



WEDNESDAY SLIDE CONFERENCE 2011-2012

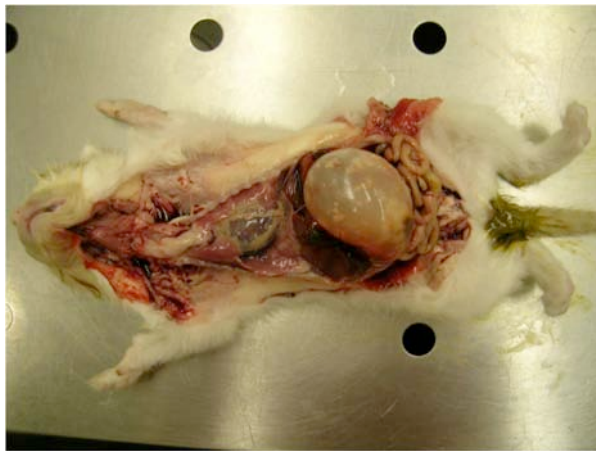
Conference 1

07 September 2011

CASE I: 60226 (JPC 3103342).

Signalment: 3-week old domestic shorthair cat (*Felis domesticus*).

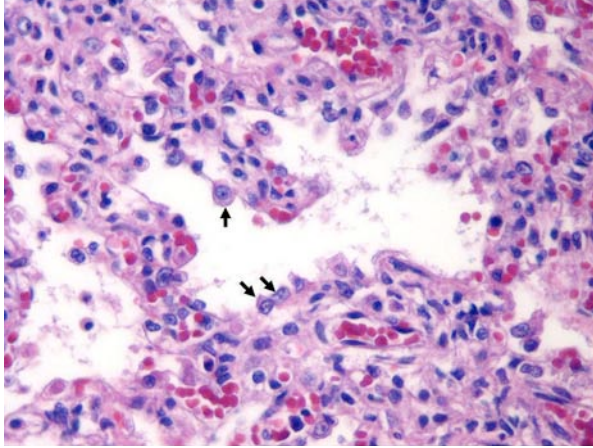
History: This kitten had upper respiratory signs for several days, then became dyspneic and died en route to the emergency clinic.



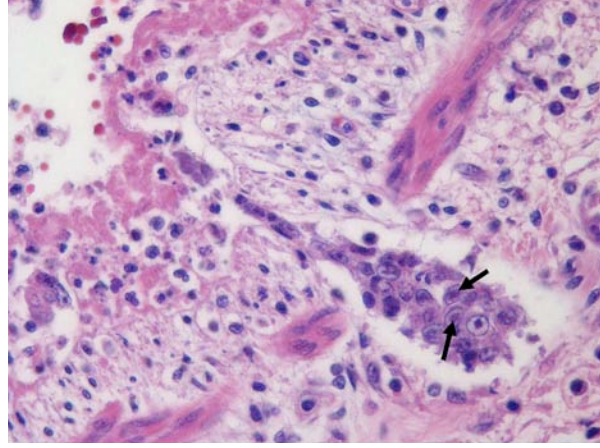
1-1. Lungs, esophagus, stomach, kitten. The lungs fail to collapse and are mottled tan and red, and the stomach is distended with air (aerophagia). Photograph courtesy of Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, www.hopkinsmedicine.org/mcp.

Gross Pathology: The lungs failed to collapse when the thoracic cavity was opened, and were mottled tan and pink, with a firm, rubbery texture. The esophagus and stomach were distended with air.

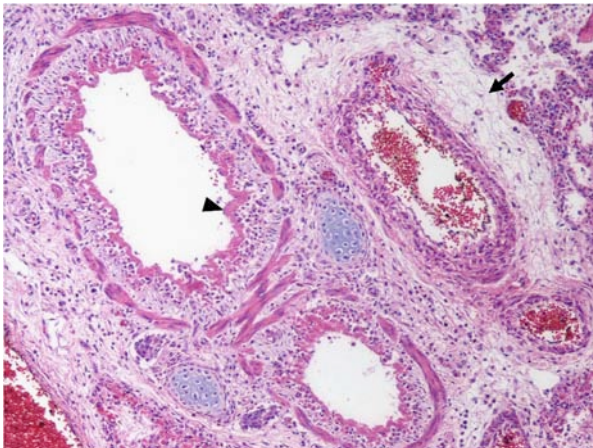
Histopathologic Description: Lung: Over 80% of the lung parenchyma is affected by severe inflammation, necrosis and perivascular and intra-alveolar edema. There are multifocal to coalescing areas of alveolar wall necrosis and replacement by dense infiltrates of macrophages and lymphocytes, mixed with abundant fibrin and cellular debris. At the periphery of the necrotic areas, alveolar septae are expanded by lymphocytes and macrophages. Pneumocytes are variably denuded or hypertrophic, protruding into the alveolar space (type II pneumocyte hyperplasia). Occasionally, there is a layer of fibrin adherent to the alveolar septa. Bronchioles and bronchi are lined by a thick layer of fibrin mixed with sloughed epithelial cells. Bronchial glands are hyperplastic. Multifocally, pneumocyte and glandular epithelial cell nuclei contain round, eosinophilic, 4-7 μ m inclusion bodies that displace chromatin to the periphery. Airways and alveolar spaces contain fibrillar eosinophilic material (fibrin), sloughed pneumocytes, red blood cells and/or large foamy macrophages. Blood vessels are congested and perivascular spaces are expanded (edema). At the periphery of the lobe, alveolar spaces are dilated with occasional alveolar wall rupture and clubbing of septae (emphysematous change). Mesothelial cells along the



