Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #8 12 October 2022

CASE I:

Signalment:
10-day-old Red Angus-Simmental calf (*Bos Taurus*) bovine

History:
Over a period of 8 years (2013–2021) a total of six Red Angus-Simmental bull calves were born alive full term with hypotrichosis and oligodontia on a ranch in the Nebraska panhandle, USA. According to the attending veterinarian, the calves appeared almost completely alopecic. Animals tended to die in the first week of life, probably related to being born in spring with associated cold temperatures. The owners kept one calf (current case) alive by housing him indoors, wrapped in an insulated coat. A skin sample was taken to exclude inter-uterine infection with bovine viral diarrhea virus (BVDV), with negative results. The calf was alert and nursed normally. He was euthanized at 10 days of postnatal life in order to characterize cutaneous lesions and to establish whether lesions were present elsewhere, particularly in respiratory tract and oral cavity.

Gross Pathology:
The calf was almost completely devoid of large, guard-type hairs at birth, excepting distal parts of the limbs and tail, eyelids, chin and tragus. Vibrissae were present on the muzzle and chin. Short fine hairs consistent with undercoat were present over the trunk, neck, head and upper two thirds of the limbs. The nasolabial plate was flat and dry. All incisors were absent grossly, a finding that was confirmed radiographically. According to the owner and the attending veterinarian, the other five affected bull calves had a similar phenotype, including the absence of visible teeth. Hooves were unremarkable. Obvious ocular lesions were absent.

Laboratory Results:
A biopsy of aural skin was negative for bovine viral diarrhea virus by antigen ELISA.

Microscopic Description:
Haired skin-subcutis from flank. There is a complete absence of large caliber hair folli-

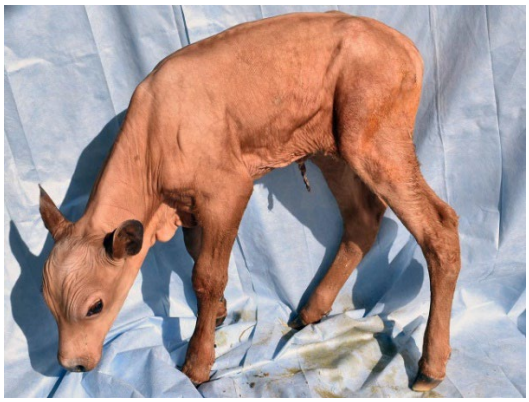

Figure 1-1. Presentation, calf. The calf is partially alopecic with no large guard-type hairs, excepting distal parts of the limbs and tail, eyelids, chin and tragus. Vibrissae were present on the muzzle and chin. The nasolabial labial plate is dry. (Photo courtesy of: Wyoming State Veterinary Laboratory; 1174 Snowy Range Road; Laramie; WY 82070; http://www.uwyo.edu/wyovet/)

cles containing medullated hair shafts. Existing follicles are uniformly small (diameter of 35 - 40 µm) and contain non-medullated, lightly pigmented hairs of unremarkable appearance. Each follicle retains a normal relationship with apocrine glands, arrector pili, and sebaceous glands. There is a mild diffuse lymphohistiocytic inflammation in upper dermis.

Contributor's Morphologic Diagnoses:
1. Severe diffuse congenital hypotrichosis, with absence of primary hair follicles.
2. Mild diffuse superficial lymphohistiocytic dermatitis.

Contributor's Comment:
In addition to hypotrichosis, the affected calf had oligodontia with only 12 deciduous teeth, all of which were unerupted or barely erupted premolars at the time of euthanasia. The calf also lacked nasolabial, tracheal and bronchial-bronchiolar glands. Analysis at the Institute of Genetics in the University of Bern identified a 53 kb deletion in the X chromosome, including part of the *EDA* gene and all of *AWAT2*. Partial deletion of *EDA* is the likely basis for this form of hypotrichosis-oligodontia.[8]

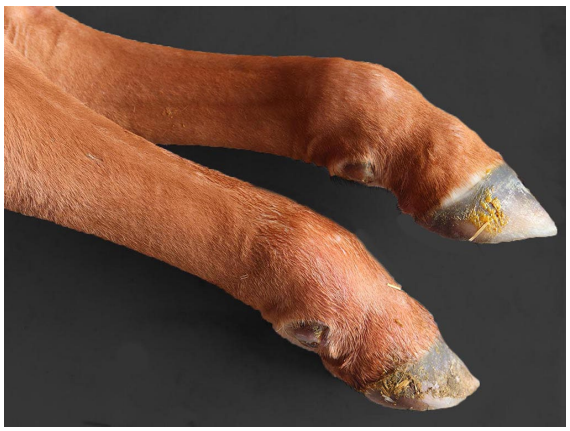

Figure 1-2. Legs, calf. Short fine hairs consistent with undercoat were present over the trunk, neck, head and upper two thirds of the limbs. (Photo courtesy of: Wyoming State Veterinary Laboratory; 1174 Snowy Range Road; Laramie; WY 82070; http://www.uwyo.edu/wyovet/)

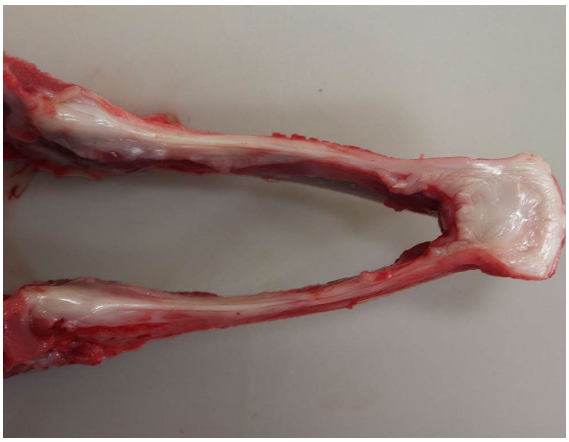

Figure 1-3. Mandible, calf. Incisors and cheek teeth are absent. (Photo courtesy of: Wyoming State Veterinary Laboratory; 1174 Snowy Range Road; Laramie; WY 82070; http://www.uwyo.edu/wyovet/)

This combination of lesions involving hair follicles, teeth and aplasia of selected glands indicates an ectodermal dysplasia.[5] Mutation in *EDA*, marked reduction in large (developmentally first-formed) hair follicles containing medullated hairs, restriction of the disorder to bull calves, and the lesions in non-cutaneous tissues is consistent with an X-linked disease. This is reported sporadically in Holstein and Holstein Friesian cattle, Japanese Black cattle, and crossbred cattle. Currently most of the published reports document one or more affected calves in commercial herds in Europe.[9] Affected calves die or are killed early in post-natal life. Some can be kept alive when fed a chopped diet after they begin to ruminate, combined with protection from weather extremes. Currently 9 episodes of the bovine condition have been documented.[9] The causative abnormality in *EDA* varies. Investigators report various small or gross deletions, insertions, inversions, splicing, and nonsense (stop-gain) mutations. The resultant phenotype is remarkably uniform, based on published accounts. Similar forms of ectodermal dysplasia due to *EDA* variants are reported in dogs, mice, and people.[3] *AWAT2* encodes an enzyme in the diacylglycerol

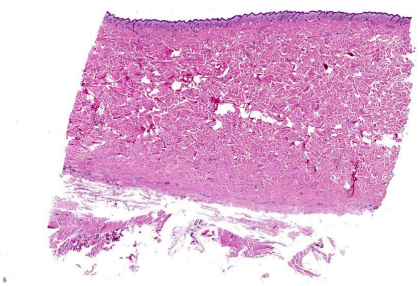

Figure 1-4. Haired skin, calf. The epidermis and superficial dermis are markedly thinned (hypoplasia). (HE, 5X)

acyltransferase family. The enzyme produces wax esters as part of the normal lipid metabolism of skin, primarily in sebocytes. The submitter did not recognize a lesion in sebaceous glands of affected calves.

This bovine ectodermal dysplasia corresponds to X-linked hypohidrotic ectodermal dysplasia-1 in human subjects (ECTD1)(OMIM 305100).[10,13] *EDA* codes for ectodysplasin, a member of the TNF-related ligand family which mediate epithelial-mesenchymal interactions during fetal development, including formation of ectodermal appendages.[11] Use of the term ECTD has been proposed for calves with analogous mutations in *EDA*.[3]

Unlike affected people and mice, there is no simple way to assess the function of sweat glands in neonatal calves. There is disagreement about whether sweat glands in affected calves are histologically normal. The submitter compared sweat glands from affected calves to those in unaffected calves from the source herd and there was no obvious difference.

Affected human patients have a triad of signs comprising sparse hair (hypotrichosis), abnormal or missing teeth (anodontia or hypodontia), and anhidrosis/hypohidrosis. Medical complications in affected people are hyperthermia due to inability to sweat normally, and recurrent respiratory infections. Affected children can be treated successfully using antenatal injections of recombinant ectodysplastin.[12] Treatment was also successful two animal models of ECTD-like syndromes (*Tabby* mice; dogs).[4,6]

It is diagnostically useful to check for oligodontia in calves with marked congenital hypotrichosis, since its presence narrows differential considerations regarding etiology. Calves that are bald at birth will be obvious to owners. It is common for veterinarians to be contacted about such calves. The first question is generally: could this be inherited? Non-genetic causes of congenital hypotrichosis exist, such as transplacental bovine diarrhea virus infection (excluded here), maternal iodine deficiency, and adenohypophyseal hypoplasia. Neonatal calves that present with hypotrichosis, oligodontia and a smooth dry nasal plate should prompt consideration of an inherited abnormally in the EDA molecular pathway. The defect most commonly involves *EDA* itself. Mutations may affect other genes in the pathway (i.e., *EDAR*, *EDARADD* or*TRAF6*), resulting in a similar phenotype.[11]

Mild changes affecting teeth, hair and sweat glands occurs in some mothers of children with ECTD1, but are generally subtle. Investigation of similar changes in carrier carriers and calves is limited.

