Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 8

13 October 2021

CASE I: J1251 (JPC 4116584)

Signalment:
Sixteen year old neutered female cat *(Felis vulgaris)*

History:
This animal was admitted to a local veterinarian with symptoms of chronic vomiting and polyuria and polydipsia. The owner was not motivated to do further diagnostic work and opted for euthanasia. The animal was brought to the veterinary faculty to be necropsied for educational purposes.

Gross Pathology:
On post-mortem examination, the four footpads of the cat were markedly swollen and very soft. There was mild hyperkeratosis and the footpads were dark red to cyanotic. On cut-section, the tissue within the footpad was diffusely mildly hemorrhagic and very soft in consistency.

Laboratory results:
No blood or other fluids were taken for further analysis.

Microscopic Description:
Footpad - The dermis is markedly expanded by multiple nodules and diffuse aggregates of inflammatory cells which are inconspicuously oriented around larger blood vessels and reach the epidermis and infiltrate in lobules of adipocytes. These infiltrates consist of large numbers of plasma cells which are oval-shaped, possess a moderate amount of basophilic cytoplasm and an eccentric small round nucleus (giving a 'fried-egg' appearance). Scattered among those plasma cells are many plasma cells which are swollen by a large amount of brightly eosinophilic globules (Russell bodies) in the cytoplasm, which pushes the nucleus to the periphery (Mott cells). Admixed with the plasma cells are moderate numbers of lymphocytes and macrophages. Within the dermis, there are irregular foci of necrosis with disruption of collagen fibers,

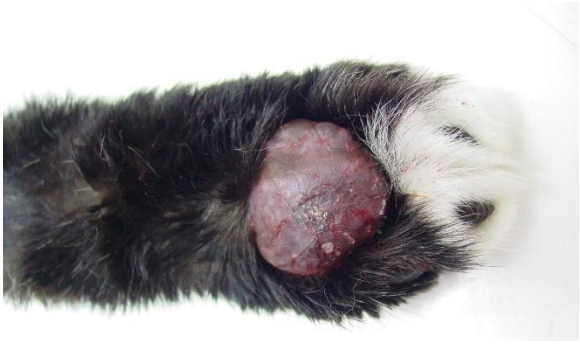

Figure 1-1. Footpad, cat. All four footpads of this cat were soft, partially collapsed, dark red, and had patch mild hyperkeratosis. (Photo courtesy of Dept of Pathology, Bacteriology and Poultry diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium http://www.ugent.be/di/en/departments?ugentid=DI05)

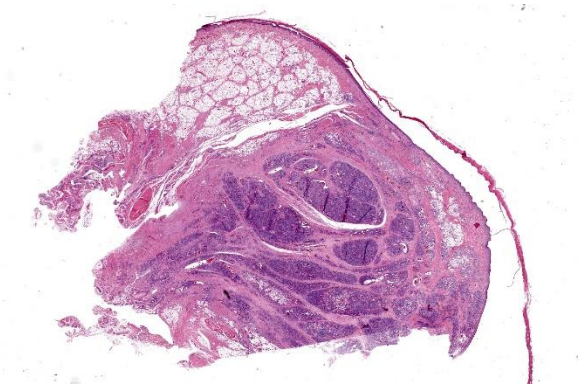

Figure 1-2. Footpad, cat. A partial section of the carpal pad is presented for examination. Approximately 75 % of the lobules have footpad adipose tissue are infiltrated and/or replaced by a cellular infiltrate. (HE, 8X)

cellular debris and karyorrhectic fragments. Scattered melanocytes are found in the superficial dermis (pigmentary incontinence). The epidermis is covered with multiple layers of orthokeratotic keratin.

Contributor's Morphologic Diagnoses: Footpad; Plasmacytic pododermatitis, severe, multinodular, chronic with mild orthokeratotic hyperkeratosis

Contributor's Comment
Identifying the tissue as a footpad without prior knowledge of where the tissue was taken, might be challenging, but the characteristic histological properties of this entity together with the presence of non-haired skin should guide one to the paws. Feline plasma cell pododermatitis (FPP) is a rare condition with unknown etiology. An immune-mediated pathogenesis seems plausible, due to resolution of lesions and clinical symptoms after administration of immunosuppressive drugs and marked hypergammaglobulinemia.[6,8] Some patients require life-long therapy.[8] Food hypersensitivity was suspected to be part of the underlying cause in some cats, as patients improved with hyposensitive diets. Seasonal waxing and waning has also been described.[8]

A large proportion of affected cats test positive for FIV, suggesting a possible role in the development of this entity.[6] Some cats exhibit concurrent plasmacytic stomatitis, renal amyloidosis or immune-mediated glomerulonephritis.[6] Unfortunately, the kidneys were not histologically examined, but had no gross abnormalities. There is no breed, sex or age predisposition.[5,6,8]

Affected footpads are markedly swollen and filled with a soft hemorrhagic tissue. There may be mild hyperkeratosis present. All four paws may be affected, but not necessarily. The gross and histologic appearance is practically pathognomonic. An interesting case of a different presentation of likely similar pathogenesis was found in a 7-month-old cat with a firm, haired rounded swelling on the bridge of the nose. Histologically, the lesion closely resembled FPP with perivascular to diffuse infiltrates of plasma cells. This animal did not show any signs of FPP.[4]

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JPC Diagnosis:
Footpad: Pododermatitis, plasmacytic, multinodular, severe.

JPC Comment:
The contributor provides a good overview of feline plasma cell pododermatitis associated literature associated with this rare condition with an unknown underlying cause. As noted within the contributor's description, this condition is often associated with Mott cells and Russell bodies, which are both known to

