Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #9 — 18 October 2023

CASE I:

Signalment:
8-year-old, male neutered Pembroke Welsh corgi, canine (*Canis lupus familiaris*)

History:
This dog developed labored breathing. An echocardiogram showed pulmonary hypertension and degenerative valve disease of the mitral, tricuspid, and pulmonic valves. The dog was started on sildenafil and pimobendan therapy and improved; however, approximately 1.5 months after the initial diagnosis the dog went into decompensated congestive heart failure and was humanely euthanized.

Gross Pathology:
The lungs were soft, wet, and mottled pink and reddish brown. The right ventricle of the heart was moderately dilated with a thickened wall. Both atrioventricular valves had moderate endocardiosis. The liver had evidence of chronic passive congestion (moderate enlargement, rounded lobar margins, meaty texture, roughened surface, and small amounts of fibrin on the capsular surface). The dog had mild ascites.

Microscopic Description:
Lung: Throughout all sections there are multiple variably sized, poorly demarcated areas within which there is increased cellularity of alveolar septa and lumina. Within these areas, alveolar septa are mildly to moderately

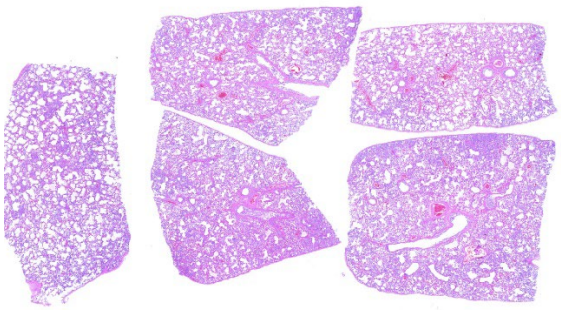

Figure 1-1. Lung, dog. Five sections of lung are submitted for examination. (HE, 5X)

expanded by variable numbers of mesenchymal cells and rare small amounts of collagen. In a few small areas, the expansion of this mesenchymal population results in markedly thickened and tortuous alveolar septa which obscure the adjacent alveolar lumina. There is frequent, regionally variable, type II pneumocyte hyperplasia with a few small areas containing markedly hypertrophied, vacuolated, and/or misshapen type II pneumocytes with up to 3 nuclei. Alveolar spaces contain large numbers of macrophages which infrequently contain finely granular, golden-brown, intracytoplasmic pigment (hemosiderin) as well as small numbers of neutrophils and lymphocytes. Infrequently, alveolar

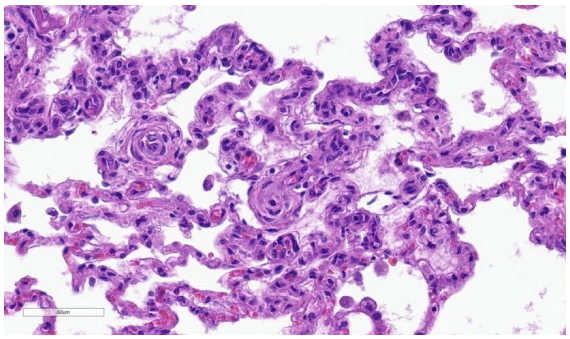

Figure 1-2. Lung, dog. There is concentric hypertrophy of the walls of tortuous pulmonary veins. Adjacent septa are hypercellular with prominent endothelial cell nuclei. (HE, 400X)

spaces contain variable amounts of fibrillar to beaded, eosinophilic material (fibrin) which often lines the alveolar septa with or without admixed cellular debris (hyaline membranes). Alveolar spaces also frequently contain finely granular, eosinophilic material (proteinaceous fluid). Within these areas as well as unaffected areas, pulmonary veins (distinguished by their location within the alveolar parenchyma) frequently exhibit remodeling, with expansion of the tunica media by plump spindle cells resulting in partial to complete occlusion of the lumen. Often associated with these remodeled veins are variably sized networks of alveolar capillaries which are moderately to markedly congested. The wall of one large pulmonary artery exhibits segmental fibrinoid necrosis.

A large number of thickened vessels within the alveolar parenchyma have external, dark purple to black elastic laminae only (as seen on Verhoeff-van Gieson stain), distinguishing them as veins, in comparison to arteries which have internal and external elastic laminae. Immunohistochemical stains for smooth muscle actin (SMA) revealed SMA immopositive mesenchymal cells expanding vein walls, consistent with smooth muscle hypertrophy/hyperplasia. The cells lining the variably congested alveolar capillaries exhibit strong immunoreactive for CD31, confirming endothelial origin.

Contributor's Morphologic Diagnoses:

1. Lung: Venous medial hypertrophy, multifocal, marked, chronic, with moderate perivenous capillary congestion.
2. Lung: Capillary hemangiomatosis, multifocal, marked, chronic, with minimal alveolar septal fibrosis.
3. Lung: Alveolar histiocytosis, multifocal, marked, chronic, with hyaline membrane formation and moderate to marked type II pneumocyte hyperplasia.

Contributor's Comment:

The combination of the findings of venous medial hypertrophy and alveolar capillary proliferation are consistent with pulmonary veno-occlusive disease (PVOD) and capillary hemangiomatosis (PCH). PVOD and PCH are rare forms of pulmonary hypertension (PH) which result from progressive remodeling and obstruction of pulmonary veins, and proliferation of thin-walled microvessels within alveolar septa, respectively.[2,6] As the two lesions often occur concurrently, the World Health Organization (WHO) recognizes that they may belong to a spectrum of the same pulmonary vascular disease.[7] In humans, the WHO recognizes five clinical groups of PH: group 1, which was recently updated to include PVOD and PCH, comprises diseases involving pulmonary arterial hypertension (PAH); group 2 represents diseases caused by left-sided heart disease; group 3 causes of PH relate to lung disease and/or hypoxia; group 4 is PH caused by pulmonary artery obstruction; and group 5 is composed of various disease with unclear and/or multifactorial mechanisms.

