CASE I – C31715-04 4 (AFIP 2988635).

Signalment: 3-year-old, male, castrated, Cocker Spaniel, *Canis domesticus*, dog.

History: The dog was presented to the referring veterinarian with a 3 week history of coughing. The dog was placed on Amoxil and prednisone. Two days later the dog began vomiting. Thoracic radiographs were performed at this time and revealed what was interpreted as peri-hilar pulmonary edema and right atrial enlargement. The dog was then treated with furosemide. Prednisone was discontinued after 4 days of treatment due to the development of polyuria and polydipsia. The dog then began seizuring, became very dehydrated and was referred to the Atlantic Veterinary College 6 days after initial presentation. On arrival, the dog was lethargic, dehydrated and a freely moveable, mid-abdominal mass was palpated. The dog was rehydrated with IV lactated ringers. Exploratory laparotomy revealed a 5 cm long segment of proximal jejunum where the intestinal wall was firm, pale yellow-tan and approximately 8-18 mm thick. There was a poorly defined approximately 3 cm in greatest diameter, mottled tan and red, firm, thickened area in the adjacent mesentery. The nearby ileocecal lymph node was moderately enlarged measuring 6 cm in greatest diameter. These tissues were surgically excised and submitted for histopathology. The dog never completely recovered from anesthesia and remained depressed with no papillary light reflex, no menace response, and was unable to stand or walk. The owners elected euthanasia 2 days later and the dog was submitted for necropsy.

Gross Pathology: The dog was in fair body condition with small visceral and subcutaneous fat stores. The lungs were diffusely dark red, rubbery, failed to collapse, and contained many scattered, poorly-defined, pale, whitish, firm foci.
There were two, 5 mm in greatest diameter, slightly raised, discrete, pale, yellow nodules visible on the epicardial surface of the right atrium. The right ventricular free wall was slightly thickened and measured 5 mm thick (compared to the left which was 1 cm thick). The site of tissue resection and skin incisions were unremarkable. Otherwise, the carcass, including the nasal passages, was grossly unremarkable.

**Laboratory Results:** Fungal culture of lung tissue contained a heavy growth of yeast identified as *Cryptococcus neoformans*.

**Histopathologic Description:** The pulmonary parenchyma contains numerous, multifocal to coalescing, nodular, interstitial aggregates of large, epithelioid macrophages interspersed with fewer lymphocytes, plasma cells, rare multinucleated foreign body type giant cells and large numbers of uninucleate yeasts. The latter organisms have 2.5-8 µm, pale, eosinophilic to slightly blue-grey, round to oval, thin-walled yeast bodies surrounded by a thick clear capsule. Mucicarmine staining reveals a thick, intensely carminophilic capsule which often has a slightly spiny outer surface surrounding the cell body of these organisms.

The thickened segment of small intestine is characterized by similar moderate inflammatory infiltrates consisting largely of epithelioid macrophages and myriads of the previously described yeasts. These foci extend in to the surrounding mesentery and largely effaced the enlarged ileocecal lymph node. The leptomeninges of the cerebrum, cerebellum and brain stem are multifocally, mildly to moderately, thickened due to similar granulomatous infiltrates accompanied by numerous yeasts. Within the cerebral cortex and the hippocampus there are patchy, poorly-defined areas where the parenchyma is mildly hypercellular, mildly vacuolated and sometimes congested. Blood vessels within these areas are often lined by plump, reactive endothelial cells. Also in these areas, neuronal cell bodies are frequently hypereosinophilic, angular, shrunken and often have pyknotic nuclei.

Multifocal, variably-sized nodular discrete granulomatous foci accompanied with numerous yeasts are also present in sections of the pancreas, spleen, cortex of the right kidney and the wall of the right atrium. In the liver, similar infiltrates often moderately to markedly expand most portal areas and are frequently scattered randomly throughout the lobules. Smaller granulomatous foci containing yeasts are also scattered within the myocardium of the left and right ventricular free walls and the thyroid glands.