1-2. Lung, kitten. Alveolar septa are expanded by lymphocytes and macrophages. There is patchy type II pneumocyte hyperplasia (arrows). Photograph courtesy of Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, www.hopkinsmedicine.org/mcp. (HE 400X)



1-3. Lung, kitten. Bronchial glandular epithelial cells often contain 4-7 um eosinophilic intranuclear inclusion bodies that peripheralize chromatin (arrows). Photograph courtesy of Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, www.hopkinsmedicine.org/mcp. (HE 400X)



1-4. Lung, kitten. Perivascular spaces are markedly expanded by edema (arrow). Bronchial epithelium is necrotic and covered by a layer of fibrin (arrowhead). Photograph courtesy of Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, www.hopkinsmedicine.org/mcp. (HE 100X)

pleural surface of the lung are multifocally hypertrophic.

Contributor's Morphologic Diagnosis: Lung: Bronchointerstitial pneumonia, acute, necrotizing and fibrinous, multifocal to coalescing, severe, with perivascular edema, type II pneumocyte hyperplasia, reactive mesothelial cells, and intranuclear inclusion bodies.

Contributor's Comment: While upper respiratory tract infection in cats is very common, pneumonia is relatively rare. Infectious causes of feline pneumonia include bacteria (*Bordetella bronchiseptica*, *Pasteurella multocida*, *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus* spp., and *Streptococcus* spp.), fungi (*Histoplasma capsulatum*), protozoa (*Toxoplasma gondii*), parasites (*Capillaria aerophila*, *Paragonimus kellicotti* and *Aelurostrongylus*

abstrusus) and viruses (feline herpesvirus-1, feline calicivirus and feline infectious peritonitis virus). In this case, the intranuclear inclusion bodies and necrosis are most consistent with a feline herpesvirus-1 infection.

Feline herpesvirus-1 (FHV-1) is an alphaherpesvirus (genus Varicellovirus) that infects members of the family Felidae. The virus is antigenically and genetically similar to canine herpesvirus-1 and phocine herpesvirus-1. Infection is typically the result of direct contact, but environmental transmission is also reported. Although experimental studies have produced transplacental infection of kittens, natural *in utero* transmission has not been documented. Viral replication primarily occurs in the nasal cavity, tonsils, and pharynx. Viremia is rare, presumably because the virus replicates best at low body temperatures. The virus typically infects respiratory epithelial cells and causes necrosis, fibrin exudation and neutrophilic infiltration. Intranuclear inclusion bodies are commonly seen during the acute phase of infection.

FHV-1 causes upper respiratory tract disease, characterized by sneezing, inappetence, fever, conjunctivitis, ocular and nasal discharge. Primary FHV-1 pneumonia is uncommon, and usually seen only in young or debilitated animals. Neurological signs, abortion, osteolytic lesions in the nasal turbinates and skin ulcers, particularly in cheetahs³ have also been reported with FHV-1 infections. Following acute disease, the virus can enter a latent phase, most likely in the trigeminal ganglion,⁴ with periodic reactivation in times of stress.

Feline calicivirus (FCV) is another important cause of respiratory disease in cats. Like FHV-1, FCV can cause pneumonia, but is more commonly associated

with upper respiratory tract disease. FCV infected cats are more likely to have oral ulceration, and less likely to have ocular lesions, compared to FHV-1 infected cats. Co-infections with FHV-1 and FCV are not uncommon. In a study of cats from eight animal shelters, the prevalence of FCV ranged from 13 to 26% and the prevalence of FHV-1 varied between 3 and 38%.¹ Recently, a FCV-associated virulent systemic disease (VSD) has been described, which, in addition to upper respiratory tract disease, is characterized by cutaneous edema, ulcerative dermatitis, and jaundice.³

JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, necrotizing, acute, diffuse, severe, with eosinophilic intranuclear inclusions, etiology consistent with feline herpesvirus-1.

Conference Comment: Pulmonary inflammation is classified in distinct morphologic categories based on distribution and pattern. Identification of a particular pattern often leads to the determination of other useful information, such as etiology, route of exposure, and pathogenesis. Morphologic categories in common usage are bronchitis/bronchiolitis, bronchopneumonia, interstitial and bronchointerstitial pneumonia, and embolic or hematogenous pneumonia.

This particular case is an example of a bronchointerstitial pneumonia because of the presence of bronchiolar epithelial necrosis as well as alveolar damage. Common causes of bronchointerstitial pneumonia are airborne viruses or toxin that damage both Clara cells and type II pneumocytes. Bronchointerstitial pneumonia is a form of interstitial pneumonia characterized by inflammation of alveolar or interlobular septa. In contrast, bronchitis and bronchiolitis involve only the airways, and bronchopneumonia is characterized by leukocytic exudates which fill bronchioles and alveoli but lacking significant bronchiolar or alveolar septal involvement.² Feline herpesvirus-1, an alphaherpesvirus, causes significant necrosis, neutrophilic inflammation and the elaboration of fibrin.