In a recent retrospective study of 15 dogs with PVOD and PCH, all dogs were older than 8 years of age (median 11 years).[4] There

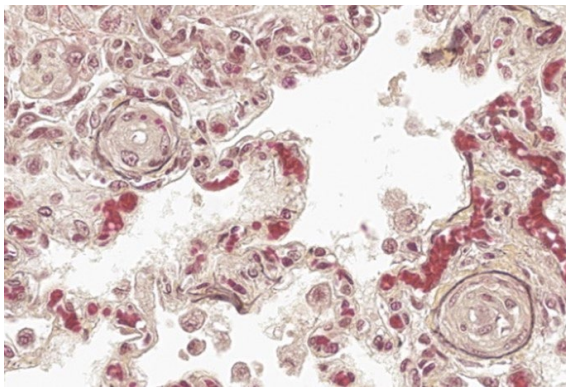

Figure 1-3. Lung, dog. An elastin stain demonstrates the lack of internal elastic lamina, establishing the primary affected vessels as veins. (Elastin, 400X)

was no clear sex or breed predilection, although the sample sizes were small. The underlying causes of these diseases in dogs remain unknown. In humans, patients with PVOD are predominantly young to middle-aged, and both genetic and non-genetic risk factors have been identified. Genetically, one study found that 13/13 familial and 5/20 sporadic cases of PVOD had a loss of function mutation of EIF2AK4; however, the link between this mutation and PVOD remains unclear.[1] Other risk factors for PVOD in humans include chemotherapeutics, exposure to organic solvents, bone marrow or stem cell transplants, neoplasia, connective tissue disorders, autoimmune diseases, sarcoidosis, HIV infection, and pulmonary Langerhans cell histiocytosis.[4] Only genetic factors have been implicated for PCH in humans.

In dogs, clinical signs involve rapidly progressive respiratory signs that include coughing, tachypnea, dyspnea, hypoxia, epistaxis, and hemoptysis.[3] Diagnostically, thoracic radiographs should demonstrate an interstitial or alveolar pattern with enlargement of the pulmonary arteries, without evidence of cardiomegaly.[4]

Gross lesions in dogs with PVOD typically involve diffusely edematous and firm to 'meaty' lungs with widespread areas of hemorrhage or congestion.[3] In a study of 11 dogs, there was no gross evidence of underlying cardiac disease and 2 dogs demonstrated compensatory hypertrophy of the right ventricle.[8]

Histologically, the hallmark feature of PVOD is extensive, patchy remodeling of small- to medium-sized pulmonary veins. This remodeling involves expansion of the vessel wall with plump spindle cells and collagen, resulting in narrowed or occluded venous lumina. As a result of this luminal occlusion, there is upstream congestion of adjacent alveolar capillaries and in some cases pulmonary arterial remodeling. PCH, which in humans is concomitantly present in 75% of PVOD cases, is characterized by proliferation of alveolar capillaries, which can extend into pulmonary arteries and veins. Additionally, there are increased numbers of alveolar macrophages which frequently contain cytoplasmic hemosiderin. In some dogs there may be small accumulations of fibrin as well as hyaline membrane formation.

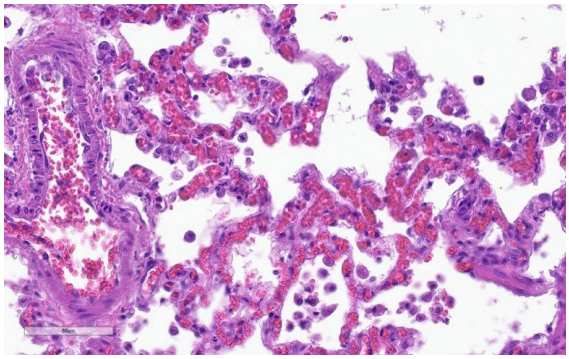

Figure 1-4. Lung, dog. There is marked segmental septal congestion due to increased capillary pressure. (HE, 400X)

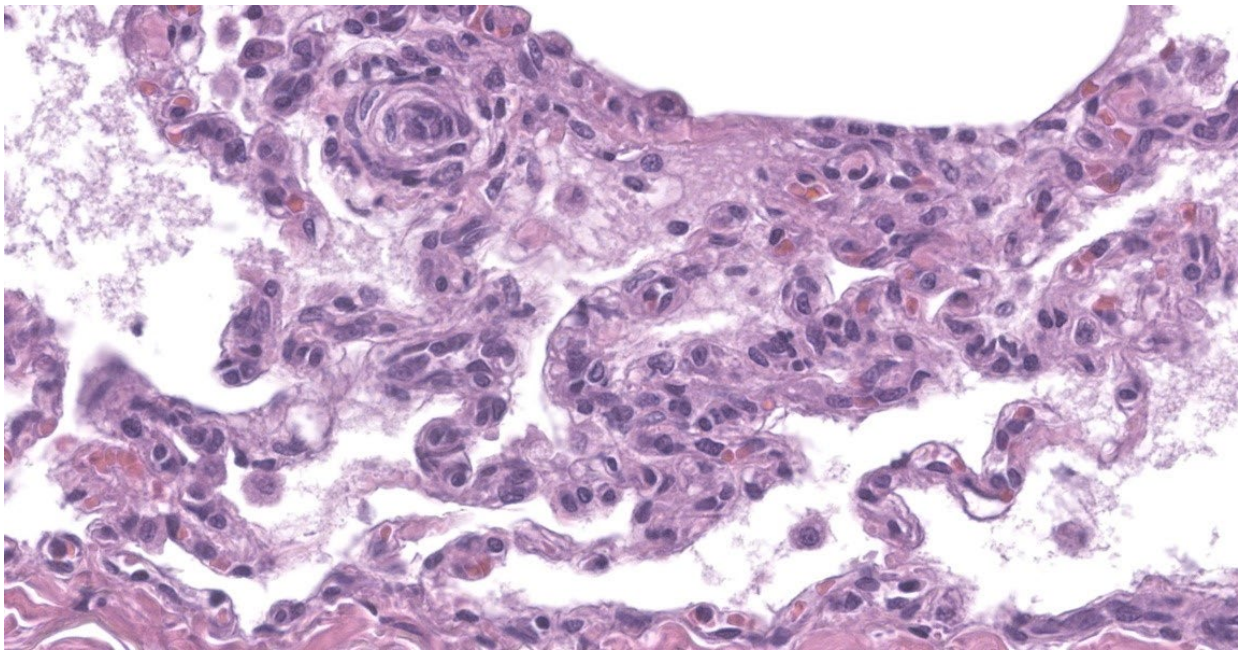

Figure 1-5. Lung, dog. Due to increased capillary pressure, there is proliferation of the capillary endothelium (capillary hemangiomatosis) (HE, 400X)

Contributing Institution:
Veterinary Diagnostic Laboratory
University of Minnesota
St. Paul, MN.
https://vdl.umn.edu/

JPC Diagnosis:
Lung: Venous intimal fibrosis and smooth muscle hyperplasia and stenosis, multifocal to coalescing, severe, with septal endothelial hypertrophy (capillary hemangiomatosis), segmental alveolar capillary engorgement, alveolar histiocytosis and siderosis, and arterial medial hypertrophy.