**Contributor’s Morphologic Diagnoses:** 1. Granulomatous interstitial pneumonia, multifocal, severe, with myriads of intralesional yeast
2. Granulomatous transmural enteritis and lymphadenitis, locally extensive, severe, with many intralesional yeast
3. Granulomatous meningitis, multifocal, moderate, with intralesional yeasts
4. Neuronal necrosis, multifocal, moderate, acute, cerebral cortex and hippocampus
5. Granulomatous splenitis, hepatitis, thyroiditis, pancreatitis, myocarditis and nephritis, multifocal, mild to moderate, with numerous intralesional yeasts

**Contributor’s Comment:** The postmortem findings in this case are consistent with widely disseminated cryptococcosis. *Cryptococcus neoformans* occurs worldwide in temperate, as well as tropical climates.¹ This opportunistic pathogen infects a wide variety of wild and domestic animals (most commonly cats and dogs), as well as humans. The organism is saprophytic in soil and is environmentally often associated with avian habitats or areas heavily contaminated with pigeon droppings.² Infection occurs most commonly via inhalation of yeasts from the environment and is not considered contagious. Nasal and/or pulmonary infection may become disseminated via direct extension and/or hematogenous routes.³ Recovery or localization of infection is dependent on a good cell mediated immune response. Most cases of disseminated human cryptococcosis are associated with concurrent immunosuppressive disease processes (e.g. AIDS) or treatments (e.g. chemotherapy or prolonged use of glucocorticoids).⁴ However, immunosuppression has not been documented in most affected cats and dogs. There was no evidence of an underlying immunosuppressive disease process nor was there a history of prolonged steroid use in this case.

The cause of coughing (the initial presenting complaint) in this dog was severe, fungal pneumonia. The right atrial enlargement noted by the referring veterinarian and mild right ventricular hypertrophy found at necropsy were likely secondary to pulmonary hypertension due to severe pneumonia. The initial seizure activity described was due to fungal meningitis (*C. neoformans* is often referred to as neurotropic). The lack of full recovery from anesthesia and postsurgical neurological clinical signs which necessitated euthanasia were due to more acute areas of neuronal necrosis in the cerebral cortex and hippocampus. This finding may have been due to hypoxia secondary to prolonged seizure activity or possibly was associated with poor oxygen exchange through the abnormal lung during surgery.

**AFIP Diagnosis:** Lung: Pneumonia, granulomatous, multifocal to coalescing, moderate, with edema and myriad intralesional yeasts, Cocker Spaniel (*Canis familiaris*), canine.

**Conference Comment:** *Cryptococcus neoformans* is a saprophytic fungus that causes disease in a wide variety of animals, but most frequently in cats, dogs,
horses and humans. As pointed out by the contributor, immunosuppression has not been documented in most affected cats and dogs. Lesions can occur in any organ, but are most common in the nasal cavity and central nervous system, followed by the integumentary system and eyes. *Cryptococcus neoformans* is a cause of mastitis in cattle. Cryptococcosis is the most frequent systemic mycosis in cats, and often begins in the nasal cavity. Dissemination to brain, eyes, skin, subcutis, and lymph nodes is common. Pulmonary involvement is rare in cats.\(^2,3,5,6\)

*C. neoformans* is the species that most commonly causes disease and is environmentally associated with avian habitats, especially pigeon droppings. *C. gattii* is also pathogenic, typically occurs in tropical and subtropical climates and is generally associated with bark and leaf litter of certain eucalyptus trees. Following an outbreak of cryptococcosis on Vancouver Island, British Columbia that affected humans, dogs, Dall’s porpoises and other mammals, it was found that *C. gattii* was the cause and was present on the bark of non-eucalypts in the area including alder, bitter cherry, cedar, Douglas fir and Garry oak. Changing climatic conditions, possibly caused by global warming, may have been involved in the spread of *C. gattii* to new ecological niches.\(^6,7\)

