Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2014-2015

Conference 5

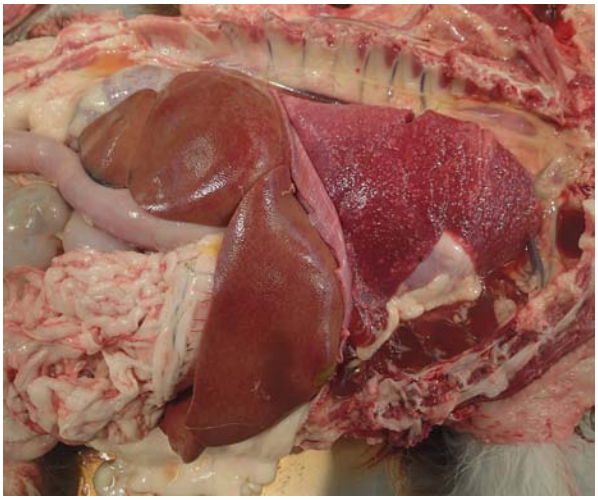
1 October 2014

CASE I: W356-13 (JPC 4034430).

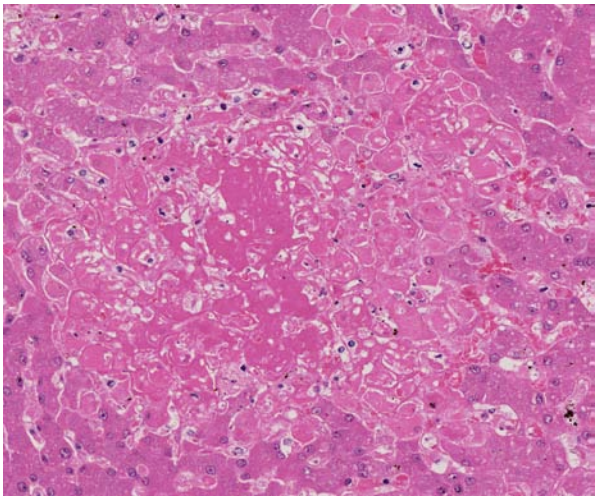
Signalment: 1-year-old male neutered Border Collie, dog, *Canis familiaris.*

History: The dog was presented at the Veterinary Hospital of the University of Melbourne with acute progressive severe respiratory distress. The dog had been diagnosed by the referring veterinarian with immune-mediated haemolytic anaemia (IMHA) two months prior to presentation and then treated with very high doses of immunosuppressants (prednisolone, cyclopsporine, azathioprine) and aspirin.

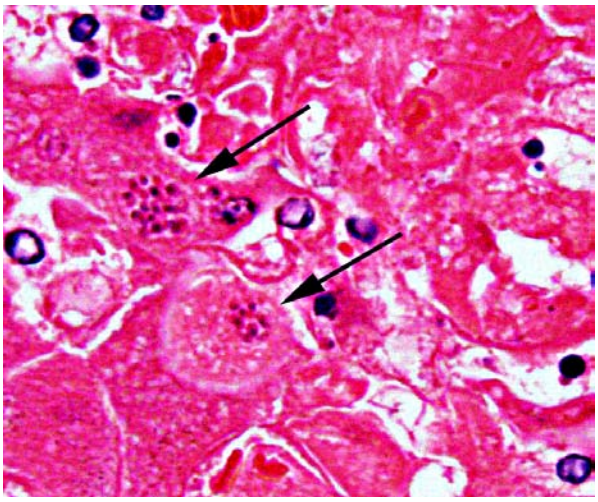
At presentation the dog had generalized heart sounds; radiographs showed a diffuse, mixed, predominantly interstitial pattern in all lung lobes. Transtracheal wash revealed neutrophilic



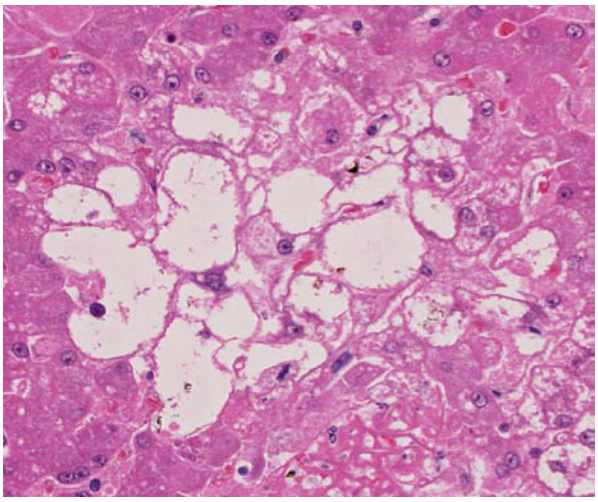
1-1. In situ photograph, dog: Lung lobes are diffusely dark red with numerous randomly distributed cream-colored nodules. The liver is enlarged and tan with rounded borders. (Photo courtesy of: Faculty of Veterinary Science, University of Melbourne www.vet.unimelb.edu.au)



1-2. Liver, dog: Foci of coagulative necrosis are randomly scattered throughout the section. (HE 172X)



1-3. Liver, dog: At the edges of necrotic foci, hepatocytes contain intracytoplasmic cysts with numerous 2-4 µm zoites. (HE 600X)



1-4. Liver, dog: Multifocally, centrilobular and midzonal hepatocytes are swollen with coalescing clear vacuoles (glycogenosis) characteristic of steroid hepatopathy. (HE 292X)

inflammation with protozoa. The dog deteriorated progressively and spontaneously arrested.

Gross Pathology: All lung lobes were diffuse dark red and had numerous randomly distributed cream-coloured nodules varying from 1-4 mm in diameter which extended throughout the lung parenchyma. The liver was enlarged with rounded borders and diffuse tan discolouration displaying fine red surface stippling.

Laboratory Results: IFAT (plasma): Positive for *Toxoplasma* sp. (titre 1:128), negative for *Neospora* sp. Immunohistochemistry was positive for *Toxoplasma* sp.

Histopathologic Description: Liver: Throughout the hepatic parenchyma there are multifocal randomly distributed areas of hepatocellular necrosis, characterized by loss of tissue architecture and replacement by eosinophilic cellular and karyorrhectic debris. In the necrotic areas, or peripheral to these, there are numerous, often clustered, tachyzoites approximately 2 µm in diameter with an indistinct internal structure that occasionally appears to have a bilobed nucleus. Multifocally groups of hepatocytes are swollen with clear, finely granular cytoplasm and peripherally displaced nucleus.

Contributor's Morphologic Diagnosis: Multifocal hepatocellular necrosis, subacute, severe, with intra-lesional protozoal organisms; multifocal hepatocellular vacuolar degeneration, moderate, chronic.