Bronchial glands are prominent in the cat lung but absent in most other domestic species. Microscopic evaluation of the lungs of felids should always include scrutiny of the bronchial glands because glandular epithelial necrosis and herpesviral inclusions are often readily apparent in these structures.

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www.hopkinsmedicine.org/mcp

References:

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5. Townsend WM, Stiles J, Guptill-Yoran L, et al. Development of a reverse transcriptase-polymerase chain reaction assay to detect feline herpesvirus-1 latency-associated transcripts in the trigeminal ganglia and corneas of cats that did not have clinical signs of ocular disease. *Am J Vet Res*. 2004;65:314-319.

CASE II: A09-650 (JPC 3136045).

Signalment: 13-year-old female Thoroughbred horse (*Equus caballus*).

History: This horse was presented for exercise intolerance. The radiographic appearance of the lungs was consistent with equine multinodular pulmonary fibrosis. The horse was euthanized and necropsied by the referring veterinarian, who submitted lung, liver and spleen for histologic examination and microbiologic tests.

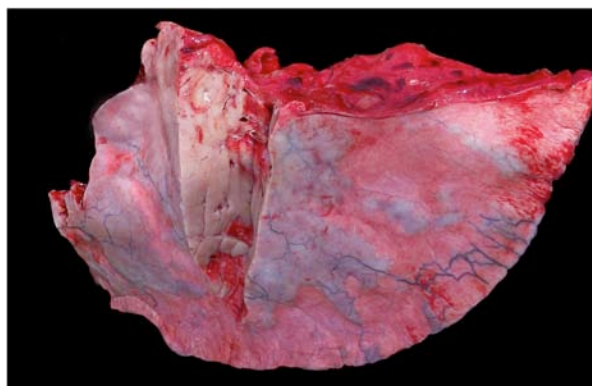
Gross Pathology: Irregularly shaped, but well-demarcated, coalescing nodules (2 to >10 cm in diameter) of firm, pale tan tissue were distributed through all lobes of the lung.

Laboratory Results: Equine herpesvirus 5 was identified antemortem in bronchoalveolar lavage fluid by PCR. Herpesvirus was isolated from the lung postmortem and also identified as equine herpesvirus 5 by PCR.

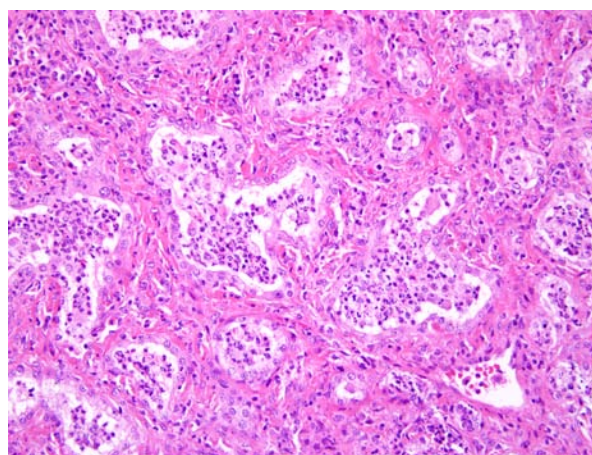
Histopathologic Description: Lung: The pulmonary nodules are well-demarcated from unaffected lung and are the result of interstitial fibrosis. Within the nodules, -alveolar septa are thickened up to 100 μ m or more by fibrous tissue composed of birefringent, orderly collagen fibers with low to moderate cellularity (well-differentiated fibroblasts) and light infiltration by lymphocytes, plasma cells and fewer neutrophils. Alveolar spaces are lined by cuboidal epithelial cells with pale vacuolated cytoplasm, and are partially filled with macrophages, neutrophils, exfoliated epithelial cells, and debris. Intranuclear eosinophilic to amphophilic inclusion bodies are easiest to find in alveolar macrophages. Bronchi and bronchioles within the nodules are filled with similar exudate and surrounded by increased fibrous tissue.

Contributor's Morphologic Diagnosis: Fibrosing alveolitis with eosinophilic intranuclear inclusions in macrophages.

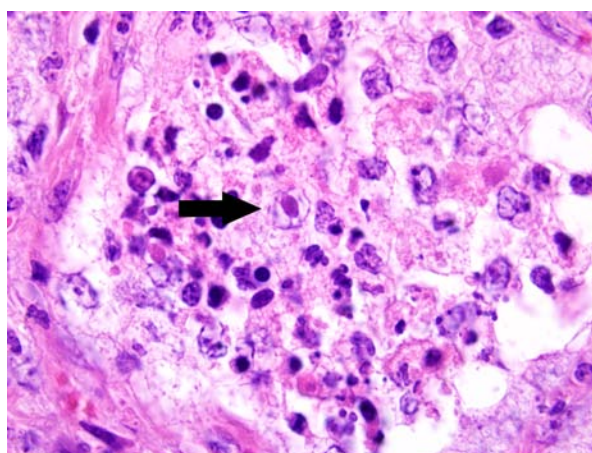
Contributor's Comment: Histologic findings resemble those described in equine multinodular pulmonary fibrosis, attributed to infection with equine herpesvirus 5 (EHV-5). Infection with this gammaherpesvirus is reportedly fairly common in Europe, parts of South America, Australia and New Zealand, but association of EHV-5 infection with multinodular pulmonary fibrosis was first described in the United States in 2007¹ and then again in 2008.² Twenty-four adult horses with multinodular pulmonary fibrosis, 4-28 years of age (mean, 14.5 yr) and with nearly equal gender distribution, were evaluated in the first article.¹ Sixteen of the horses were



2-1. Lung, horse. Irregularly shaped, but well-demarcated, coalescing nodules (2 to >10 cm in diameter) of firm, pale tan tissue were distributed through all lobes of the lung. Photograph courtesy of Purdue University, Animal Disease Diagnostic Laboratory, <http://www.addl.purdue.edu/>.