Gross lesions of *Cryptococcus neoformans* are often gelatinous due to the yeast’s mucopolysaccharide capsule. The capsule hinders phagocytosis and is a major diagnostic feature of the organism. However, unencapsulated mutants do exist. Histologically, the yeasts are round, 5-20 µm in diameter, reproduce by narrow-based budding and are usually surrounded by a 2-8 µm mucopolysaccharide capsule that stains with mucicarmine, PAS, and Alcian blue. Masses of organisms surrounded by clear capsules have a “soap bubble” appearance. The immune response varies from sparse to a prominent granulomatous depending on the presence of a capsule and the host’s immune status.\(^2,3,5,6,7\)

Other fungi that cause granulomatous pneumonia include *Blastomyces dermatitidis, Coccidioides immitis* and *Histoplasma capsulatum*. *Blastomyces, Coccidioides, and Histoplasma* are unencapsulated, unlike *Cryptococcus*. *Blastomyces* reproduces by broad-based budding, while *Cryptococcus* and *Histoplasma* reproduce by narrow-based budding. *Coccidioides* reproduces by endosporulation. Mature sporangia of *Coccidioides* are 10-80um in diameter with a double-contoured highly refractile wall and are filled with 2-5um diameter endospores. *Histoplasma* is much smaller (5-6um diameter) than *Cryptococcus* and is located intracellularly within macrophages.\(^5\)

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CASE II – P04-4470 (AFIP 2956312).

Signalment: 12-year-old, male neutered, Cocker Spaniel, *Canis familiaris*, pet dog.

History: 12-year-old neutered male Cocker Spaniel pet dog weighing 27.4 kg, with signs of diabetes mellitus; hyperglycemia (16.8 mmol/l) and polyuria/polydipsia. There was a firm mass in the caudal part of the pancreas. The dog was also affected by an axillary and inguinal alopecic hyperkeratotic dermatosis. The partially excised pancreas containing the nodular mass was sent in for histological evaluation. No skin biopsies were available.

Gross Pathology: Tissue section approximately 5 x 1 cm, pale, firm, multinodular

Histopathologic Description: Pancreas: The pancreatic parenchyma is partly effaced by a multinodular non-encapsulated partially circumscribed focally infiltrating hypercellular mass consisting of moderately pleomorphic neoplastic cells
arranged in small multi-layered trabeculae and islands separated and supported by well vascularized delicate fibrous septa.

The polygonal tumor cells have round to oval vesicular nuclei, which vary moderately in size with finely stippled chromatin containing a single central conspicuous medium-sized nucleolus and regularly displaying mitotic figures (ranging from 2-4 per HPF). Tumor cells have abundant pale basophilic, finely granular cytoplasm with mainly indistinct cell borders.

Extensive tumorous vascular invasion can be observed within lymphatics and blood vessels in the pre-existing pancreatic hilar stroma and in the extra-pancreatic mesenteric adipose tissues.

Some portions of the tumor display a moderate amount of fibrovascular stroma with extensive central tumorous necrosis and hemorrhage with the presence of iron pigment and/or ceroid bearing macrophages.

The interlobular pancreatic septa are slightly edematous containing congested blood vessels with moderate neutrophilic leucocytosis.

In the intact pancreatic parenchyma, there is diffuse prominent cytoplasmic medio-to macro-vacuolation of the exocrine acinar cells and intercalated ductular cells, and also to a lesser extent affecting the endocrine islet cells. Multifocally there are poorly delineated areas in which the aforementioned cytoplasmic vacuolation is more pronounced with a tendency of loss of acinar detail.

**Immunohistochemistry:** Tumor cells stain strongly positive for glucagon and show no positivity for insulin, ACTH, gastrin or somatostatin. Pancreatic islets of Langerhans stain slightly positive for insulin.