Contributor's Comment: *Toxoplasma gondii* is a coccidian protozoan that is found throughout the world and infects an extensive range of intermediate hosts in which it causes both clinical and more commonly, subclinical disease. Domestic and wild felids are the only known definitive hosts and also serve as intermediate hosts.[1]

Infection occurs by ingestion of sporulated oocysts excreted in the feces of felids, by ingestion of tissues of intermediate hosts that contain encysted bradyzoites or tachyzoites, and less frequently by vertical transmission. Once ingested, sporozoites excyst and multiply in the intestinal epithelial cells as tachyzoites. Tachyzoites can either disseminate and infect cells throughout the body resulting in the necrosis and less commonly non-suppurative inflammation characteristic of toxoplasmosis, or encyst in tissues as bradyzoites. Following ingestion of tissue cysts by an intermediate host, bradyzoites will excyst, become tachyzoites, and the cycle continues.[1] Necrosis is caused by rupture of infected cell membranes by rapidly dividing tachyzoites. Inflammation is typically not associated with the cysts and can be minimal in association with the tachyzoites.[2] Tissue cysts are presumed to persist throughout the life of the host. Even though a high percentage of animals are serologically positive for toxoplasmosis, only a few animals develop clinical disease. Immunosuppression commonly causes reactivation of latent infections caused by *T. gondii* and is usually characterized by

involvement of the central nervous system, occasionally the lungs and infrequently other organs, including the skin.[4]

The tachyzoites, bradizoites and tissue cysts of *Neospora caninum* and *Toxoplasma gondii* appear essentially identical by standard light microscopy. Immunohistochemistry and PCR are commonly used to confirm the diagnosis. Even though IHC using polyclonal antibodies against *T. gondii* is considered sensitive, positive results might not be conclusive, due to moderately low specificity of polyclonal antibodies that can cross-react between *T. gondii* and *N. caninum.*[5]

Ultrastructure is also used to distinguish between *T.gondii and N.caninum.* Some of the most prominent ultrastructural differences occur in the number, appearance and location of rhoptries, looped-back rhoptries, micronemes, dense granules, small dense granules and micropores. The tissue cysts of both parasites are basically similar, being surrounded by a cyst wall and not compartmentalised by septa. The cyst wall of N. *caninum* is usually irregular and substantially thicker (<0.5 mm), than those of *T. gondii* which are typically smooth and 0.5 mm thick.[6]

JPC Diagnosis: 1. Liver: Hepatitis, necrotizing, random, multifocal, moderate, with edema and intrahepatocytic, intrahistiocytic, and extracellular zoites.
2. Liver, hepatocytes: Glycogenosis, centrilobular and midzonal, multifocal, moderate.

Conference Comment: While most often associated with abortion in domestic animals, this case of *Toxoplasma gondii* serves as a reminder of its ubiquitous nature. All homeothermic animals are susceptible to infection, and the organism may be present in a wide range of organ systems.[2] Infection is also often associated with immunosuppression, as demonstrated in this case. Of note, marsupials are thought to be particularly susceptible to both *Toxoplasma* and *Neospora.*[3]

Toxoplasmosis is a significant contributor to abortion in domestic animals due to both cotyledonary and caruncular necrosis of the placenta. Other characteristic lesions include interstitial pneumonia, lymphadenitis, myocarditis, nonsuppurative meningitis, ophthalmitis and hepatic necrosis.[2]

T. gondii is an obligate intracellular parasite, and its growth and replication only occur in target cells and eventually cause cell death. At the core of its infectivity is the ability to cross barrier systems, including intestinal mucosa, the blood-brain barrier, the blood-retina barrier and the placenta. This process involves parasite motility, likely in the tachyzoite stage, and interactions between parasite adhesins and target cell receptors. The cellular binding occurs via the parasite surface proteins SAGs, which are expressed in abundance on tachyzoites, and SAG1 receptors in addition to laminin and lectin on target cells.[7]

The contributor accurately details the distinguishing characteristics between *Toxoplasma* and *Neospora* on electron microscopy. Both parasites induce similar lesions and appear identical with histopathology; however, *Toxoplasma* is always found within a parasitophorous vacuole while *Neospora* may not be. Other organisms which may be found within parasitophorous vacuoles include *Sarcocystis*, though these are primarily in skeletal or cardiac muscle.[2]

The glycogenosis noted in the hepatocytes is secondary to the administration of exogenous corticosteroids. Glycogenosis may be histologically differentiated from lipidosis as the vacuoles seen in glycogenosis are coalescing rather than discrete. In most cases, steroid-induced gluconeogenesis causes increases in both hepatocellular lipid and glycogen, but glycogensosis is often the predominant histologic change.

We favor the use of two morphologic diagnoses in this case, one for the protozoal infection and one for the glycogenosis, as they are part of two distinct, albeit related processes.

Contributing Institution: Faculty of Veterinary Science, University of Melbourne www.vet.unimelb.edu.au

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CASE II: 10536 (JPC 4048507).

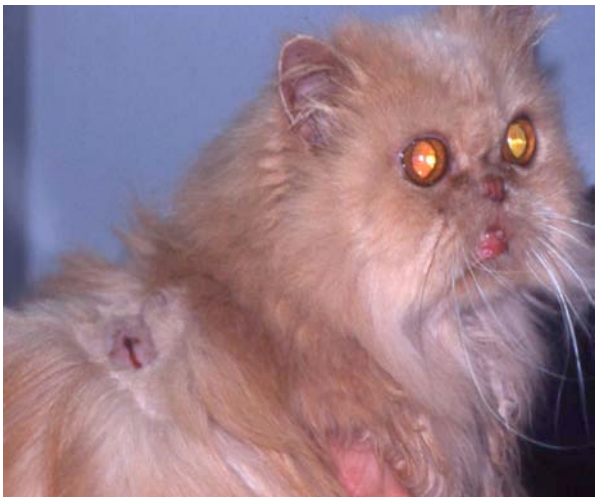
Signalment: 9-year-old, spayed female Persian cat, *Felis catus.*

History: The cat developed a soft dermal nodule in the right thoracic region; the overlying skin was unremarkable. The cat was brought to the referring veterinarian due to rapid (2-week) enlargement of the nodule with ulceration (Fig. 1) 8 months after initial presentation. Cytology of the material was characterized by abundant necrotic debris, foamy reactive macrophages, degenerated neutrophils and occasional fragments of fungal hyphae. The nodule was surgically excised and processed for histology and electron microscopy. The histopathology revealed a pyogranulomatos inflammation centered on PAS positive fungal hyphae. After 3 years, the cat is still free of disease without any additional therapy. An electron micrograph at 12,000x magnification is provided.

Gross Pathology: Nodular lesion in dorso-lateral thorax with draining tracts oozing sero-sanguineous material

Laboratory Results: No fungal isolation was attempted by the referring veterinarian. CBC and urine analysis were unremarkable.

Electron Microscopy Description: Dermis, 12,000 magnification. On the electron micrograph, there are longitudinal and transverse sections of fungal hyphae within the cytoplasm of a reactive macrophage. On the right-hand top corner, two nuclei with slightly irregularly infolded nuclear envelopes, a prominent nucleolus, randomly scattered dispersed chromatin and multifocal aggregates of marginated chromatin are evident. The cytoplasm is abundant and contains mitochondria, stacks of rough endoplasmic reticulum and free ribosomes (binucleated macrophage).