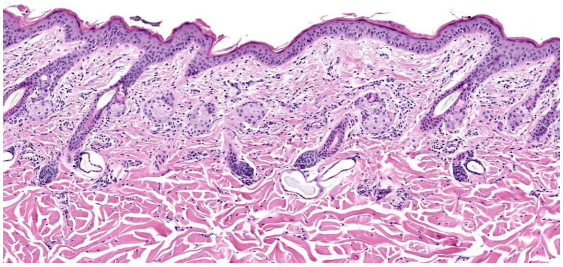

Figure 1-5. Haired skin. The epidermis is hypoplastic, with one cell for each of the basal layer, stratum spongiosum, and stratum corneum. All follicles are in telogen. Hair shafts within telogen follicles are thin with no medulla; deeper telogen follicles are twisted. Sebaceous glands are normal, and there are few lymphocytes and plasma cells within the dermis. (HE, 200X)

Contributing Institution:
Wyoming State Veterinary Laboratory; 1174 Snowy Range Road; Laramie; WY 82070; http://www.uwyo.edu/wyovet/

JPC Diagnosis:
1. Haired skin: Hypotrichosis, diffuse, severe with trichodysplasia.
2. Haired skin: Dermatitis, lymphohistiocytic, superficial and perivascular, mild.

JPC Comment:
This week's moderator, Dr. Charles Bradley from the University of Pennsylvania, emphasized that it is not possible based on histopathologic examination alone to rule out non-genetic causes of hypotrichosis. Other differentials in calves which be considered include iodine deficiency; toxins (*Veratrum album* in Japanese cattle), adenohypophyseal hypoplasia in Guernsey and Jersey cattle, and intrauterine infection with bovine pestivirus (bovine viral diarrhea virus; ruled out in this case using ELISA testing).

Currently, there are nine X-linked *EDA* mutations causing ectodermal dysplasia in various breeds of cattle.[1] Additionally, there is one documented autosomal recessive mutation in the *EDA* receptor gene associated with ectodermal dysplasia in Charolais cattle.[8]

Humans, mice, and cattle with ectodermal dysplasia may lack of normal glands of the respiratory tract, such as bronchial and bronchiolar glands, which compromises mucociliary clearance and predisposes affected animals to respiratory infections, including sinusitis and pneumonia.[1,7,8] Additionally, EDA is crucial for the development and function of lacrimal and Meibomian glands, corneal homeostasis, and corneal wound healing, and recurrent conjunctivitis has been documented in dogs with ectodermal dysplasia and EDA-deficient mice.[1,7,8]

Recently, it has been reported that several mice and rat strains with EDA deficiency also have significant Zymbal's gland hypoplasia, with fewer hair follicles and smaller sebaceous glands in the external ear canal.[2] Affected rodents have increased prevalence of otitis externa, and the authors concluded this was likely due to sebum deficiency.[2] Strains with Zymbal's gland hypoplasia included EDA-deficient mice, EDA receptor (EDAR) deficient mice, and EDAR-associated death domain (EDARADD) deficient rats.[2] The EDA-deficient mice and EDARADD deficient rats were also predisposed to otitis media due to deficiency of nasopharyngeal submucosal glands associated with the auditory tube.[2]

References:

1. Capuzzello G, Jacinto JCP, Hafliger IM, et al. A large deletion encompassing exon 2 of the *ectodysplasin A* (*EDA*) gene in a British blue crossbred calf with hypohidrotic ectodermal dysplasia. *Acta Veterinaria Scandinavica*. 2022; 64(23):1-8.
2. Del-Pozo, Headon DJ, Glover JD, et al. The EDA deficient mouse has Zymbal's gland hypoplasia and acute otitis externa. *Dis Model Mech*. 2022; 15(3):1-31.
3. Drögemüller C, Distl O, Leeb T. X-linked anhidrotic ectodermal dysplasia (*ED1*) in men, mice, and cattle. *Genet Sel Evol.* 2003;35: S137-145.
4. Gaide O, Schneider P. Permanent correction of an inherited ectodermal dysplasia with recombinant EDA. *Nat Med*. 2003;9:614-618.
5. Hargis AM, Ginn PE. The integument. In: Zachary JF, McGavin MD, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. Elsevier Inc. 2012.
6. Mauldin EA, Gaide O, Schneider P, Casal ML. Neonatal treatment with recombinant ectodysplasin prevents respiratory disease in dogs with X-linked ectodermal

dysplasia. *Am J Med Genet A*. 2009;149A:2045-2049.
7. Moura E, Rotenberg IS, Pimap CT. X-Linked hypohydrotic ectodermal dysplasia - General features and dental abnormalities in affected dogs compared with human dental abnormalities. *Top Companion Anim Med.* 2019, 35, 11-17.
8. O'Toole D, Häfliger IM, Leuthard F, et al: 2021, X-linked hypohidrotic ectodermal dysplasia in crossbred beef cattle due to a large deletion in *EDA*. *Animals (MDPI*). 2021;11:657.
9. OMIA - Online Mendelian Inheritance in Animals database search engine. Accessed 27 May 2021. https://www.omia.org/home/
10. OMIM – Online Mendelian Inheritance in Man database search engine. Accessed 27 May 2021. https://www.omim.org/about
11. Sadier A, Viriot L, Pantalacci S, Laudet V. The ectodysplasin pathway: from diseases to adaptations. *Trends Genet*. 2014;30:24–31.
12. Schneider H, Faschingbauer F, Schuepbach-Mallepell S, et al. Prenatal correction of X-linked hypohidrotic ectodermal dysplasia. *N Engl J Med*. 2018;378:1604-1610.
13. Wright JT, Fete M, Schneider H, et al. Ectodermal dysplasias: Classification and organization by phenotype, genotype and molecular pathway. *Am J Med Genet*. 2019, 179, 442-447.

CASE II:

Signalment:
3 year, 7 month old female four-toed hedgehog (*Atelerix albiventris*)

History:
This adult female hedgehog had a brief (2-3 day) history of reduced appetite and unsteady gait before being found dead.

Gross Pathology:
Gross examination revealed a large, solitary, well-demarcated, 1.3 cm x 1.2 cm x 1.8 cm mass in the left axillary region, approximately 0.2 cm lateral to the second left teat. An approximately 3.0 cm x 1.0 cm x 0.4 cm segment of the mass projected cranially through the subcutis, adjoining the axillary lymph node. On cut section, the mass was firm and tan with coalescing red segments. The skin overlying the mass was partially alopecic and hyperkeratotic, while the skin of the pinnae and dorsum exhibited moderate hyperkeratosis and flaking. Internally, two, approximately 2 mm diameter, tan, firm nodules were present in the dorsal aspect of the right lung and there was enlargement of a dorsocranial mediastinal lymph node (1.2 cm x 0.4 cm x 0.3 cm) which was mottled red and tan. Moderate thoracic effusion, approximately 2-3 ml of brown translucent fluid, was present. The spleen was mildly enlarged (5.6 cm x 1.5 cm x 0.4 cm) and diffusely dark purple.

Laboratory Results:
PCR targeting the ITS2 region of the fungal genome (5.8S – 28S), performed on FFPE tissue from the axillary mass, amplified two bands. The first, a 256 bp sequence, was 97.6% identical to GenBank No.

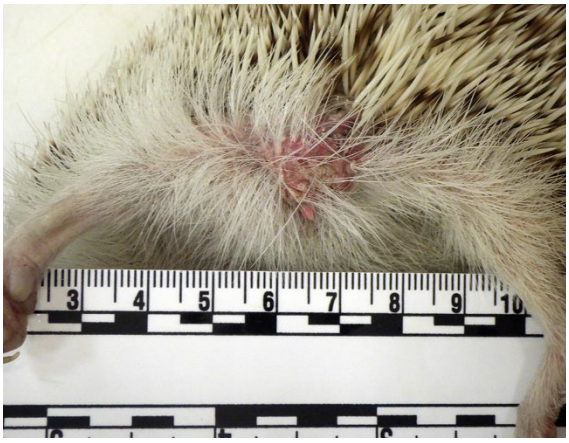

Figure 2-1. Axilla, hedgehog. There is a 1.3 cm nodule within the axillary subcutis. (Photo courtesy of: Wildlife Conservation Society, Zoological Health Program; https://oneworldonehealth.wcs.org; www.wcs.org)

NR_149340, *Trichophyton erinacei* strain ATCC 28443 (Blastn analysis, NCBI). Analysis of this sequence using the ISHAM (International Society for Human and Animal Mycology) Barcoding Database (https://its.mycologylab.org/; accessed June 2021) revealed this sequence to be greater than 99.4% identical to two strains of *T. erinacei*. 367 bp of trimmed DNA sequence was also obtained, and Blastn analysis showed this sequence to be 100% identical to several isolates of *Malassezia restricta*, including GenBank No. EU915456.