**Contributor’s Morphologic Diagnoses:**

**Contributor’s Comment:** Several types of pancreatic endocrine tumors in animals and man are recognized. In dogs they include insulinoma (β-cells), glucagonoma (α-cells) and gastrinoma (gastrin-producing non-β-cell tumor). Some islet cell tumors show mixed immunohistochemical reactivity, including somatostatin (δ-cells). Additionally, in humans, VIPomas (which produce vasoactive intestinal peptide) are
seen. In this case, the tumor cells only showed immunohistochemical positivity for glucagon. The preexisting pancreatic islets showed only faint positivity for insulin (periphery of islets) when compared to the strongly staining positive control of a normal dog. The preexisting pancreatic islets did not stain for glucagon.

This dog was also affected by an axillary and inguinal alopecic hyperkeratotic dermatosis as can be seen in conjunction with liver disease and with pancreatic disease; this is known as hepatocutaneous syndrome or superficial necrolytic dermatitis (SND). SND in canids and in humans (necrolytic migratory erythema (NME)) is a paraneoplastic syndrome associated with functional pancreatic endocrine tumors that secrete glucagon (glucagonoma) resulting in hyperglycemia. The pathogenesis of SND is still obscure. Persistent gluconeogenesis, due to hyperglucagonemia, is associated with hypoaminoacidemia and seems to be a likely pathogenic factor. In the cat, paraneoplastic flank alopecia is associated with pancreatic neoplasms.


Conference Comment: Almost all islet cell tumors in dogs are malignant and generally microscopic metastasis has occurred by the time of surgical diagnosis. Carcinomas of the pancreatic islets commonly occur in the duodenal (right) lobe of the pancreas. Metastasis most commonly occurs to the liver, mesentery, omentum, and regional lymph nodes. In contrast, islet cell tumors in humans and ferrets are usually benign. Islet cell carcinomas tend to be larger than adenomas, invade into and through the fibrous capsule of the pancreas, and are multilobular. Mitotic figures are usually infrequent.12,13

Glucagonomas in humans cause the glucagonoma syndrome, which is characterized by necrolytic migratory erythema, glossitis, stomatitis, anemia, weight loss, mild diabetes mellitus, hypoaminoacidemia, deep vein thrombosis and depression. In dogs, glucagonomas have been associated with superficial necrolytic dermatitis, lethargy, anorexia, hyperglycemia and hypoaminoacidemia. The large majority of cases of canine superficial necrolytic dermatitis are associated with severe vacuolar hepatopathy rather than glucagonoma.13,14,15

Other neoplasms of islet cell origin which can be differentiated on the basis of clinical signs and/or immunohistochemical stains include insulinoma and gastrinoma. Typical clinical findings associated with insulinoma include marked hypoglycemia, recurrent disorientation or seizures associated with exercise, stress or fasting. Animals recover with administration of glucose. Gastrinomas secrete...
excess gastrin producing Zollinger-Ellison syndrome, which is characterized by gastric hypersecretion resulting in gastric hyperacidity and gastric and duodenal ulceration.\textsuperscript{12, 15}

The cytoplasmic vacuolation of multiple pancreatic cell types in this case was probably caused by hyperglycemia. This finding suggested that the islet cell carcinoma might be a glucagonoma.

Readers are encouraged to review WSC 20, Case 1, 2006-2007 for a summary of superficial necrolytic dermatitis.

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**References:**
CASE III – N05-238 (AFIP 2984014).

Signalment: 11-year-old, female spayed, domestic short-haired feline (*Felis domesticus*).

History: The patient was an indoor cat which resided in a household of four cats. All four cats began sneezing acutely. By the following evening, the patient was coughing and began open mouth breathing with her neck extended. On the third day, the patient was inappetant, lethargic, and was drooling excessively in addition to the open mouth breathing. The patient was taken to the veterinarian where she was sedated with telazol for radiographs. After sedation, the patient went into extreme respiratory distress and was taken to NCSU-CVM Veterinary Teaching Hospital for emergency treatment. The patient was placed in an oxygen cage for observation and to decrease stress and was noted to be unable to expand her chest (working clinical diagnosis of diaphragm paralysis secondary to the zolazepam). Intubation and hand ventilation was initiated. The zolazepam portion of the telazol was reversed with flumenzanil and the patient regained the ability to move her chest; however, the severe dyspnea was still present. The patient was current on rabies vaccination only. FeLV and FIV status are not known.