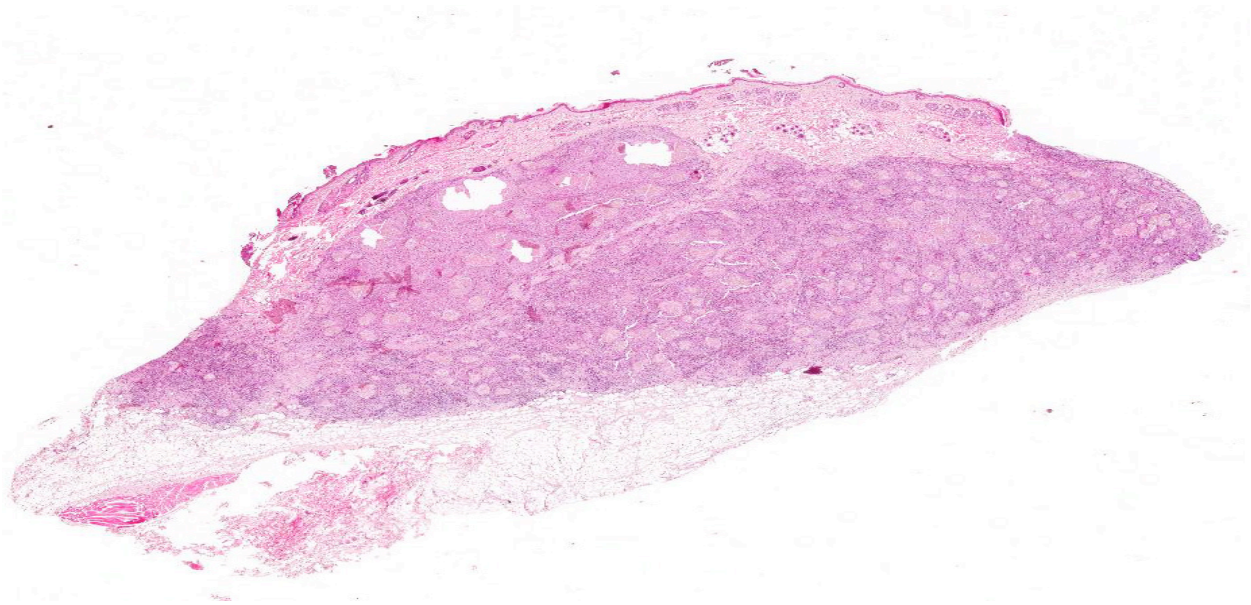
2-1. Presentation, cat: The cat presented with a nodular lesion in the dorsolateral thorax with a draining tract and a serosanguineous exudate. (Photo courtesy of: DIVET - Department of Veterinary Science and Public Health, http://www.divet.unimi.it/ecm/home)

The fungal hyphae are septate and characterized by a thick homogeneously moderately electrondense fibrillar cell wall with an underlying thin, highly electron-dense plasma membrane enclosing moderate amount of granular, moderately electron-dense cytoplasm. The latter contains few elongated to round mitochondria, moderate numbers of round single-membrane bound electron lucent vacuoles, scattered ribosomes and rare myelin figures. Two nuclei with central to paracentral prominent nucleoli and a nuclear envelope are present. Fungal structures are surrounded by a variably thick, moderately to highly electron-dense, fibrillar material (accumulation of immunoglobulins – Splendore-Hoeppli reaction), multifocally radiating from the hyphal surface.

Contributor's Morphologic Diagnosis: Macrophagic (granulomatous) dermatitis with fungal hyphae and Splendore-Hoeppli formation consistent with pseudomycetoma.

Contributor's Comment: Fungal organisms are eukaryotes with structurally defined cell, cytoplasm and nucleus. According to ultrastructural descriptions, most fungal cells contain free ribosomes, mitochondria, vesicles involved in endocytosis and exocytosis and lipid. The fungal vacuole is similar to the plant vacuole has enzymatic functions. Nuclei are smaller than those of vertebrate cells with a defined nuclear membrane and a nucleolus. DNA is largely located in the nucleolus.[6]

Specifically, electron microscopy of *M. canis* has been well described.[17] *M. canis* hyphae have a cell wall consisting of an outer thin, electron-dense layer and an inner and broader fibrillar electron-lucid layer. Septa are produced from the inner and broader fibrillar electron-lucid layer and are characterized by septal pores. The plasma membrane is a thin, electron-dense delimiting membrane contiguous with the inner surface of



2-2. Haired skin, cat: The dermis is expanded by coalescing poorly formed pyogranulomas. (HE 6.3X)

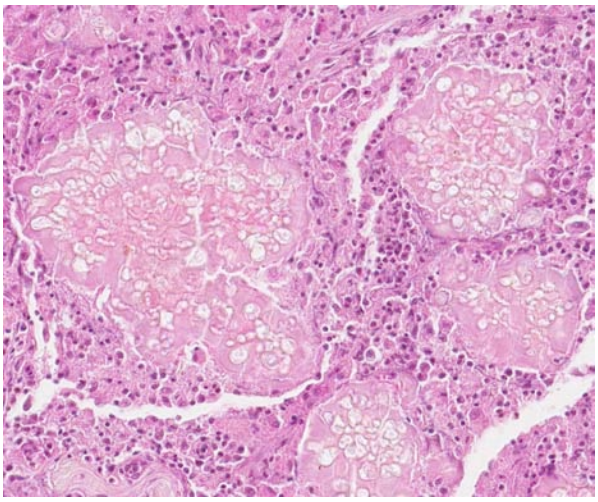
the cell wall. In the cytoplasm, a large central vacuole surrounded by an electron-dense tonoplast enclosing electron-lucent flocculent material is visible. Mitochondria having no polarity are scattered throughout the cytoplasm of the hyphae and can be filamentous or spherical. Mitochondria have double membrane and extensive cristae that might extend across the organelles. Hyphae have sparse narrow tubular endoplasmic reticulum and a large tonoplast. Glycogen is abundant and often clumped and lipid inclusions are also present. Single membrane bound vesicles with central bodies are also present at the margins of the hyphae. Hyphae are often bi- to multinucleated (as in the image provided). The nuclear membrane has a double envelope with intervening nuclear pores. Additional features are the presence of single membrane bound vesicles with central bodies with high electron opacity.

Clinical, gross and microscopic findings were representative of a deep dermatophyte infection consistent with feline dermatophytic pseudomycetoma. The disease associated with *Microsporum canis* has been described also in dogs,[1,8] horses[12] and humans.[2,7,10,14] Dermatophytic pseudomycetomas are uncommon to rare, deep cutaneous to subcutaneous fungal infections that produce tissue grains or granules. These tissue grains are composed of fungal aggregates embedded in amorphous eosinophilic material representing antigen–antibody complexes (Splendore–Hoeppli reaction).