2-2. Lung, horse. Interalveolar septa are expanded by abundant collagen fibers. Alveoli are often filled with an exudate of alveolar macrophages, neutrophils and necrotic debris, and are lined by hypertrophied Type II pneumocytes. (HE 200X)



2-3. Alveolar macrophages occasionally contain an eosinophilic intranuclear inclusion body that marginates chromatin (arrow). (HE 1000X)

Thoroughbreds. Salient gross lesions were restricted to the lungs and bronchial lymph nodes, and usually appeared as numerous coalescing fibrotic nodules that involved most of the lung. A less common macroscopic presentation was as multiple discrete and larger fibrotic nodules, separated by unaffected pulmonary parenchyma.

Histologically, well-organized, mature fibrous tissue expand the interalveolar septa with preservation of alveolar architecture.¹ Lymphocytes infiltrate the fibrotic interstitium. Alveolar spaces are lined by cuboidal cells, and contain neutrophils and macrophages. A few of the alveolar macrophages have eosinophilic intranuclear inclusions.

Multinodular pulmonary fibrosis is histologically distinct from the pulmonary interstitial fibrosis of silicate pneumoconiosis, which is associated with granulomatous inflammation, and from idiopathic pulmonary fibrosis, which more commonly affects foals than adults and is attributed to diffuse alveolar damage.

In the second report, equine multinodular pulmonary fibrosis was tentatively diagnosed antemortem, as in this case, on the basis of clinical presentation and radiologic findings (nodular pulmonary interstitial pattern) with supportive PCR detection of EHV-5 from bronchoalveolar lavage specimens. Inappetance, weight loss, fever, cough, and respiratory distress were common to all 5 cases in that study.²

To date, Koch's postulates have not been fulfilled to establish EHV-5 as the definitive cause of equine multinodular pulmonary fibrosis. However, the virus is consistently associated with this unique pulmonary lesion, so pathologic findings of nodular pulmonary fibrosis with herpetiform inclusions and supportive PCR analysis should prompt consideration of EMPF in adult horses with respiratory distress and nodular interstitial pneumonia.

JPC Diagnosis: Lung: Pneumonia, interstitial, fibrosing, focally extensive, severe, with marked type II pneumocyte hyperplasia, neutrophilic and histiocytic alveolitis, and rare intrahistiocytic eosinophilic intranuclear inclusions.

Conference Comment: It is worth noting that EMPF presents grossly in two distinct manifestations: the more common diffuse to coalescing form with little unaffected lung parenchyma, and a discrete nodular form in which fibrotic nodules are separated by grossly unaffected lung. There is marked lymphadenomegaly of the bronchial lymph nodes resulting from lymphoid hyperplasia with sinus histiocytosis.

An interstitial pneumonia of donkeys has been reported which is associated with asinine herpesvirus.¹ This disease differs from EMPF in that it is a diffuse inflammatory disease with syncytial cell formation without viral inclusions; interstitial fibrosis is considered a secondary component.

Conference participants noted that pleural arteries were often hypertrophied and surrounded by abundant collagen. This is likely due to increased intrapulmonary blood pressure due to the diffuse fibrosis, which inhibits adequate blood flow through large portions of the affected lung.

Conference participants also discussed a differential diagnosis that included paraquat and diquat toxicosis, which causes fulminant pulmonary fibrosis, although due to the dwindling availability of these compounds, this differential is becoming exceedingly rare. Another possibility is exercise-induced pulmonary hemorrhage, which also has large areas of pulmonary fibrosis, but is characterized by numerous hemosiderophages, and lacks intranuclear inclusion bodies.

Alveolitis, a term commonly used in human respiratory pathology, was used in our morphologic diagnosis because of the striking inflammation centered on alveolar lumens as separate from the fibrosing interstitial pneumonia, which is characteristic of EMPF. This histologic finding is expected with interstitial pneumonias in which there is abundant protein exudation, as well as viral-induced leukocyte chemotaxis. A common feature of EMPF is the preservation of an "alveolar-like" architecture,² which are often filled with neutrophils and macrophages; hence the designation of alveolitis in addition to fibrosing interstitial pneumonia.

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References:

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3. Wong DM, Belgrave RL, Williams KJ, et al. Multinodular pulmonary fibrosis in five horses. *J Am Vet Med Assoc.* 2008;232:898-905.

CASE III: C6352-11 (JPC 4002424).

Signalment: 9-year-old intact male beagle dog (*Canis familiaris*).

History: This dog, submitted by a Humane Society that had received several complaints from residents that the dog was neglected and left outdoors during the winter, was found dead and frozen in an outdoor enclosure.