Gross Pathology: Within the left cranial and caudal lung lobes and the right cranial and middle lung lobes, there were multifocal, poorly demarcated, dark pink to purple, slightly collapsed, semi-firm to rubbery regions, interpreted as pneumonia. There was a moderate amount of yellow mucoid material within the trachea and large airways. Cytology of the tracheal material revealed moderate numbers of neutrophils and macrophages within a proteinaceous and mucinous background. Involving approximately 50-60% of the mucosal surface of the esophagus, there were numerous multifocal to coalescing, tan, 2-4 mm diameter, ulcers.
**Histopathologic Description:** There is diffuse acute interstitial pneumonia characterized by alveolar septal necrosis and fragmentation, and mild expansion of the septa with fibrin, edema, and small numbers of macrophages and neutrophils. There is intra-alveolar accumulation of edema, fibrin with scattered hyaline membrane formation, alveolar macrophages, small to moderate numbers of neutrophils, and small amounts of hemorrhage. Throughout all sections of lung examined, there are multifocal to coalescing regions of marked necrosis of the bronchi and bronchioles with often complete loss of the epithelium and infiltration of the surrounding alveoli and septa with moderate numbers of macrophages and neutrophils. The bronchial and bronchiolar necrosis extends into the surrounding tissue with necrosis of adjacent bronchial glands and pulmonary parenchyma. Within remaining bronchiolar epithelial cells and bronchial glandular epithelial cells, there are scattered eosinophilic intranuclear inclusions surrounded by a clear nuclear halo and margined chromatin (herpesviral inclusions).

**ESOPHAGUS:** Multifocally, there is ulceration of the esophageal mucosa with necrosis of the underlying submucosa and infiltration by moderate numbers of neutrophils. Within the epithelium adjacent to the regions of ulceration, there are scattered eosinophilic intranuclear inclusions surrounded by a clear nuclear halo and margined chromatin (herpesviral inclusions).

**Contributor’s Morphologic Diagnoses:**
1. **LUNGS,** Multiple lobes:
   a. Multifocal to coalescing, severe, acute necrotizing and suppurative bronchiolitis and bronchopneumonia with intraepithelial herpetic intranuclear inclusions.
   b. Diffuse, moderate to severe, acute interstitial pneumonia.
2. **ESOPHAGUS:** Multifocal to coalescing, marked, subacute, ulcerative and neutrophilic esophagitis with intraepithelial herpetic intranuclear inclusions.

**Contributor’s Comment:** The primary lesion in this patient is a herpesvirus induced severe necrotizing pneumonia, bronchitis and bronchiolitis with intraepithelial herpetic intranuclear inclusions consistent with a fulminant fatal herpesviral infection. Similar inclusions are identified within the esophageal mucosa adjacent to regions of ulceration.

Feline viral rhinotracheitis caused by feline herpesvirus-1 (FHV-1) is primarily an infection of the upper respiratory system in cats. Infected cats exhibit sneezing, coughing, oral respiration, and salivation, similar to clinical signs identified in the presented case. Infected cats usually recover in 7-14 days, although there can be high mortality in kittens, debilitated, or immunosuppressed cats. Lesions are typically limited to the upper respiratory tract including nasal cavity, pharynx, soft palate, tonsils, and conjunctiva. Rare infections proceed to a fulminant fatal...
pneumonia characterized by a severe necrotizing bronchitis and bronchiolitis with an interstitial pneumonia.¹

The ulcerative esophagitis in this patient is thought to be related to the herpesviral infection due to the presence of intranuclear inclusions with a similar histomorphology. Ulcerative esophagitis in cats is most commonly associated with calicivirus infection. Esophageal ulcers are not specifically mentioned in association with feline herpesviral infections, although oral ulcers and pharyngitis have been reported.