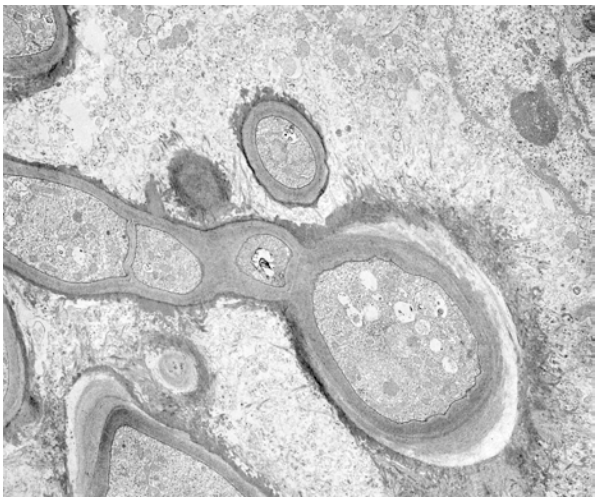
Feline pseudomycetomas have been reported mostly in Persian cats.[3,4,5,8,9,11,15] Sporadic cases have been reported in Himalayan, Domestic Shorthair, and in Maine Coon cats.[8,11,13,16] Genetic predisposition has been hypothesized to play a role in the development of these lesions since the Persian breed seems predisposed also to conventional dermatophytosis.[8] Age and gender predilections have not been observed.

The frequent localization of the lesions in the dorsal trunk, most commonly in outdoor cats, suggests a traumatic implantation of organisms from hair follicles with dermatophytic colonization by biting or fighting.[8] Some authors hypothesize that mycelial elements reach the dermis from spontaneously ruptured hair follicles in association with dermatophyte infection. Once in the dermis, fungi aggregate and induce a granulomatous reaction. Positive dermatophyte cultures from normal-appearing areas distant from the dermatophytic pseudomycetoma indicate that affected cats may previously have been inapparent carriers. Inapparent carriage of *Microsporum canis* is common in Persian and Himalayan cats.[5,8]

Grossly, dermatophytic pseudomycetomas are characterized by one or more subcutaneous nodules that occur most commonly over the dorsal trunk or tail base.[5,8,9,11] Lesions are firm,



2-3. Haired skin, cat: The pyogranulomas are centered on aggregates of fungal hyphae with thick, nonparallel walls, rare irregular non-dichotomous branching, and up to 25 µm diameter bulbous swellings embedded in an amorphous eosinophilic material (Splendore-Hoeppli reaction). (HE 192X)



2-4. Haired skin, cat: Ultrastructural examination of fungal hyphae demonstrates several cross sections of a thick lamellar cell wall enclosing granular cytoplasm with moderate numbers of mitochondria and vacuoles and transverse septations. The hyphae are surrounded by electron-dense fibrillar Splendore-Hoeppli material. (EM X12,000)

irregularly shaped nodules that gradually enlarge and coalesce in the dermis or the underlying subcutaneous tissue. Lesions may fistulate and discharge a seropurulent to necrotic material. Systemic clinical signs are uncommon, but lymphadenomegaly may be present. Intra-abdominal dermatophytic granulomatous peritonitis sharing many features with pseudomycetoma has been reported in Persian cats.[3,15,18]

Although dermatophytic pseudomycetoma fulfils most of the criteria for true mycetomas (nodular inflammation with fibrosis, fistulae draining from deep tissue, presence of tissue grains), fewer hyphal elements are present, and the lesions apparently lack the cement substance that holds true mycetoma grains together.[8]

Microscopically, lesions are located mostly in the dermis where aggregates of grey, refractile and highly pleomorphic fungal hyphae are characteristically found. These are tangled and delicate, and contain numerous large, clear, bulbous, thick-walled dilatations, resembling spores. Smaller swellings within the hyphae create a vacuolated or bubbly appearance to these structures. The fungal aggregates are imbedded in amorphous eosinophilic material to form large tissue grains, or granules that are also visible grossly. Splendore–Hoeppli reaction is brightly eosinophilic and locates around the periphery of organized aggregates of organisms. Granules are cuffed by and intermingled with large macrophages, giant cells, and variable, sometimes numerous neutrophils. Macrophages have abundant, granular cytoplasm. In some cases, fragments of hyphae are present within individual macrophages beyond the boundaries of tissue grains or granules. Reactive fibroblasts and collagen may surround or dissect the lesions often creating lobules composed of multiple granules and their attendant inflammation.[8,11]

Cytology has proven useful in the diagnosis of pseudomycetoma.[9,13,19] However, histopathology and fungal culture of biopsy specimens are required for a definitive and specific diagnosis. Organisms can be stained with periodic acid-Schiff, Gomori methenamine silver, Grocott stains and Fonata-Masson.[8,10] *Microsporum canis* has been isolated in typical cases of dermatophytic pseudomycetomas where fungal culture has been performed.[8] PCR from paraffin embedded tissues is also useful for *M. canis* identification and has been utilized in cases of feline, canine and equine lesions.[12] Immunohistochemistry using rabbit anti-*Microsporum canis* antiserum identified this agent also in two canine cases.[1]

Dermatophyte pseudomycetomas are considered difficult to manage clinically and the prognosis is considered poor in cats.[3,4,8] The lesions often recur after surgical excision alone[5] although in this case no recurrence was observed. There are contrasting reports regarding poor[4] or successful response of feline pseudomycetomas following terbinafine treatment.[13] Little response to

griseofulvin, ketoconazole or itraconazole has also been reported.[5,13,18] However, a combination of surgical excision with adjunctive long term medical therapy has recently been reported to be successful.[5,18]

Clinical differential diagnoses should include cryptococcosis and other systemic mycoses, sporotrichosis, cutaneous infections of other opportunistic fungi, and neoplasia. The marked breed predilection for Persian cats is helpful in increasing the index of suspicion for dermatophytic pseudomycetoma. Histologically, most of the systemic and opportunistic fungi affecting cats and dogs are smaller and more uniform in appearance and do not form granules or grains in tissue.[8] *Trichophyton mentagrophytes* has been reported to cause dermatophytic granulomatous inflammation in cats but the lesions do not have the typical histologic features of dermatophytic pseudomycetoma, and are characterized by a more diffuse tissue reaction with associated heavy colonization of the keratin of hair follicles and epidermis.

JPC Diagnosis: Haired skin: Dermatitis and panniculitis, granulomatous, focally extensive, severe, with Splendore-Hoeppli and numerous fungal hyphae.

Conference Comment: The presentation of this case provides a challenging perspective on an otherwise routine histopathologic diagnosis. Provided only the EM image, many participants were able to recognize a fungal hyphae within the cytoplasm of a macrophage; however, when given the accompanied glass slide during the conference, the characteristic granules of a pseudomycetoma due to *Microsporum canis* infection were readily identified.

The contributor provides an eloquent discussion on this entity, highlighting the characteristic ultrastructural, histopathologic and gross findings while adeptly discussing clinical presentation, management and appropriate differentials worthy of consideration.

Contributing Institution: DIVET Department of Veterinary Science and Public Health
http://www.divet.unimi.it/ecm/home

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CASE III: D13-55496 (JPC 4048072).

Signalment: 12-year-old intact male Pug, dog (*Canis familiaris*).