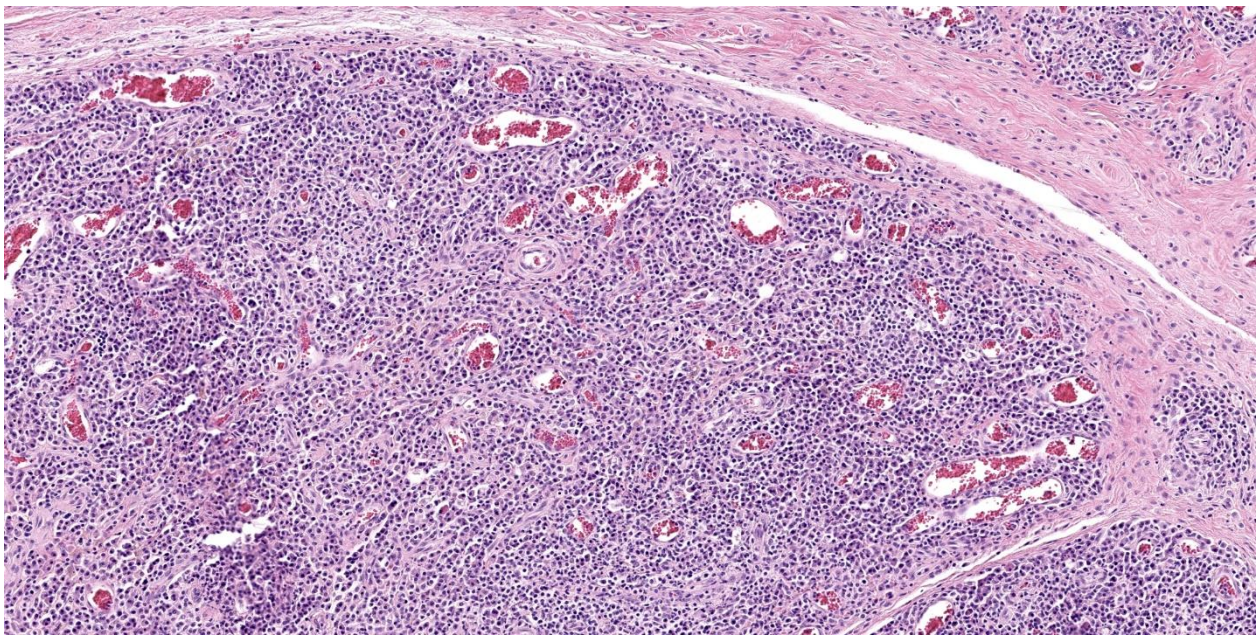

Figure 1-3. Footpad, cat. The lobular adipose tissue and embedded eccrine glands are replaced by a dense infiltrate composed primarily of plasma cells. (HE, 125X)

occur in reactive and neoplastic plasma cell disorders.[1,2,6]

The term "Mott cell" is derived from a surgeon, F.W. Mott, who described "morular cells" (Latin: morus "mulberry) with spherical inclusions packed into the cytoplasm while describing sections of brain tissue from monkeys experimentally infected with various species of genus *Trypanosoma* while investigating "sleeping sickness" in 1905.[7] He recognized the cells were plasma cells and indicative of chronic inflammation.[7] However, the first description of these cells is most likely attributed to William Russell. Russell was a pathologist at the School of Medicine at the Royal Infirmary in Edinburgh. Thinking he had discovered the organism that caused cancer, he addressed Pathological Society of London in December 1890 and described large spherical inclusions within cells that often occupied the entire cytoplasm and compressed the nucleus. We now know these inclusions, which may occur in both plasma cell neoplasms and reactive conditions, are derived from immunoglobulin.[1,2]

The term "Russell body" is inconsistently used between countries and even within countries, with some pathologists using the term to only refer to a single large spherical inclusion displacing the nucleus whereas others use the term to refer to the multiple inclusions within Mott cells. Hand-drawn illustrations of Russell's article reveals he observed multiple spherical inclusions within single cells. Mott cells therefore contain Russell bodies, as reflected in the contributor's microscopic description in this case.[1,2]

"Immunomodulatory-responsive lymphocytic-plasmacytic pododermatitis" has been proposed to denote a similar condition in domestic canines, with a report describing the condition in 20 adult dogs of various breeds. There was chronic (≥ 6 months) inflammation confined to the pedal skin in each case, with lesions present on all four feet in 18/20 cases. Lesions were histopathologically characterized by epidermal hyperplasia, hyperkeratosis, spongiosis, dermal edema, and perivascular aggregates

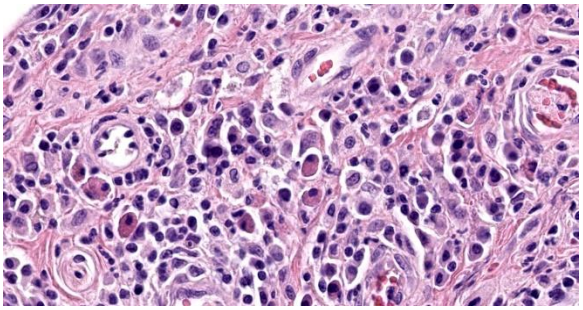

Figure 1-4. Footpad, cat. Mott cells replete with large eosinophilic cytoplasmic Russell bodies are scattered throughout the infiltrate. (HE, 750X)

of lymphocytes and plasma cells. Affected dogs had significantly elevated serum IgG and IgM concentrations. None of the dogs responded to antimicrobial therapy administered over an 8-week period, had evidence of ectoparasitism, satisfied criteria for atopic dermatitis, nor responded to a dietary trial using a novel protein source. Each dog responded to immunosuppressive doses of prednisone or cyclosporine.[3]

During the conference, the moderator noted areas within the section with scant refractile anisotropic material associated with epithelioid macrophages and rare multinucleated giant cells. Although advanced diagnostics are required for confirmation, this is a common observation in cases of feline plasma cell pododermatitis and is consistent with a local foreign body reaction to embedded cat litter.

References:

1. Bain BJ. Russell bodies. *Am J Hematol*. 2009;84(7):439.
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3. Breathnach RM, Baker KP, Quinn PJ, McGeady TA, Aherne CM, Jones BR. Clinical, immunological and histopathological findings in a subpopulation of dogs with pododermatitis. *Vet Dermatol*. 2005;16(6):364-372.
4. Declerq J, Debosschere H, Nasal swelling due to plasma cell infiltrate in a cat without plasma cell pododermatitis. *Veterinary Dermatology;* 2010;21;412-4
5. Dias Pereira P, Faustino AM, Feline plasma cell pododermatitis: a study of 8 cases. *Veterinary Dermatology;* 2003;14(6);333-7.
6. Mauldin EA, Peters-Kennedy J. Integumentary system. In: Maxie MG ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. Philadelphia, PA: Elsevier Ltd. 2016: 613-614.
7. Mott FW. Observations on the brains of men and animals infected with various forms of trypanosomes. Preliminary note. *Proceedings of the Royal Society of London Series B-Containing Papers of a Biological Character*. 1905;76:235–242.
8. Werner A, Zetwo A, Feline Plasma Cell Pododermatitis*, Clinican's Brief*; 2015

CASE II: PV406-20 (JPC 4162140)

Signalment:
Eight-year-old female spayed Border collie, canine.

History:
Alopecia and hyperpigmentation for a few months. Gradually presented with marked

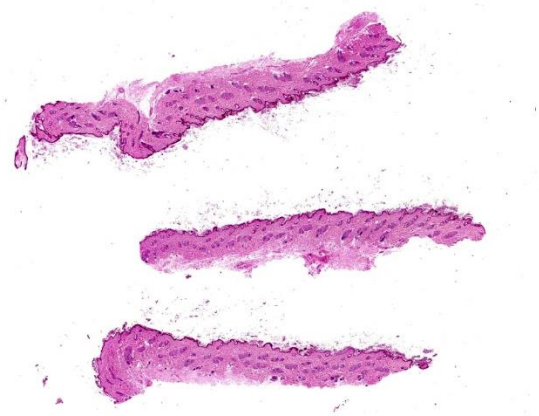

Figure 2-1. Haired skin, dog. Three sections of haired skin are submitted for examination; there are no subgross lesions. (HE, 5X)

areas of alopecia of black skin. Negative for demodecosis. No response to antimycotic treatment for two weeks. Biopsies of skin from the leg and from the side of the mouth.