Microscopic Description:
Axillary Skin: Markedly expanding and effacing the subcutis, and multifocally effacing skeletal muscle and extending into the dermis is a densely cellular inflammatory infiltrate. The infiltrate is composed of loose nodules of neutrophils, macrophages and multinucleated giant cells with occasional eosinophils, surrounded by a rim of epithelioid macrophages and a thin band of fibrous connective tissue (pyogranulomas). There is multifocal lysis of leukocytes within the center of some pyogranulomas. Similar inflammatory cells, accompanied by lymphocytes, plasma cells and scattered eosinophils and mast cells, fill the space between nodules, leaving little more than small islands of remnant adipose. Within pyogranulomas and within the cytoplasm of scattered multinucleated giant cells are low to moderate numbers of fungal elements. Round to ovoid yeast-like structures ranging in diameter from approximately 5 µm up to approximately 15 µm in diameter which display occasional budding and often form tight clusters and knots are most prevalent. Rare elongate cytoplasmic protrusions are present and some yeast-like structures have thick, refractile capsules with an internal granular appearance. Chains of round or more elongate yeast-like structures, each individual unit separated from the next by septation with a waist-like indentation, are also present, as are branching true hyphae. A dense fibrous capsule which contains relatively few lymphocytes and plasma cells multifocally separates the inflammation from the surrounding tissue. In some areas, inflammation extends into the superficial dermis, where higher numbers of eosinophils and mast cells are present. Clear space (edema), pale basophilic granular material and/or inflammatory cells expand lymphatic vessels throughout the dermis. Endothelial cells lining vessels in areas of inflammation are enlarged with prominent nuclei with open chromatin. Degeneration, necrosis and regeneration are present in the inflamed skeletal muscle deep to the dermis. The overlying epidermis exhibits mild orthokeratotic hyperkeratosis and there are scattered aggregates of yeast within the keratin (approximately 3 µm diameter).

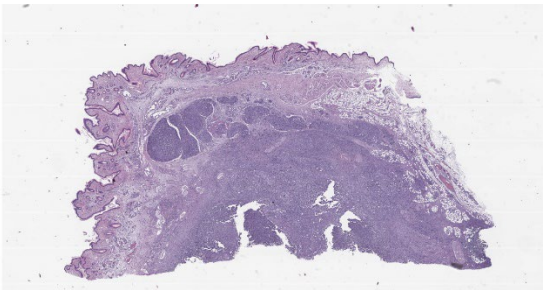

Figure 2-2. Haired skin, hedgehog. There is an extensive focus of pyogranulomatous inflammation within the deep dermis and subcutis. (HE, 12X)

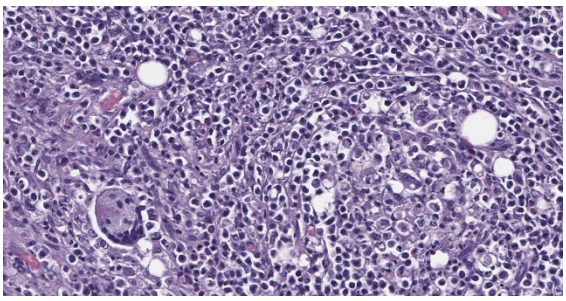

Figure 2-3. Haired skin, hedgehog. The inflammatory nodule is composed of innumerable neutrophils, macrophages, lymphocytes, and plasma cells, with numerous multinucleated macrophages including some ranging up to 60um, enmeshed in strands of collagen. (HE, 376X)

Contributor's Morphologic Diagnoses:
Skin, axillary: Cellulitis, dermatitis, pyogranulomatous to mixed, chronic, locally extensive, marked, with intralesional and intracorneal fungal elements.
Skin, axillary: Hyperkeratosis, orthokeratotic, chronic, diffuse, mild, with intracorneal yeast

Contributor's Comment:
Systemic fungal infection identified in this four-toed hedgehog (*Atelerix albiventris*) was most severe in the left axillary subcutis, where a large inflammatory mass was present, but also involved the lungs and multiple lymph nodes. Histology of the lungs and lymph nodes was similar to that described in the axillary region, with the additional findings of bronchiolitis and vasculitis in the lung, and vasculitis and rare intralesional bacteria (gram-negative bacilli) in the lymph nodes. Fungi within lesions were largely round to ovoid yeast-like forms of varying size, with occasional thick capsules and internal granularity, and multifocally forming chains. The appearance was somewhat reminiscent of *Blastomyces* species. Branching hyphae and chains of more elongate structures, representing abnormal arthroconidiation or pseudohyphae were also present, as were rare elongate structures similar to germ tubes. PAS and GMS staining markedly enhanced visualization of fungi. Pan-fungal PCR utilizing primers targeting the ITS2 region amplified two separate and discrete bands, both of which were sequenced and were consistent with *Trichophyton erinacei* and *Malassezia restricta*, respectively; *T. erinacei* was suspected to be the primary etiology.

Trichophyton erinacei, formerly *T. mentagrophytes* var. *erinacei* and of the *T. benhamiae* complex, is a common isolate from hedgehog skin, with both clinical disease and subclinical carrier states being identified in

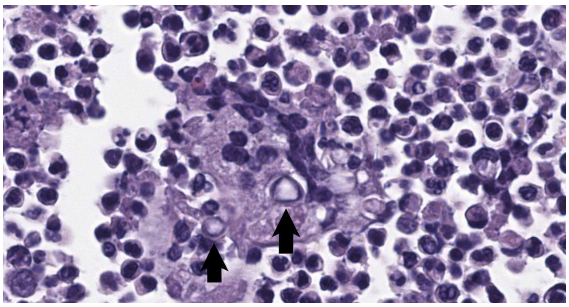

Figure 2-4. Haired skin, hedgehog. Macrophages and multinucleated cells often contain phagocytized 8-14 µm yeasts (arrows). A pseudohypha or germ tube is present adjacent to the yeast at left. (HE, 576X)

free-ranging and pet European (*Erinaceus europaeus*) and four-toed hedgehogs.[1] In one study investigating dermatophytosis in European hedgehogs at a wildlife center in France, over 79% of the *T. erinacei* culture-positive animals were asymptomatic and overall 20% of animals without skin disease were culture positive for *T. erinacei*.[7] When present, disease associated with *T. erinacei* in hedgehogs presents with scaling, crusting and alopecia, including loss of spines; the head is often the focus of infection.[1,2,7] While the histologic appearance of dermatophytosis in hedgehogs is not well described, it is expected to consist of fungal hyphae limited to the keratin layers and the hair/spine shafts, with mild inflammation and proliferative changes to include epidermal hyperplasia and hyperkeratosis, as described in other species.[5,9] In addition to the large axillary inflammatory mass, this patient had a focal area of fungal dermatitis on the foot which was reddened grossly (no flaking or crusting) and histologically exhibited orthokeratotic hyperkeratosis with intracorneal hyphae. This was interpreted as a potential second, more typical, area of *T. erinacei* dermatomycosis, although fungal identification was not pursued at this site.

Dermatophytosis is relatively common in both domestic animals and humans. It is caused by keratinophilic fungi, typically belonging to one of three genera: *Trichophyton*, *Epidermophyton* and *Microsporum*.[3,9] Der-

matophyte species are further classified according to natural history; these categories include the zoophilic species which primarily infect animals but can be transmitted to humans, the anthropophilic species which primarily infect humans, and the geophilic species which are typically found in the soil but can also infect animals and humans.[3,9] *Trichophyton erinacei* is an example of a zoophilic dermatophyte, and one that may be considered an emerging pathogen based on increases in human and pet infections.[2] Dermatophytes can be readily transmitted between animals and between animals and humans via direct contact with infected animals or indirect exposure to shed hairs (made brittle and easily broken by infection) and desquamated keratin containing infective spores.[9] While normal, intact skin resists infection, minimal damage to the epidermis can predispose to infection.[3] As alluded to above, the typical presentation consists of superficial lesions with fungi restricted to the stratum corneum of the epidermis and hair follicles and/or the hair shafts themselves. Deep infection by dermatophytes is rare in humans but can be prominent in animals, and includes both kerion (solitary inflammatory nodules, often with draining tracts) and pseudomycetoma (subcutaneous inflammatory nodules). Development of deep infection can result from implantation or furunculosis of affected follicles.[9]

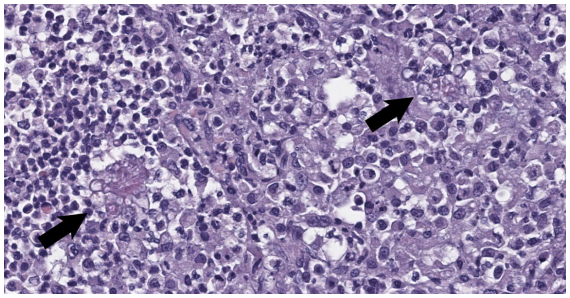

Figure 2-5. Haired skin, hedgehog. Clusters of 8-14 µm yeasts and pseudohyphae are scattered throughout the inflammatory nodules (arrows). (HE, 576X)

Deep and/or systemic/disseminated trichophytosis in hedgehogs due to *T. erinacei* has not been described. In the sections examined from this case, folliculitis was not observed, nor was there specific evidence of furunculosis, such as hair or keratin within lesions. The presence of eosinophils, most notably in the dermis, was suggestive of furunculosis, however, and this remains a possibility, especially given the apparent chronicity of the case. One of the most interesting features in this case was the fungal morphology. *Trichophyton* species are typically hyphal when found superficially[5] and morphologic forms like those seen in the current case have not been described with *T. erinacei*. However, *T. rubrosum*-associated deep mycoses in humans can contain atypical forms of the fungus, including *Blastomyces*-like yeast forms as seen here, as well as short, thickened hyphal fragments and arthroconidia.[8,10,11,12] Descriptions of atypical dermatophyte morphology have to date been restricted to immunocompromised patients, in whom deep dermatophytoses are typically described, and have been limited to *T. rubrosum* in humans. While immune compromise was not confirmed in this hedgehog, marked lymphoid depletion in the spleen could indicate some degree of underlying immunodeficiency; the bone marrow was unremarkable. As in humans, superficial dermatophytosis was suspected to precede the deep infection in this hedgehog and more typical fungal hyphae were identified within the stratum corneum via GMS staining.