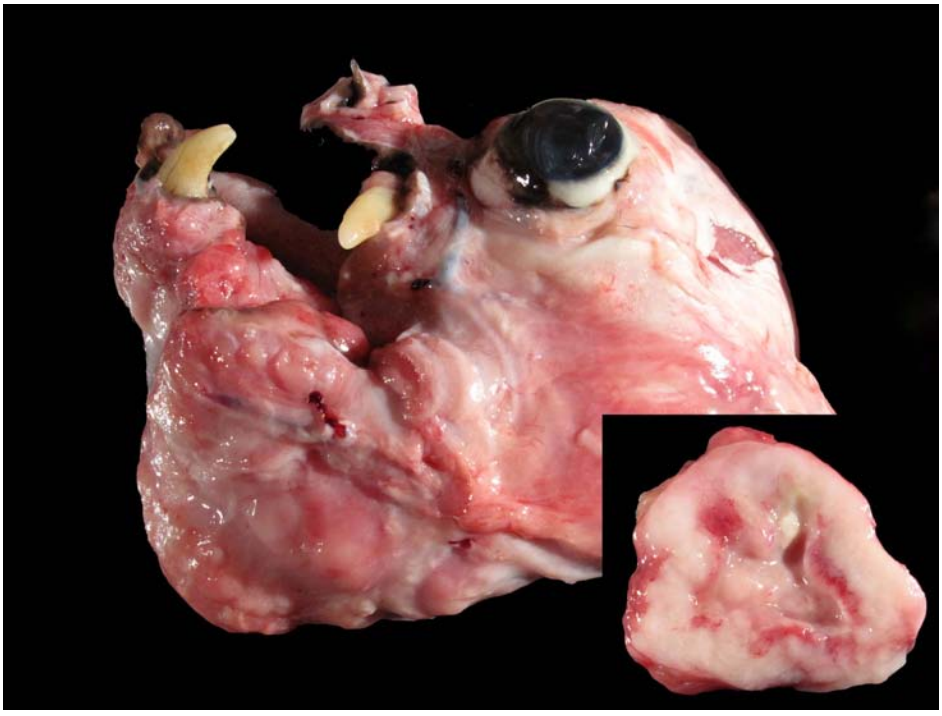
History: The dog had a recent history of an oral melanoma, edema (not further specified), and cavitary effusion (not further specified). The dog had multiple biopsies of the oral mass that was on the left mandible, which was confirmed to be malignant melanoma. The mass with incomplete margins was removed along with teeth 304-308. The dog was ultimately euthanized.

Gross Pathology: The entire body of the dog was submitted in a state of fair to good postmortem preservation. The dog was in a good body condition with a moderate amount of subcutaneous and intraabdominal adipose tissue. There were no visible teeth in the 300 arcade (lower left) within the mandible. There was a locally invasive 5.5 cm x 4 cm x 5.5 cm firm, tan to white, multilobular mass encompassing the left side of the oral cavity involving both the mandible and maxilla. The mass surrounding the mandible extended caudally from the rostral aspect of the body of the mandible to the ramus, slightly across the midline in the caudal intermandibular region, and dorsally surrounding the caudal most aspect of the maxilla including the remaining maxillary molar (presumably tooth 210). The mass completely surrounded the mandible; however, it was not attached to the mandible. The left mandible was markedly thinned with a complete, mid-body, transverse fracture present. On cut section, the mass was tan to white and semifirm with a tan, soft, gelatinous central region and occasional cavities that oozed a small amount of yellow to green, semi-viscous, opaque material.

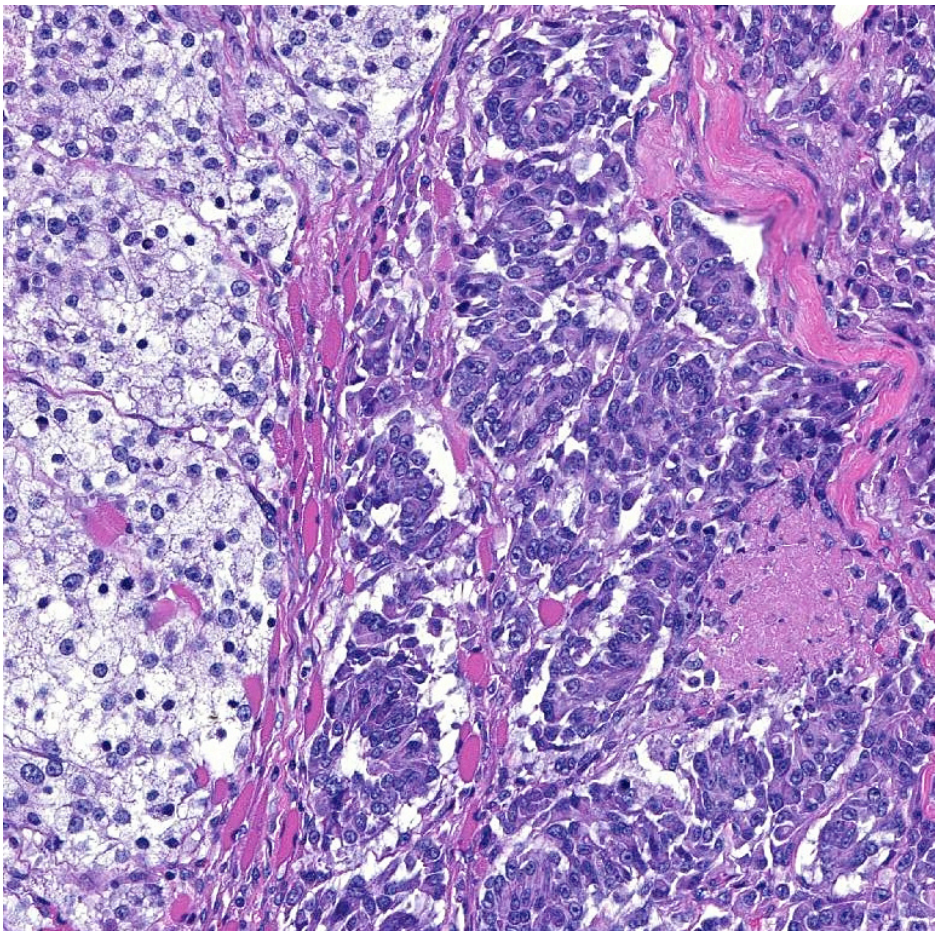
The lungs were diffusely mottled, tan to dark red and crepitant. There were multifocal, round, tan, slightly raised nodules ranging from pinpoint to 4 mm diameter throughout all lung lobes, but affecting less than 1% of the pulmonary parenchyma. One pedunculated tan nodule was present on the right caudal lobe. On cut section the pulmonary nodules were tan.

Laboratory Results: There were no ancillary tests performed.