Gross Pathology:
Wedge biopsies of skin.

Laboratory results:
No laboratory findings reported.

Microscopic Description:
Serial sections of the skin samples submitted are evaluated. The epidermis appears to be thinner than normal. There is mild diffuse hyperkeratosis. Hair follicles are in all stages of growth. Small to moderate numbers of lymphocytes are forming aggregations around hair follicles, infiltrating anagen bulbs. There is occasional swelling of acanthocytes of the bulb epithelium and pyknosis of epithelial cells in occasional anagen bulbs.

Contributor's Morphologic Diagnoses:
Lymphocytic folliculitis (bulbitis), findings most compatible with alopecia areata.

Contributor's Comment:
Alopecia areata is uncommon in dogs and very rare in cats. It may be focal, multifocal or generalized (*alopecia universalis*). It is mostly characterized by well-defined patches of hair loss, usually non-scarring and non-inflammatory.[2,3,4] The patches of alopecia most commonly present in the skin of the head or face. Legs may also be affected. Leukotrichia is occasionally observed. The areas of alopecia may become variably pigmented and may be bilaterally symmetrical. Occasionally, alopecia areata may be confined to the dark-haired areas of multicolored hair coats.[3] There is no apparent age predilection and there are no studies on breed predisposition, but it is thought that German shepherds, dachshunds and beagles may be predisposed.[4]

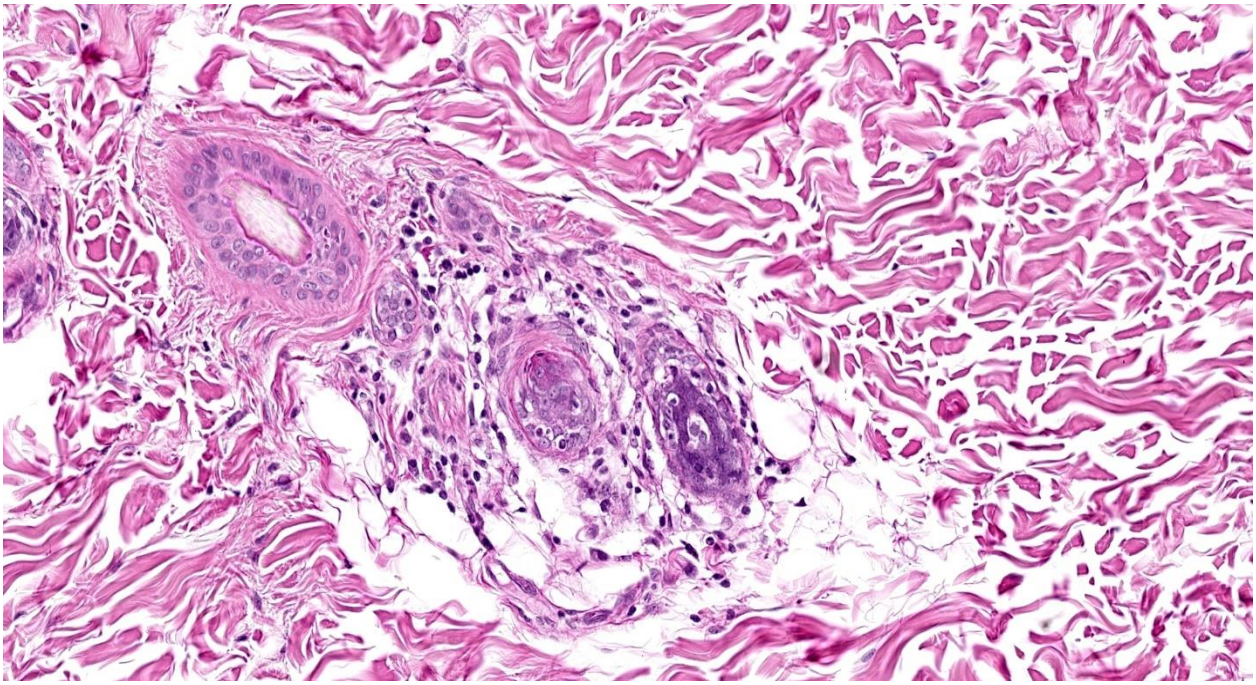

Figure 2-2. Haired skin, dog. Hair follicles and adnexa and infiltrated by low to moderate numbers of lymphocytes, with few neutrophils. In addition to lymphocytes infiltrating the hair bulb, there is multifocal pyknosis. (HE, 230X)

In alopecia areata cycling is interrupted. It is believed that the lesions are caused by an immune-mediated mechanism directed against hair follicles in humans, nonhuman primates, dogs, cats, horses and cattle, which may be modulated by genetics and hormones.[3,4] Environmental factors such as stress, vaccination, infection and diet may affect the development of the disease.[4]

The histological findings initially may present by accumulation of lymphocytes ("swarm of bees") in and around the inferior segment of anagen hair follicles. But more commonly subtle aggregations of lymphocytes, macrophages and even some plasma cells are observed around the inferior portion of the hair follicle (peribulbar).[2] Occasionally neutrophils may be observed.[4] Peribulbar mucin deposition and pigmentary incontinence may also be seen.[4] Sebaceous glands are normal.[2]

Immunostaining to detect CD3+ and CD8+ lymphocytes may be helpful in some cases.
In chronic cases the histological findings consist of a predominance of catagen and telogen hair follicles as well as follicular atrophy.[1] Therefore, early sampling and submission of multiple biopsies is strongly recommended for accurate diagnosis.[4]

Diagnosis is fairly straightforward if bulbitis is observed histologically. Differential diagnosis should include other syndromes with mural/isthmus folliculitis, such as pseudopelade, as in some severe cases, the lymphocytic infiltration of alopecia areata may progress towards the isthmus of the hair follicle, and isthmus and mural folliculitis of other syndromes may progress towards the bulb. It is important to note though, that in other syndromes, the follicular bulb is not affected.[1]

The differential diagnosis is of prognostic importance because hair loss in alopecia areata is usually transient. Alopecia in pseudopelade is usually permanent.

Reorientation and/or step sectioning of skin samples should be considered in cases where the clinical presentation is highly suspicious and subtle bulbitis can't be observed histologically.[1,4]

The prognosis for alopecia areata is good, with 60% of the cases having spontaneous and complete hair regrowth. However, sometimes the hair regrowth is white.[1,2,3]
Treatment with immunosuppressive doses of prednisone is often effective.[3]

Contributing Institution:
The Weizmann Institute of Science
http://www.weizmann.ac.il/

JPC Diagnosis:
Haired skin, anagen follicles: Bulbitis, lymphocytic, multifocal, mild.

