Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2012-2013

Conference 20

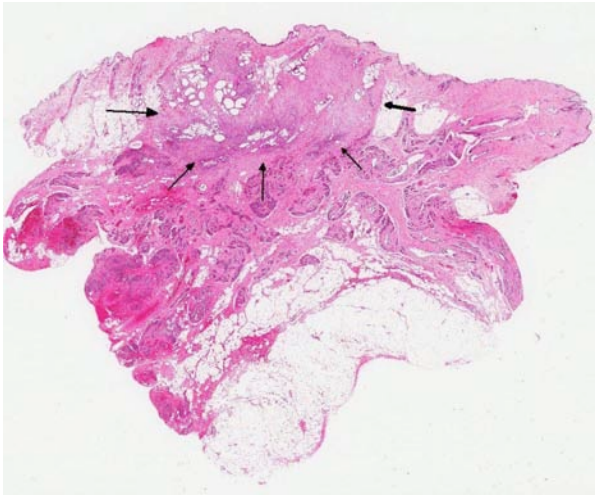
03 April 2013

CASE I: S2011-0023 (JPC 4019875).

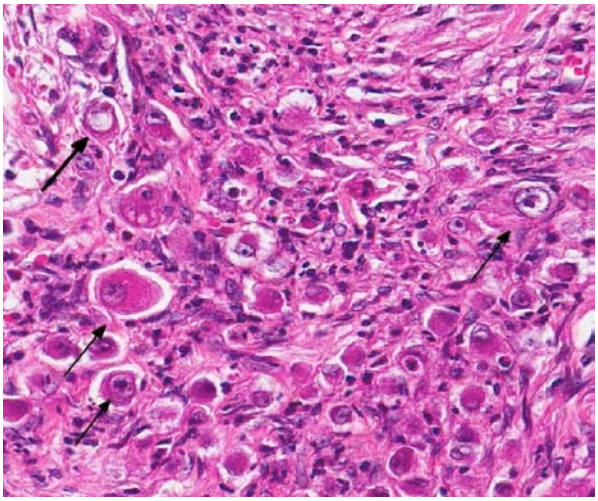
Signalment: 7-year-old Maltese bitch *(Canis familiaris)*.

History: A 7-year-old Maltese bitch that had been ovariohysteretomized at age 6 years was found by the owner to bear a nodular mass at the right 2nd mammary gland in the past two weeks. Upon clinical examination, the mass was subcutaneous, solid, firm, and movable near the right second mammary gland. Malignant mammary tumor was suspected and lumpectomy was recommended. The tumor mass was removed one week later.

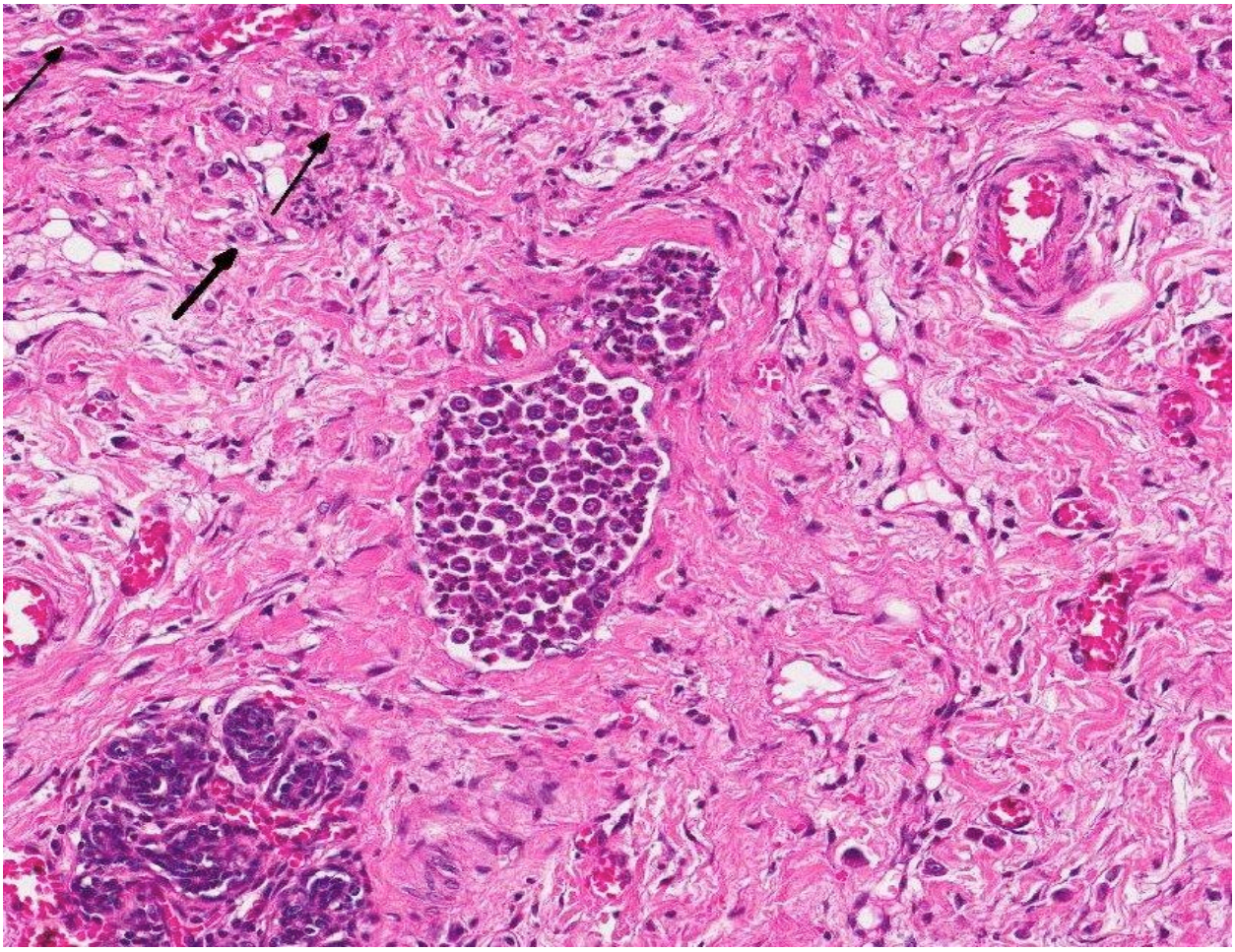
Gross Pathology: Grossly, the mass was approximately 3.0 x 3.0 cm and appeared light red on the cut surfaces.



1-1. Mammary gland, dog: There is a poorly demarcated, infiltrative neoplasm within the superficial dermis (arrows). Surrounding the mass are numerous densely basophilic mammary ducts. (HE 0.63X)



1-2. Mammary gland, dog: Within the mass, neoplastic cells (arrow) show marked pleomorphism, and are scattered individually throughout the desmoplastic stroma. Neoplastic cells show no predilection to recapitulate mammary gland. (HE 288X)



1-3. Haired skin, mammary gland: Pleomorphic neoplastic cells fill dilated lymphatics adjacent to the primary mass. The primary contains numerous individualized neoplastic cells (arrows). Hyperplastic mammary ducts are present below. (HE 288X)

Histopathologic Description: Mammary gland: Within the interlobular connective tissue, separating and surrounding pre-existing and mildly hyperplastic ducts, there is a poorly cellular, infiltrative, unencapsulated, poorly demarcated neoplasm. In a large portion of the neoplasm, neoplastic cells line mammary ducts, proliferating up to 5 cells deep and expanding the ductal lumen. Neoplastic cells are polygonal with distinct cell borders and a moderate amount of eosinophilic cytoplasm. Nuclei are irregularly round with finely stippled chromatin and 1-2 large eosinophilic nucleoli. There is moderate anisocytosis and anisokaryosis. Mitotic figures average 1/400x field. Apoptotic cells are common within ducts, and many ducts lined with neoplastic cells contain low numbers of neutrophils admixed with cellular debris, and ducts are surrounded by low to moderate numbers of lymphocytes, with fewer plasma cells, histiocytes, and hemosiderin-laden macrophages. Within the superficial dermis, neoplastic cells have escaped ducts, and are distributed in an individualized fashion, rarely forming nests and acini on a dense fibrous stroma. In this area, neoplastic cells range up to 50 μm in diameter, exhibit marked anisokaryosis, and are often separated and surrounded by dense bands of fibrous connective tissue, and in some areas, plump myofibroblasts. They often fill and expand lymphatics, and surrounding tissue is often edematous.

