterinary Pathology, Freie Universität Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html).

vessels (perivascular cuffing). Cells frequently form merging granulomas characterized by nodules with abundant central partially epithelioid macrophages and few degenerate neutrophils, surrounded by lymphocytes, plasma cells, and multiple multinucleated giant cells admixed with plump, reactive fibroblasts and small amounts of loose collagenous connective tissue (fibrosis). The overlying mucosa is thickened, forming irregular rete ridges (hyperplasia) and infiltrated by neutrophils, partially forming serocellular crusts. In some areas, the mucosa is lost (ulceration) and the submucosa expanded by increased numbers of small caliber vessels, accompanied by activated fibroblasts admixed with loose collagenous connective tissue (granulation tissue).

<u>Additional special stains were used for histology:</u>

Ziehl-Neelsen stain for acid fast bacteria: negative

PAS reaction: negative

Contributor's Morphologic Diagnosis:
Penis/prepuce: Balanoposthitis, severe, chronic-active, multifocal to coalescing, granulomatous with epithelial hyperplasia, partial ulceration, granulation tissue formation, lymphocytic vasculitis and numerous multinucleated giant cells.

Lymph node: Lymphadenitis, mild, multifocal, chronic-active, granulomatous with numerous multinucleated giant cells.

Contributor's Comment:
The pathological findings are characterized by a systemic granulomatous inflammation without indications of a specific cause as no particular pathogen related lesions were present. The picture is therefore consistent with Equine idiopathic systemic granulomatous disease (ISGD), also referred to as "Equine sarcoidosis", "Equine generalized granulomatous disease", "Equine systemic granulomatous disease", "Equine histiocytic disease" or "Equine histiocytic dermatitis."[7]

The etiology of ISGD is unknown, though it has similarities to human sarcoidosis which is presumed to be a multifactorial disease due to

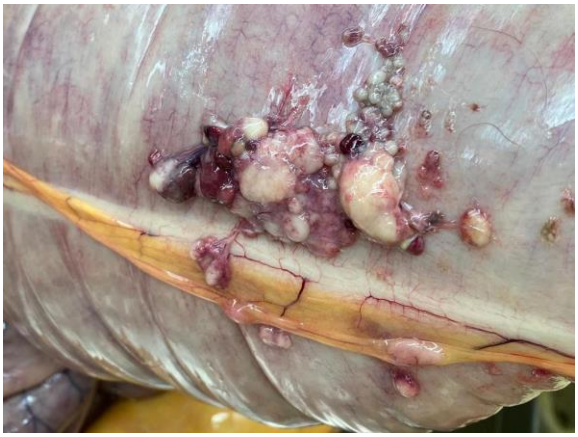

Figure 3-2. Cecum, pony. The penis was reddened and edematous, the mucosa exhibited multifocal nodular thickenings and superficial yellow deposits (left and center). Similar changes were seen in the prepuce (right). (*Photo courtesy of:* Department of Veterinary Pathology, Freie Universität Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

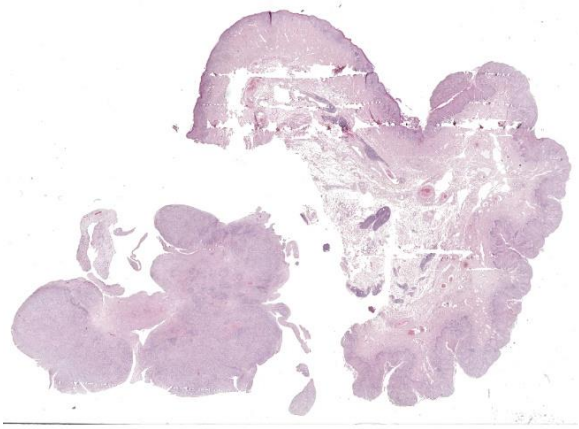

Figure 3-3. Penile mucosa and cecum, pony. A section of penis and one of cecum (left) is submitted tor examination.The mucosal epithelium is markedly hyperplastic, and there is a dense inflammatory infiltrate within the submucosa. (HE, 5X)

an abnormal host response to antigens.[5] Etiological investigations failed to identify specific agents. Similar lesions were documented after ingestion of *Vincia villosa* ('hairy vetch') by horses and more likely cattle, but not all by ISGD affected horses had been exposed to it.[9]