JPC Comment:
As the contributor notes, PVOD and PCH are rare causes of pulmonary hypertension in humans and are exceedingly rare and only recently described in dogs. This case provides an excellent example of the characteristic histologic features of these conditions and the contributor provides an excellent summary of the current state of knowledge surrounding this unusual entity.

Pulmonary hypertension can be caused by four broad mechanisms: increased pulmonary blood flow, increased pulmonary vascular resistance, increased pulmonary venous pressure, or some combination of these factors.[5] The American College of Veterinary Internal Medicine has recently published a proposed classification scheme that categorizes pulmonary hypertension based roughly on these broad casual mechanisms.[5] The classification scheme includes many well-known causes of pulmonary hypertension including left-sided heart disease, pulmonary disease, hypoxia, congenital left-to-right cardiovascular shunts, and *Dirofilaria immitis* or *Angiostrongylus vasorum* infection.[5] Under this rubric, PVOD and PCH are nested in their own subcategory under the pulmonary arterial hypertensive diseases, a categorization that may be initially confusing since the vascular lesions of PVOD preferentially affect the pulmonary veins; however, PVOD is classified as a distinct cause of pulmonary *venous* hypertension whose clinical expression is severe pulmonary *arterial* hypertension.[8]

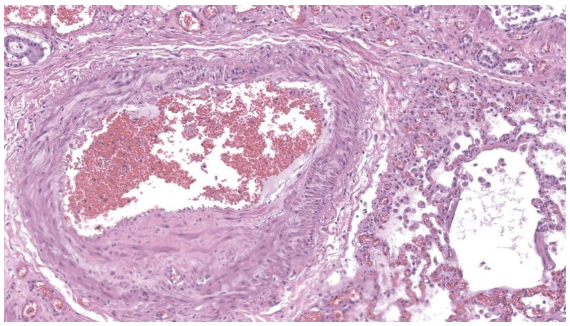

Figure 1-6. Lung, dog. Multifocally, pulmonary arterioles demonstrate asymmetric myointimal hyperplasia. (HE, 400X)

The placement of PVOD and PCH in their own subcategory within the proposed classification scheme is neither merely descriptive nor academic, but instead highlights a key difference in the response of PVOD and PCH patients to typical pulmonary arterial hypertension treatments. The treatment mainstays for these conditions include the use of type-5 phosphodiesterase (PDE5) inhibitors, the most well-known of which are sildenafil and tadalafil. Human PVOD and PCH patients treated with PDE5 inhibitors can develop acute, massive, and fatal pulmonary edema due to the inability of occluded vasculature to accomodate the increased blood flow caused by these potent vasodilators.[2] Though this effect has not been definitely proven in dogs, the consensus statement extrapolates from human literature and recommends administering PDE5 inhibitors only in hospital where patients can be carefully monitored for the development of pulmonary edema.[5]

This week's conference moderator was Dr. Kurt Williams, Director of the Oregon Veterinary Diagnostic Laboratory and pulmonary system enthusiast. Dr. Williams waxed poetic at the start of the conference about the ability of a slide to tell a story about pathogenesis. He encouraged conference participants to spend time understanding that story and then to structure slide descriptions and interpretations to convey it accurately to others.

In this case, the story being told centers on the vasculature, so an accurate description should describe the vessels in full, to include which branches of the vascular tree and what caliber of vessels are being affected. In cases such as PVOD, where occlusive changes can make it difficult to distinguish vein from artery, a Verhoeff-van Gieson stain, which highlights vascular elastic lamina, can be helpful in characterizing the vessels; veins will have only an external elastic lamina, while arteries will have both internal and external elastic laminae. In the absence of special stains, anatomy can be useful, as arteries are typically adjacent to airways, while veins tend to branch out more diffusely into the parenchyma.

Dr. Williams noted that the hemosiderin-laden macrophages, or "heart failure cells," associated with PVOD typically have an abundance of hemosiderin far in excess of that normally found in garden-variety heart failure. Conference participants remarked on the generally unimpressive nature of these cells in this case, while Dr. Bruce Williams cautioned against the use of this term in communications meant for clinicians, as the term could cause the inappropriate administration of positive ionotropes which are contraindicated and potentially fatal in PVOD patients.

As the contributor notes (and this case exemplifies), capillary hemangiomatosis typically accompanies PVOD. Dr. Williams noted that the capillaries in capillary hemangiomatosis are not simply congested but also larger than normal. For this reason, Dr. Williams prefers the more dramatic term, "capillary engorgement" to "capillary congestion." Finally, Dr. Williams noted several vessels that contained foci of intimal thickening with longitudinally-oriented blood vessels embedded within. This change is characteristic of hypoxic injury in the lung and can be seen in

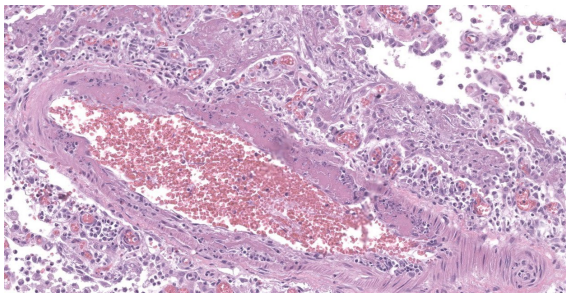

Figure 1-7. Lung, dog. Occasional pulmonary arterioles demonstrate extrusion of protein and fibrin into their walls. (HE, 200X)

other contexts, such as in high mountain/brisket disease in cattle.