JPC Comment:
The contributor provides a concise review of alopecia areata, a condition that affects multiple species, including humans. In the United States, this condition affects 4.5 million people. Amongst the worldwide human population, the prevalence is up to 0.2% with a calculated lifetime risk of 2%. Although children are rarely affected, 66% of those affected are younger than 30 years of age and only 20% are older than 40 years of age. The condition is also associated with in increased risk of other autoimmune disorders, such as lupus erythematosus, vitiligo, and autoimmune thyroid disease.[1]

Normal hair growth occurs via a repetitive cyclic process. Healthy hair follicles transition from a period of very rapid growth, pigmentation, and hair-shaft production (anagen) to a short, apoptosis-driven phase of

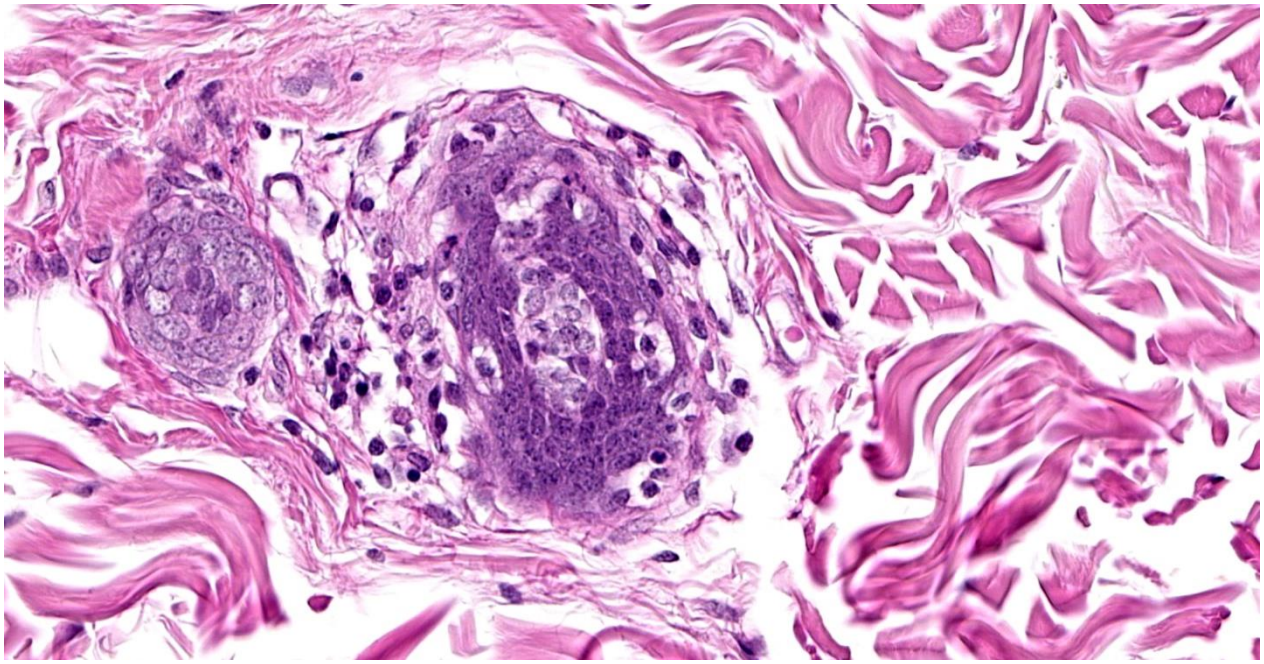

Figure 2-3. Haired skin, dog. Infiltrating T-cells and pyknotic nuclei resemble a "swarm of bees" attacking an anagen hair follicle. (HE, 570X)

involution (catagen). Following catagen, hair follicles undergo a period of relative quiescence (telogen) before reentering anagen. This regenerative cycle is made possible by an abundance of keratinocyte and melanocyte stem cells predominantly located in the hair bulb. Hair follicle cycling and regeneration are stem-cell dependent and hair-shaft production and pigmentation are accomplished by differentiated progeny of these stem cells. These rapidly proliferating keratinocytes and pigment producing melanocytes reside in the anagen hair matrix, the primary target of inflammation in alopecia areata.[1]

A key feature of alopecia areata is inflammatory cells only attack anagen hair follicles, resulting in their premature reentry into the catagen phase. The hair shaft is rapidly shed from the follicle as a result of inflammation induced dystrophy. However, the follicle often retains the ability to regenerate and continue cycling since hair follicle stem cells are not usually lost, which is in contrast to other disorders that may result in scarring.[1] Histologic sections obtained from chronic and clinically static cases often demonstrate predominately telogen follicles and follicular atrophy, which are non-diagnostic and may result in the misdiagnosis of an endocrine skin disorder.[3]

The pathogenesis of alopecia areata is not completely understood although it is presumably an autoimmune alopecic inflammatory disorder directed against hair follicles.[2] Notably, several autoantigens associated with pigment production are immunogenic. One theory is alopecia areata occurs as the result of melanogenesis-associated autoantigens generated during active hair shaft pigmentation (in addition to other anagen associated hair follicle autoantigens) attracting autoreactive CD8+ T cells. Clinically, this theory is supported in that alopecia areata is occasionally confined areas of darkly pigmented hair. Under normal conditions, a key immunologic feature of the normal hair follicle is its creation of an environment of relative immune privilege, suppressing an auto-

immune attack on intrafollicularly expressed autoantigens. This relative site of immune privilege results primarily from the suppression of surface molecules required for presenting autoantigens to CD8+ T lymphocytes (i.e. MHC class Ia antigens). Notably, the down-regulation of MHC class I molecules increases the risk of the hair follicle being attacked by natural killer (NK) cells, as these are primed to recognize and eliminate MHC class I negative cells. However, healthy hair follicles also counter this process by down regulating ligands that stimulate the activation of NK cell receptors (NKG2D) while also secreting molecules that inhibit NK cell and T cell functions, such as transforming growth factors β1 and β2, α melanocyte–stimulating hormone, and macrophage migration inhibitory factor.[1]

The moderator discussed the advantage of using immunohistochemical stains to aid in the diagnosis of alopecia areata, particularly in regard to the identification of CD3 positive lymphocytes within the hair bulb, which was observed in this case. Although CD79a lymphocytes were also observed, these cells were present within the adjacent perifollicular dermis, not within the follicular epithelium.

References:

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4. Miller, W.H. et al. Autoimmune and immune-mediated dermatoses. In: *Muller & Kirk's Small animal dermatology;* 7th edition. Elsevier. 2013:462-463.