Clinical onset is variable. Cutaneous lesions in ISGD may present as an exfoliative dermatitis or less commonly, as a nodular lesions.[5,6] Additionally, internal organs are often involved in the course of a generalized disease with the lung, liver, gastrointestinal tract, spleen, kidney, bones, and central nervous system being affected in decreasing frequency. Although lymph nodes are often involved, a peripheral lymphadenopathy is mostly absent. In the case presented here, regional lymph nodes were enlarged and histopathology revealed multifocal sometimes nodular infiltrates by macrophages and giant cells were detectable. In generalized disease, most horses develop a wasting syndrome.[6,7] Diagnosis is primarily done by exclusion of infectious diseases (like dermatophilosis, dermatophytosis), autoimmune diseases (like pemphigus foliaceus, systemic lupus erythematosus), allergic reactions (like cutaneous adverse drug reaction, contact dermatitis), miscellaneous conditions (like seborrhea, multisystemic eosinophilic epitheliotropic disease), neoplasia (like epitheliotropic lymphoma), and toxins (like hairy vetch).[5] Characteristic pathohistological findings are so called sarcoidal granulomas affecting the skin or internal organs. Multinucleated histiocytic giant cells are typical and numerous.

Contributing Institution:

Department of Veterinary Pathology, Freie Universität Berlin

http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html

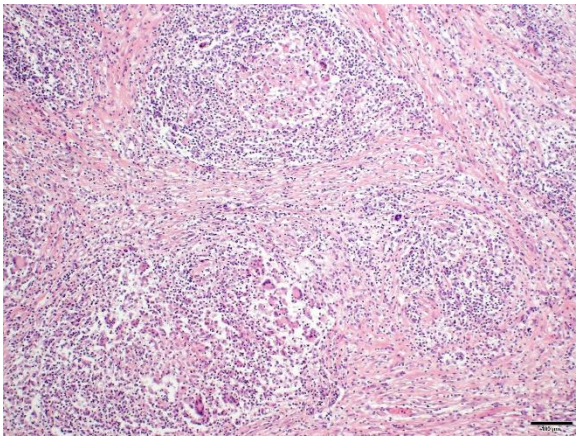

Figure 3-4. Penile mucosa, pony. The submucosa is expanded by coalescing, poorly formed granulomas. (*Photo courtesy of:* Department of Veterinary Pathology, Freie Universität Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html) (HE, 100X)

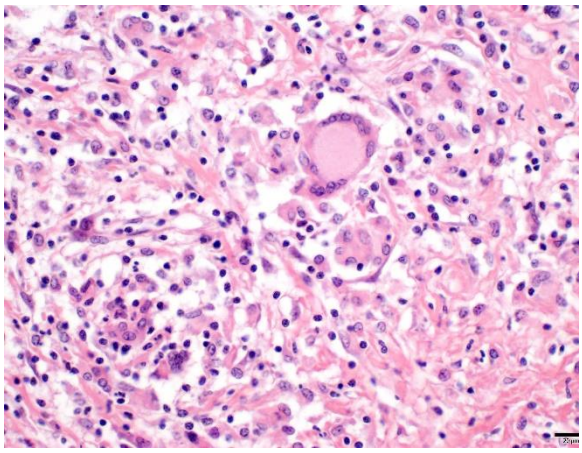

Figure 3-5. Penile mucosa, pony. Submucosal granulomas contain moderate foreign body and Langhans's type multinucleated giant cell macrophages admixed with numerous epithelioid macrophages and fewer lymphocytes and plasma cells. (*Photo courtesy of:* Department of Veterinary Pathology, Freie Universität Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html) (HE, 400X)

JPC Diagnosis:
Mucous membrane, penis: Balanoposthitis, lymphohistiocytic, chronic, diffuse, moderate with marked epidermal hyperplasia and ulceration and lymphocytic perivasculitis.

Fibrovascular tissue (presumed peritoneum): Peritonitis, granulomatous, chronic, diffuse, severe.