Contributor's Morphologic Diagnosis: Canine mammary anaplastic carcinoma.

Contributor's Comment: Canine mammary tumors are the most common neoplasm in female dogs, and anaplastic carcinoma is the most malignant form. The occurrence of anaplastic carcinomas is uncommon; no case has ever been reported in Taiwan.

The present case is a 7-year-old Maltese bitch with a history of ovariohysterectomy performed a year before the tumor occurrence. No other external abnormalities were observed. Lumpectomy rather than mastectomy was performed. This bitch was still alive at the time this manuscript was prepared (8 months post-surgery) despite the malignant features observed on histopathology. Continuous follow up for "two year survival" will be interesting.

Classification of canine mammary tumors has been complicated and debatable. The earliest classification scheme for canine and feline mammary tumors was seen in 1961, in Moulton's *Tumor in Domestic Animals.* The classification at that time was fairly simple; however, over the years, the classification scheme has further advanced to more detailed and complex ones, including the 1974 WHO edition of International Histological Classification of Tumors of Domestic Animals, and the 1999 Armed Forces Institute of Pathology's classification. In 2011, a new classification scheme was proposed.[2]

JPC Diagnosis: Mammary gland: Anaplastic mammary carcinoma.

Conference Comment: Of the canine mammary tumors, anaplastic carcinomas are the most malignant, and thus carry the worst prognosis. As illustrated so well in this case, anaplastic carcinomas often exhibit diffuse invasion of interlobular connective tissue and elicit a marked desmoplastic response with concomitant proliferation of myofibroblasts. Lymphatic invasion and metastasis to regional lymph nodes and lung is also common. Interestingly, pulmonary metastasis appears radiographically as an interstitial pattern, rather than a nodular one.

As the name implies, neoplastic cells are pleomorphic, round to polygonal, with moderate to abundant eosinophilic cytoplasm, and round to oval nuclei which are sometimes indented. Neoplastic cells are often individualized or grouped in small nests. Features of malignancy include frequent multiple prominent nuclei, severe anisokaryosis and anisocytosis and a high mitotic rate; occasional multinucleated neoplastic cells are also present.[1]

Conference participants discussed use of the term "inflammatory carcinoma," which should not be used as a morphologic diagnosis, as it refers to a clinical entity that can be associated with several types of malignant mammary carcinomas, including anaplastic carcinomas. The hallmark histologic lesion for this condition is invasion of neoplastic emboli into dermal lymphatics[2] and despite the name, and the clinical appearance of a reddened mammary neoplasm, which is warm to the touch, there is often little to no microscopic inflammation associated with the neoplasm.

In addition, inflammatory carcinomas are highly angiogenic.[1] Tumor angiogenesis can be accomplished via several mechanisms, including production of vessels through endothelial precursor cells from the bone marrow or from endothelial cells in preexisting vessels, by sprouting angiogenesis, by intussusceptive angiogenesis, and by vessel co-option. More recently, a new mechanism, vasculogenic mimicry (VM) has been identified. Vasculogenic mimicry consists of the *de novo* generation of microvascular channels by genetically deregulated aggressive tumors cells without participation of endothelial cells. The resulting channels are not true blood vessels, although they function to distribute plasma and blood cells to the neoplasm, and are thought to play a role in metastasis. VM has been identified in several types of malignant tumors in humans, including inflammatory breast carcinoma and ductal breast carcinoma, as well as in several types of inflammatory mammary carcinomas in dogs, with its occurrence seen most frequently in anaplastic carcinomas. Highly malignant neoplastic cells in canine mammary cancer have been observed to resemble endothelial cells that histologically, immunohistochemically, and ultrastructurally resembled VM as described in human tumors. Specifically, the endothelial-like cells (ELCs) in VM are positive for epithelial markers and for the same markers used for the rest of the tumor cells may be positive for vimentin, but negative for smooth muscle actin and desmin, and show absence of specific immunoreaction with endothelial markers. Ultrastructurally, ELCs lack Weibel-Palade bodies (which are characteristic of endothelial cells) and have desmosomes (the type of junctions between epithelial cells) instead of fascia occludens (endothelial cell- to-cell junctions). Tumor and/or blood cells contained in the channels formed by ELCs are not inside a vacuole as in emperipolesis or phagocytosis; instead, they are inside an actual space formed by

the cytoplasmic membranes of ECLs. It is thought that ECLs can also form channels that mimic lymph vessels rather than blood vessels; however, further studies are needed to identify the mechanisms of cancer cells developing channels of VM as well as confirm the presence of lymphatic VM.[1]

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CASE II: FMVZ USP Case II (JPC 4019407).

Signalment: 15-month-old, intact female cat (*Felis catus)*.

History: A 15-month-old, female cat of indeterminate breed with history of good general condition presented with multiple ulcerated necrotic nodular skin lesions in the nasal planum, ears, forelimbs, cervical and lumbosacral regions. The cat had been treated orally with itraconazole for a few weeks based on isolation of *Sporothrix schenckii* from lesions. After approximately six weeks the cat was sent to necropsy.

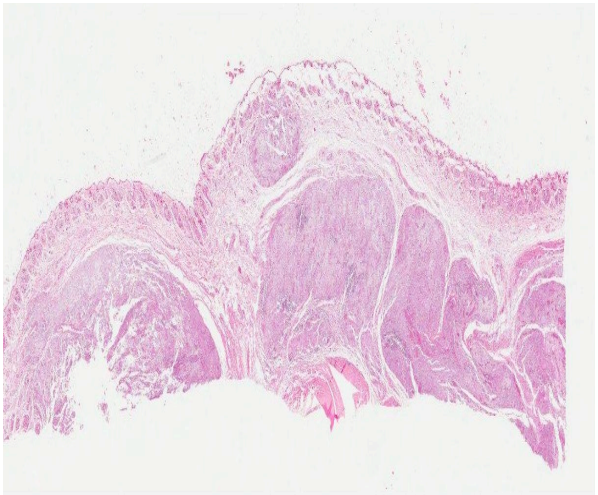
Gross Pathology: The animal was in poor body condition, weighting less than 2 kg, with reduced amounts of internal body fat. On the right cheek, ears, cervical region, digits of both forelimbs and in the lumbosacral region there were multiple nodular and ulcerative gummy reddish skin lesions measuring 1 to 7 cm in diameter. The ulcerated skin lesions were covered by hemorrhagic crusts and had irregular edges. Cut surfaces of lesions were white to yellowish, irregular, friable and well demarcated nodules or plaques.

Laboratory Results: Skin fragments collected during gross examination resulted in growth of *Sporothrix schenckii* in Sabouraud's dextrose agar.