Malassezia restricta, identified via PCR, was considered an incidental finding. This is a yeast that is part of the normal skin flora of humans and for which there are no descriptions of abnormal morphologies, or hyphal elements, in tissue. Yeast were present on the surface of the skin which may have been responsible for the positive PCR result. Confirmation of the fungal species within the

deep lesions would require immunohistochemistry, in-situ hybridization or other advanced diagnostic modalities, none of which were pursued in this case.

Additional findings of note in this hedgehog included vacuolation in brain and spinal cord, predominantly affecting the white matter in the latter with more generalized distribution in the brain. This was similar to lesions described with "wobbly hedgehog syndrome (WHS)," a somewhat common idiopathic condition in hedgehogs and WHS could have contributed to those signs. This was a slightly unusual case in that WHS clinical signs are typically insidious in onset, rather than the acute onset in the current case, and other causes of vacuolation were not ruled out. It is possible that this lesion was incidental and the perceived neurologic signs the result of patient weakness and debilitation due to systemic fungal disease. There was also acute renal tubular degeneration and necrosis attributed to pulmonary disease and tissue hypoxia, and chronic hepatic and biliary changes suggestive of prior biliary obstruction.

This case highlighted the importance of ancillary testing for fungal disease, including culture and/or molecular diagnostics, rather than reliance on tissue fungal morphology alone.

Contributing Institution:
Wildlife Conservation Society, Zoological Health Program;

- https://oneworldonehealth.wcs.org
- www.wcs.org

JPC Diagnosis:
Haired skin: Dermatitis and cellulitis, pyogranulomatous, focally extensive, severe, with numerous fungi.
Haired skin: Hyperkeratosis, orthokeratotic, diffuse, mild, with superficial yeast.

JPC Comment:
The contributor provides a thorough report of *Tricophyton erinacei* infection in hedgehogs. Similar to *T. erinacei*, hedgehogs have a high prevalence of *mecC*-methicillin resistant *S.* aureus (MRSA), with carriage rates of 60-64% in various studies in wild European hedgehogs (*Europeaus erineaus*).[4] The *mecC* gene encodes penicillin-binding protein 2c (PBP2c) and confers resistance to most beta-lactam antibiotics. Most *mecC*-MRSA infections occur in Europe, and it was initially isolated in bulk milk tanks and human infections in Europe.[4] Dairy cattle were thought to be the reservoir, and it was believed that administration of beta-lactam antibiotics selected for resistant bacteria.[4,6] While recent studies confirm that antibiotic exposure has indeed selected for *mecC*-MRSA resistance, a different antibiotic source has been incriminated: the cohabitant *T. erinacei*.

Penicillin-producing dermatophytes and penicillin-resistant *S. aureus* were first documented in hedgehogs in the 1960s, and it was recently demonstrated that *T. erinacei* from wild hedgehogs encodes and expresses the same antibiotic-encoding genes as *Penicillium chrysogenum*, with some isolates actively producing benzylpenicillin.[4] A subsequent *in vitro* study showed that the fungus has greater growth inhibition on *mecC*-deleted MRSA mutants compared to *mecC*-

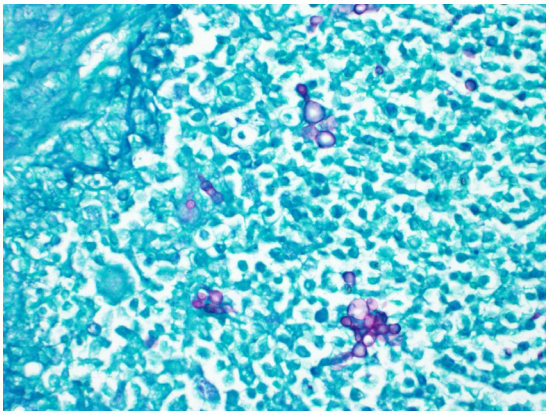

Figure 2-6. Haired skin, hedgehog. Yeasts form pseudohyphae and multidirectional chains. (PAS, 400X)

MRSA.[6] These results strongly suggest that *T. erinacei* has imposed a selective pressure on *S. aureus* which favors resistance. Furthermore, phylogenetic studies have indicated that resistance of many *mecC*-MRSA lineages was acquired prior to the modern use of antibiotics. This is supported by the fact that *T. erinacei* isolates from hedgehogs in New Zealand have produced the same antibiotic resistance in *mecC*-MRSA, and these hedgehogs were imported from Europe in the 1800s.[6]

There is wide diversity but geographic clustering of *mecC*-MRSA isolates in hedgehogs.[4] Most isolates lack the genetic variations required to infect humans and cattle, implying that methicillin-resistance did not originate in these other species.[6] Furthermore, human and hedgehog isolates within the same geographic region have similar patterns of genetic variation.[6] Coupling these facts with the high prevalence of *mecC*-MRSA in hedgehogs, researchers have concluded that hedgehogs are the natural reservoir for *mecC*-MRSA, and, for this strain, antibiotic resistance developed due to selective pressure from *T. erinacei* and predates medical use of antibiotics.[6]

References:

1. Abarca ML, Castella G, Martorell J, Cabanes FJ. *Trichophyton erinacei* in pet hedgehogs in Spain: Occurrence and revision of its taxonomic status. *Med Mycol*. 2017;55:164-172.
2. Cmokova A, Kolarik M, Dobias R, et al. Resolving the taxonomy of emerging zoonotic pathogens in the *Trichophyton benhamiae* complex. *Fungal Diversity*. 2020;104:333-387.
3. Donnelly TM, Rush EM, Lackner PA. Ringworm in small exotic pets. *Sem Av Ex Pet Med*. 2000;9:82-93.
4. Dube F, Soderlund R, Salomonsson ML, Troell K, Borjesson S. Benzylpenicillin-producing *Trichophyton erinacei* and methicillin resistant *Stapylococcus aureus* carrying the *mecC* gene on European hedgehogs – A pilot study. *BMC Microbiology.* 2021; 21:212-222.
5. Gregory MW, English MP. *Arthroderma benhamiae* infection in the Central African hedgehog, *Erinaceus albiventris*, and a report of a human case. *Mycopathologia*. 1975;55:143-147.
6. Larsen J, Raisen CL, Ba X, et al. Emergence of methicillin resistance predates the clinical use of antibiotics. *Nature*. 2022; 602:135-141.
7. Le Barzic C, Cmokova A, Denaes C, et al. Detection and control of dermatophytosis in wild European hedgehogs (*Erinaceus europaeus*) admitted to a French wildlife rehabilitation centre. *J Fungi*. 2021;7:74-87.
8. Lillis JV, Dawson ES, Chang R, White CR. Disseminated dermal *Trichophyton rubrum* infection – an expression of dermatophyte dimorphism? *J Cutan Pathol*. 2010;37:1168-1169.
9. Mauldin EA, Peters-Kennedy J. Integumentary system. In: Maxie, MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals Volume 1*. 6th ed. Elsevier; 2016.
10. Nir-Paz R, Elinav H, Pierard GE, et al. Deep infection by *Trichophyton rubrum* in an immunocompromised patient. *J Clin Microbiol*. 2003;41:5298-5301.
11. Squeo RF, Beer R, Silvers D, Weitzman I, Grossman M. Invasive *Trichophyton rubrum* resembling blastomycosis infection in the immunocompromised host. *J Am Acad Dermatol*. 1998;39:379-380.
12. Talebi-Liasi F, Shinohara MM. Invasive *Trichophyton rubrum* mimicking blastomycosis in a patient with solid organ transplant. *J Cutan Pathol*. 2017;44:798-800.

CASE III:

Signalment:
5-year-old spayed female European Shorthair cat (*Felis catus*)

History:
The cat had a chronic history of diffuse exfoliative dermatitis with multifocal alopecia, in particular in the region of head, trunk and limbs with scaling, hair casts, flakes, easy detachable hairs and moderate itching.

Based on clinical history the main differential diagnoses were: demodicosis, dermato-phytosis, exfoliative dermatitis (thymoma associated or not), and sebaceous adenitis.

Gross Pathology:
Four skin biopsies (8 mm punch and surgical scissors excision) were performed at the level of the neck (right side), costate, right thigh.

Laboratory Results:
Microbiology: negative.
Deep skin scraping: negative.
PAS stain: negative.

Microscopic Description:
Haired skin. Haired skin is affected by a moderate inflammatory change involving hair follicles and obscuring sebaceous glands. Follicular walls are transmurally infiltrated, mainly in the region of isthmus and extending to the other portions, by a high number of lymphocytes and a lower number of macrophages and rare neutrophils. Keratinocytes of the outer root sheath frequently have vacuolated cytoplasm and rarely are shrunk-en, hypereosinophilic with pyknotic nuclei (apoptosis). In the superficial dermis a mild perivascular to interstitial inflammatory infiltrate composed, in a similar amount, of mast cells, lymphocytes, plasma cells and eosinophils is present associated with scant interstitial edema.