JPC Comment:
Case 3 was challenging for participants in that the exact location sampled is not obvious from the slide alone. The presence of large, multinucleated giant cell macrophages was a helpful feature that led participants to consider equine sarcoidosis for this case. Because sarcoidosis is a diagnosis of exclusion, excluding other causes of granulomatous inflammation (e.g. fungal, mycobacterial, pythiosis, foreign body reaction, *Actinobacillus*) must be performed. In addition to the acid-fast stain performed by the contributor, we performed modified Gram stains (Brown-Brenn, Brown-Hopps), GMS, and PAS Light Green which were negative for organisms. IHC for IBA1, CD3, and CD20 were also helpful is establishing the distribution of these inflammatory cells and excluding T-cell rich B-cell lymphoma as a possibility as well. Previously, equid gammaherpesvirus 2 was implicated as a potential cause of this condition,[3] which is a reasonable connection given that lymphohistiocytic inflammation is associated with gammaherpesviruses across species (e.g. malignant catarrhal fever). That stated, EHV-2 has not been a consistent finding in all cases of sarcoidosis and it is possible that the development of granulomatous inflammation may be the result of any number of antigens.[8]

Understanding of sarcoidosis is largely extended from human medicine, to include the entity name itself. Patients with sarcoidosis appear to have increased genetic susceptibility, to include variance in MHC class II genes[1] such as HLA-DRB1 that is associated with acute sarcoidosis and Löfgren syndrome. M1 macrophages are activated by a highly polarized T helper 1 (Th1) cytokine milieu including IFN-γ and TNF-α which correlate with the Langhans multinucleated giant cells seen in our case. Interestingly, not all of these macrophages may be classically activated. Cytokine studies of sarcoidosis patients have identified macrophages with increased IL-13 expression (i.e. M2 macrophages) that may play a role in later stages of the disease.[2] Additionally, M2 macrophages also form multinucleated giant cells (foreign body type), which were also observed in the present case.

As a final point, equine sarcoid should not be confused with sarcoidosis as they are entirely separate from one another. The term 'sarcoid' denotes a raised plaque or nodule in the skin (literally, sarcoma-like) which can be a feature of sarcoidosis, though other cutaneous

presentations can include crusting and scaling.[8] Equine sarcoids differ histologically from sarcoidosis in that they have increased density of dermal fibroblasts which form interlacing bundles and whorls within the dermis.[4] Bovine papillomavirus type 1 and type 2 are associated with the development of equine sarcoids[4] though the association of viruses in the development of sarcoidosis, if any, is uncertain.

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CASE IV:

Signalment:
10-week-old, female, C57BL/6 mice (*Mus musculus*)

History:
Mice arrived from vendor with no abnormalities observed upon entrance. Lab members shaved animals to prepare for subcutaneous tumor injection 2-week post-arrival and noted abnormal skin (bumpy, thick with patchy fur growth). No other health concerns. Lab stated this condition has only been observed with mice from this vendor. Three mice were submitted for skin histopathology.

Gross Pathology:
All mice from this cohort had skin lesions consisting of patchy alopecia and irregular/bumpy skin foci in the dorsal flank area with extension to the limbs and (rarely) abdomen.

Microscopic Description:
Haired skin: Three skin sections from different mice in the cohort are examined. In all sections, there are focal ulcers and erosions. Multifocally, longitudinal follicle sections show hair shafts with twist severely in the infundibulum and/or disintegrating of hair in the superficial dermis and as it emerges on the surface. Rarely, hair shafts can be seen

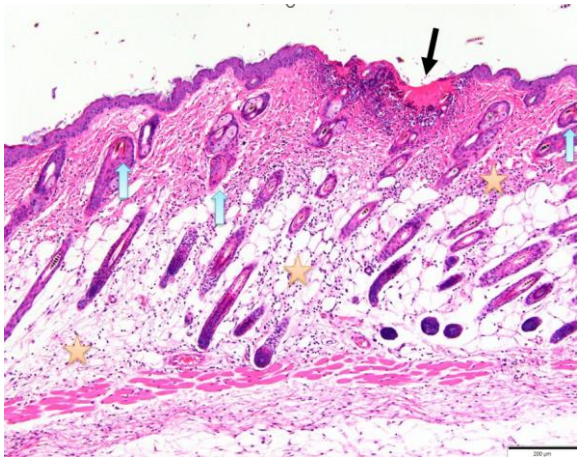

Figure 4-1. Haired skin, mouse. There is a focal ulcer (black arrow), several intrafollicular twisted, bent, and fragmented hair shafts (blue arrow), and diffuse chronic dermatitis (stars) in this section. (HE, 100X) (*Photo courtesy of:* Division of Laboratory Animal Resources, University of Pittsburgh, http://www.dlar.pitt.edu/)