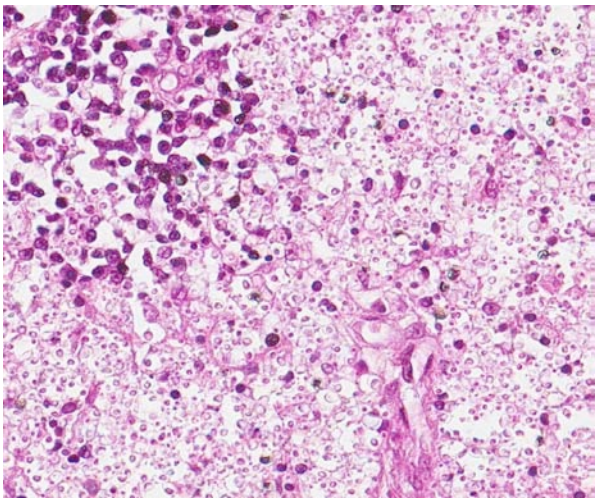
Histopathologic Description: Histopathologic evaluation of sections of the forelimb nodule revealed on low power a nodular to diffuse dermatitis that extended into the deep dermis. The overlying epidermis of the sample was intact and atrophic. The superficial dermis was edematous and in the deep dermis there were epithelioid macrophages intermingled with rare lymphocytes, plasma cells and neutrophils. At higher magnification myriads of small, 2 to 6 µm diameter, elongated or dot-like, cigar-shaped to oval yeasts consistent with *Sporothrix schenckii* were observed. The organisms were pink in periodic acid–Schiff stain (PAS) and lie in vacuoles often grouped within macrophages, but were also free in the tissue. The blood vessels within the lesion were dilated and congested.

Contributor's Morphologic Diagnosis: Skin: Dermatitis, nodular to diffuse, granulomatous, marked, with myriads of cigar-shaped to oval *Sporothrix schenckii* yeast, undefined breed, cat.

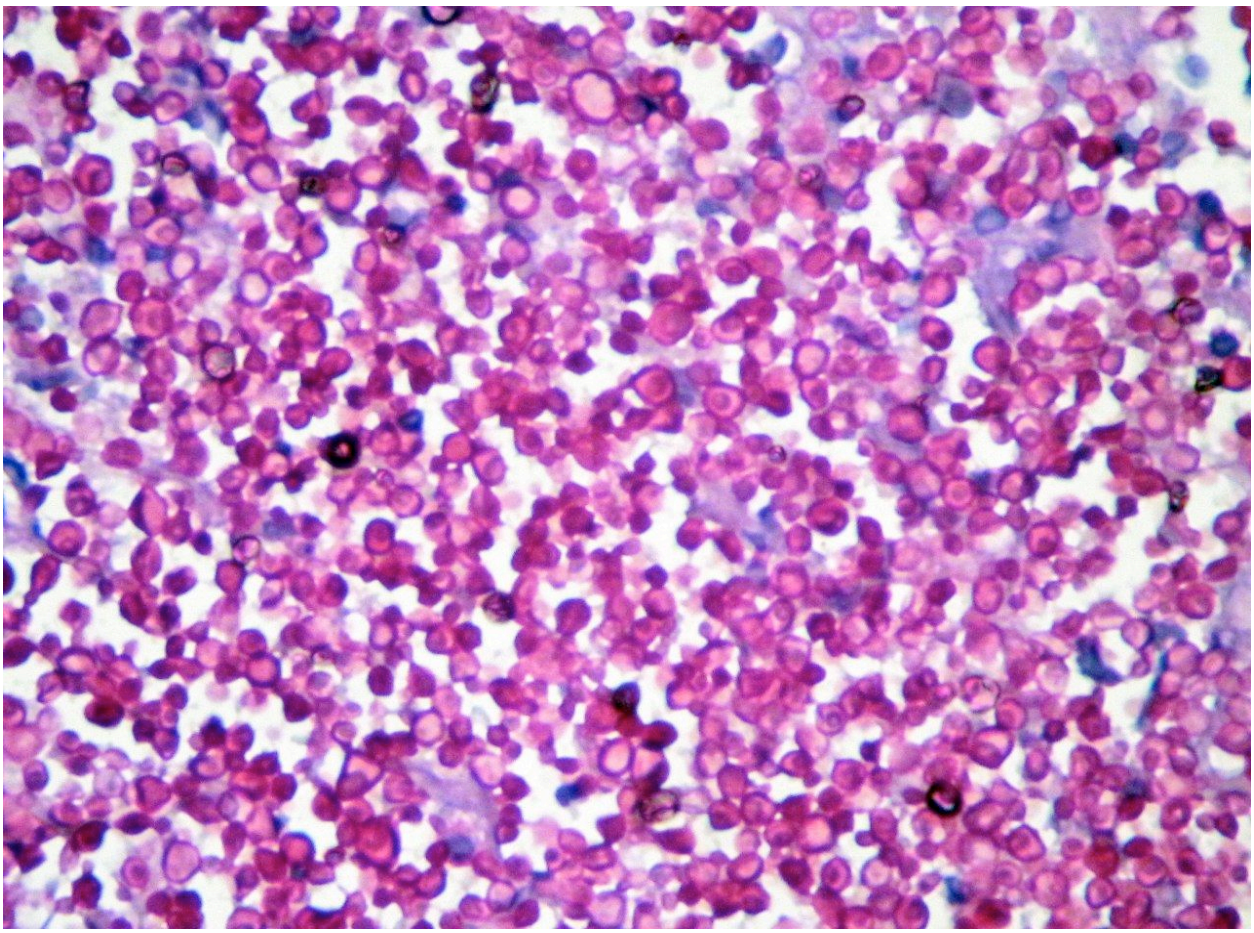
Contributor's Comment: Sporotrichosis is an ergodermatosis anthropozoonotic or saprozoonotic dermatopathy caused by the thermally dimorphic fungus *Sporothrix schenckii*, whose main sources of infection are domestic cats, plants and soil. *Sporothrix schenckii* is distributed worldwide, and at present it is rare in Europe, but frequent in the Americas, Africa, Japan, and Australia. The main areas of endemicity are located in Japan, India, Mexico, Brazil, Uruguay, and Peru. In Latin America, it is the most common subcutaneous mycosis in humans.



2-1. Haired skin, cat: The dermisis is expanded by multifocal to coalescing granulomas which extend from the dermis into the subcutis. (HE 0.63X)



2-2. Haired skin, cat: Within the granulomas, macrophages are greatly expanded by abundant mildly pleomorphic 5-8 µm yeast with a clear capsule, consistent with Sporothrix schenkii. There are small nodules of lymphocytes and plasma cells scattered through the granulomas (upper left). (HE 400X)



2-3. Haired skin, cat: A periodic acid-Schiff preparation highlights the yeast cell wall. (PAS 400X) (Photo courtesy of the Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87, CEP 05508 270, Cidade Universitária, São Paulo/ SP – Brasil.)

S. schenckii belongs to the kingdom Fungi and is a eukaryotic organism that has no mobility, is heterotrophic, and presents chitin on its cell wall. For several years, this fungus was included in division *Eumycota*, subdivision *Deuteromycotina*, class *Hyphomycetes*, order *Moniliales*, and family *Moniliaceae*. After a substantial fungal taxonomy revision, this fungus was replaced in division *Ascomycota*, class *Pyrenomycetes*, order *Ophiostomatales*, and family *Ophiostomataceae*.