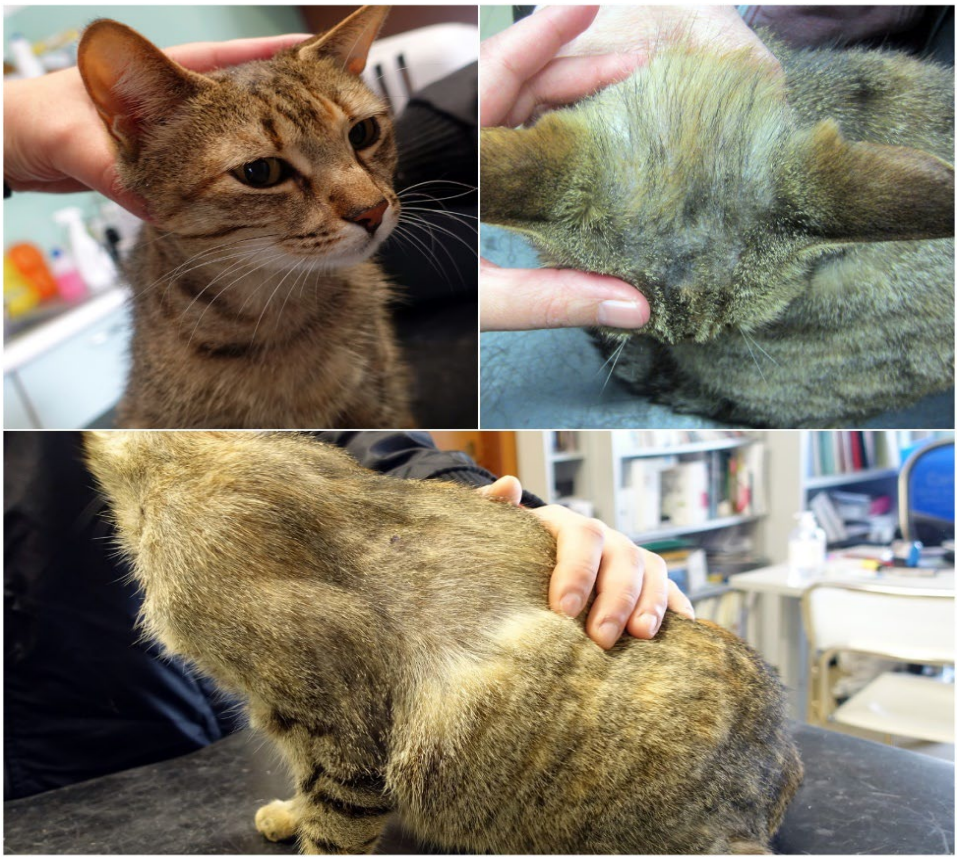

Figure 3-1. Presentation, cat. "Lola" has patchy alopecia, especially on the head and flank.on the head and flank. (Photo courtesy of: San Marco Veterinary Clinic and Laboratory, Pathology division, Viale dell'Industria 3, 35030, Veggiano (PD), Italy, https://www.clinicaveterinari-asanmarco.it/))

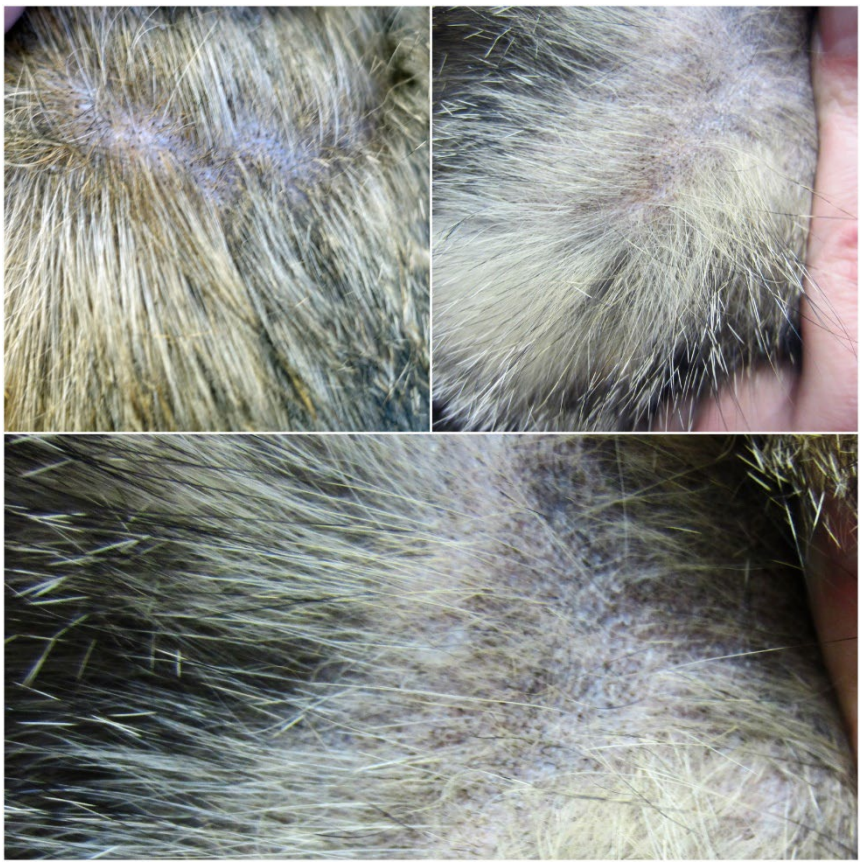

Figure 3-2. Presentation, cat. Hairs are easily detached, and there is skin flaking and hair casts. (Photo courtesy of: San Marco Veterinary Clinic and Laboratory, Pathology division, Viale dell'Industria 3, 35030, Veggiano (PD), Italy, https://www.clinicaveterinariasanmarco.it/)

Contributor's Morphologic Diagnoses:
Haired skin:
-moderate chronic lymphocytic mural folliculitis with loss of sebaceous glands
-mild perivascular to interstitial superficial dermatitis lymphoplasmacytic, with mast cells and eosinophils

Contributor's Comment:
Histopathological findings are consistent with a sebaceous adenitis associated with mural folliculitis. Sebaceous adenitis is an uncommon disease in dogs and rabbits and extremely rare in cats.

Sebaceous adenitis in dogs, although it has idiopathic origin, in standard poodles and akitas can be inherited through an autosomal recessive gene with variable expression. Hypothesis made for its pathogenesis include a cornification disorder, a cell-mediated destruction of sebaceous glands, an anatomic defect in sebaceous glands leading to lipid leakage and a foreign body response, and a metabolic defect in lipid metabolism leading to a cornification abnor-mality and sebaceous gland destruction.[1,3,6]

Histology of canine sebaceous adenitis varies depending on the duration of disease and the breed affected. In the early stages histological lesions are characterized by a granulomatous to pyo-granulomatous inflammation targeting the sebaceous glands while, in the chronic stage, which are more frequently submitted to laboratories, appears in an almost total loss of sebaceous glands. Severe epidermal orthokeratotic hyperkeratosis and follicular keratosis are invariably present. In Standard Poodles, the late stage of disease may be characterized by follicular atrophy and an absence of sebaceous glands, while in Vizslas inflammation

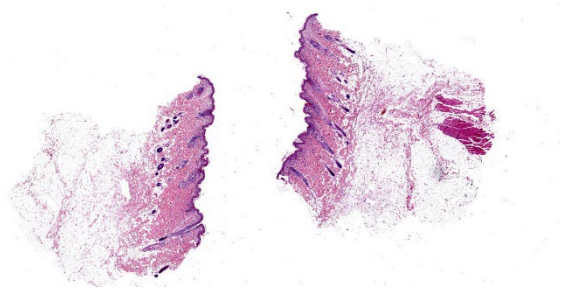

Figure 3-3. Haired skin, cat. Two sections of haired skin are submitted for examination. (HE, 14X)

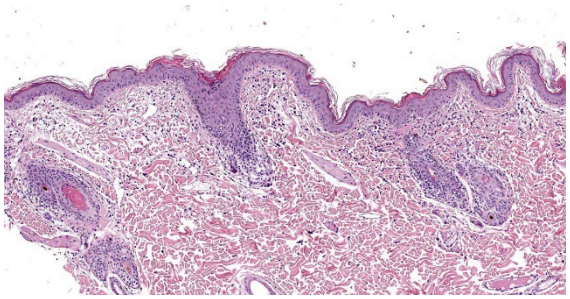

Figure 3-4. Haired skin, cat. Each follicle is infiltrated by numerous lymphocytes. (HE, 108X)

is more consistently prominent at all stages of the disease and the main differential diagnosis, in this case, is leishmaniasis.[1,3]

In rabbits, in addition to what is described in dogs, an interface dermatitis and an interface folliculitis are often present, suggesting that sebaceous adenitis may be only one aspect of a more generalized disorder.[10]

In cats, it is rarely described and it occurs as a chronic progressive disease with non-itchy scaling, crusting, alopecia and skin depigmentation in regions of the face, cervical and trunk which are the same features found in the case described except for pruritus. The latter has been described to be related to secondary infections and/or *Malassezia* spp. overgrowth.[2,4,6]

In our case, the definitive diagnosis was established histologically. The main lesions were the loss of sebaceous glands and a mural folliculitis, prominent in the isthmic region. The former is frequently described in sebaceous adenitis in different species; the latter is a reaction pattern of cats that has been associated with several diseases, included sebaceous adenitis but also with allergic dermatoses.[7] In this case, once dermatophytosis and demodicosis were excluded with special stains, negative cultures and deep skin scraping, histological lesions associated with clinical presentation led to the diagnosis of sebaceous adenitis with mural folliculitis. Treatments proposed include cyclosporin therapy; in one case, topical fatty acid supplementation was also reported.[2,4] Our case improved quickly with a cyclosporin therapy.