Although FeLV and FIV status in this patient are not known, we suspect some underlying cause of immunosuppression.

We did consider a possible severe fatal feline caliciviral infection in this case. Feline caliciviral pneumonia is generally less severe than herpesviral pneumonia and often requires a secondary bacterial infection to become significant. Also, the presence of the typical herpesviral intranuclear inclusion bodies within both the lungs and esophagus make a diagnosis of herpesviral pneumonia and esophagitis most likely in this case.² Virulent systemic feline calicivirus infection typically includes cutaneous ulceration of the pinnae, footpads, nares, and skin, along with subcutaneous edema, alopecia, and other systemic lesions such as bronchopneumonia and hepatic necrosis.³ Cutaneous ulcerations and marked subcutaneous edema were not identified in this patient.

AFIP Diagnoses: 1. Lung: Pneumonia, bronchointerstitial, necrotizing, diffuse, severe, with fibrin, edema, syncytia, and epithelial intranuclear inclusion bodies, etiology consistent with feline herpesvirus, domestic shorthair (Felis domesticus), feline.
2. Esophagus: Esophagitis, necro-ulcerative, multifocal, marked, with fibrin, edema, and intraepithelial inclusion bodies.

Conference Comment: Feline herpesvirus 1 (FHV-1) is a double-stranded DNA alpha-herpesvirus that causes feline viral rhinotracheitis. All species of felidae are believed to be susceptible. Infection with FHV-1 is naturally acquired through oral, nasal, or conjunctival routes by either direct contact or from aerosolized oronasal secretions of virus-shedding infected cats. After an incubation of 24-48 hours, the onset of typical clinical signs of serous to mucopurulent nasal and conjunctival discharge occurs, accompanied by fever, sneezing, coughing, oral respiration, profuse salivation and corneal ulcers. While oral ulceration may also be present in FHV-1, this lesion is more typical of feline calicivirus infections. Skin ulcers and
dermatitis syndrome in domestic cats and cheetahs, and nervous signs have been
described, but are likely rare sequels to infection.\textsuperscript{1,4,6}

Viral replication occurs primarily in the epithelium of the nasal cavity, oropharynx,
conjunctiva, tonsils, and, to a lesser extent, the trachea. Shedding of viral particles
may begin as early as 24 hours post infection and may last as long as one to three
weeks, though most active viral replication and cell necrosis occur between two to
seven days post infection. During this time, herpessviral intranuclear inclusions are
most often present in infected epithelial cells and occasionally within endothelial
cells. Inclusions are rarely detected beyond seven days after infection and cannot
be relied on for diagnosis. Because viral replication is normally restricted to areas
of lower body temperature, such as the upper respiratory passages, viremia is rare,
and resolution of disease normally takes about two to three weeks. FHV-1 remains
latent in carriers in the trigeminal ganglia.\textsuperscript{1,4,5}

Uncommonly, generalized disease may follow initial upper respiratory tract infection
in debilitated or immunocompromised animals and in neonatal kittens. In these
cases, viremia may be present. Mortality due to FHV-1 is rare in domestic cats;
however, when fulminating cases of viral infection occur, there is often widespread
necrotizing bronchitis, bronchiolitis, and interstitial pneumonia with edema. Viral
infection may predispose to fatal secondary bacterial bronchopneumonia with
bacteria such as \textit{Pasteurella multocida}, \textit{Bordetella bronchiseptica}, \textit{Streptococcus}
sp., and \textit{Mycoplasma felis}.\textsuperscript{1,4,5}

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CASE IV – C30551-06 (AFIP 3034589).

Signalment: 1.5-year-old, male/neutered, Doberman Pinscher, canine.

History: The patient presented with a 3 month history of lethargy, shifting leg lameness, and waxing and waning fever. The lameness was getting progressively worse, and the dog was very depressed and anorexic at the time of referral. Vaccinations were current. The dog was given a monthly heartworm preventative and was routinely treated for the control of fleas and ticks.