penetrating the follicle wall with fragments free in the dermis and hypodermal fat layer surrounded by a mixed inflammatory cell infiltrate consisting of neutrophils, macrophages, rare multinucleated giant cells, and occasional lymphocytes forming a foreign body granuloma (trichogranuloma). Other areas have a more diffuse chronic lymphoplasmacytic infiltrate in the dermis and hypodermal fat. The overlying epidermis exhibits mild to moderate acanthosis, hypergranulosis, and orthokeratotic hyperkeratosis. Resolved ulcers often contain underlying superficial dermal granulation tissue proliferation, fibroplasia, and increased connective tissue.

Contributor's Morphologic Diagnosis:
Haired skin: Dermatitis, chronic, hyperplastic, ulcerative, with hair shaft twisting, fragmentation, trichogranuloma formation, and superficial dermal scarring.

Contributor's Comment:
Mice of the strain C57BL/6 and those on a C57BL/6 background develop ulcerative dermatitis (UD), a disease of unknown etiology that leads to significant morbidity.[4,10] In this entity, ulcerations present on the dorsal scapulae, torso, shoulder, and face from pruritis-induced self-trauma, and may be single or multifocal in distribution.[1,4,10] A recent large-scale studies in mice reveal that chronic ulcerative dermatitis is still the primary non-tumoral cause of euthanasia in both sexes (39.1% in males and 35.4% in females).[2] In the case of these mice, the lesions did not fit the typical clinical, gross, or histopathology lesions characteristic of UD. Specifically, no pruritis was noted in any of the cohort clinically, and lesions were initially noted when fur was removed. Histopathologically, small ulcerations were noted, however, the changes to the hair follicles, shafts, and secondary inflammation of the dermis were the most characteristic findings.

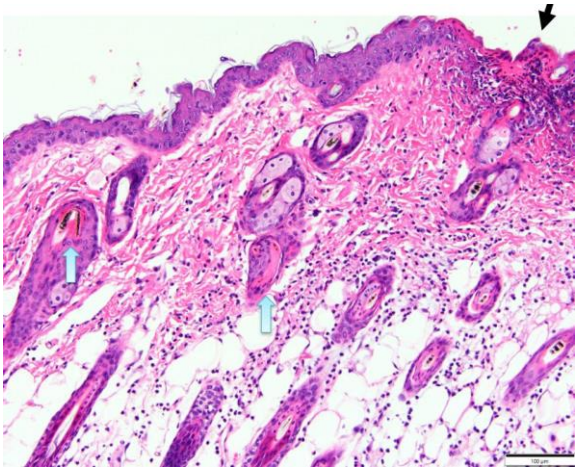

Figure 4-2. Haired skin, mouse. Higher magnification of the field in image 4-2 with twisted hair shafts (blue arrows) with surrounding chronic dermal infiltrates and a surface ulcer (black arrow). (HE, 100X) (*Photo courtesy of:* Division of Laboratory Animal Resources, University of Pittsburgh, http://www.dlar.pitt.edu/)

Many of the scarring alopecias in humans and other species, are based on primary sebaceous gland pathological features.[7] Although sebaceous gland loss can be seen in some areas with chronic scarring in these mice, the primary lesions are centered on the follicles. An entity was characterized in 2011 in C57BL/6J mouse substrains describing a primary follicular dystrophy (PFD) which leads to trichogranulomas, chronic scarring of the dermis, and alopecia.[8] The changes in the primary follicles include fragmentation and twisting of the hair shaft as we saw in these mice. In 2017, a large scale screening study of knockout mice revealed sporadic trichogranulomas are common in mutant mice, however, these are not consistently seen in all mice of a specific line. PFD was noted at a much lower frequency.[9] Although the etiology of PFD remains unclear, C57BL/6J mice were found to have defects in vitamin A metabolism.[9] The skin takes up circulating retinol and can either store it in the form of retinyl esters or metabolize it to retinoic acid.[5,8] Two enzymes are present in the skin that can oxidize retinol to retinal. These include the medium chain alcohol dehydrogenase type 4 (ADH4) and the short chain dehydrogenase/reductases epithelial retinol dehydrogenase (DHRS9).[3,8] DHRS9 is microsomal and can oxidize both free and CRBP-bound retinol.[3,8] Upregulation of DHRS9 in C57BL/6J and C57BL/6Tac but not C57BL/6NCr or C57BL/6Crl mice provides a potential explanation for why the first two strains have a much higher frequency of dorsal skin alopecia.[8]