CASE III: P16-282 (JPC 4082545)

Signalment:
4-year-old female horse (*Equus caballus*)

History:
Pigmented hairless mass in the skin of the pinna

Gross Pathology:
1 cm diameter white mass in the skin

Laboratory results:
No laboratory findings reported**.**

Microscopic Description:
The dermis contains a well-delineated nodule composed of lymphocytes that form follicles, some with germinal centers. Dispersed throughout the lymphoid tissue are collections of macrophages, neutrophils and multinucleate giant cells. The macrophages and giant cells contain pigmented fungal

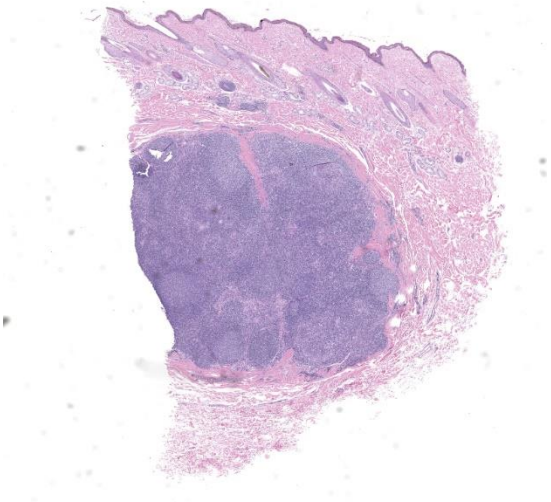

Figure 3-1. Ear pinna, horse. There is a 3cm nodular focus of inflammation in the deep dermis. (HE, 5X)

organisms, forming round, yeast-like bodies, 10-30 microns in diameter, and thin septate hyphae.

Contributor's Morphologic Diagnoses: Cutaneous phaeohyphomycosis.

Contributor's Comment:
Phaeohyphomycosis is an infection with the pigmented fungi of the family *Dematiaceae*.[4] This family includes several genera of which *Alternaria*, *Cladophialophora*, and *Curvalaria* are the most common ones occurring in animals. Infections have been reported in skin, brain, nasal cavity, mucosal surfaces and systemically. These organisms are saprophytes that reside in soil, water, and decaying vegetable matter. They gain entrance into the tissue through a wound. A compromised immune system may be another factor associated with infection.

The skin is the most common site of phaeohyphomycosis in the horse and *Alternaria* is the most common genus isolated.[2] The infection in the horse often has a pronounced lymphocytic reaction as in this case.

Contributing Institution:
College of Veterinary Medicine
Virginia Tech
Blacksburg, VA 24061
www.vetmed.vt.edu

JPC Diagnosis:
Haired skin: Dermatitis, pyogranulomatous and lymphocytic, nodular, severe, with dematiaceous yeasts and hyphae.

JPC Comment:
Dematiaceous fungi are most commonly found in the environment as plant pathogens, saprophytes, or colonizing rocks. Although these melanized fungi rarely infect vertebrates, opportunistic infections occur worldwide and have been reported in

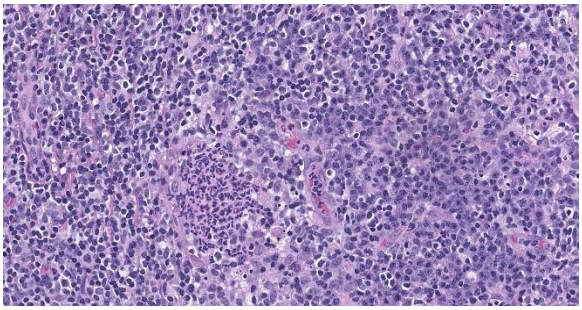

Figure 3-2. Ear pinna, horse: The nodule is composed of poorly demarcated foci of pyogranulomatous with central areas of aggregated neutrophils, with a surrounding layer of uni- and multinucleated macrophages admixed with lymphocytes and fewer plasma cells. (HE, 380X)

amphibians, reptiles, birds, fish, humans, and domestic animals causing a wide range of clinical conditions. These organisms are not considered as emerging opportunists in immunocompromised or otherwise debilitated vertebrates; in contrast, infections occur in healthy individuals.[5]

In domestic species, phaeohyphomycosis has been reported most frequently in cats and occasionally in horses, dogs, cattle, and goats. Gross lesions typically consist of single or multiple subcutaneous nodules that may be pigmented and are often mistaken for melanocytic neoplasms. Affected felines typically develop solitary lesions on the face and paws whereas horses tend to develop multiple nodules located on different parts of the body. Histologically, phaeohyphomycosis is characterized by pigmented hyphae within tissue that are associated with nodular to diffuse pyogranulomatous dermatitis and panniculitis.[3]

Factors that likely contribute to their pathogenicity include the presence of melanin and carotene, formation of thick cell walls, presence of yeast-like phases, thermotolerance, adhesion, hydrophobicity, and production of siderophores.[5]

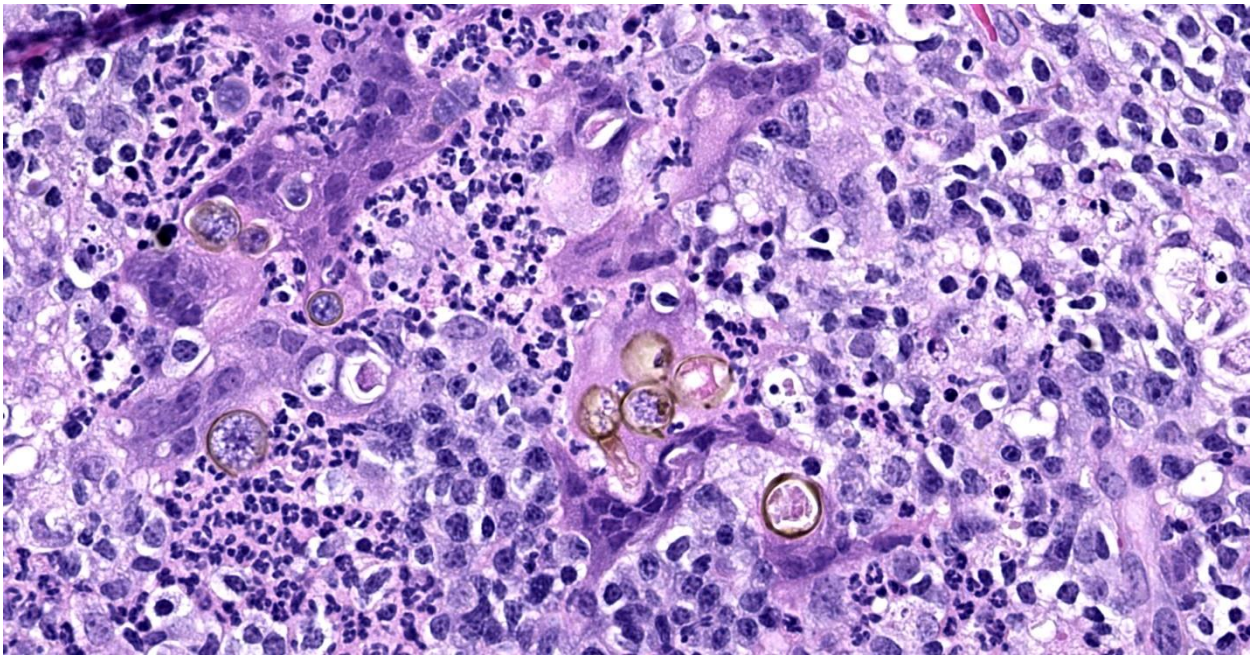

Figure 3-3. Ear pinna, horse: Scattered throughout the nodule, cross sections of 10 µm fungal hyphae and yeasts with a dark brown cell wall are entrapped within multinucleated macrophages. (HE, 560X)