Histopathologic Description: Compressing the adjacent salivary gland and submucosa as well as infiltrating and dissecting between the adjacent skeletal muscle and collagen bundles, and occasionally infiltrating the basal epithelial layer (junctional activity – variable by section), there is a densely cellular, multilobular, poorly demarcated, unencapsulated, infiltrative neoplasm composed of two populations of cells. One population of cells is arranged in cords, trabeculae, and packets and supported by a fine fibrovascular stroma. The neoplastic cells are polygonal to columnar with variably distinct cell borders, a moderate amount of eosinophilic cytoplasm, and one round to oval nucleus with finely



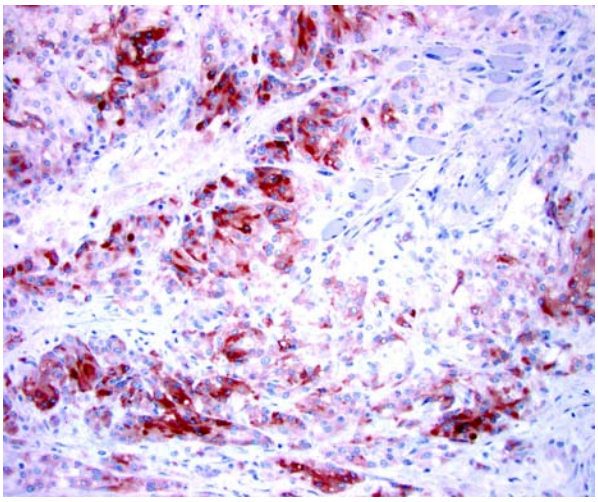
3-1. Head, dog: There was a locally invasive, 5.5 cm x 4 cm x 5.5 cm firm, tan to white, multilobular mass encompassing the left side of the oral cavity involving both the mandible and maxilla. Inset: Cut section of the mass. (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu)



3-2. Soft tissue, jaw: Melanocytes within the neoplasm have two distinct appearances with one population being polygonal with abundant clear cytoplasm (left) and a second being spindled with abundant granular basophilic cytoplasm. (HE 240X)

stippled chromatin and one to three prominent nucleoli. The second population of cells is arranged in tightly arranged packets and supported by a fine fibrovascular stroma. The neoplastic cells are polygonal with indistinct cell borders, a large amount of clear to lightly eosinophilic, vacuolated cytoplasm, and one round to oval nucleus with finely stippled chromatin and one to two variably prominent nucleoli. Overall the neoplastic cells exhibit a moderate degree of anisocytosis and anisokaryosis with a mitotic rate of approximately 35 in 10 high power fields (400x). Multifocally within the neoplasm, the stroma is brightly eosinophilic and hyalinized. Multifocally, there are variably sized regions of coagulative to lytic necrosis characterized by eosinophilic and basophilic karyorrhectic cellular debris and a pale eosinophilic background with faded cellular outlines and nuclear details. Along the periphery of the neoplasm and admixed within the regions of necrosis, there are moderate numbers of degenerate and non-degenerate neutrophils, nuclear streaming, a small amount of deeply basophilic granular material (mineral), and small numbers of mixed bacterial colonies. The bacterial colonies are characterized as basophilic cocci and eosinophilic short rods. Scattered throughout the neoplasm are moderate numbers of individual apoptotic/necrotic cells with pyknotic nuclei and karyorrhectic debris. Throughout the neoplasm, numerous small and medium caliber blood vessels are expanded by moderate numbers of erythrocytes (congestion). Multifocally throughout the neoplasm, there are small to moderate aggregates of extravasated erythrocytes (hemorrhage). Along the periphery of the neoplasm and extending into the surrounding collagen bundles, there are small aggregates of lymphocytes and plasma cells. Within the adjacent muscle, there is myofiber degeneration and necrosis characterized by myofiber loss, variation in myofiber size, and loss of cross-striations. Within these same regions along the periphery of the neoplasm, there is moderate collagenolysis characterized by a loss of organization, collagen bundle fragmentation, loss of eosinophilia and increased basophilia. There are multifocal areas of pigmentary incontinence. Multifocally there is a loss of the overlying oral epithelium (ulceration).

Immunohistochemistry for Melan-A, S100, chromogranin A, and synaptophysin were prepared at the University of Minnesota Veterinary Diagnostic Laboratory. Less than 2%



3-3. Soft tissue, jaw: Neoplastic cells are weak to moderately immunopositive for melanA. (Photo courtesy of: Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu)

of neoplastic cells within the periphery of each lobule had strong intracytoplasmic immunopositivity and less than 10% of the neoplastic cells throughout the mass had weak to moderate intracytoplasmic immunopositivity for Melan-A. Approximately 50% of the neoplastic cells had weak intracytoplasmic immunopositivity for Chromogranin A. The neoplastic cells were immunonegative for S100 and Synaptophysin.

Contributor's Morphologic Diagnosis: Oral cavity, amelanotic melanoma with neuroendocrine differentiation, focally extensive (with metastasis to lungs and left mandibular pathologic fracture).

Contributor's Comment: The two main types of melanocytic tumors are melanocytoma and malignant melanoma.[8] These tumors are derived from melanocytes that originate as melanoblasts from neural crest ectoderm.[8] Melanocytomas are the benign neoplasm of melanocytes and are most often heavily pigmented.[8] As the name suggests, malignant melanoma is the malignant tumor of melanocytic origin and is often used synonymously with melanoma. Melanocytic tumors can be found in multiple locations; however, the integument (cutaneous) and oral cavity are common sites. Cutaneous tumors in dogs are often benign. The cutaneous form of melanoma is commonly seen in gray horses and swine.[3]

In dogs, malignant melanomas are the most common oral tumor and have variable breed and gender predilection.[3,7-9] These tumors are often heavily pigmented and grossly appear black; however, in some cases, these tumors are variably pigmented or completely unpigmented (amelanotic), and appear grossly white.[3,7,8] The degree of pigmentation does not indicate biologic behavior or aid in prognosis.[3,8] Malignant melanomas often metastasize, with the regional lymph nodes being the most common site, but also commonly spread to distant sites (ex. lung) through the blood and lymphatics.[3,9] Histologically, the cell morphology of malignant melanomas can range from round/polyhedral/epithelioid cells to spindloid cells to mixed (a combination of the two)[3,8]; however, one defining histologic feature of the these tumors is often junctional activity[4], which can be seen as tumor infiltration crossing between the basal epithelium of the mucosa and the submucosa.

Most often, well-differentiated round/polygonal/epithelioid cell melanocytic tumors with abundant pigmentation are easily diagnosed; however, amelanotic and spindloid appearing tumors are quite often diagnostically challenging. Special stains [Fontana-Masson, 3,4-dihydroxyphenylalanine (DOPA)], immunohistochemistry, and/or electron microscopy can be used to aid in the diagnosis of amelanotic tumors.[1,3,5,7,8,10] Amelanotic melanomas are generally immunopositive for tryrosinase-related proteins 1 and 2 (TRP-1 and TRP-2), Melan-A, melanocytic antigen PNL2, HMB-45, microphthalmia transcription factor (MiTF), S100, tyrosine hydroxylase, tyrosinase, and vimentin[3,5,7-10]; however, according to Smedley et al. PNL2, Melan-A, TRP-1 and TRP-2 as a group provide the most diagnostic information for canine oral amelanotic melanomas.

In human medicine, melanocytic tumors are often further classified allowing for integration of morphology, location, and biologic behavior; however, this classification system is not currently applied in veterinary medicine.[8] According to Eyden et al., while not currently a subclassification of malignant melanoma, neuroendocrine differentiation is a rarely seen variant. The three currently reported human cases of this variant had predominantly epithelioid cell morphology, variable pigmentation, and immunopositivity either in the primary tumor or metastases for both melanocytic and neuroendocrine markers. Ultrastructurally, the

assessed cases (2 of 3) revealed neuroendocrine granules and no melanosomes.[6] Although electron microscopy was not performed on the current case, the cell morphology, architecture, and immunophenotype are consistent with an amelanotic melanoma with neuroendocrine differentiation; which has currently not been reported in dogs.