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CASE II:

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

History:
This dog presented with an approximately 3-month history of chronic coughing. Thoracic radiographs revealed a multifocal alveolar pattern extending to the caudodorsal lung fields. The alterations appeared to have progressed compared to a radiograph taken 7 weeks prior. The tracheobronchial lymph nodes were enlarged but appeared to be of similar size as noted in the previous radiograph. Due to poor prognosis, humane euthanasia was elected.

Gross Pathology:
The lungs were diffusely mottled red to dark red with numerous coalescing firm, tan nodules embedded in the pulmonary parenchyma of all lobes. Representative tissue samples floated in 10% neutral buffered formalin. The tracheobronchial lymph nodes were enlarged, ranging in size from 1x2x1 cm to 1.7x4.0x1.5 cm. On the cut surface of these nodes, there were multiple variably sized, random, light to dark brown areas. The liver had several pinpoint to 0.3 cm diameter tan foci throughout.

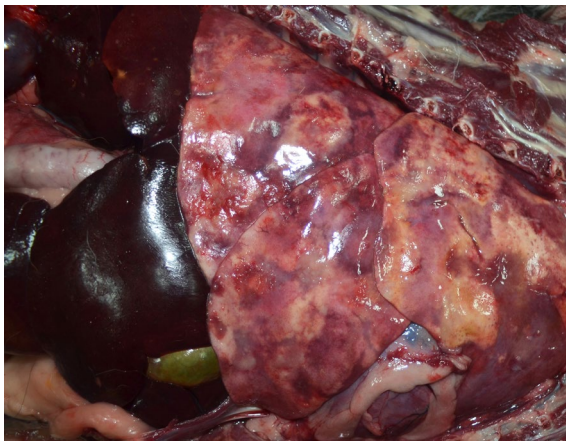

Figure 2-1. Lung, dog. The lungs were diffusely mottled red to dark red with numerous coalescing, firm tan nodules embedded in the pulmonary parenchyma of all lobes. (*Photo courtesy of:* Louisiana Animal Disease Diagnostic Laboratory (LADDL), School of Veterinary Medicine, Louisiana State University, http://www1.vetmed.lsu.edu/laddl/index.html)

Laboratory Results:
Bacteriology (Aerobic culture), lung: No bacteria isolated.

Mycology (Fungal culture), lung: No fungi isolated.

Microscopic Description:
Lung: Corresponding to the coalescing firm tan nodules noted grossly, there is multifocal to coalescing infiltration of the pulmonary parenchyma by large number of mixed inflammatory cells consisting predominantly of macrophages and eosinophils, fewer neutrophils, lymphocytes, and plasma cells, and occasional multinucleated giant cells, amidst a background of multifocal polymerized fibrin deposits, occasional mild hemorrhage, multiple plump type II pneumocytes, and spindle cells. In some areas, the inflammation concentrates in the bronchiolar and perivascular interstitium, variably extending into the airways (often as epithelium-lined fibrous polypoid structures) and the vascular wall. No microorganisms are apparent, and Gomori's methenamine silver (GMS) staining is negative. Within the remaining areas of the pulmonary parenchyma, the alveoli often contain moderately increased numbers of alveolar macrophages and fibrin strands. In addition, in some sections the pleural surface is multifocally expanded by villous fibrous proliferation with a mixed, predominantly eosinophilic inflammatory infiltrate.

Additional histologic findings (sections not submitted) include marked eosinophilic and granulomatous lymphadenitis of the tracheobronchial lymph nodes, mild eosinophilic and granulomatous portal hepatitis, and mild to moderate extramedullary hematopoiesis in the spleen.

Contributor's Morphologic Diagnosis:
Lung: Pneumonia, eosinophilic and granulomatous, multifocal to coalescing, marked, chronic (consistent with eosinophilic pulmonary granulomatosis).

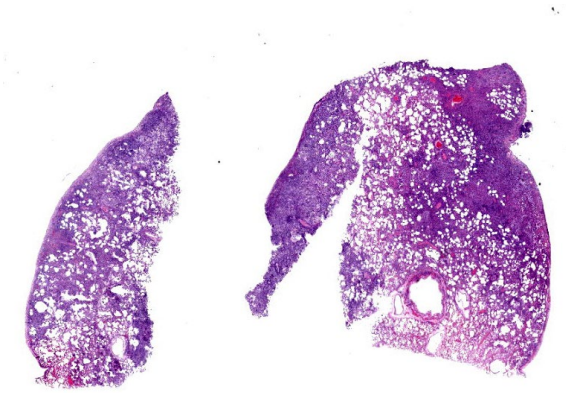

Figure 2-2. Lung, dog. Two sections of lung are submitted for examination. There are multifocal to coalescing areas of consolidation, particularly in subpleural areas. (HE, 5X)