Gross Pathology: The dog was thin (body condition score = 2/5) with easily palpable ribs and only a small amount of subcutaneous fat. There were small amounts of visceral fat and no evidence of serous fat atrophy in the bone marrow. The lungs had dark red mottling with lobes on the right side appearing darker than those on the left. Additionally, there were multifocal to coalescing tan-white areas (~10% of overall lung volume) that were firmer than the surrounding lung tissue and were slightly collapsed. These areas were mainly distributed along the margins of the lung with the largest area (2 cm X 2 cm) having a dark red center. The stomach was filled with food and had a roughly 2 X 3 cm area of congestion on the serosal and mucosal surfaces. The liver was dark reddish-brown and the gall bladder was mildly distended with bile. Two, roughly 0.5 cm diameter, raised, dark red nodules were noticed on opposite poles of the spleen (consistent with nodular hyperplasia). The kidneys appeared bilaterally dark red.

Laboratory Results: Scant growth of mixed flora including *Bacillus* sp. was found on lung tissue culture.

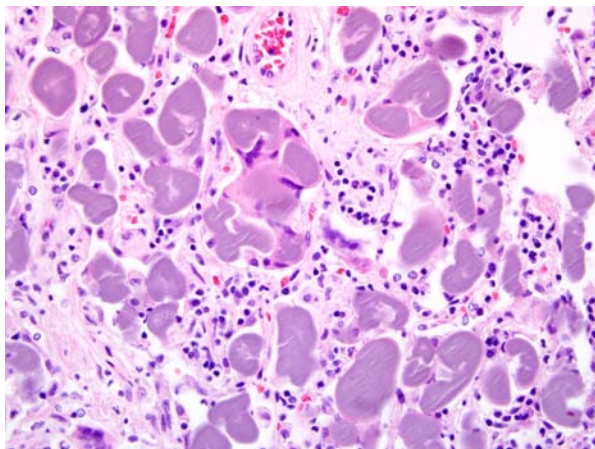
Histopathologic Description: Lung: The firm areas in the lungs correspond histologically to regions with diffuse alveolar fibrosis and infiltration of small to

moderate numbers of predominately lymphocytes and plasma cells. Additionally, almost all of the alveolar spaces and many of the airways in this region are filled with aggregates of amorphous, relatively homogeneous (hyalinized) amphophilic material forming laminated bodies. These bodies are often surrounded by macrophages and occasional multinucleated giant cells. The material is uniformly PAS-positive and portions of the material, particularly at the margins, are birefringent under polarized light. The surrounding lung parenchyma is often mildly atelectatic and often markedly congested. In regions further away from the affected areas there is mild patchy congestion of the alveolar interstitium and infiltrates of small to moderate numbers of predominately macrophages within alveolar spaces.

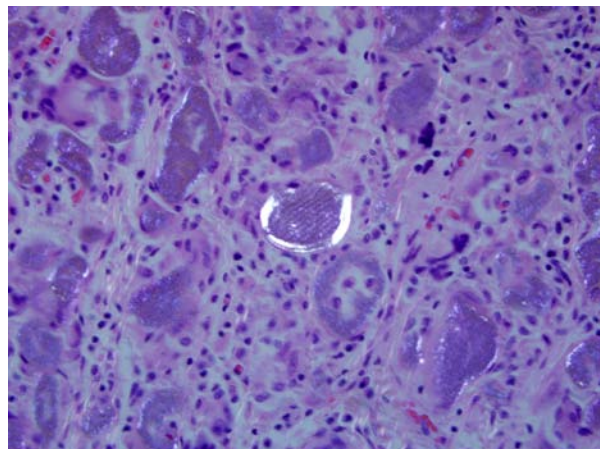
Contributor's Morphologic Diagnosis: Lung: Pneumonia, interstitial, lymphoplasmacytic, histiocytic and fibrosing, multifocal, chronic, severe with intra-alveolar hyaline material (pulmonary hyalinosis).

Contributor's Comment: The lung lesions are characteristic of a condition known as pulmonary hyalinosis of dogs.¹⁻³ Pulmonary hyalinosis is a type of alveolar filling disorder, which is characterized by accumulations of abnormal material in airways. Other disorders in this group include endogenous lipid pneumonia, alveolar proteinosis and alveolar phospholipidosis and alveolar microlithiasis.¹

The cause of pulmonary hyalinosis is unknown. Some pathologists consider it an incidental finding in older dogs³, while others postulate that it is a response to inhalation of dust or pneumotoxicants such as silica in uranium ore dust¹ or to non-specific aspirated material.² This dog did not have any evidence of aspiration and it is unknown whether the unusual material in the lung



3-1. Alveolar spaces are filled by homogenous, amphophilic material that is within multinucleated macrophages. (HE 200X)



3-2. The material, is birefringent under polarized light.

was also present in the enclosure or environmental surroundings.

Unfortunately, the definitive cause of death in this dog remained inconclusive. The most significant findings were changes found in the lungs and kidneys and the overall poor body condition. The pulmonary lesions involved only a relatively small portion of the overall volume of lung (about 10%), however where the lesions were present, the normal architecture was completely obliterated. Additionally a moderate, chronic, multifocal-segmental, membranoproliferative glomerulonephritis was detected microscopically. Although no biochemical testing of renal function and urinalysis were done in this dog, there was no evidence of uremic lesions at necropsy, which could have indicated uremia or renal failure. However, as glomerular disease can cause protein loss, this lesion could have potentially contributed to the poor body condition of this dog.