S. schenckii must not be considered a single species. A recent molecular study demonstrated that *S. schenckii* is a complex of cryptic species, denominated the *S. schenckii* complex. Within this complex, at least five species are considered of interest due to their pathogenic potential. Gene sequencing has revealed the following six species in the complex: *S. albicans*, *S. brasiliensis*, *S. globosa*, *S. luriei*, *S. mexicana* and *S. schenckii*. These cryptic species were further subdivided into a number of smaller groups that appear to be reproductively isolated in nature. This suggests not only that the existing *S. schenckii* populations are in the process of divergence but also that all of the resulting lineages are undergoing separation into distinct taxa. In Brazil there are four species recognized as pathogens for human and animals: *S. albicans*, *S. brasiliensis*, *S. luriei*, and *S. schenckii*.

Although the genetic separation is considerable among the three major monophyletic clades (i.e., the Spanish clade, the Brazilian clade, and the clade made up of the rest of the South American isolates), each of them shows a high level of clonality. Primitive populations were probably isolated by the separation of the continents, and the formation of natural barriers facilitated their speciation as they became adapted to hosts endemic to the different regions. However, although geographical separation of the main

clades is clearly evident, the different genotypes present within them are not related to geography, which seems to indicate that there has been interbreeding within these isolated populations.

In the environment, at temperatures ranging from 25 to 30°C, *S. schenkii* grows as filamentous mold; it forms white to cream-colored colonies which become brown to black in weeks and produces dark or hyaline conidia arranged along hyphae with a bouquet-like appearance. In the wild, the fungus is a saprophyte on living and decaying vegetation. Soil is fundamental for mycelium development. At 37°C, *S. schenkii* grows as yeast-like cell.

S. schenckii virulence factors are thermotolerance and the ability to synthesize melanin that enhances resistance to macrophage phagocytosis, allowing the first steps of infections in mammalian hosts; melanization also promotes the formation of multifocal granuloma. Another virulence factor is expression of integrins or adhesion lectin-like molecules that recognize fibronectin from the host. The fibronectin adhesins are located on the surface of yeast cell and the existence of these adhesins would favor adherence to host tissues and fungal dissemination. Components of the *S. schenckii* cell wall that act as adhesins and immunogenic inducers, such as a 70-kDa glycoprotein, are apparently specific to this fungus and they seem to participate in adhesion to the dermal extracellular matrix. The main glycan peptido-rhamnomannan cell wall component is the only *O*-linked glycan structure known in *S. schenckii*, and it causes depression of immune response until the sixth week of infection. Yeast cells also synthesize ergosterol peroxide that is a protective mechanism to evade reactive oxygen species during phagocytosis and may represent a virulence factor.

Differences in virulence between clinical and environmental strains were reported, but no correlation was found with the different clinical forms of sporotrichosis. In several *in vitro* antifungal susceptibility studies of clinical isolates of *S. schenckii*, a wide range of susceptibility to different drugs has been demonstrated. This suggests that these isolates could represent different species. If true, knowledge of their various responses to antifungal agents would be critical for appropriate patient management.

Classically, the infection is acquired through traumatic implantation of *S. schenckii* present in organic matter. The most frequent clinical presentations are the cutaneous and subcutaneous forms with or without regional lymphatic involvement. But, inhalation of the conidia can lead to pulmonary infection which, rarely, may also spread to bones, eyes, central nervous system and viscera. Another mode of transmission is through animal bites and scratches.

Sporotrichosis usually occurs in isolated cases or in small family or professional outbreaks. Epidemics are rare and, when they occur, are commonly related to a single source of infection. Interhuman transmission is rare and human sporotrichosis has sporadically been related to the scratch or bite of infected animals. The role of felids in the transmission of the mycosis to humans has, however, gained importance. There was no description of epizootics before a cat-transmitted epidemic was reported in Rio de Janeiro, Brazil, in 1980. Since then, successive reports of epizootics from different geographical regions have characterized a new risk group for acquisition of sporotrichosis, composed of cat owners and veterinarians.

It should be noted that the highest number of cases that occurred during Rio de Janeiro epizootics were in an area characterized by underprivileged socioeconomic conditions and precarious health services. The typical human patients were female, mainly housewives, which is normal if we consider that members of this group are those most frequently exposed to the fungus because they care for cats. Some authors explained the wide dissemination of the disease with factors related to the behavior of cats which, although cohabiting with human beings, do not always stay in the house but also circulate in the neighborhood, often getting involved in fights with other animals and coming into contact with soil and plants.

It is possible that environmental factors, increased urbanization, and improved diagnostics partly explain the alterations in the profile of the disease. Furthermore, since sporotrichosis is not a reportable disease in most countries, such as Brazil, there is little information on the incidence, and the known data are those generated by case publications.

Following inoculation, the fungus penetrates into deeper layers of tissue where it converts into the yeast like form (37°C). It can remain in the dermis and subcutaneous tissue at the inoculation site, spread up to regional lymphatics and produce lymphangitis and lymphadenitis, or disseminate systemically through blood vessels. Furthermore, in cats, the high frequency of respiratory signs and pulmonary and nasal mucosal lesions, in addition to the isolation of *S. schenckii* from bronchoalveolar lavage and from the lungs of necropsied animals, suggests the epidemiologic importance of the inhalation route in the infection. Multiple skin lesions can occur because of self-trauma, grooming, and hematogenous dissemination from the lungs or perhaps from the initial skin lesion.

The lesions are characterized by a wide variety of morphologies: nodules, tubercles, pustules, cysts, gummy lesions, ulcers, vegetative lesions, and plaques, accompanied or not by lymphangitis.

Virulence is one of the factors thought to play a role in the development of sporotrichosis, but there are discordant results concerning disease evolution in experimental sporotrichosis with *S. schenckii.* Clinical isolates from cutaneous and disseminated infection indicate that host immune responses also substantially participate in the progress of sporotrichosis. The immunological mechanisms involved in prevention and control of *S. schenckii* infections are still not very well understood. However, they probably include both humoral and cellular responses, which appear to be triggered by distinct antigens. Surface cell antigens, especially some lipids, inhibit the phagocytosis process; while the humoral response is induced by secreted fungal proteins, the exoantigens are not involved in the cellular response. The innate immune response also plays a role in the pathogenesis of sporotrichosis.

The classification of clinical presentations used for humans includes several forms: lymphocutaneous, fixed cutaneous, mucocutaneous, extracutaneous, and disseminated. These categories are difficult to transpose to sporotrichosis in dogs and cats because they frequently have more than one of these forms simultaneously. Although the cutaneous lymphatic form is the most frequently seen clinical presentation in humans, this is not the case with cats and dogs. The most common lesions in dogs and cats are skin nodules and ulcers, with frequent mucosal involvement. The initial lesions are firm subcutaneous nodules that slowly soften, generally draining purulent or seropurulent content, and progress to form exudative ulcers with slightly elevated well-defined rims. In addition, dogs and cats may have extracutaneous, mainly respiratory signs, such as sneezing, nasal discharge, and dyspnea, followed by lymphadenomegaly. Other clinical signs that may be observed are anorexia, vomiting, weight loss, cough, fever, and dehydration.

According to the location of the lesions, sporotrichosis can be classified into three forms in animals. The primary cutaneous form consists of multiple scattered raised alopecic, ulcerated, crusted nodules or plaques that remain confined to the point(s) of entry of the organism. It is thought that this form results from a high degree of host immunity, preventing spread of infection. Nodules may become ulcerated and associated with seropurulent exudate and crust formation. The normal grooming behavior of cats may result in autoinoculation and spread of lesions to distant sites. The cutaneous form may have a very chronic course. An unusual case of sporotrichosis in a dog consisted of otitis externa characterized by multiple cutaneous nodules which persisted for more than five years.