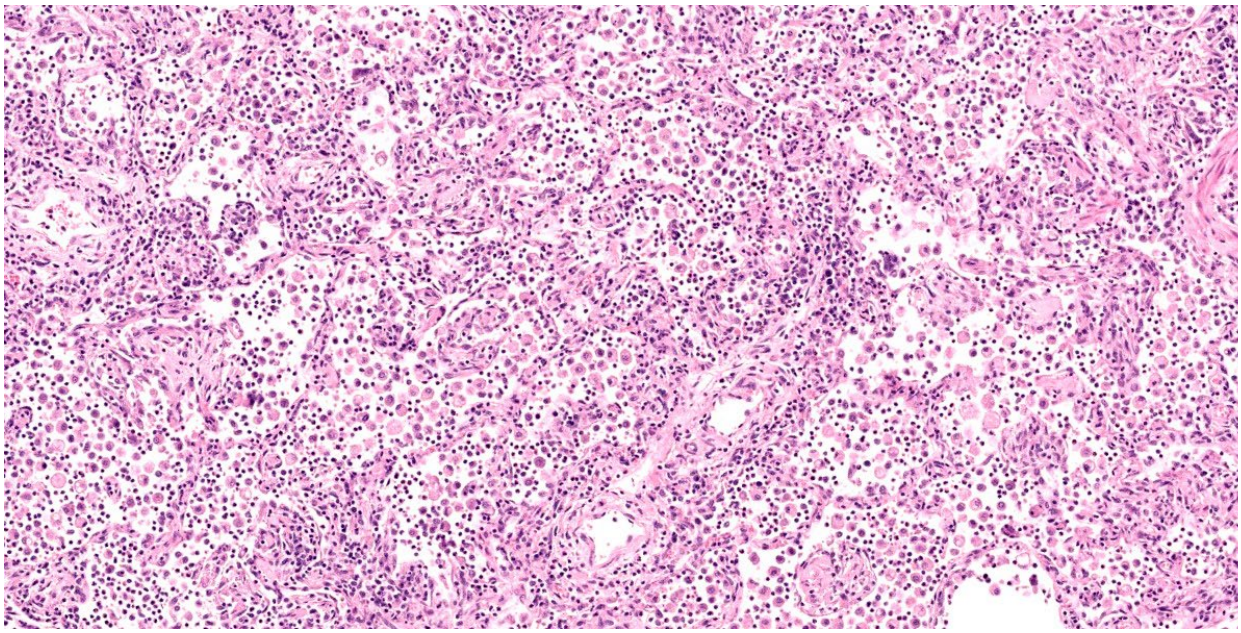

Figure 2-3. Lung, dog. In consolidated areas, alveoli are filled with a markedly cellular exudate, and septa are fibrotic. (HE, 81X)

Contributor's Comment:

Eosinophilic pulmonary granulomatosis (EPG) is an uncommon, idiopathic disease of dogs with clinical signs of progressive coughing, exercise intolerance, variable dyspnea, and eosinophilia.[2,3,6,8,13] EPG is differentiated from the other eosinophil-rich conditions, such as eosinophilic pneumonia and eosinophilic bronchopneumopathy (EBP), by the formation of nodules and masses composed of eosinophils, macrophages, and various combinations of other leukocytes within fibrous tissue.[1] Some authors have speculated that EPG might represent a progressed form of EBP, which is a more commonly diagnosed idiopathic condition in young dogs.[1,2,6,7,9,12] Although eosinophilic airway inflammation and peripheral eosinophilia may be seen in both conditions, EBP is microscopically characterized by airway and pulmonary involvement without granuloma formation or lymph node involvement.[2,8] Clinically, EBP responds to immunosuppressive treatment far better than EPG.[2,6,8]

The cause and pathogenesis of EPG have yet to be determined.[4,8] Multiple reports in the veterinary literature have discussed an association between *Dirofilaria immitis* infection and EPG.[1,2,4-6] However, affected dogs do not always have gross, microscopic, or serologic evidence of dirofilariasis.[1,2,6,8,10,13] In this case, there was no gross or microscopic evidence of concurrent or previous *D. immitis* parasitism. In addition, bacterial and fungal cultures of the lung were negative.

Typical gross findings of EPG include firm, consolidated pulmonary parenchyma with multifocal to regionally extensive discrete nodules.[2,5,6,10] Similar nodules may be found in the regional lymph nodes, heart, liver, and spleen.[1,2,5] Microscopically, the nodules are predominantly composed of eosinophils, macrophages, epithelioid macrophages, and small numbers of neutrophils, lymphocytes,

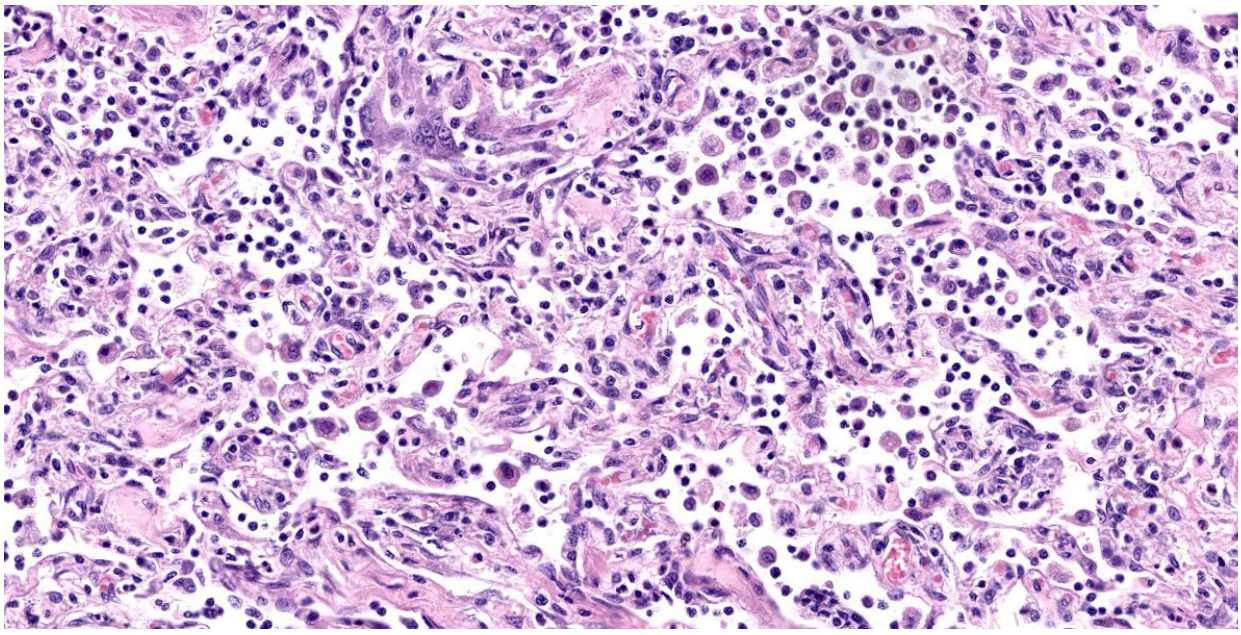

Figure 2-4. Lung, dog. Higher magnification of pulmonary parenchyma. There is an organizing chronic pneumonia with an infiltrate large numbers of macrophages and eosinophils with occasional multinucleated giant cell macrophages. Alveolar septa are expanded by protein yet to be resorbed (covered by proliferating epithelium and abundant collagen and fibroblasts. (HE, 381X)

and plasma cells, and are separated and surrounded by variably thick fibrous connective tissue.[1,2,5,6,10] In some cases, eosinophilic and/or granulomatous inflammation is also described in the trachea, kidney, stomach, and small intestine.[1] In this case, the tracheobronchial lymph nodes and the liver had similar but milder inflammation as that in the lung, likely all part of the same disease process.