Although the dog was thin at the time of death, it was not emaciated, as visceral and marrow fat stores were still present, and there was no histologic evidence of hepatocyte or pancreatic atrophy. Abundant foodstuffs were present in the stomach, however the long-term food consumption and therefore energy intake of this dog was unknown. Increased food energy is required in animals living in a cold environment to maintain adequate body temperature, so inadequate food intake would also have to be considered as a possible contributing factor to this dog being very thin.

JPC Diagnosis: Lung: Pneumonia, interstitial, granulomatous, chronic, focally extensive, severe, with abundant intra-alveolar hyaline material.

Conference Comment: This unusual canine condition was originally considered a result of aspiration pneumonia and analogous to a condition in humans known as pulmonary nodular granulomatosis, which was often found in nursing home patients with impaired swallowing abilities who were fed soft, legume-based diets. Hyaline rings were experimentally produced in animal lungs by injecting broth of lentils.⁴ Lentils consist of grains of starch within honeycomb-like cotyledons; the cotyledons, which are composed of cellulose, incited the hyaline rings. The resulting lesion was termed “lentil pulse pneumonia.”

In 1972, Dr. Leonard Billups of the Armed Forces Institute of Pathology reported that the canine condition now known as pulmonary hyalinosis was not composed of plant material as in pulse pneumonias; the hyaline bodies in dogs (known colloquially as “Billups bodies”) did not stain with silver impregnation stains as leguminous cotyledons do.²

Pulmonary hyalinosis may be differentiated from other conditions based upon histologic and staining features. The hyaline bodies are intracellular in macrophages and multinucleated giant cells, occasionally calcify, are birefringent, positive for periodic acid-Schiff (PAS), crystal violet and oil red O, and ultrastructurally consist of a whorled arrangement of lamellar membranes suggestive of degenerate cells.^{1,5} Corpora amylacea are rounded, basophilic hyaline masses that are often concentrically laminated. Corpora amylacea are not birefringent, do not calcify, and are weakly PAS positive. Pulmonary microliths are often distributed throughout the lung, and the calcospherites that compose this lesion are concentrically laminated, have radial striations, are calcified, and are intensely positive with PAS and colloidal iron stains, and unlike pulmonary hyalinosis, the material is extracellular.⁶

Contributor: Atlantic Veterinary College, University of Prince Edward Island
<http://www.upei.ca/avc>

References:

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CASE IV: 10A780 (JPC 4002925).

Signalment: 8-year-old male Indian rhesus macaque (*Macaca mulatta*).

History: This animal had a significant swelling in the left lower jaw with some drainage. The left mandible was very mobile with manual palpation. Radiographs indicated the left mandible was displaced by a large mass. The animal was not responsive to antibiotic treatment. Euthanasia was elected due to the poor prognosis.

Gross Pathology: This animal had normal body condition with adequate subcutaneous and abdominal adipose tissues. Expanding and effacing the left mandible, and displacing molar teeth was a solid, soft, white, 9x6x7 cm mass. The left mandibular lymph node was x3 enlarged. There was a focal gingival ulcer at the lower left jaw.

Laboratory Results: Bacterial culture from gingival ulcer: Normal flora Immunohistochemistry of pan-cytokeratin: odontogenic epithelial cells: +++ Immunohistochemistry of vimentin: mesenchymal cells: +++.

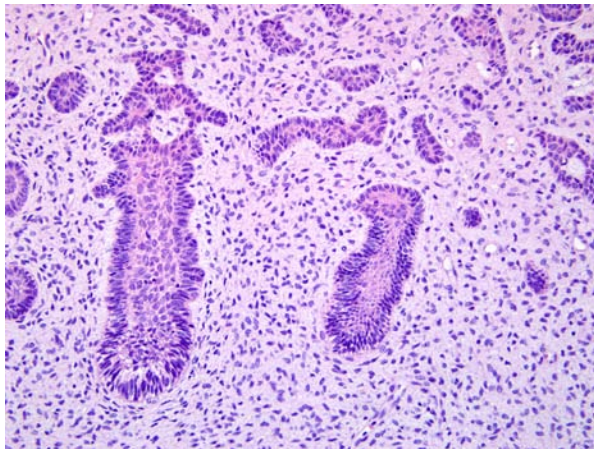
Histopathologic Description: Left mandible: Compressing, disrupting and infiltrating the alveolar bone, and extending into the cut borders is a partially encapsulated, well circumscribed neoplasm composed of islands, cords and strands of odontogenic epithelium separated by a large amount of edematous connective tissue. The islands and cords are often lined by 1-2 layers of distinct peripheral palisading, tightly packed, tall columnar epithelial cells with apical nuclei and basal cytoplasmic clearing. The centers of islands are loosely arranged by small spindle to stellate cells

(stellate reticulum) with prominent intercellular spaces on a pale myxomatous matrix. The odontogenic epithelial cells have distinct cell borders, moderate amounts of pale eosinophilic fibrillar cytoplasm, a pale, oval to elongate, basillar nucleus, with finely stippled chromatin, and 1-2 distinct nucleoli. The neoplastic stellate cells have distinct cell borders, scant eosinophilic fibrillar cytoplasm, and an oval to elongate nucleus, with finely stippled chromatin and a variably distinct nucleolus. Occasionally, islands are surrounded by a variably thick layer of hyaline material. Surrounding the odontogenic epithelial cells are large amounts of primitive connective tissue comprised of loose, collagen-poor primitive stroma, with abundant extracellular matrix containing plump fibroblasts, sometimes with a stellate appearance, with dendritic branches resembling the dental papilla. Mitoses are rare in all cell populations. There are many microcyst formations in the epithelial and ectomesenchymal components. Fibrosis is also noted in multiple areas between the alveolar bone and neoplasm. There are multifocal areas of osteonecrosis and new bone formation. Dental hard tissue formation is not observed.