The cutaneous-lymphatic form involves the skin, subcutaneous tissue, and associated lymphatics. Lesions begin as firm round nodules at the site of entry, usually on an extremity, and spread proximally along lymphatics. Lymphatic vessels become thick and corded and a series of secondary nodules forms as the infection progresses. The nodules may break open and discharge seropurulent material. Lesions may cavitate and expose extensive areas of underlying muscle and bone. Regional lymphadenopathy is common. This is the most common form in horses. Lesions generally involve the proximal forelimbs, chest, and thigh but usually no regional lymph node involvement is evident. Dogs usually have the cutaneous or cutaneous-lymphatic form. The head, pinnae, and trunk are involved most frequently. In cats, lesions are usually located on the head, distal limbs, and base of the tail. The initial draining puncture wounds may be indistinguishable from cat-inflicted fight wound infections.

The extracutaneous/disseminated form may involve a single extracutaneous tissue, such as osteoarticular sporotrichosis, or multiple internal organs. It develops as a sequela to cutaneous lymphatic infection or following inhalation of the fungus. The disseminated form of sporotrichosis occurs most frequently in cats, and no immunosuppressive factors are usually identified. In experimentally induced sporotrichosis in cats, organisms were shown by culture to have disseminated to viscera in 50% of the cases. Cats with disseminated sporotrichosis are often febrile, depressed, and anorexic.

In cats, as the case described, unlike humans, the low frequency of typical formed granulomas and the richness of fungal elements found in the histopathology of the skin demonstrate the increased susceptibility of animals to *S. schenckii.* Some investigators believe that the severity of feline sporotrichosis can be related to immunosupression caused by co-infection with feline immunodeficiency virus (FIV) or feline leukemia virus (FeLV), although reports of co-infection with FIV/FeLV and *S. schenckii* are very rare which renders this hypothesis unlikely.

Microscopically, sporotrichosis is usually a nodular to diffuse pyogranulomatous or granulomatous inflammatory reaction involving the dermis and subcutaneous fat. The epidermis is acanthotic or ulcerated. Neutrophils, epithelioid macrophages, multinucleated giant cells, and fewer lymphocytes and plasma cells can form discrete granulomas or extensive sheets of inflammation replacing dermal and subcutaneous tissues. Fibrosis is variable, and necrosis may be extensive. Yeast(s) surrounded by a stellate radial corona of brightly eosinophilic material (asteroid body/Splendore-Hoeppli reaction) are seen in some cases. The yeasts appear as round, oval, or elongated ("cigar"-shaped) single or budding cells which measure 2-6 µm or more in diameter for the round and oval forms.

Although *S. schenckii* may be seen in tissue with the routinely used hematoxylin and eosin (H&E) stain, other special stains such as Gomori methenamine silver (GMS) or periodic acid-Schiff (PAS) can be employed to enhance fungal detection. Atypical *S. schenckii* cells can appear spherical and surrounded by a PAS-positive capsule, resembling *Cryptococcus* cells.

Isolation of the fungus from the nails and oral cavity of cats supports evidence indicating that transmission can occur through a scratch or bite, while isolation from the nasal fossae and cutaneous lesions indicates the possibility of infection through secretions. Sporotrichosis can be diagnosed through a correlation of clinical, epidemiological, and laboratory data. Laboratory analysis for the determination of sporotrichosis includes direct examination of specimens such as tissue biopsy specimens or pus from lesions. In case of disseminated infections, other specimens, such as sputum, urine, blood, and cerebrospinal and synovial fluids can be analyzed, depending on the affected organs.

The differential diagnoses should be considered in accordance with the diversity of clinical forms and the morphology of the lesions. The main differential diagnosis is cutaneous leishmaniasis, cryptococcosis, mycobacterioses and skin neoplasias. Sporotrichosis can also mimic cutaneous bacterial infections, sarcoidosis, lupus vulgaris, tuberculosis, and scrofuloderma, among others in humans. These conditions should be differentiated by history, areas of endemicity, and lab tests.

Sodium iodide (NaI) or potassium iodides (KI) were considered the drugs of choice in human and canine sporotrichosis; however, serious adverse effects have limited their use. Itraconazole (ITZ) is considered the drug of choice in feline and human sporotrichosis treatment because of its greater effectiveness and safety when compared to other antifungal agents. Sporotrichosis in cats is more difficult to treat than in dogs, requiring a prolonged period of therapy. The number of affected noncontiguous anatomic regions, the general medical condition, and the degree of compromise to the immune system influence the treatment outcome. The cooperation and persistence of the owners is instrumental in attempting a successful response to therapy. When sporotrichosis is not treated for an adequate time, it often recurs, usually with respiratory signs. In these cases the clinical cure is difficult; respiratory signs are associated with treatment failure and death. There is a report of ITZ-resistant strains of *S. scherenkii*.

People handling cats with potential sporotrichosis should follow biosecurity measures. In addition,

separation of sick cats from other animals in the same environment is indicated. Care must be taken to avoid cuts or penetrating injuries when working with infected cats, and protective outerwear should be worn. Proper physical restraint or sedation of noncooperative patients must be done to allow complete examination of lesions and the collection of biologic material for laboratory analysis.

Decontamination and cleaning of cages or transport containers must be done with hypochlorite (1%), diluted 1:3 in water, for at least 10 minutes. If possible, sun drying is also beneficial. Examination tables should be cleaned after contact with infected animals and disinfected with sodium hypochlorite solution (1%), followed by alcohol 70% for at least 10 minutes using disposable paper towels. Additionally, floors and walls must be cleaned and disinfected daily with sodium hypochlorite solution (1%). For public health purposes and to control epidemic cat-transmitted sporotrichosis, an effective and viable therapeutic regimen applied to cats under field conditions is necessary.

Moreover, public awareness programs on sporotrichosis prophylaxis are required, encouraging the following: responsible ownership, castration, cremation of dead cats, confinement of cats inside the home, limitation of the number of cats per household, regular cleaning of the dwelling, and proper health care for the animals.

This case is interesting because of the possibility of dealing with an ITZ resistant strain. Even if other possibilities (like inadequate treatment) cannot be excluded, such cases deserve special attention because of the discussed antropozoonotic potential and epidemic situation in some cities.

JPC Diagnosis: Haired skin: Dermatitis, pyogranulomatous, multifocal to coalescing, severe with numerous intrahistiocytic yeasts.

Conference Comment: The contributor provides a very thorough and informative summary of sporotrichosis. Conference participants compared the histopathologic findings in this case with those found in cases of dermatitis associated with *Histoplasma capsulatum,* noting the lesions and organisms can be very similar in appearance. As the contributor states, sporotrichosis can also mimic leishmaniasis, cryptococcosis, and mycobacterioses, as well as other non-infectious conditions. Therefore, participants noted that, although histopathology is a useful auxiliary test for sporotrichosis, the gold standard for definitive diagnosis of *Sporothrix schenckii* remains culture, as was performed in this case.

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CASE III: T12 20790 (JPC 4019451).

Signalment: 8-year-old, female spayed Jack Russell mix *(Canis familiaris)*.