The diversity of the reported affected canine breeds does not suggest a breed predisposition. Although 6 of the 26 dogs with EPG included in a literature review were German Shepherd or German Shepherd crossbred dogs, the authors speculated that this might simply represent the popularity of the breed.[1] There does not appear to be sex predilection for EPG in dogs.[1]

Brown Norway rats, which have been used to study the pathogenesis of asthma, may also develop a spontaneous eosinophil-rich granulomatous pneumonia in the absence of any experimental procedure. While lesions have been observed in rats of various ages, they seem to affect particularly young adult animals. Both sexes are susceptible. Typical gross findings are multiple 1-3 mm diameter, pale tan to gray to red foci scattered throughout the pulmonary parenchyma. Microscopic findings are characterized by multifocal to diffuse granulomatous pneumonia composed of epithelioid cells with or without prominent multinucleated giant cells. There may be marked perivascular and peribronchiolar edema with mixed leukocytic infiltrates rich in eosinophils. Detection of possible infectious agents by serology, bacterial culture, and special stains on affected lungs have all been negative.[11]

Treatment of EPG includes administration of immunosuppressive and cytotoxic drugs,

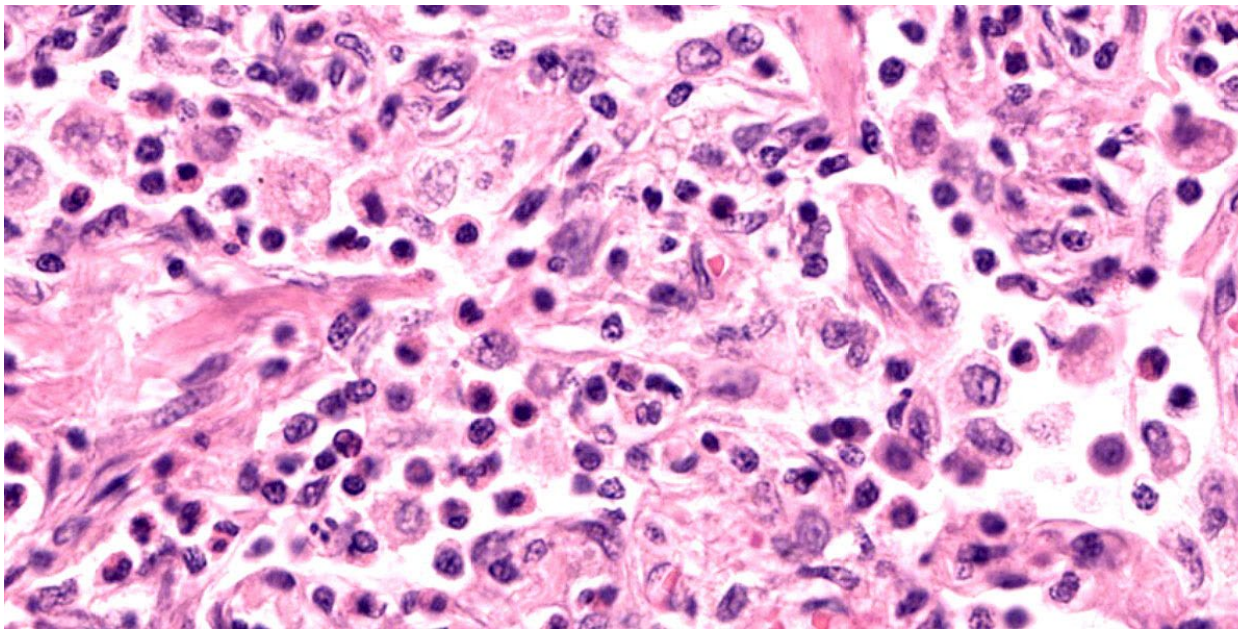

Figure 2-5. Lung, dog. The inflammatory infiltrate is composed primarily of eosinophils and macrophages. (HE, 850X)

based on a presumed immune-mediated pathogenesis.[6,7] Immunosuppressive doses of prednisone, azathioprine, and cyclophosphamide are among the documented treatments; the efficiency of other immunosuppressive drugs (e.g., cyclosporine) is not known.[2,6-8,10] The prognosis is poor due either to partial response to treatment or rapid recurrence of respiratory clinical signs after cessation of treatment.[2,6,10]

Contributing Institution:
Louisiana Animal Disease Diagnostic Laboratory (LADDL)
School of Veterinary Medicine
Louisiana State University
http://www1.vetmed.lsu.edu/laddl/index.html

JPC Diagnosis:
Lung: Pneumonia, eosinophilic, organizing, chronic, multifocal to coalescing, severe.