Both innate and adaptive immune responses are required for effective containment of dematiaceous fungal infections. Although melanin may facilitate the organism's evasion of the host's defense mechanisms, it has also been reported to play a role in activating both the humoral and cellular immune responses. In addition, melanin may play a role in the activation of Toll-like receptor (TLR)-4 and induce production of the proinflammatory cytokine interleukin (IL)-8. Host defense predominantly relies upon ingestion and elimination of fungal cells by the cells of the innate immune system, especially neutrophils and macrophages. Many cases are associated with a characteristic pyogranulomatous reaction, usually indicating incomplete or frustrated phagocytosis, which means phagocytic cells are unable to efficiently phagocytize fungal cells, leading to in-situ persistence.[5]

Pigmented fungi may be readily apparent during histological examination, however, histochemical stains such as Grocott-Gomori's methenamine silver (GMS) and Fontana-Masson's enhance the detection of fungal walls and melanin, respectively. Regardless, identification of most dematiaceous fungi cannot be achieved based on histomorphologic features alone. Culture is often required for definitive identification[3]; however, culture isolates are frequently misidentified.[5] This emphasizes the important role of molecular identification of these rare mycoses and the benefits of sequencing

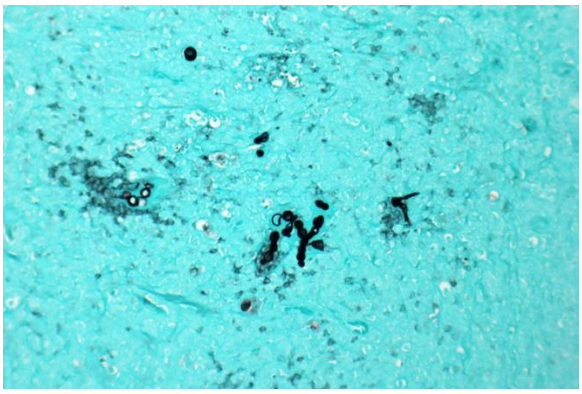

Figure 3-4. Ear pinna, horse: The morphology of the fungal hyphae are demonstrated better with a GMS stain. (GMS, 400X) (Photo courtesy of College of Veterinary Medicine, College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061, www.vetmed.vt.edu)

as the gold standard for accurate identification.[5]

As noted by the contributor, the organisms responsible for phaeohyphomycosis are numerous and include (but are not limited to) the following genera: *Alternaria*, *Bipolaris*, *Cladosporium*, *Curvularia*, *Exophiala*, *Phialophora*, and *Wangiella*.[3]

Conference participants briefly discussed the value of using special stains, such as GMS, to aid in the differentiation of phaeohyphomycosis from chromoblastomycosis. Both conditions are caused by dematiaceous fungal etiologies. Chromoblastomycosis is characterized by 4-15µm diameter, round, pigmented yeasts that are also known as "sclerotic bodies", "chromo bodies", and "Medlar bodies". In contrast, phaeohyphomycosis is characterized by pigmented fungi with both mycelial and yeast morphologies in tissue sections.[3]

References:

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CASE IV: 18N045 (JPC 4160777)

Signalment:
6 to 8-week-old female athymic nude mouse (*Mus musculus*, Foxn1^{nu}, background strain not reported)

History:
This athymic nude mouse had been injected subcutaneously with a human-derived tumor line by an approved rodent vendor. Several weeks later, the mouse was delivered to our facility. One week after delivery the mouse was reported for moderate to severe scaly dermatitis. No other clinical signs were reported.

Gross Pathology:
Skin: There was moderate ulcerative and proliferative dermatitis extending over most of the dorsum, hind limbs, and tail. Ulcers were most severe at the base of the tail. There was an approximately 1cm subcutaneous mass on the right flank (pre-sumed to be the injected tumor). As *C. bovis* was the presumed etiologic agent, the mouse was euthanized.

Laboratory results:
Skin swabs samples were PCR positive for *Corynebacterium bovis.*

Microscopic Description:
Skin: Severe widespread neutrophilic dermatitis, characterized by marked transdermal neutrophilic inflammation and multifocal epidermal necrosis. There is marked widespread ortho- and parakeratosis with scattered intraepidermal clefts, some of which contain large numbers of acantholytic

cells, and/or neutrophils. The epidermis is hyperplastic. There is widespread single cell necrosis as well as marked disorganization of the epidermal layers. Hair follicles and surface epidermis are both affected.

A Gram stain revealed clusters of gram-positive cocci within the keratin layers with equivocal short gram-positive rods.

Contributor's Morphologic Diagnoses: Dermatitis: Chronic, severe, neutrophilic with intradermal clefts and abscesses, acantholytic cells, hyper-and parakeratosis, and gram-positive cocci.

Contributor's Comment:
Upon gross examination, the dermatitis in this mouse was initially presumed to be due to *Corynebacterium bovis*, consistent with the PCR results. *C. bovis* is a common colonizer of the skin in laboratory mice, and is associated with hyperkeratosis, para-keratosis, and epidermal hyperplasia with minimal to absent inflammation. The gross lesion is particularly apparent in nude mice because of the absence of a normal hair coat, but other immunosuppressed mouse strains as well as immunocompetent strains can also be colonized and develop lesions.[1] When it was first identified as the cause of scaly skin disease (also called *Corynebacterium*-associated hyperkeratosis) in nude mice, it was considered a pathogen of high morbidity but low mortality[5], but in recent years, infection with *C. bovis* has been associated with research complication including immunological anomalies, wasting disease, and failure of engraftment of tumor cells.[6] For this reason, some institutions exclude this organism, and PCR detection sometimes leads to depopulation of research colonies.

In the present case, histologic examination revealed severe inflammatory lesions that are not characteristic of *C. bovis* colonization of mice. Severe inflammation with ulceration, intradermal abscesses, crusting, and other damage to the epidermis and dermis are not usual findings. In addition, the presence of colonies of gram-positive coccoid bacteria in the keratin layer were suggestive of infection with *Staphylococcus* spp. The lesions in this case are similar to those described by Russo et al.[4] in which an inflammatory dermatitis in a nude mouse was associated with culture of