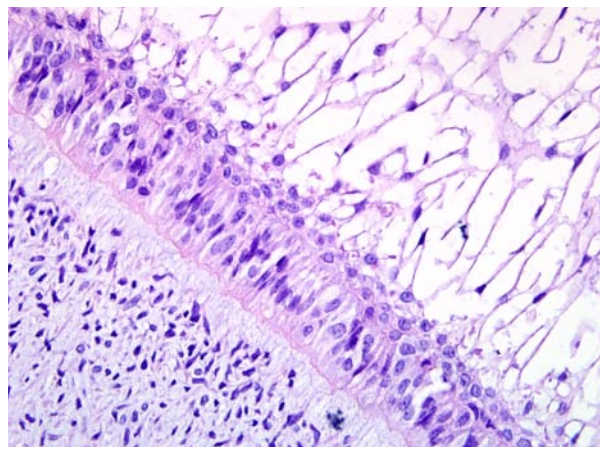
The odontogenic epithelial cells are strongly positive for cytokeratin, and ectomesenchymal tissue is strongly positive for vimentin by immunohistochemistry.

Contributor's Morphologic Diagnosis: Left mandible: Ameloblastic fibroma.

Contributor's Comment: Submitted are neoplastic tissues from two paraffin blocks. Our description is based on the tissue containing alveolar bone. The other tissue does not have adjacent alveolar bone, but major neoplastic lesions are present.



4-1. The neoplasm is composed of islands and cords of odontogenic epithelium on an abundant fibrous stroma with stellate cells in loose streams. (HE 200X)



4-2. Islands of odontogenic epithelium have a peripheral palisading layer of columnar cells with anti-basilar nuclei and clearing of the basilar pole. Internal cells (upper right) are connected by long intercellular bridges. (HE 400X).

In humans, ameloblastic fibroma (AF) belongs to a family of tumors with mixed proliferating odontogenic epithelium and a cellular mesenchymal component that resembles dental papilla. Members of this family include AF, ameloblastic fibrosarcoma (ameloblastic sarcoma), ameloblastic fibro-odontoma, odontoameloblastoma and odontoma, with or without dental hard tissue formation.^{1,7,8} AF is generally intraosseous, mostly in young patients, more male than female.^{3,6} The mandible is the predominant site of occurrence and the posterior mandible is affected more commonly than the maxilla.⁶

Grossly, AF occurs as solid, soft tissue mass with or without a capsule. Histologically, epithelial cells are forming anastomosing cords and/or islands. The cells at the periphery of the epithelial islands are columnar cells, with palisading nuclei and reverse nuclear polarity, similar to ameloblasts. The cells in the center of the epithelial islands had a stellate reticulum-like appearance, similar to the stellate reticulum of the enamel organ. The ectomesenchymal component comprised loose connective tissue, with abundant extracellular matrix containing young, plump fibroblasts, sometimes with a stellate appearance, with dendritic branches resembling the dental papilla. Microcyst formation is rarely seen. If there is dentin formation, the lesion should be diagnosed as ameloblastic fibrodentinoma; if there is also enamel formation, it should be diagnosed as ameloblastic fibro-odontoma.¹

AF is a rare tumor in animals and has been reported in young horses and dogs, but it is the most common odontogenic tumor in cattle. This neoplasm is similar to the lesions in humans and primarily composed of loose, collagen-poor primitive stroma resembling dental pulp. Odontogenic epithelial cells form cords and sheets, resembling enamel organ epithelium.⁴

AF must be distinguished from ameloblastomas, particularly those with desmoplastic changes. Distinct from AF, odontogenic epithelial cells in ameloblastoma are surrounded by mature fibrous connective tissue rather than primitive mesenchymal stroma.^{4,7} Differentiation of ameloblastic fibrosarcoma from AF is also critical. Ameloblastic fibrosarcoma is the malignant counterpart of AF characterized by marked cytologic atypia, increased cellularity with diminution of the epithelial component, high numbers of mitoses and aggressive behavior.¹⁰ Ameloblastic fibro-odontoma has features of AF, but, in addition, they have enamel and dentin deposition.⁴

Anti-pan-cytokeratin antibody is suggested to detect odontogenic epithelial cells. Odontogenic epithelial cells are positive for cytokeratin, but negative to

vimentin by immunohistochemistry. The mesenchymal component is positive for vimentin.²

JPC Diagnosis: Mandible (per contributor): Ameloblastic fibroma.

Conference Comment: Understanding the normal process of the development of tooth structures and the interaction of epithelial and mesenchymal elements is helpful in understanding the various odontogenic tumors and their classifications. The tooth bud is an aggregation of cells that are derived from the ectoderm of the first branchial arch and the ectomesenchyme of the neural crest and is organized into the enamel organ, the dental papilla and the dental follicle.