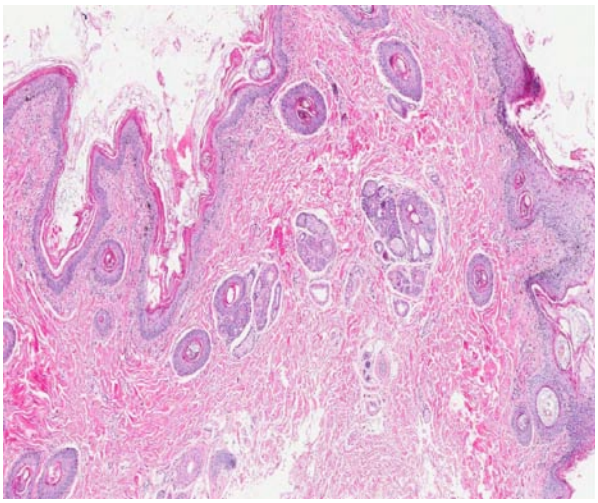
History: The referring veterinarian described pustule formation progressing to ulceration in the axillary and inguinal regions, ventral abdomen and around the vulva.

Gross Pathologic Findings: NA.

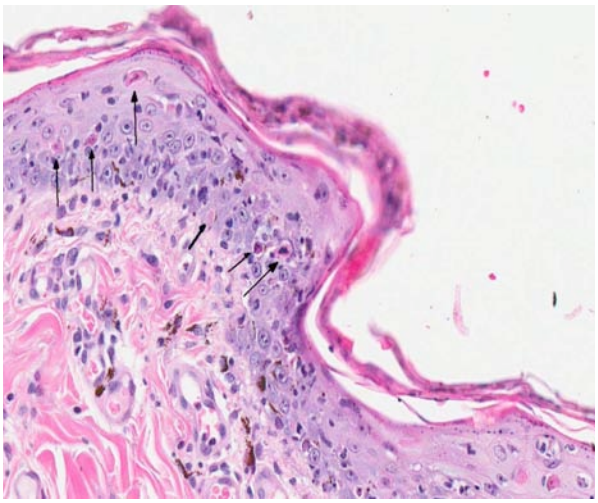
Histopathologic Description: Haired skin: There are multiple focal plaque-like lesions alternating with small areas of normal skin. The epidermis is characterized by mild parakeratosis that often spans several hair follicles. The epidermis has mild to moderate thickening with apoptotic cells present in high numbers in all levels. Some of the apoptotic cells are surrounded by lymphocytes (satellitosis). Numerous intraepithelial or exocytosing lymphocytes are present. There is vacuolation along the basal layer with moderate pigmentary incontinence. These changes extend into the infundibular portion of the hair follicle sheaths. A mild diffuse interface and perivascular infiltrate of lymphocytes, plasma cells and histiocytes is present. Several hair follicles are in growth arrest and contain keratin plugs. Sebaceous glands are small and absent from a few follicles. In the area of the isthmus of the follicles with small or absent sebaceous glands, there is pyogranulomatous inflammation and vacuolation. Apocrine glands are mildly dilated. No fungal organisms are detected in routinely stained sections. A small colony of bacteria is associated with hair in the ostia of a single follicle. Rare coccoid bacteria are noted in the surface keratin debris.

Contributor's Morphologic Diagnosis: Haired skin: Dermatitis, interface, subacute, multifocal, moderate with apoptosis, mild parakeratotic hyperkeratosis, folliculitis and adnexal destruction (sebaceous gland)

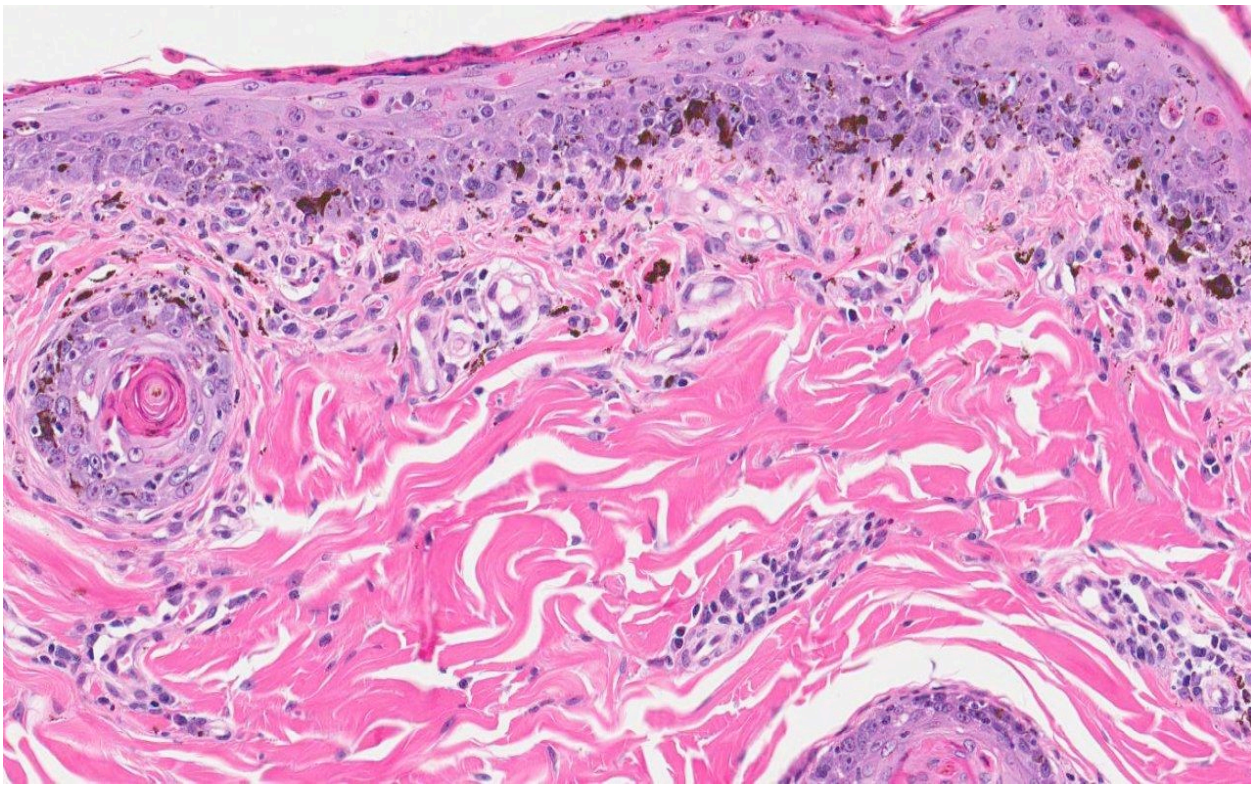
Contributor's Comment: The clinical history, lesion distribution and histologic lesions are consistent with erythema multiforme (EM). The two major differential diagnoses for immune-mediated skin disease with interface dermatitis and apoptosis are erythema multiforme (EM) and all forms of lupus erythematosus (LE). The apoptosis observed in LE tends to be limited to the basal cell layer where vacuolation also occurs. The classic lesion in EM is apoptosis involving any cellular layer of the epidermis along with lymphocyte satellitosis.[4] There are usually aggregates of lymphocytes and histiocytes along the dermal-epidermal junction. Vacuolation and apoptosis of the basal cell layer occur, and there is variable pigmentary incontinence. Areas of ulceration are typically associated with additional infiltrates of neutrophils, eosinophils and plasma cells.