JPC Comment:
As the contributor notes, there is a long-standing debate, referenced in every review of EPG, of a potential association between *D. immitis* infection and the development of the eosinophilic granulomas that characterize the disease. In the earliest cases, reported in the 1980's, *D. immitis* infection was common.[2,5,10] Since that time, however, *D. immitis* infection has become uncommon in reported cases, perhaps indicating the development and widespread adoption of heartworm prophylaxis in the intervening years.[1] In fact, a recent study notes that, of the 19 cases of EPG reported before 1988, 8 were infected with *D. immitis*; of the 7 cases reported since, none had evidence of *D. immitis* disease.[1]

Many cases of EPG present with concurrent peripheral eosinophilia and basophilia. The presence of basophilia is a particularly uncommon clinical pathology finding that tends to narrow a differential list significantly. Among the causes for peripheral basophilia are allergic diseases, including eosinophilic granulomas; neoplastic diseases such as mast cell tumors, thymomas, and lymphomatoid granulomatosis; drug reactions; stress (in birds); and parasitism, including *Dirofilaria immitis*.[14] The fact that EPG and *D. immitis*

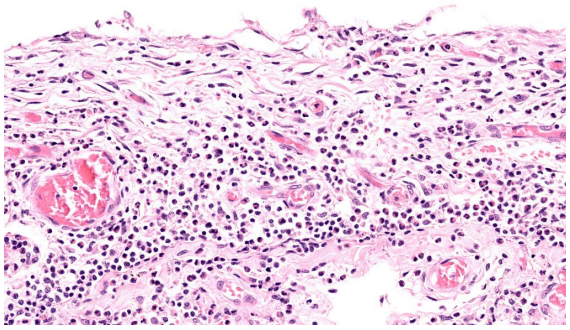

Figure 2-6. Lung, dog. The inflammatory infiltrate extends into the pleura. (HE, 318X)

infection both provoke this uncommon clinical pathology further stokes suspicion that a casual link underlies this association. Whether the eosinophilia and basophilia is useful diagnostically and whether there is a causal link between *D. immitis* infection and EPG are currently both open questions that require further investigation.[1]

There is speculation that EPG might be a more progressed form of eosinophilic bronchopneumopathy (EBP), an uncommon, typically steroid responsive condition of young dogs.[3] The eosinophilic bronchitis with epithelial hyperplasia, ulceration, and/or squamous metaplasia that characterize this condition tends to destroy airway walls and leads to bronchiectasis in 25% of cases.[3] EBP is currently thought to be immune-mediated, but a diagnosis of EBP requires that more specific conditions be ruled out. These include the tracheobronchial parasites *Crenosoma vulpis, Eucoleus aerophilus,* and *Oslerus osleri*; the lung worms *Angiostrongylus vasorum* and *Filaroides hirthi*; *Dirifilaria immitis* infection; and neoplasia, including pulmonary carcinoma, histiocytic sarcoma, lymphoma, and lymphomatoid granulomatosis.[3]

Conference discussion initially focused on the lesion distribution, which is predominantly parenchymal and is curiously concentrated subjacent to the pleura. Dr. Williams prefers to describe this distribution, which most closely resembles an interstitial pattern but isn't an exact fit to any of the establish patterns, as simply pneumonia. Conference participants also remarked on the lack of a clear nodular pattern that is typical of EPG.

A particular striking histologic feature in this slide is the irregular, eosinophilic, densely cellular material found protruding into airways and multifocally adhered to alveolar walls. Though most conference participants interpreted this as alveolar fibrosis, Dr. Williams interpreted these areas as ancient remnants of previously suffered bouts with an extremely exudative inflammatory process. The fibrous tissue left behind represents attempts by the lung to repair the damage, referred to as organizing pneumonia. In less affected areas of lung, alveoli are multifocally mineralized, a lesion that likely represents a more recent stage of injury, providing multiple temporal stages in the disease process in one slide.

Participants also discussed the nature of the inflammatory infiltrate at length. While eosinophils are clearly the main driver of disease in this case, the remainder of the inflammation was characterized by the conference participants as mixed, with large numbers of plasma cells, lymphocytes, and histiocytes.

Based on these discussions, the JPC morphologic diagnosis highlights the eosinophilic nature of the inflammation and eschews the reference to granulomatous due to the heterogenous nature of the infiltrate. Conference participants also felt that the multifocal foci of organizing pneumonia was an important histologic feature to highlight as it provides a window into pathogenesis and an indicator of chronicity.

References:

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CASE III:

Signalment:
4-year-old, Angus cow, bovine *(Bos taurus*)

History:
Four adult Angus cows and an adult Hereford bull died suddenly a few days after being moved to lush pasture.

Figure 3-1. Lung, ox. The lungs failed to collapse when the thorax was opened. They are reddened, and interlobular septal expansion is prominent with interlobular emphysema in the caudal lobes. (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

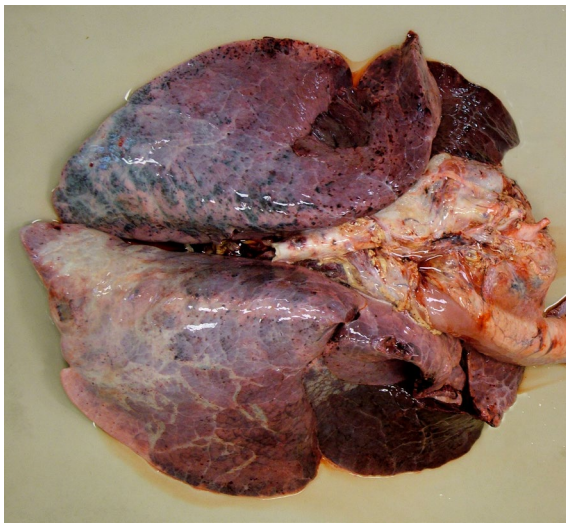

Figure 3-2. Lung, ox. When removed from the thorax, the lungs fail to collapse, there is extensive consolidation of all lobes, and leaked fluid pools beneath them. (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services)

Gross Pathology:
The lungs did not collapse when the thorax was opened. The majority of the lung parenchyma was red, heavy, wet and meaty with emphysema and edema in the interlobular septa and moderate numbers of petechiae on the surface. Approximately 10-30% of the lung parenchyma in the cranioventral lung fields was dark red to plum colored, firm and consolidated.

Laboratory Results:
Pasteurella multocida was isolated on bacterial culture of the consolidated lung.

PCR testing of the lung was negative for bovine viral diarrhea (BVD) virus, bovine herpesvirus-1 (IBR), bovine respiratory syncytial virus (BRSV), *Mycoplasma bovis*, bovine respiratory coronavirus, and bovine influenza virus (BIV).

Microscopic Description:
The lung is diffusely congested. In the majority of the lung, the alveoli are filled with edema and fibrin mixed with variable numbers of macrophages, small numbers of neutrophils and rare multinucleated giant cells. The fibrin occasionally forms hyaline membranes that are associated with the alveolar wall. The alveolar septa are thickened by fibrin, edema, macrophages, lymphocytes and swollen type II pneumocytes that epithelialize the alveolar wall. The interlobular septa and pleura are thickened by edema and emphysema.