JPC Diagnosis: Fibrovascular tissue adjacent to salivary gland: Malignant amelanotic melanoma.

Conference Comment: Melanomas are often referred to as "the great imitator" because of their ability to display a variety of cytologic features thanks to their common embryologic roots with both neural and epithelial tissues. This case nicely illustrates the point, due to variation is cellular appearance across various regions of the neoplasm. In some sections, there is a portion of the neoplasm which exhibits junctional activity, a feature which lends additional credence to the morphologic diagnosis on HE section. The list of other neoplasms that share this characteristic with melanomas is short, usually limited to histiocytomas and epitheliotropic (T-cell) lymphomas.

Nuclear atypia and mitotic activity is directly correlated with prognostic behavior in melanomas at all locations. Immunohistochemistry has recently been evaluated as an additional prognostic tool. Ki67, a protein expressed only in growth and mitotic phases of the cell cycle, is considered a measure of tumor growth fraction and its expression has been correlated with outcome in canine melanomas comparable to nuclear atypia and mitotic index.[2] Additionally, immunodetection of RACK1, a protein expressed at high levels in melanocytes, may be a specific marker aiding in not only identifying melanomas but also offering progonostic value as its expression is also consistently correlated with nuclear atypia and mitotic index.[4]

Contributing Institution: Veterinary Diagnostic Laboratory
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CASE IV: N12-119 (JPC 4032704).

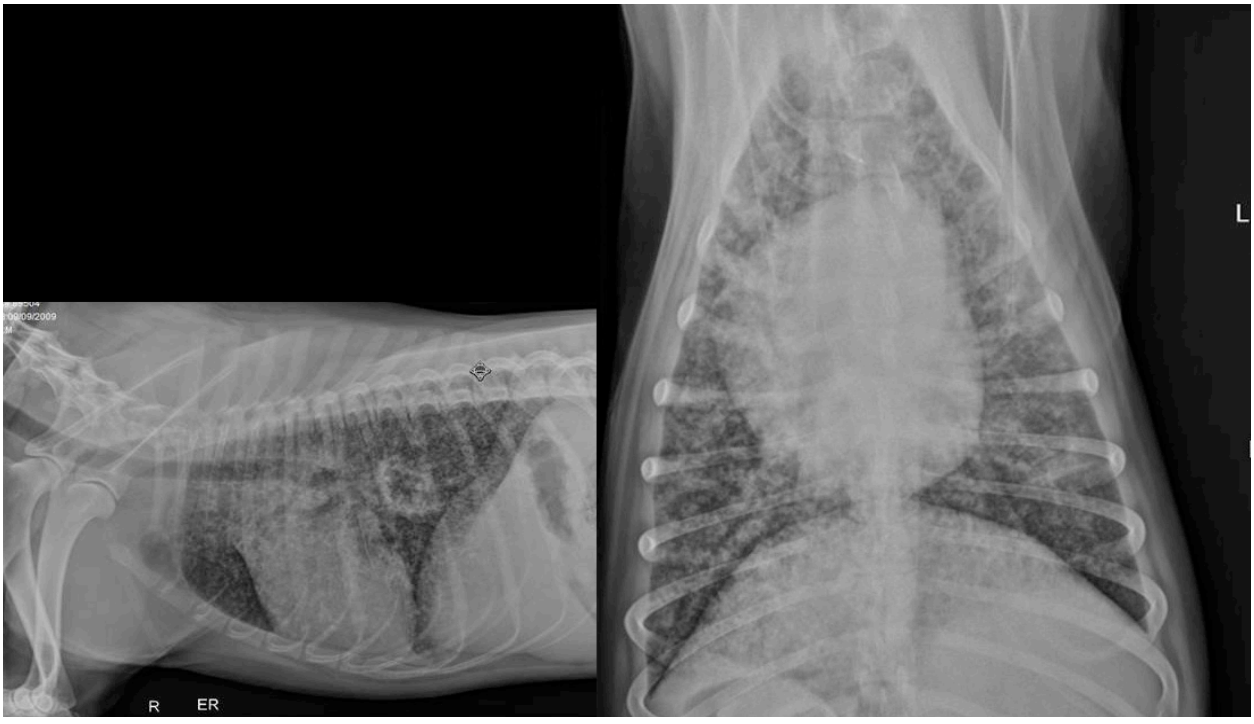
Signalment: 3-year-old male Australian shepherd (*Canis lupus familiaris*), canine.

History: A three-year-old male Australian Shepherd presented to the University of Florida Emergency Service for coughing, right hind-limb lameness, and a fever which did not respond to antibiotic therapy. He was adopted two months prior to presentation from a breeder in Illinois with no history of prior illness. He lives at home on a farm with several other adult dogs. They are primarily outdoor dogs and have free access to two acres. Two weeks prior to presentation, the patient became febrile and started coughing. He was placed on Baytril, chloramphenicol and prednisone, however his cough did not resolve. He was then taken to a specialist and prescribed fluconazole. Physical examination revealed harsh lung sounds bilaterally, right hind limb lameness with no neurological deficits, enlarged right popliteal lymph node, dry non-productive cough, and elevated temperature (temp 104.3). Thoracic radiographs were obtained and revealed diffuse miliary interstitial pulmonary pattern bilaterally. Owner elected for humane euthanasia.

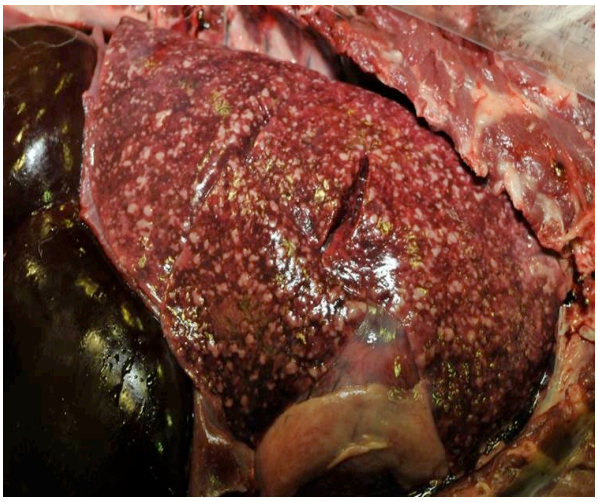
Gross Pathology: LUNGS: The lungs are diffusely mottled light tan to dark red and contain too–numerous-to-count light tan, irregular, firm, granulomatous nodules in all lung fields. The granulomas range in size from 0.2 x 0.1 x 0.1cm to 5.0 x 2.5 x 2.5cm (left caudal lung lobe). The granulomas are slightly raised above the parenchymal surface and extend throughout all cut surfaces. There are only 2-3 of these larger than 5 mm diameter. The remaining parenchyma is diffusely dark red, wet, and heavy. All sections float low in formalin.

Laboratory Results: None.