Contributing Institution:
San Marco Veterinary Clinic and Laboratory Pathology division
Viale dell'Industria 3, 35030
Veggiano (PD), Italy
https://www.clinicaveterinariasanmarco.it/

JPC Diagnosis:
1. Haired skin: Sebaceous adenitis, lymphocytic, diffuse, marked with sebaceous gland loss and multifocal mural folliculitis.
2. Haired skin: Dermatitis, mastocytic and eosinophilic, superficial and perivascular, mild.

JPC Comment:
One hallmark gross lesion of sebaceous adenitis is the follicular cast, as demonstrated in figure 3-2, which appear grossly as flaky concretions surrounding the bases of hair shafts, though this is less common in short haired dogs.[9]

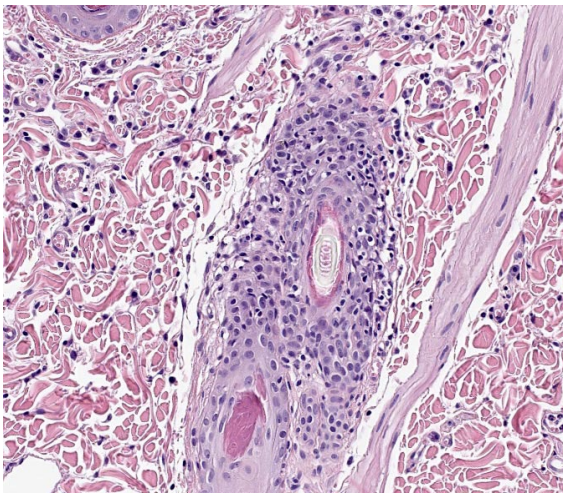

Figure 3-5. Haired skin, cat. Lymphocytes efface sebaceous glands and infiltrate the outer root sheath of the follicular infundibulum. (HE, 357X)

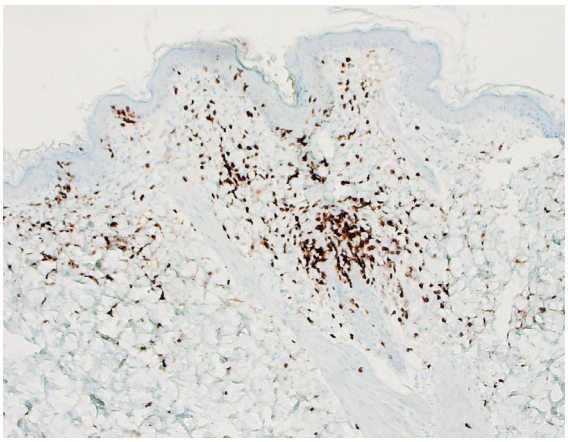

Figure 3-6. Haired skin, cat. Lymphocytes infiltrating follicles and adnexa demonstrate strong cytoplasmic immunopositivity for CD-3. (anti-CD3, 200X)

As the contributor mentioned, a main gross differential in this cat is exfoliative dermatitis. Histologically, the mural folliculitis of exfoliative dermatitis is focused on the infundibulum and isthmus and is frequently accompanied by secondary sebaceous adenitis or absence of sebaceous glands, which may mimic sebaceous adenitis.[9] Exfoliative dermatitis can be differentiated, however, by its variable lymphocytic interface dermatitis with hydropic degeneration of basal keratinocytes, suprabasilar keratinocyte apoptosis, and orthokeratotic hyperkeratosis.[9]

Standard poodles are predisposed to a sebaceous adenitis, in addition to a few other autoimmune diseases, including primary hypoadrenocorticism. This high incidence of these two diseases have been linked to an artificial genetic bottleneck which occurred in the mid 20th century resulting from the closed breed registry and intensive breeding of a handful of show-winning dogs, including Sir Gay, Wycliffe Jacqueline, Bel Tor, Carillon, and a few other dogs with equally grandiose names. [5] A study of pedigrees and DLA (MHC) markers in standard poodles from the US, Canada, and Europe showed that this mid-century bottleneck decreased genetic diversity within 70% of the standard poodle population and concentrated the genetic polymorphisms underlying sebaceous adenitis and primary adrenocorticism. [5] Geneticists recommend careful mate selection based on pedigree and genetic testing to improve the diversity within the breed and decrease incidence of disease within this breed.[5] As there is no currently available DNA test for sebaceous adenitis, the Orthopedic Foundation for Animals (OFA) recommends histologic evaluation of biopsies every 1 to 2 years in breeding animals greater than 12 months of age to assist in removing affected animals from the breeding population.[8]

References:

1. Bardagì M, Fondevila D, Zanna G et al. Histopathological differences between canine idiopathic sebaceous adenitis and canine leishmaniosis with sebaceous adenitis. *Veterinary Dermatology*. 2010; 21: 159-165.
2. Glos K, von Bomhard W, Bettenay S et al. Sebaceous adenitis and mural folliculitis in a cat responsive to topical fatty acid supplementation. *Veterinary Dermatology*. 2016; 11:57-60.
3. Gross TL et al. *Skin diseases of the dog and cat*. Second edition. Blackwell Publishing; 2005.
4. Noli C, Toma S. Three cases of immune-mediated adnexal skin disease treated with cyclosporine. *Veterinary Dermatology*. 2006;17: 85–92.
5. Pederson NC, Brucker L, Tessier NG, et al. The effect of genetic bottlenecks and inbreeding on the incidence of two major autoimmune diseases in standard poodles, sebaceous adenitis and Addison's disease. *Canine Genetics and Epidemiology*. 2015 (2):14-32.
6. Possebom J, de Farias MR, de Assunção DL et al. Sebaceous Adenitis in a Cat. *Acta Scientiae Veterinariae*. 2015; 43:1-5.
7. Rosenberg AS, Scott DW, Hollis N et al. Infiltrative lymphocytic mural folliculitis: a histopathological reaction pattern in

skin-biopsy specimens from cats with allergic skin disease. *Journal of Feline Medicine and Surgery*. 2010; 12: 80-85.
8. Sebaceous adenitis. OFA. Accessed October 12, 2022. https://ofa.org/diseases/other-phenotypic-evaluations/sebaceous-adenitis/
9. Welle MW, Linder KE. The Integument. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease.* St. Louis, MO: Elsevier. 2022; 1246-1247,1261.
10. White SD, Linder KE, Shultheiss P et al. Sebaceous adenitis in four domestic rabbits. *Veterinary Dermatology*. 2000; 11: 53-60.

CASE IV:

Signalment:
4-year-old, intact male, mix breed canine (*Canis lupus familiaris*)

History:
A 4-year-old, intact male, medium-sized (~15 Kg of body weight), mix breed dog was presented to the veterinary clinic with multiple (5) skin nodules in the right knee, thoracic region, dorsal aspect of the nose, and right and left thoracic limbs. In the initial clinical examination, the dog was normothermic, bright, alert and responsive, had good body condition and muscle tone, adequate hydration state, normal capillary refill time, and adequate coloration of the mucous membranes.

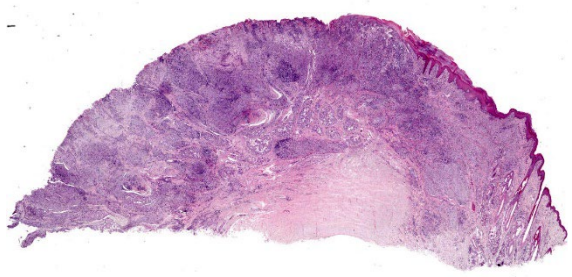

Figure 4-1. Pawpad and haired skin, dog. A single section of pad and adjacent haired skin is submitted for examination. The fat pad and eccrine glands of the pad is effaced by coalescing poorly formed granulomas. (HE, 5X)

The dog was treated empirically and unsuccessfully with enrofloxacin and prednisone. After one month of treatment, the animal was presented again to the clinic with vomiting, diarrhea, and partial loss of vision. The larger mass, located in the left thoracic limb, was surgically biopsied for histologic examination.

Gross Pathology:
A firm, raised skin nodule covered by ulcerated and alopecic epidermis.

Laboratory Results:
No laboratory results reported.

Microscopic Description:
Haired skin. Diffusely expanding the dermis and subcutaneous tissue and elevating the overlying epidermis, there is a dense inflammatory cellular infiltrate composed predominantly of activated and epithelioid macrophages, occasional multinucleated giant cells, lymphocytes, plasma cells, and fewer neutrophils. Admixed with the inflammatory cells, there are numerous extracellular and intrahistiocytic microorganisms and pyknotic and karyorrhectic cellular debris (necrosis). Microorganisms (algae) are mildly pleomorphic, mostly spherical to ovoid, and range between 4 and 17 µm in diameter, with a 2 to 4 µm, often surrounded by a clear halo. Algal cells contain granular flocculent amphophilic material and a distinctly basophilic nucleus. Some algal cells present internal septation with daughter cells (endosporulation). The overlying epidermis is extensively ulcerated or lost, and the superficial dermis covered by extravasated fibrin and necrotic cellular debris, there is extensive fibrosis with perpendicularly-oriented blood vessels lined by plumped epithelium (neovascularization/granulation tissue), scattered fibrin thrombi in small-caliber superficial venules and microhemorrhage.