3-1. Haired skin, dog: The epidermis is diffusely hyperplastic and hyperkeratotic. The parakeratotic hyperkeratosis extends into hair follicles. (HE 4X)

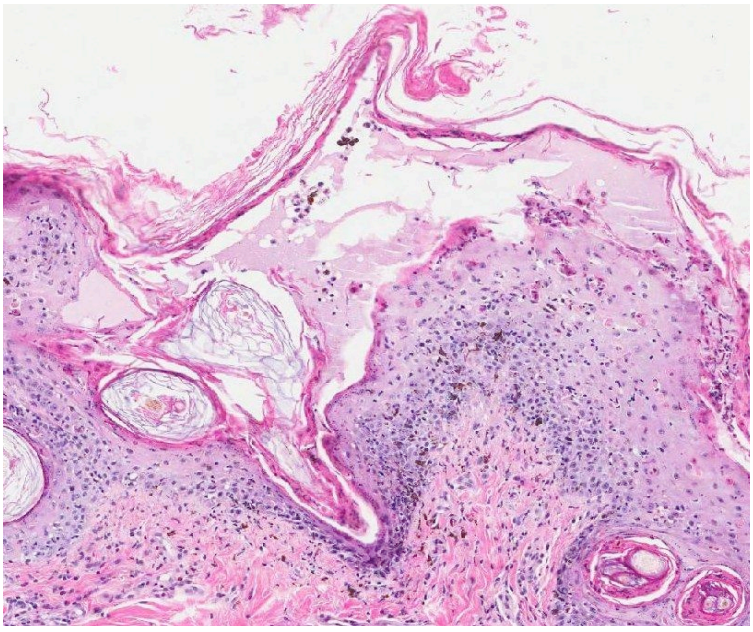


3-2. Haired skin, dog: There are numerous apoptotic epithelial cells within all levels of the epidermis (arrows) and follicular infundibulum (not pictured). The epidermis is hyperplastic and hyperkeratotic. The dermal-epidermal interface is blurred and contains lymphocytes, histiocytes, plasma cells, and melanomacrophages (thick arrow). (HE 40X)



3-3. Haired skin, dog: There is a mild interface dermatitis composed of low numbers of lymphocytes, histiocytes, plasma cells, and macrophages at the dermal epidermal junction as well as hair follicles. Melanomacrophages in the subjacent dermis ("pigmentary incontinence") attest to damage of the basal layer. Apoptotic keratinocytes are scattered throughout the epidermis. (HE 400X)

Erythema multiforme is a relatively uncommon skin disease reported in dogs, cats, horses and cattle that has been associated with a number of different triggers.[2,3] The pathogenesis of EM is not completely understood; however, this disease is thought to be a T-cell-mediated hypersensitivity reaction against a variety of antigens including drugs, viruses, bacteria and food substances.[1,3,5-9] EM has also been described as a paraneoplastic condition associated with a variety of tumor types.[1,4,10]



3-4. Haired skin, dog: Suprabasilar clefting is present. Apoptotic keratinocytes are scattered at all levels thought the epidermis. (HE 360X)

The most commonly incriminated drugs include trimethoprim-potentiated sulfonamides, penicillins and cephalosporins.[4,7,8] The dog in this case was medicated with trimethoprim-sulfa for cystitis prior to the onset of the cutaneous lesions.

Clinically, dogs present with an acute onset of erythematous annular macules, elevated circular plaques and papules that are most commonly involve the glabrous skin of the inguinal and axillary regions.[4] Mucocutaneous junctions, oral mucosa, ears and paw pads are also commonly affected. Two

classifications for EM have been adopted from human nomenclature. EM minor includes patients with no more than one mucosal surface affected and <10% of the body surface affected. EM major is used for cases in which more than one mucosal surface and between 10% to 50% of the body surface is affected. A third syndrome, Stevens-Johnson syndrome, is reserved for patients with >50% of the body surface affected.

JPC Diagnosis: Haired skin: Apoptosis, transepidermal, epidermal and follicular, multifocal, with necrosis, hydropic degeneration, subepidermal clefting, orthokeratotic hyperkeratosis, and neutrophilic and lymphohistiocytic interface dermatitis.

Conference Comment: As the contributor notes in the above informative summary, EM is thought to be a T-cell mediated hypersensitivity in which the host's cellular immune response is directed against keratinocyte-associated antigens. Such reactions have been reported to be associated with certain drugs, bacterial infections (staphylococcal folliculitis, pseudomonal otitis externa), food products, and epitheliotropic viral infections. In particular, conference participants discussed the association between EM and canine parvovirus type 2b (CPV-2b). There have been a few cases of EM reported in dogs associated with CPV-2b infection, including in a 2-month-old Great Dane puppy and, more recently, a litter of English Setter puppies.[2,6] It has been suggested that infection of stem cells and transient amplifying keratinocytes occurs following hematogenous dissemination of CPV-2b, thus leading to initiation of EM. The recent report suggests that, along with the multiple other possible initiating factors, CPV-2b should be considered as a potential initiator of EM in dogs.[6]

Contributing Institution: University of Georgia Tifton Veterinary Medical Diagnostic Laboratory

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CASE IV: A2011-01 (JPC 4002941).

Signalment: 10-year-old, spayed female mixed breed, canine *(Canis familiaris)*.

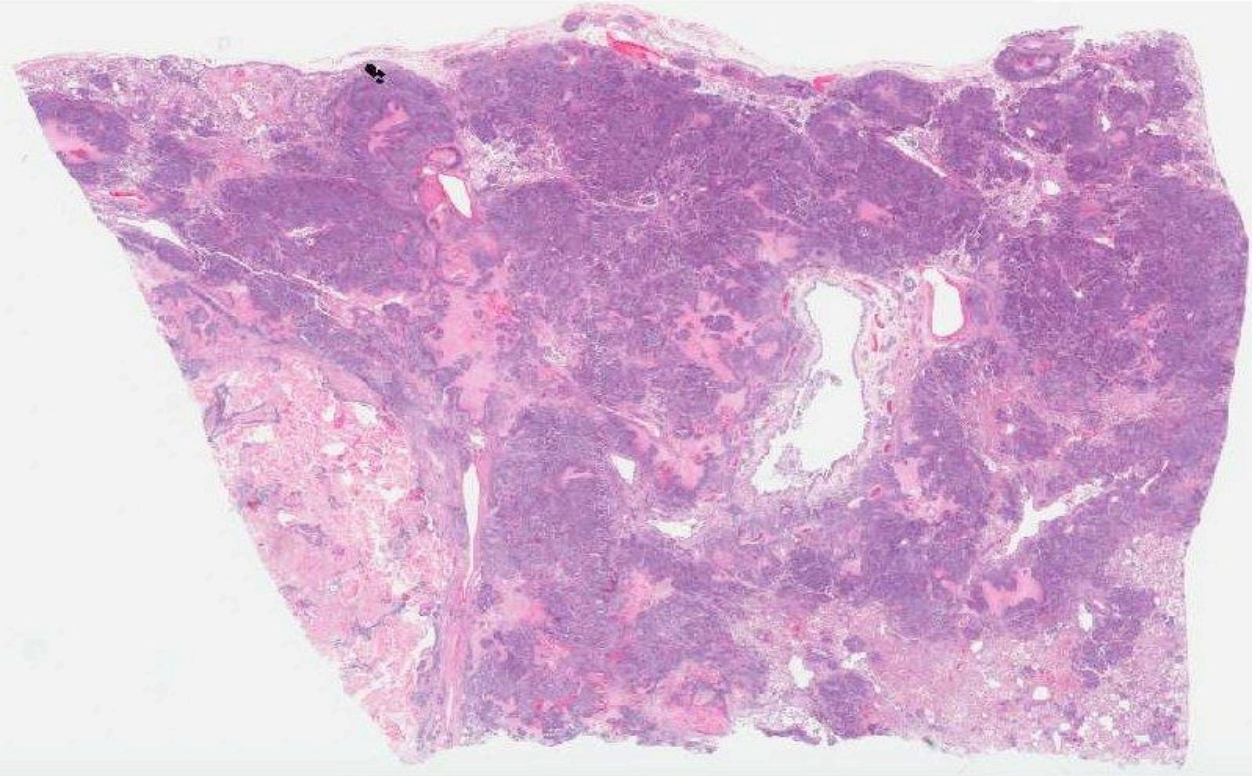
History: This animal had a cutaneous mass removed from the inter-scapular region three years previously which was diagnosed as a malignant pilomatricoma. The animal presented recently with labored breathing and was euthanized due to numerous pulmonary soft-tissue opacities radiographically. Portions of lung were submitted for histopathology.