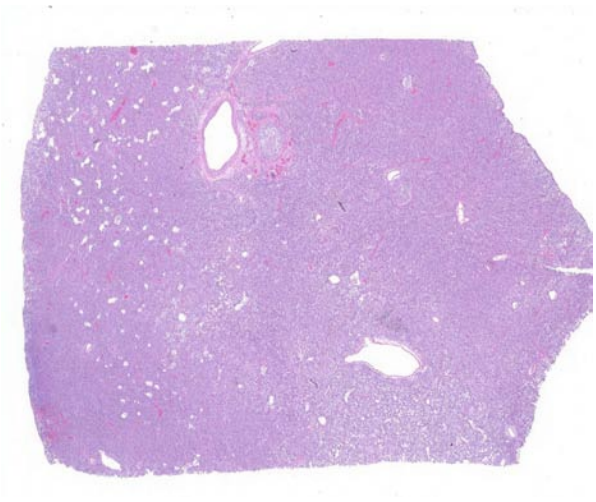
Histopathologic Description: Lung: The alveolar capillaries are diffusely congested and there is lightly eosinophilic, homogenous material within 95% of the alveolar air spaces (alveolar edema). Approximately 40% of the lung parenchyma is effaced by pyogranulomatous inflammation. The interstitium is disrupted by numerous large, multifocal to coalescing pyogranulomas that are composed of a central area of necrotic debris and degenerate neutrophils, surrounded by a layer of macrophages and occasional multinucleated giant cells admixed with and surrounded by abundant degenerate neutrophils. Within the granulomas are numerous



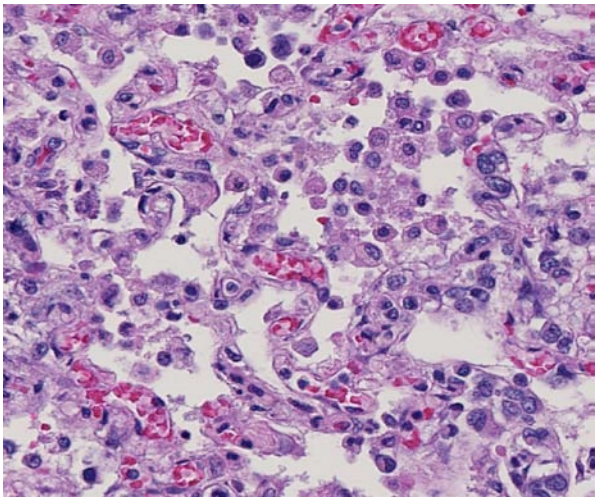
4-1. Thoracic radiographs, dog: Lateral and ventrodorsal radiographs reveal a diffuse military pattern throughout all lung fields. (Photo courtesy of: University of Florida College of Veterinary Medicine, Department of Infectious Disease and Pathology)



4-2. Lung, dog: Lungs are diffusely mottled light tan to dark red and contain innumerable less than 5mm granulomas in all lung fields. (Photo courtesy of: University of Florida College of Veterinary Medicine, Department of Infectious Disease and Pathology)



4-3. Lung, dog: There is diffuse consolidation of the section, to include airways of all sizes. (HE 6.3X)



4-4. Lung, dog: Alveolar septa and alveolar spaces are expanded by large numbers of foamy and rarely multinucleated macrophages, and is multifocal type II pneumocytes hyperplasia. (HE 400X)

intracellular and extracellular 8-15 µm diameter argyrophilic round yeast with a 2-3 µm thick clear to basophilic, refractile capsule and a central, basophilic, granular nucleus (GMS stain). Yeasts frequently exhibit broad-based budding. There is mild to moderate, multifocal perivascular edema surrounding large vessels.

Contributor's Morphologic Diagnoses: 1. Granulomatous interstitial pneumonia, chronic, diffuse, severe, with fungal organisms consistent with *Blastomyces dermatitidis*, lung.
2. Granulomatous lymphadenitis, multifocal, severe, with fungal organisms consistent with *Blastomyces dermatitidis,* cranial mediastinal, tracheobronchial, and right popliteal lymph nodes.

Contributor's Comment: *Blastomyces dermatitis* is a dimorphic, saprophytic fungus most commonly associated with the Mississippi and Ohio River valleys.[6] The primary route of infection is inhalation, with young male dogs of hunting and working breeds most commonly affected.[3] Clinical signs follow physiologic dysfunction of the affected tissue and infection is frequently associated with underlying immunosuppression.[3] Although *Blastomyces dermatitidis* does infect both dogs and humans and is therefore considered zoonotic, infection is thought to originate from inhalation of spores from the soil.[6] No cases of direct transmission from animals to humans reported have been reported; however, dogs are up to 10 times more likely to acquire infections and are considered a sentinel species.[4] Due to its aggressive pathologic nature and potential of infection by inhalation, culture of the organism is not recommended due to the risk of transmission to staff.[5]

Infection begins with the inhalation of mycelial fungal conidia from contaminated soil. Following inhalation, conidia undergo nonspecific phagocytosis and killing mediated by polymorphonuclear leukocytes (PMN), monocytes, and alveolar macrophages. The infection is most commonly self-limiting; however, in select cases the primary infection is not eliminated and conidia are converted to the yeast phase at body temperature.[3] The yeasts are encapsulated by a thick cell wall and are more resistant to phagocytosis and killing. Local

replication occurs via asexual budding and systemic dissemination via hematogenous and lymphatic routes is common.[2] Dissemination to multiple organ systems has been reported including cutaneous, ocular, CNS and bone.[2]

Although 85% of fungal pneumonia is reported to be attributed to blastomycosis, other differentials for fungal pneumonia should be considered and include: *Histoplasma capsulatum*, *Coccidioides immitis*, *Cryptococcus neoformans* and *Aspergillosis*. Differentiation and definitive diagnosis is best determined by the combination of geographic location, clinical presentation and histopathologic findings.[6]

JPC Diagnosis: Lung: Pneumonia, interstitial, granulomatous, diffuse, severe, with patchy type II pneumocyte hyperplasia.

Conference Comment: Despite examining multiple sections, repeating fungal stains and consulting with the JPC Department of Infectious Disease, we were unable to definitively demonstrate the double-contoured, broad-based budding yeast of *Blastomyces* in the submitted slides. The clinical history, gross findings and histologic lesions are all consistent with *Blastomycosis*; thus we don't dispute the contributor's diagnosis and rather attribute it to the variation that comes with cutting hundreds of slides.

Blastomycosis is one of the dimorphic fungi, whose transition from the mycelial form, known as conidia, to the yeast form is thermally dependent and takes place in tissue where temperature exceeds 37ºC. Conidia are readily phagocytosed by neutrophils and macrophages while yeast are more resistant, thus this conversion enables its pathogenicity. Also facilitating infection is the appropriately-named surface protein BAD-1 which mediates adhesion to host cells and modulates the inflammatory response by suppression of TNF-α production.[3]

While the lung is the most commonly affected site, *Blastomyces dermatitidis* can cause a disseminated infection where lesions are commonly found in lymph nodes, eyes, skin, subcutaneous tissues, bones and joints.[3] It is also one of the listed causative agents of granulomatous disease associated with hypercalcemia. The mechanism behind hypercalcemia has not been proven; however, it is suspected the population of macrophages produce 1,25 dihydroxycholecalciferol. Also known as calcitriol, it is the active form of vitamin D which increases calcium uptake from the gastrointestinal tract.[5]

Contributing Institution: University of Florida College of Veterinary Medicine
Department of Infectious Disease and Pathology

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