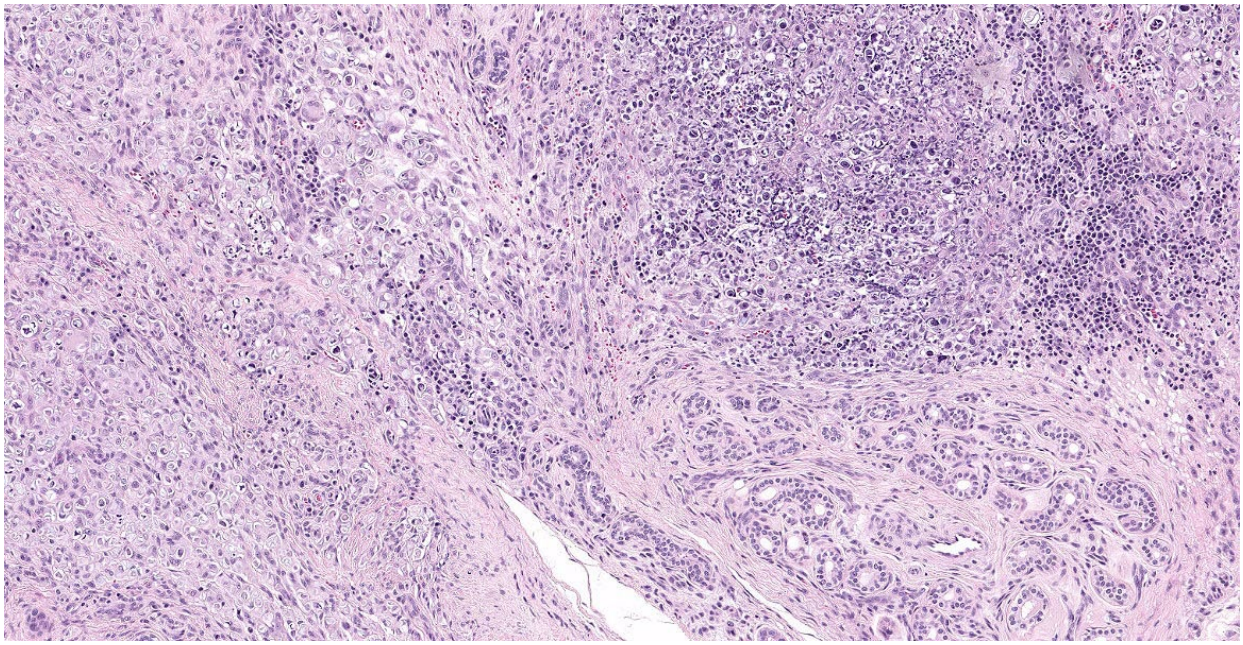

Figure 4-2. Pawpad and haired skin, dog. Poorly formed granulomas efface the pawpad adipose tissue. Eccrine glands are present at lower right. (HE, 126X)

Algal cells are highlighted by GMS stain and PAS reaction.

Contributor's Morphologic Diagnoses:
Haired skin: severe granulomatous and ulcerative dermatitis/panniculitis, with multinucleate giant cells and myriads of intrahistiocytic and extracellular algal microorganisms morphologically resembling *Prototheca* spp., *Canis lupus familiaris*.

Contributor's Comment:
Differential diagnoses on clinical grounds in this case included multifocal/multicentric neoplasia with cutaneous involvement, and granulomatous/pyogranulomatous dermatitis/panniculitis caused by infectious agents, mostly bacterial or fungal infections. The microscopic examination of the biopsy with HE, GMS and PAS stains revealed severe granulomatous dermatitis with intralesional algae morphologically consistent with *Prototheca* spp. as depicted in the submitted slides.
Additionally, as part of the diagnostic investigation, formalin-fixed paraffin-embedded skin was processed for molecular identification of *Prototheca* spp. at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. For molecular confirmation and speciation, a polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP) was performed as previously described.[14] The agent was identified as *P. zopfii* genotype 1.

Thus, the diagnostic investigation in this case allowed for unequivocal confirmation of *P. zopfii* genotype 1 infection in a dog, by a combination of morphological and molecular methods.

Protothecosis is a rare disease caused by environmental algae of the genus *Prototheca*, saprophytes of worldwide distribution that can infect mammals, and result in either localized or systemic, life-threatening disease, particularly in immunocompromised individuals.[15,21] *Prototheca* spp. is a non-photosynthetic, achlorophyllic (colorless), aerobic alga in the family *Chlorellaceae*. Seven *Prototheca* species have been described to date,[11] of these *P. zopfii* and *P. wickerhamii* have

been more commonly implicated in pathogenic infections in humans and animals,[21,2,16] while the novel species *P. miyajii* was isolated recently from a human patient with systemic protothecosis.[11] Within *P. zopfii* species, two different genotypes (1 and 2) are currently recognized; genotype 2 is the most commonly involved in different clinical forms.[2,13]

Prototheca spp. have been associated with various syndromes in different species. The most commonly recognized forms of human protothecosis are disseminated, cutaneous, and olecranon bursitis.[12] In cattle, mastitis is the more common manifestation,[13] while rhinitis and sinusitis have been described in horses.[18] In dogs, protothecosis is usually associated with colitis and/or disseminated disease, sometimes with ocular,[19] or central nervous system involvement.[3,10] The infection restricted only to the skin is rare in this species,[5] although it appears to be the most common form in the cat.[7] The cutaneous form in the dog is usually associated with *P. wickerhamii* infection,[21,16,4] although it has recently been described in a case of *P. zopfii* genotype 2 infection.[2] The case presented here represents the first documentation of *P. zopfii* genotype 1 infection in a dog.[20]

The pathogenesis of protothecosis in dogs is not yet fully elucidated. Ingestion has been regarded as the most likely route of transmission in most cases of disseminated protothecosis by *P. zopfii*;[21] however, cutaneous entrance through skin trauma or penetration through mucosal surfaces have also been documented.[15,21,3,10]

Reports on genotypic characterization of *P. zopfii* strains infecting dogs in South America were limited to one description of the genotype 2 in Brazil,[17] which had also been implicated in cases of bovine mastitis in this country.[14] This genotype has also been detected in diseased dogs in Italy,[2] and the USA.[9] A large epidemiological, phenotypic and molecular analysis of 350 clinical *Prototheca* isolates, including 342 bovine strains from around the world, 6 canine strains (3 from Germany, 2 from Brazil and 1 from USA) and 2 human

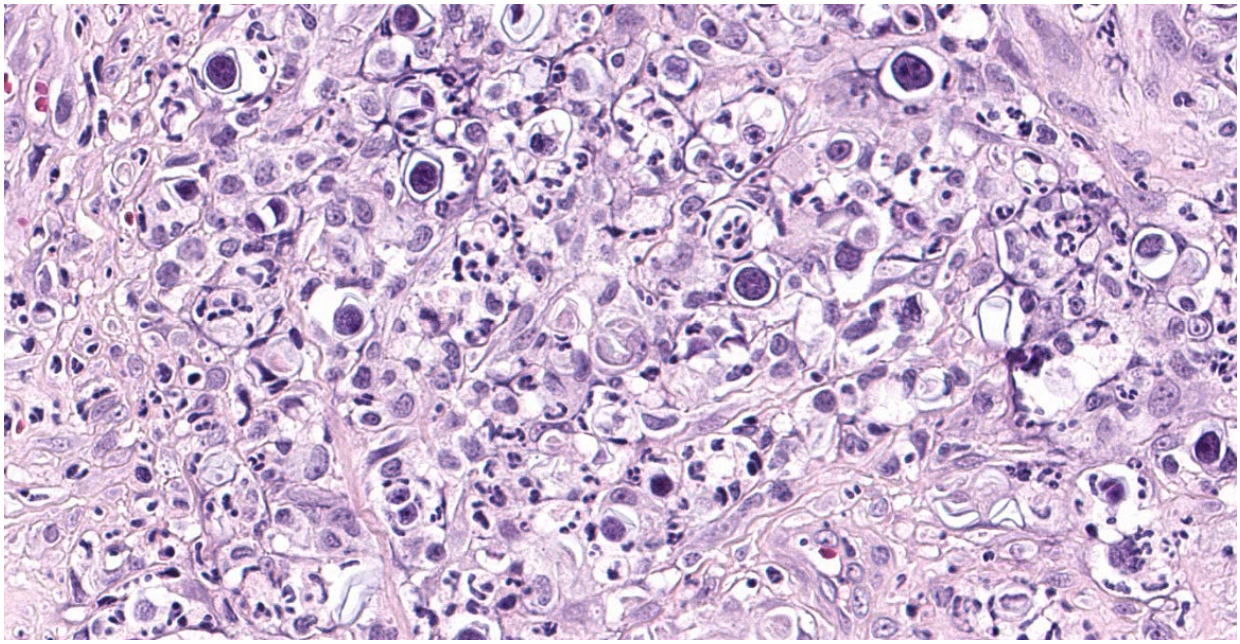

Figure 4-3. Pawpad and haired skin, dog. Numerous algae, including endosporulating forms and empty cell walls are phagocytized by epithelioid macrophages and multinucleated giant cells. (HE, 400X)