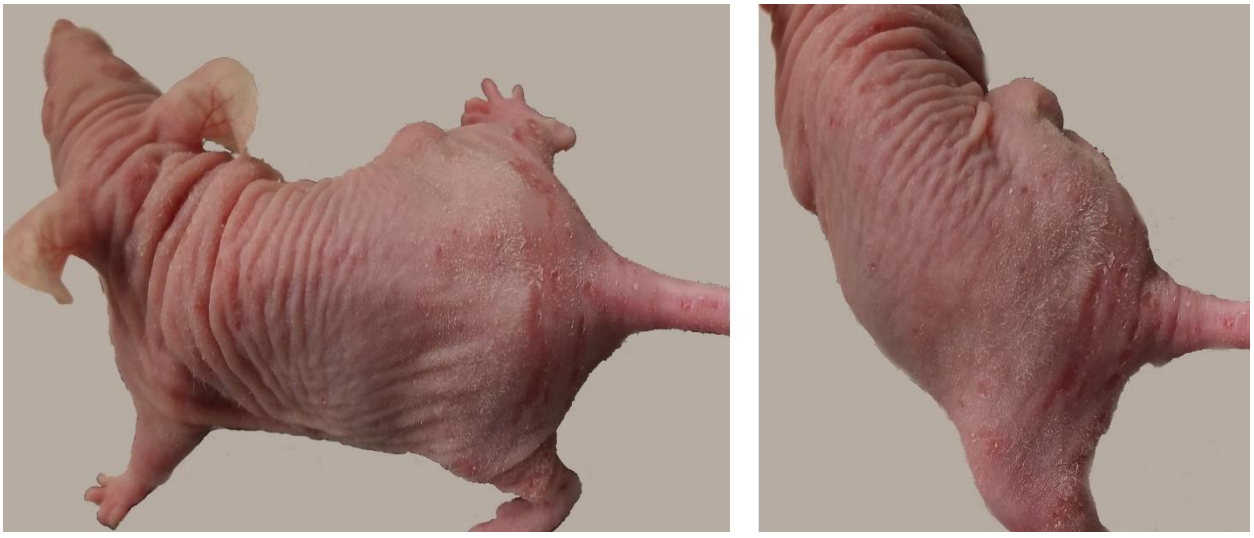

Figure 4-1. Haired skin, mouse. There was moderate ulcerative and proliferative dermatitis extending over most of the dorsum, hind limbs, and tail with multifocal and crust formation. (Photo courtesy of: In Vivo Animal Core, Unit for Laboratory Animal Medicine, University of Michigan Medical School, https://ncrc.umich.edu/research/scientific-resources/ivac)

Staphylococcus xylosus. In that report, lesions were similar to the current case, with individual keratinocyte necrosis, ulceration, and crust formation, but intracorneal pustules and acantholytic cells were not described. *C. bovis* was not isolated in that case, but large numbers of *Staphylococcus xylosus* were isolated and clusters of gram-positive cocci in the keratin layer were demonstrated in histologic sections of skin. Based on the bacteriologic and histologic findings, a diagnosis of dermatitis due to *S. xylosus* was made. Like the current case, the published case was a single mouse in a group of unaffected mice. In that report, PCR was not performed.

The causative or contributory role of *C. bovis* in our case was complicated by the unusual histologic features and the absence of bacteriologic confirmation. Simple *C. bovis* colonization is associated with mild hyperkeratosis and scattered short gram-positive rods that in the keratin layer. Inflammation is minimal or not present. In this case, there was marked inflammation with some features of immune-mediated disease, including acantholytic cells both within intracorneal pustules and individually within the stratum spinosum, and disruption of the stratum basale. Gram staining revealed clusters of gram-positive cocci, most consistent with *Staphylococcus sp*. Whether or not *C. bovis*

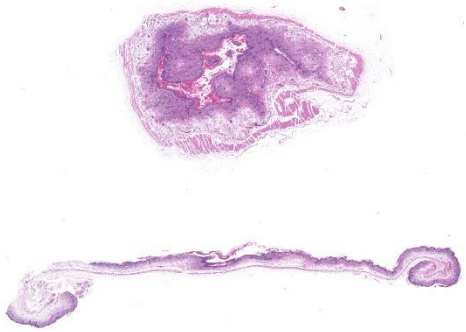

Figure 4-2. Haired skin, mouse. Two sections of haired skin are submitted for examination. At subgross magnification, mild thickening of the epidermis and hyperkeratosis are discernable. (HE,

contributed to the lesions in this mouse could not be established. However, the ulcerations noted grossly and the atypical inflammatory and necrotizing lesions in the skin suggested that a different or additional infectious agent was present. Unfortunately, the skin was not cultured, and the presence or absence of viable *C. bovis* or other bacteria could not be established.

Contributing Institution:
In Vivo Animal Core, Unit for Laboratory Animal Medicine
University of Michigan Medical School
https://ncrc.umich.edu/research/scientific-resources/ivac

JPC Diagnosis:
Haired skin: Dermatitis, neutrophilic and histiocytic, perivascular, moderate, with hyperkeratosis, acanthosis, apoptotic keratinocytes, and intracorneal bacilli and cocci.

JPC Comment:
As noted by the contributor, *Corynebacterium bovis* presents a significant challenge to in the research setting. As an example, a pre-clinical research laboratory was recently affected by broad dissemination of *C. bovis*, resulting in cutaneous infections in murine inventories that diminished patient-derived chronic myelomonocytic leukemia engraftment in immunodeficient mice, resulting in reduced confidence in preclinical data validity. In an effort to exclude this organism and reduce risk of such invalidations, many institutions conduct facility-wide *C. bovis* PCR murine and environmental surveillance, cull positive mice, and sterilize of equipment and rooms via methods such as vaporized hydrogen peroxide.[2]

C. bovis is an opportunistic, lipophilic, gram-positive coryneform rod often found within the stratum corneum. The organism resides