The enamel organ is composed of the outer enamel epithelium, inner enamel epithelium, stellate reticulum and stratum intermedium, which give rise to ameloblasts that produce enamel.

The dental papilla contains cells that develop into dentin-forming odontoblasts, and mesenchymal cells within are responsible for formation of tooth pulp.

Tooth development is commonly divided into the following stages: the bud stage, the cap, the bell, and maturation. The bud stage is characterized by the appearance of a tooth bud without a clear arrangement of cells. The tooth bud itself is the group of cells at the end of the dental lamina, and is formed when cells of the oral ectoderm proliferate faster than cells of other areas, and is best described as an in-growth of oral ectoderm. This dividing tissue is surrounded and stimulated by ectomesenchymal growth. When it is present, the dental lamina connects the developing tooth bud to the epithelium of the oral cavity. Eventually, the dental lamina disintegrates into small clusters of epithelium and is resorbed. This invagination of ectodermal tissues is the progenitor to the later ameloblasts and enamel while the ectomesenchyme is responsible for the dental papilla and later odontoblasts.⁸

The first signs of an arrangement of cells in the tooth bud occur in the cap stage. A small group of ectomesenchymal cells stops producing extracellular substances, which results in an aggregation of these cells into the dental papilla. The tooth bud grows around the ectomesenchymal aggregation, taking on the appearance of a cap, and becomes the enamel organ. A condensation of ectomesenchymal cells called the dental follicle surrounds the enamel organ and limits the dental papilla, and later forms the surrounding alveolar bone and periodontal ligament.⁵

The dental organ is bell-shaped during the bell stage, and the majority of its cells are stellate reticulum.

Cells on the periphery of the enamel organ separate into three layers. Cuboidal cells on the periphery of the dental organ are the outer enamel epithelium, the columnar cells of the enamel organ adjacent to the dental papilla are the inner enamel epithelium, and the cells between the inner enamel epithelium and the stellate reticulum form the stratum intermedium. The rim of the dental organ where the outer and inner enamel epithelium joins is called the cervical loop. In summary, the layers in order of innermost to outermost consist of dentin, enamel, which is formed by inner enamel epithelium, or ameloblasts, as they move outwards and upwards, inner enamel epithelium and stratum intermedium, which are the specialized stratified cells that support the synthetic activity of the inner enamel epithelium.⁵

Hard tissues, including enamel and dentin, develop during the next stage of tooth development, which is the crown, or maturation stage. Mitosis stops during the crown stage at the location where the cusps of the teeth form, and the first mineralized hard tissues form at this location. At the same time, the inner enamel epithelial cells change in shape from cuboidal to columnar. The nuclei of these cells move closer to the stratum intermedium and away from the dental papilla. The adjacent layer of cells in the dental papilla increases in size and differentiates into odontoblasts, and form dentin. The odontoblasts secrete an organic matrix into their immediate surrounding which contains the material needed for dentin formation. As odontoblasts deposit organic matrix, they migrate toward the center of the dental papilla. Thus, unlike enamel, dentin starts forming in the surface closest to the outside of the tooth and proceeds inward. Cytoplasmic extensions are left behind as the odontoblasts move inward, which form the unique, tubular microscopic appearance of dentin.⁵

After dentin formation begins, the cells of the inner enamel epithelium secrete an organic matrix against the dentin. This matrix immediately mineralizes and becomes the tooth enamel. Outside the dentin are ameloblasts, which continue the process of enamel formation; therefore, enamel formation moves outwards, adding new material to the outer surface of the developing tooth. Reciprocal induction governs the relationship between the formation of dentin and enamel; dentin formation must always occur before enamel formation. Generally, enamel formation occurs in two stages: the secretory and maturation stages. Proteins and an organic matrix form partially mineralized enamel in the secretory stage, and the maturation stage completes enamel mineralization.⁵

In the secretory stage, ameloblasts release enamel proteins that contribute to the enamel matrix, which is then partially mineralized by the enzyme alkaline

phosphatase. Odontoblasts differentiate from cells of the dental papilla. They begin secreting an organic matrix around the area directly adjacent to the inner enamel epithelium, closest to the area of the future cusp of a tooth. The organic matrix contains collagen fibers with large diameters (0.1–0.2 μm). The odontoblasts begin to move toward the center of the tooth, forming an extension called the odontoblast process. Thus, dentin formation proceeds toward the inside of the tooth. The odontoblast process causes the secretion of hydroxyapatite crystals and mineralization of the matrix. This area of mineralization is known as mantle dentin.⁵

There was some confusion among conference participants between the diagnosis of ameloblastic fibroma and ameloblastoma. Ameloblastic fibromas are well demarcated and consist primarily of a loose, matrix-poor primitive mesenchyme and sheets or cords of poorly formed odontogenic epithelium. Those ameloblastic fibromas with more advanced dental differentiation i.e. deposition of dentin or enamel matrix, are categorized as ameloblastic fibro-odontomas or ontoameloblastomas.

Ameloblastomas are usually discrete tumors with irregular islands and strands of odontogenic epithelium and an abrupt transition to mature fibrous stroma. They can occur in the gingival soft tissue (peripheral ameloblastoma) or deep in the bone of the jaw (central ameloblastoma). Additionally, keratinization can occur and be abundant within the odontogenic epithelium.

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