Gross Pathology: The dog was humanely killed. The dog had significant loss of muscle mass and was emaciated, evidenced by minimal body fat stores. Peripheral lymph nodes including mandibular, prescapular, axillary, cranial mediastinal, and popliteal were moderately enlarged and soft. The liver was massively enlarged, extending approximately 6 cm caudal to the costal arch, and had a pale reticulated lobular pattern and friable consistency. The spleen was similarly massively enlarged and had a meaty consistency exuding very little blood on the cut surface. Numerous small thin-walled tortuous blood vessels extended from the portal vein to the left renal vein and caudal vena cava, consistent with acquired portosystemic shunts. Both kidneys had a slightly pitted capsular surface. Numerous approximately 1 mm diameter red foci were scattered throughout the cortex. The ribs folded rather than breaking. The femoral bone marrow had replacement of marrow by firm white tissue that sank in formalin. This change was prevalent throughout the diaphysis of the bone.

Laboratory Results: The CBC revealed a hyperproteinemia (8.3), a leukopenia (5.3) characterized by a neutropenia (1007) and monocytopenia (106), and a profound non-regenerative anemia (RBC 1.77, Hgb 3.8, HCT 11.4) with an increased number of nucleated RBC (128). Abnormalities in the serum chemistry included a low carbon dioxide (18.7), mild hypercalcemia (11.3), hypoalbuminemia (1.7), hyperglobulinemia (4.8), low ALT (8), and high cholesterol (436). Evaluation of the blood smear revealed variably sized blast cells that had round, centrally located nuclei, indistinct nucleoli, and deeply basophilic cytoplasm. Several of the blast cells had cytoplasmic projections or blebs. The morphologic features of these blast cells were suggestive of a megakaryocytic lineage. The platelet count was within the reference interval; however, several giant, atypical platelets (macrothrombocytes) were observed. Bone marrow aspiration was performed but was not diagnostic; only peripheral blood contamination was obtained.
**Histopathologic Description:** The bone marrow is diffusely filled with a population of neoplastic cells interspersed with abundant fibrous tissue and intermittent bony trabeculae with evidence of marked osteolysis. The neoplastic cells are pleomorphic polygonal cells with prominent anisocytosis and anisokaryosis and variable chromatin patterns and amounts of cytoplasm. Larger cells have large, lobulated and sometimes ring-shaped nuclei or multiple small nuclei, often with dense ropey chromatin, and abundant eosinophilic cytoplasm with distinct cytoplasmic margins. Smaller blast-type cells also occur and blend with a background of fibroblasts and intervening fibrillary collagen. Bony trabeculae are often scalloped and margined by osteoclasts in Howship's lacunae. Similar large multi-nucleated cells resembling megakaryocytes, as well as smaller blast type cells, fill some capsular and medullary sinuses and often efface the architecture of lymph nodes. The spleen is similarly filled with neoplastic megakaryocytes and immature precursors intermingled with small residual populations of lymphocytes. Virtually no distinction of red and white pulp can be seen. The sinusoids of the liver are diffusely filled with neoplastic megakaryocytes. Atrophy of hepatic cords is often prominent in centrilobular regions. Alveolar septal capillaries are prominently thickened by intraluminal neoplastic megakaryocytes. Small numbers of similar cells occur in pulmonary vessels and some pulmonary arteries have prominent medial hypertrophy and tortuosity. Glomeruli throughout the kidney have global thickening of glomerular capillary loops and moderate hypercellularity. This correlates with frequent intraluminal hyaline casts in tubules.