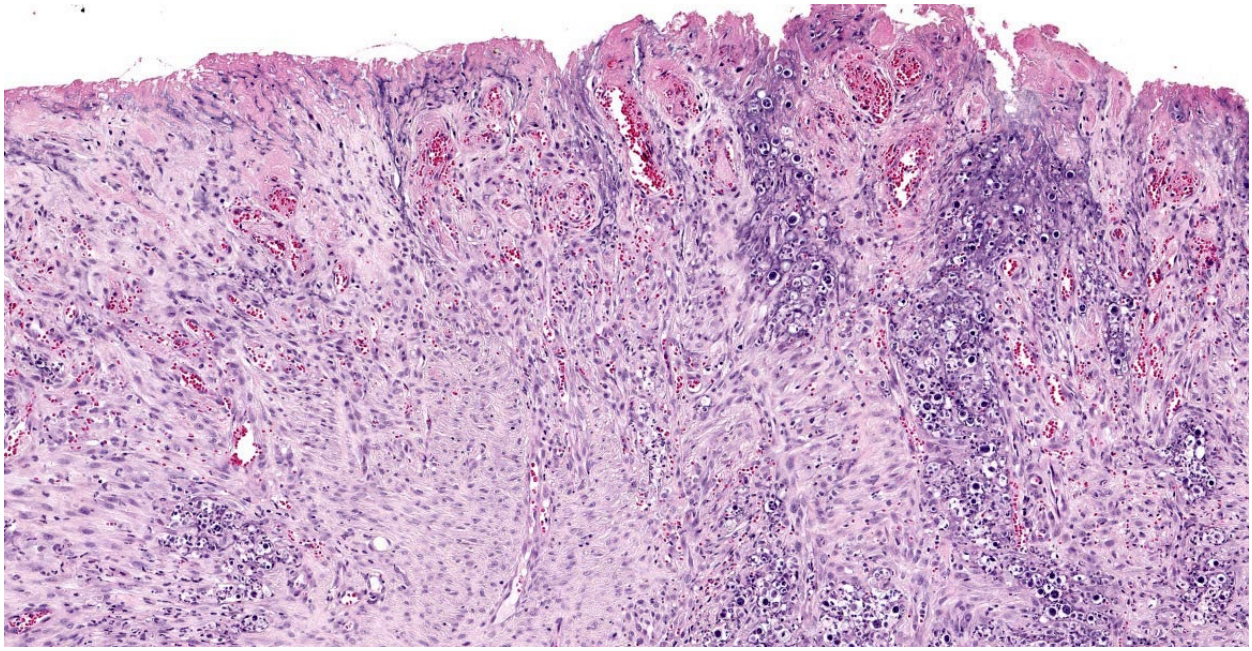

Figure 4-4. Pawpad and haired skin, dog. There is granulation tissue and fibrosis subjacent to the ulcerated pad, and algae-laden macrophages reach up to the ulcerated surface. (HE, 123X)

strains (1 from Austria and 1 from China), revealed that 90.6% of the bovine isolates and 100% canine and human isolates were *P. zopfii* genotype 2.[1] *P. zopfii* genotype 1 was only found in 2 (0.5%) of the 342 bovine isolates, both from Germany.[1] Despite this overall low frequency of detection in clinical cases, *P. zopfii* genotype 1 has recently been linked to human protothecosis,[6] which opposes to previous knowledge that considered this genotype as non-pathogenic.[8]

Contributing Institution:
Plataforma de Investigación en Salud Animal, Instituto Nacional de Investigación Agropecuaria (INIA), Uruguay, www.inia.uy

JPC Diagnosis:
Pawpad and haired skin: Pododermatitis, ulcerative and granulomatous, focally extensive, severe, with numerous intrahistiocytic and extracellular algae.

JPC Comment:
Since this case was submitted in 2018, *Prototheca* taxonomy has expanded to include 15 species, 6 of which are known to cause disease in humans or animals.[12] Additionally, *Prototheca zopfii* genotypes 1 and 2 have been established as their own species: *P. ciferrii* and *P. bovis*, respectively.[12]

In addition to the species described above, *P. wickerhamii* can infect the nose and face of goats causing proliferative and ulcerative nodules of pyogranulomatous inflammation admixed with sporangia which may extend into the nose or underlying bone.[19] Disseminated infection in goats has not been reported, and it appears sheep are either extremely rarely infected or not infected by *Prototheca*.[19]

In contrast, sheep can be infected by a related chlorophyll-containing green algae, *Chlorella*.[19] Infection may be limited to the liver and mesenteric lymph nodes or may be systemic, and the algae produces characteristic green discoloration that persists during formalin fixation but is lost during processing.[19] *Chlorella* sporangia also contain PAS-positive cytoplasmic granules (chloroplasts) that *Prototheca* lacks. *Chlorella* infection has

also been documented in humans, cattle, a dromedary camel, and a dog.[19]

References:

1. Ahrholdt J, Murugaiyan J, Straubinger RK, Jagielski T, Roesler U. Epidemiological analysis of worldwide bovine, canine and human clinical *Prototheca* isolates by PCR genotyping and MALDI-TOF mass spectrometry proteomic phenotyping. *Med Mycol*. 2012;50(3):234–243.
2. Carfora V, Noris G, Caprioli A, Iurescia M, Stravino F, Franco A. Evidence of a *Prototheca zopfii* genotype 2 disseminated infection in a dog with cutaneous lesions. *Mycopathologia*. 2017; 182:603–608.
3. Font C, Mascort J, Marquez M, Esteban C, Sanchez D, Durall N, Pumarola M, Lujan-Feliu-Pascual A. Paraparesis as initial manifestation of a *Prototheca zopfii* infection in a dog. *J Small Anim Prac.* 2014; 55:283–286.
4. Ginel PJ, Perez J, Molleda JM, Lucena R, Mozos E. Cutaneous protothecosis in a dog. *Vet Rec*. 1997;140: 651–653.
5. Gross TL, Ihrke PJ, Walder EJ, Affolter VK. Infectious nodular and diffuse granulomatous and pyogranulomatous diseases of the skin. In: Gross TL, Ihrke PJ, Walder EJ, Affolter VK, editors. Skin diseases of the dog and cat. Oxford, UK: Blackwell Publishing Inc; 2005:272–319.
6. Hirose N, Hua Z, Kato Y, Zhang Q, Li R, Nishimura K, Masuda M. Molecular characterization of *Prototheca* strains isolated in China revealed the first cases of protothecosis associated with *Prototheca zopfii* genotype 1. *Med Mycol*. 2018; 56(3):279–287.
7. Huth N, Wenkel RF, Roschanski N, Rösler U, Plagge L, Schöniger S. *Prototheca zopfii* genotype 2-induced nasal dermatitis in a cat. *J Comp Pathol*. 2015; 152:287–290.
8. Irrgang A, Murugaiyan J, Weise C, Azab W, Roesler U. Well-known surface and extracellular antigens of pathogenic microorganisms among the immunodominant proteins of the infectious microalgae *Prototheca zopfii*. *Front Cell Infect Microbiol*. 2015; 5:67.
9. Lane LV, Meinkoth JH, Brunker J, Smith SK 2nd, Snider TA, Thomas J, Bradway D, Love BC. Disseminated protothecosis diagnosed by evaluation of CSF in a dog. Vet Clin Pathol. 2012;41(1):147–152.
10. Marquez M, Rodenas S, Molin J, Rabanal RM, Fondevila D, Añor S, Pumarola M. Protothecal pyogranulomatous meningoencephalitis in a dog without evidence of disseminated infection. *Vet Rec.* 2012; 171:100.
11. Masuda M, Hirose N, Ishikawa T, Ikawa Y, Nishimura K. *Prototheca miyajii* sp. nov., isolated from a patient with systemic protothecosis. *Int J Syst Evol Microbiol*. 2016; 66:1510–1520.
12. Masuda M, Jagielski T, Danesi P, et al. Protothecosis in Dogs and Cats – New Research Directions. *Mycopathologia*. 2021;186:143-152.
13. Mayorga J, Gómez JFB, Martínez APV, Estrada VFM, Welsh O. Protothecosis. *Clin Dermatol*. 2012; 30:432–436.
14. Moller A, Truyen U, Roesler U. *Prototheca zopfii* genotype 2-the causative agent of bovine protothecal mastitis? *Vet Microbiol*. 2007; 120:370–374.
15. Morandi S, Cremonesi P, Capra E, Silvetti T, Decimo M, Bianchini V, Alves AC, Vargas AC, Costa GM, Ribeiro MG, Brasca M. Molecular typing and differences in biofilm formation and antibiotic susceptibilities among *Prototheca* strains isolated in Italy and Brazil. *J Dairy Sci*. 2016; 99(8):6436–6445.
16. Pal M, Abraha A, Rahman MT, Dave P. Protothecosis: an emerging algal disease of humans and animals. *Int J Life Sci Biotechnol Pharm Res*. 2014; 3:1–13.

17. Papadogiannakis EI, Velonakis EN, Spanakos GK, Koutinas AF. Cutaneous disease as sole clinical manifestation of protothecosis in a boxer dog. *Case Rep Vet Med*. 2016;1–4.
18. Ribeiro MG, Rodrigues de Farias M, Roesler U, Roth K, Rodigheri SM, Ostrowsky MA, Salerno T, Sigueira AK, Fernandes MC. Phenotypic and genotypic characterization of *Prototheca zopfii* in a dog with enteric signs. *Res Vet Sci.* 2009;87(3):479–481.
19. Riet-Correa F, do Carmo PMS, Uzal FA. Protothecosis and chlorellosis in sheep and goats: a review. *Jour Vet Diag Invest.* 2021; 33(2): 283-287.
20. Schöniger S, Roschanski N, Rösler U, et al. *Prototheca* species and *Pithomyces chartarum* as causative agents of rhinitis and/or sinusitis in horses. *J Comp Pathol*. 2016; 155:21–125.
21. Shank AM, Dubielziq RD, Teixeira LB. Canine ocular protothecosis: a review of 14 cases. *Vet Ophthalmol.* 2015; 18:437–442.
22. Silveira C, Cesar D, Keating K, DeLeon-Carnes M, Armién AG, Luhers M, Riet-Correa F, Giannitti F. A case of *Prototheca zopfii* genotype 1 infection in a dog (C*anis lupus familiaris*). *Mycopathologia*, 2018, in press.
23. Stenner VJ, Mackay B, King T, et al. Protothecosis in 17 Australian dogs and a review of the canine literature. *Med Mycolo.* 2007; 45:249–266.