Gross Pathology: In multifocal to coalescing regions, there is extensive replacement of the pulmonary parenchyma by soft, pale tan to red, variably necrotic masses.

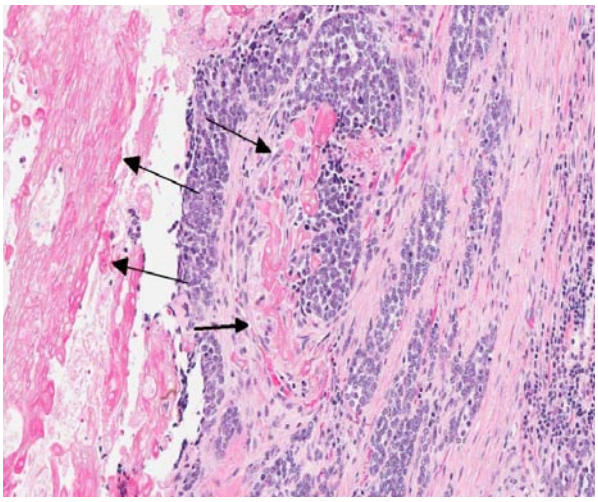
Histopathologic Description: The normal pulmonary architecture is extensively effaced by neoplastic epithelial cells, necrosis, and debris. The basaloid cells are arranged in coalescing cords and clusters supported by a fibrovascular to inflammatory stroma. The cells have indistinct cell borders and small amounts of eosinophilic cytoplasm and central round to ovoid nuclei with coarse, irregular chromatin and prominent nucleoli. There are scattered foci of squamous differentiation. The cells exhibit moderate to marked anisocytosis and anisokaryosis. Mitoses are common and range from 3-10 per 40X field. The cell clusters occasionally have central necrosis or central keratinized "ghost cells." Tumor emboli are common in vessels and lymphatics.

Contributor's Morphologic Diagnosis: Lung: Metastatic pilomatricoma.

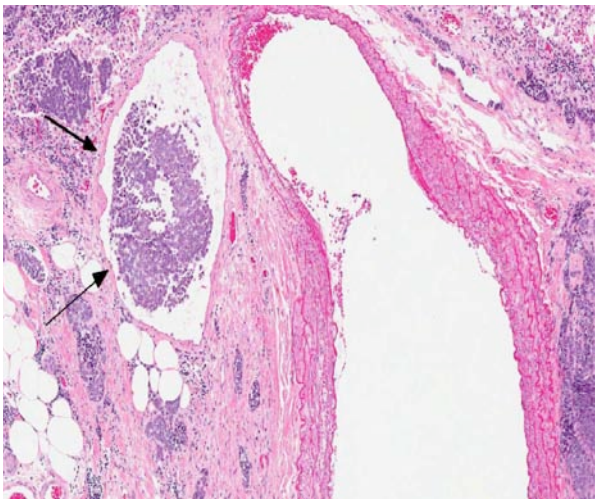
Contributor's Comment: Pilomatricoma is a benign cutaneous tumor that arises from the germinative cells of the follicular matrix, or hair bulb exhibiting only matrical differentiation.[1,2,4] It is an uncommon neoplasm of dogs and comprises approximately 1% of canine skin tumors.[1,3] Pilomatricomas most commonly occur on the back, neck, thorax, and tail. The tumors are generally well-delineated, firm, multilobular intradermal masses that often contain areas of grey-white chalky material on cut section.[2] Young, adult dogs are typically affected. Poodles, Kerry Blue Terriers, Old English Sheepdogs, Soft-



4-1. Lung, dog: 85% of the section is effaced by an infiltrative, densely cellular neoplasm. (HE 0.63X)



4-2. Lung, dog: Islands of neoplastic polygonal cells resembling basal epithelium are centered around areas of abrupt keratinization containing ghost cells (arrows). (HE 40X)



4-3. Lung: Neoplastic cells are contained within vessels and lymphatics (arrows). (HE 84X)

coated Wheaten Terriers, and other breeds with continuously growing coats are over-represented which is believed to be related to the continuously-growing coat and subsequently greater numbers of mitotically active anagen follicles in these breeds.[1,4]

The histological features include a well-delineated dermal and/or subcutaneous epithelial tumor composed of multiple, variably sized cystic structures supported by a collagenous stroma. The structures are lined with several layers of basaloid keratinocytes which resemble the matrix cells of the anagen hair bulb with a moderate to high mitotic activity. Scattered squamous differentiation can be seen. As the neoplastic epithelial cells differentiate towards the center of the lesion they exhibit matrical keratinization ("ghost" or "shadow" cells) and the center of the cysts accumulate large aggregates of ghost cells and debris. The central debris can degenerate and become mineralized with foci of osseous metaplasia.[2,4]

Malignant pilomatricomas are rare and can be distinguished from the benign variety by their poorly delineated, infiltrative nature, increased mitotic activity, and increased ratio of basaloid cells to keratinized ghost cells. Lymphatic invasion is often evident along the periphery of the lesion. Metastasis has been reported to local lymph nodes, bone, and lung.[1,2,3]

JPC Diagnosis: Lung: Metastatic pilomatricoma.

Conference Comment: Recently, Martano et al. reported on the histopathological and immunophenotypic characteristics of malignant pilomatricoma with metastasis to bone in an 11-year-old mongrel dog.[5] Malignant pilomatricoma was diagnosed in an ulcerated mass from the dog's neck and a nodular bone mass, based on the histopathological features of irregularly-shaped, lobulated islands of basaloid cells undergoing abrupt keratinization to shadow cells, a high mitotic rate, and nuclear atypia, as well as metastasis to bone. Anti β-catenin immunohistochemistry revealed strong nuclear immunoreactivity of neoplastic basaloid cells, with few transitional cells exhibiting membrane-bound immunoreactivity. In humans, although the extent of membrane, cytoplasmic and nuclear expression of β-catenin varies in most skin tumors, basaloid cells in pilomatricoma and malignant pilomatricoma usually demonstrate intense diffuse nuclear immunoreactivity with β-catenin antibody. Furthermore, pilomatricoma in humans are often associated with deregulation of the Wnt/β-catenin pathway due to mutations in the *CTNNB1* gene. In normal cells, β-catenin can be located at the cell surface where it links E-cadherin to the actin cytoskeleton to create cell-to-cell junctions. Cytosolic β-catenin is phosphorylated by the APC-AXIN-GSK3β destruction complex, and subsequently destroyed via ubiquitination (known as the APC/ β-catenin pathway).[6] Wnt signaling blocks the destruction complex, allowing β -catenin to accumulate and translocate to the nucleus, where it complexes with transcription factors that up-regulate

transcription of c-*MYC, cyclin D1,* and other genes that increase cellular proliferation. Dysregulation of the APC/ β-catenin pathway has been found to play a role in the development of several neoplasms, including colon tumors and hepatocellular carcinomas in addition to pilomatricomas.[5,6] Based on the findings in this study, Martano et al. suggest that, similar to humans, β-catenin is involved in the pathogenesis of malignant pilomatricoma in dogs, and therefore may be a useful diagnostic marker.[5]

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