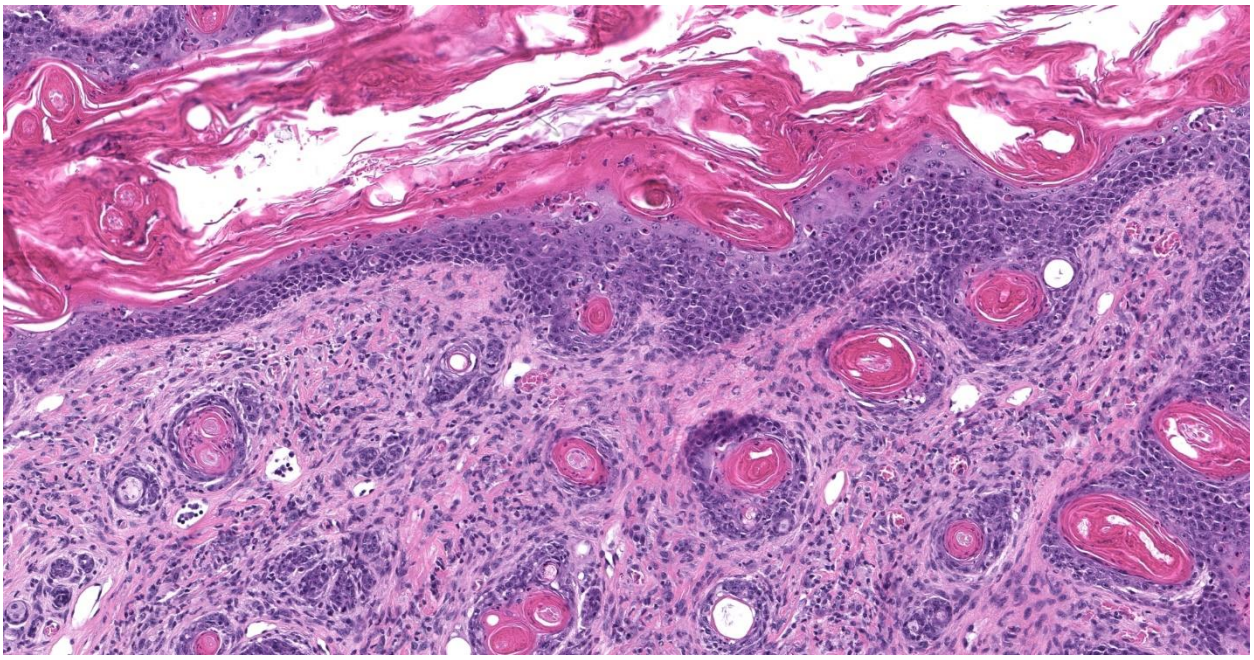

Figure 4-3. Haired skin, mouse: The hyperkeratosis is moderate and extends down into the follicular infundibula. There are scattered pustules within the scale, and the epidermis is thickened and mildly disorganized. There are individualized and clusters of apoptotic keratinocytes and moderate numbers of neutrophils and macrophages in the superficial dermis and neutrophils within the overlying epidermis. (HE, 186X)

on the skin of mice, particularly affecting immunodeficient strains such as *nu/nu*, *Prkdc*^{scid}, SCIDbeige, and others. Persistent infections result in the development of an orthokeratotic, hyperkeratotic, acanthotic dermatitis that subsequently generates *C. bovis*-infected keratin flakes that can be spread by airborne and fomite transmission within facilities.[2,3]

The exact mechanism by which *C. bovis* colonizes murine skin and induces the previously described lesions has not been described. Historically, immunocompetent and immunodeficient mice have been known to be vulnerable to transient infection but only immune deficient strains, such as nude and SCID strains, were thought to develop "persistent" infections that progress into clinically apparent hyperkeratosis following natural infection. However, a reported natural outbreak of *C. bovis* in a laboratory resulted in persistent infections in not only immunodeficient but also some immuno-competent strains, including transgenic mice expressing the human papillomavirus (HPV) E6 oncoprotein and epidermal mutant *dep/dep* mice. Epidermal mutant *dep/dep* mice remained persistently PCR positive for >45 days and developed clinically apparent hyperkeratosis.[2] *Corynebacterium* species cannot produce their own lipids and are well suited to reside within lipid-rich sebum and the stratum corneum. Therefore, a possible explanation for persistent infections within the immunocompetent *dep/dep* strain was the presence of excessive sebum from hyper-plastic sebaceous glands and abundant lipids of the interfollicular epidermis which created a favorable environment for the organism's survival.[2]

Cutaneous dysbiosis with an overrep-resentation of *Corynebacterium* species as a result of altered skin homeostasis has also been reported in mice. Therefore, a possible explanation for persistent infection in immunocompetent transgenic mice that

express the HPV-E6 oncoprotein may be due to altered skin homeostasis as the result of a human keratin 14 promoter directed E6 oncogene expression in the hair follicle and epidermal basal layer, resulting epidermal hyperplasia.[2]

Definitive diagnosis of *Corynebacterium bovis* can be achieved through culture or PCR of skin swabs or feces, although the organism can be isolated from the oral cavity, skin, and heart blood of infected mice. Cultures should be held for up to seven days due to the organism's slow growth.[3]

Differential diagnoses include low ambient humidity in addition to *Staphylococcus* xylosus, as previously described by the contributor in a recent report.

Both *Staphylococcus aureus and S. xylosus* are known to cause another syndrome in mice known as ulcerative dermatitis.[3] Some strains, such as B6 manifest excessive scratching behavior which facilitates colonization, most often by *S. aureus.* Similarly, athymic nude mice and B6-Nos2^{tm1Lau} (NOS2) strains are affected by *S. xylosus.* Following colonization, the bacteria produce virulence factors such as hemolysis, nucleases, proteases, lipases, hyaluronidase, collagenase, and exotoxins that result in focal small to large chronic ulcerative lesions, most often around the head and neck, but also on the trunk and tail base. Histologically, these

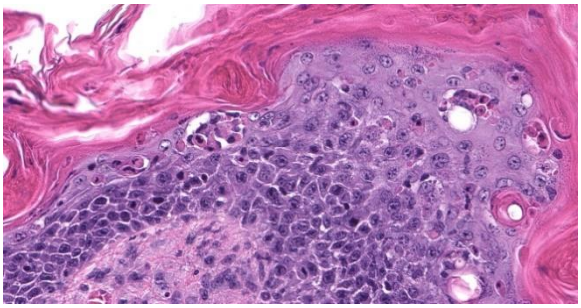

Figure 4-4. Haired skin, mouse: There are clusters and individualized apoptotic cells within the stratum spongiosum. (HE, 400X)

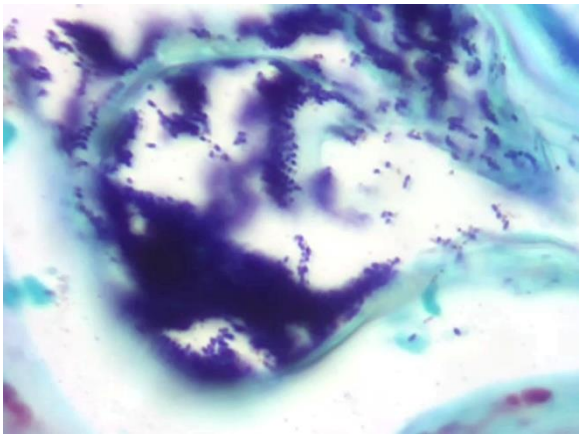

Figure 4-5. Haired skin, mouse. A tissue Gram stain demonstrates gram-positive cocci within the keratin layer. (Gram, 1000X) (Photo courtesy of: In Vivo Animal Core, Unit for Laboratory Animal Medicine, University of Michigan Medical School, https://ncrc.umich.edu/research/scientific-resources/ivac)

infections are characterized as prominent colonies of gram-positive cocci within a superficial exudate and coagulative necrosis of the underlying epidermis and dermis which resemble 1st, 2nd, and 3rd degree burns with varying degrees of leukocytic infiltration and granulation tissue.[3]

Conference participants agreed with the contributor's assessment that the histologic features observed in this case were atypical for simple *C. bovis* colonization and suspect an additional etiologic agent, such as *Staphylococcus* spp. contributed toward this lesion's pathogenesis. As previously noted, *C. bovis* is typically associated with mild hyperkeratosis with minimal to mild inflammation in contrast to the robust inflammation observed in this case.

References:

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6. Vedder AR, Miedel EL, Ragland NH, et al. Effects of *Corynebacterium bovis* on Engraftment of Patient-derived Chronic-Myelomonocytic Leukemia Cells in NSGS Mice. *Comp Med*. 2019;69(4):276-282. doi:10.30802/AALAS-CM-18-000138.