The histologic lesions seen in mice with primary follicular dystrophy resemble the human disease currently termed central centrifugal cicatrical alopecia (CCCA).[8] Premature desquamation of the inner root sheath occurs in CCCA leading to marked thinning of the outer root sheaths with reactive perifollicular inflammation, and eventually entry of the hair fiber into the dermis with resulting destructive chronic granulomatous inflammation.[6,8,9] Although this entity does not have all features of CCCA in people, the degenerative features of the inner root sheath in PFD mirror those changes in CCCA.[8]

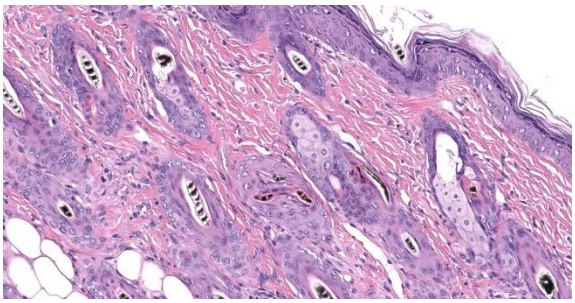

Figure 4-3. Haired skin, mouse. Hair shafts are twisted, fragmented, and poorly formed. (HE, 255X)

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JPC Diagnosis:
Haired skin: Follicular dysplasia, multifocal, moderate with ulcerative dermatitis, trichogranulomas, and superficial fibrosis.

JPC Comment:
The final case of this conference proved tricky for participants. Although these sections of skin featured dermatitis that was ulcerative, the cause was follicular dysplasia with ulcerative dermatitis developing similarly to the CCCA that the contributor describes. From low magnification, the twisting of hair shafts and abnormal orientation of hair follicles relative to the epidermis (figure #) are key details. Under higher magnification, dystrophic hairs are hypereosinophilic

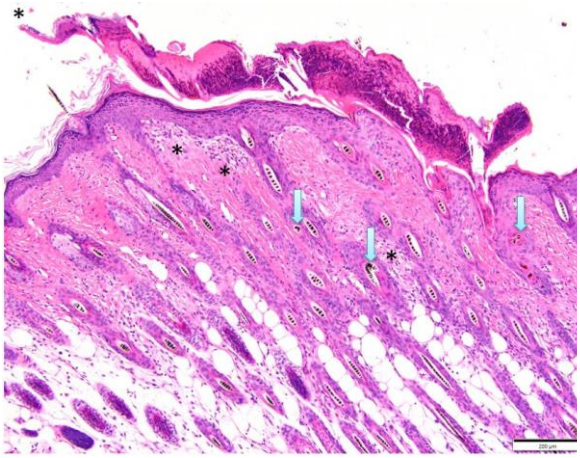

Figure 4-4. Haired skin, mouse. In addition to twisted and fragmented hair shafts (arrows), this section shows a large surface crust, underlying epithelial hyperplasia, and superficial dermal fibrosis and fibroplasia (*)(HE, 100X) (*Photo courtesy of:* Division of Laboratory Animal Resources, University of Pittsburgh, http://www.dlar.pitt.edu/)

due to poor cuticle maturation as well. Although subtle, these sections of skin also have increased space (widening) between abnormal hair follicles as well. In contrast, UD is characterized by marked lymphohistiocytic and neutrophilic inflammation of the dermis, epidermis, and deeper tissues (adipose, muscle, nerves) that can progress to fibrosis in chronic cases.[2] The overlying epidermis is also often hyperplastic. These features of UD are notably absent in this case however. Other causes of ulcerative skin disease and alopecia in mice include barbering, feeder/waterer-associated dermatitis, ectoparasites (e.g. *Myobia musculi*), self-trauma, fighting, and chemotherapy agents. Primary deficiencies of genes associated with hair development as was likely in this case should also be considered.

Lastly, conference participants discussed some of the unique features of mouse skin. Mice lack apocrine glands and rete ridge formation. They also have synchronized hair cycles such that growth occurs in waves (i.e. adjacent follicles in section are in the same phase) though overall mice tend to be anagen-heavy. In this case, there is disruption of this synchronization of hair development due to alopecia and inflammation.

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