**Contributor’s Morphologic Diagnosis:** Megakaryocytic leukemia with myelofibrosis and metastatic sites in spleen, liver and lymph nodes

**Contributor’s Comment:** Acute myeloid leukemias (AML) are neoplastic myeloproliferative disorders (MPD) that arise from hematopoietic precursors, including granulocytic, monocytic, erythrocytic, and megakaryocytic cell lines.\(^1\) Megakaryocytic leukemia is a rare subtype of AML, both in humans and animals, and is designated as AML-M7. While chronic leukemias are characterized by infiltration of the bone marrow with more mature neoplastic hematopoietic cells, AMLs, in contrast, are characterized by large numbers (\(\geq 20\%\)) of blast cells in the bone marrow.\(^2\) Megakaryoblasts are pleomorphic, and can potentially resemble lymphoblasts or myeloblasts. Thus, a diagnosis of AML-M7 based on morphology alone can only be accomplished if the blasts demonstrate some degree of differentiation. Cytoplasmic blebs or platelet shedding help identify megakaryoblasts. When the megakaryoblasts are poorly differentiated, it is difficult to distinguish AML-M7 from acute myeloid leukemia, acute lymphoid leukemias, and pure erythroid leukemia simply from evaluation of blood smears or bone marrow aspirates.\(^2\) Thus, cytochemical, ultrastructural, and immunophenotypic features may be required for a definitive diagnosis. Immunophenotyping is a very
useful diagnostic tool for determination of cell origin in poorly differentiated leukemias. In dogs with AML-M7, the blasts will be positive for CD41 (GPIIb/IIIa), CD61 (GPIIIa), and factor VIII-related antigen.\textsuperscript{2}

Myelofibrosis develops in some cases of AML-M7 in dogs, similar to human patients with megakaryocytic leukemia.\textsuperscript{1} A critical role for megakaryocytes in the pathogenesis of myelofibrosis has been recognized in human patients with idiopathic myelofibrosis (IMF). IMF is a clonal hematopoietic disorder characterized by atypical megakaryocytes, severe myelofibrosis, and splenic extramedullary hematopoiesis.\textsuperscript{1} Megakaryocytic overproduction of fibrogenic cytokines, particularly platelet derived growth factor (PDGF) and transforming growth factor-\(\beta\) (TGF-\(\beta\)), are thought to be responsible for the myelofibrosis observed in IMF and other disorders of the megakaryocytic lineage, including AML-M7 and MDS with prominent dysmegakaryopoiesis.\textsuperscript{3} It has been demonstrated that the PDGF contained within the platelet \(\alpha\) granules are capable of inducing fibroblast proliferation.\textsuperscript{3} The release of PDGF from megakaryocytes, however, is not thought to be completely responsible for the observed myelofibrotic stroma observed in megakaryocytic disorders. PDGF does not have angiogenic properties or the capability of inducing gene transcription of laminin, fibronectin, or the collagens. Thus, other growth factors must be involved; TGF-\(\beta\) is probably the most important additional growth factor involved.\textsuperscript{3} TGF-\(\beta\), also stored in platelet \(\alpha\) granules, regulates the synthesis of the extracellular matrix in myelofibrotic disorders.\textsuperscript{3} It accomplishes this by increasing the expression of genes for fibronectin, collagens type I, III, and IV production, in addition to chondroitin/dermatan sulphate proteoglycans. TGF-\(\beta\) is also pro-angiogenic and decreases the production of collagenase-like enzymes that are responsible for degrading extracellular matrices. Ultimately, these properties of TGF-\(\beta\) result in an increase in extracellular matrix.

**AFIP Diagnoses:** 1. Bone marrow: Acute megakaryoblastic leukemia (AML M7) with myelofibrosis, Doberman Pinscher (*Canis familiaris*), canine. 2. Lymph node and liver: Acute megakaryoblastic leukemia, metastatic.

**Conference Comment:** The contributor provides a thorough overview of megakaryocytic leukemia, a rare subtype of acute myeloid leukemia. This condition is designated acute megakaryoblastic leukemia (AML M7) in the WHO classification. CD61 immunohistochemical staining performed at the AFIP revealed multifocal cytoplasmic immunoreactivity of the neoplastic megakaryocytes supporting the diagnosis of megakaryoblastic leukemia.\textsuperscript{5}

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