Contributor's Morphologic Diagnosis:
Lung: Interstitial pneumonia, acute, diffuse, severe, with hyaline membrane formation, type II pneumocyte hyperplasia, edema, and emphysema (acute bovine pulmonary edema and emphysema)

Contributor's Comment:
The clinical history and the gross and microscopic lesions are consistent with the respiratory disease known as acute bovine pulmonary edema and emphysema (ABPEE). ABPEE is a toxic interstitial pneumonia of cattle.[3,5] The disease most commonly occurs in adult cattle.

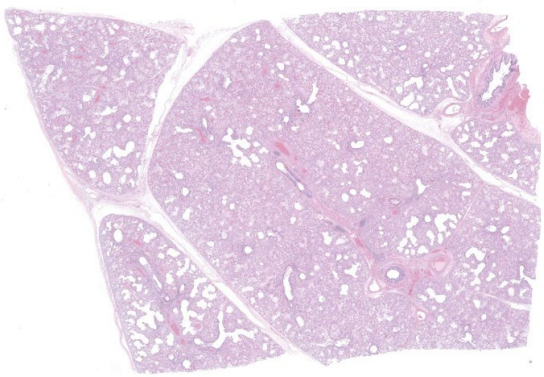

Figure 3-3. Lung, ox. At subgross magnification, there is diffuse lobular consolidation and marked expansion of interlobular septa. (HE, 5X)

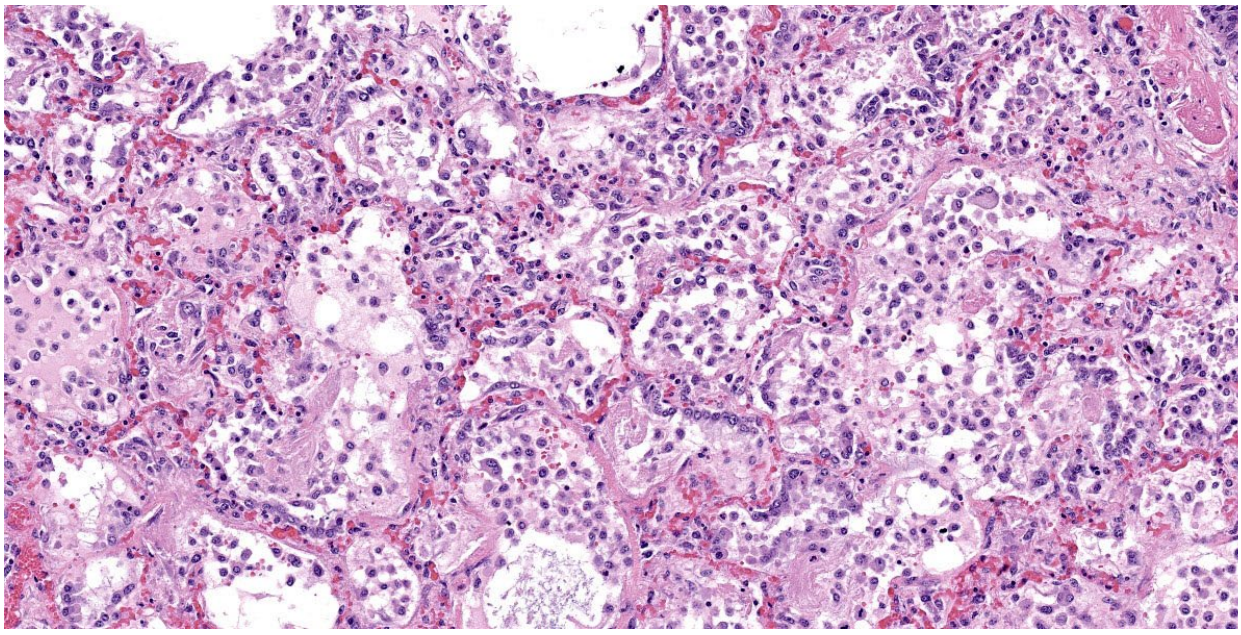

Figure 3-4. Lung, ox. Diffusely, alveoli are filled with edema and numerous alveolar macrophages. Alveolar septa are expanded by edema and multifocally lined by type II pneumocytes. (175X)

Calves appear to be resistant to the disease. ABPEE typically occurs in the fall 4-10 days after cattle are moved from summer pastures to lusher fall pastures, but it can occur any time of the year when cattle are moved to lush pastures.

ABPEE is caused by L-tryptophan within plants being converted to 3-methylindole by rumen microbes.[1,2,3,5,6,7] The 3-methylindole formed in the rumen is absorbed into the bloodstream, eventually making it to the lungs.[3,5] There is evidence that suggests 3-methylindole preferentially binds in the lung of susceptible species compared to other organs such as the liver.[7] Within the lung, 3-methylindole is converted to unknown toxic metabolites by mixed function oxidases, mainly cytochrome p450 in the nonciliated bronchiolar epithelial cells.[3,5] The toxic metabolite of 3-methylindole causes necrosis of bronchial epithelium and type I pneumocytes, endothelial cell swelling, and increased vascular permeability. The increase in vascular permeability leads to edema and thickening of the alveolar wall. Type II pneumocyte hyperplasia occurs in response to necrosis of type I pneumocytes.

Typical gross lesions of ABPEE in cattle include pale to red, edematous, meaty and rubbery lungs that fail to collapse when the thorax is opened.[3,5] The lungs are often emphysematous. Microscopically, there is interstitial and alveolar edema with hyaline membrane formation in alveoli. There is often interstitial emphysema. Eosinophils and neutrophils may be present within alveolar septa. In more chronic cases, there is diffuse type II pneumocyte hyperplasia and alveolar fibrosis. Secondary suppurative bronchopneumonia can occur. Differential diagnoses include 4-ipomeanol from ingestion of *Fusarium solani*-contaminated sweet potatoes, ingestion of purple mint (*Perilla frutescens*), ingestion of rapeseed and kale (*Brassica* spe-

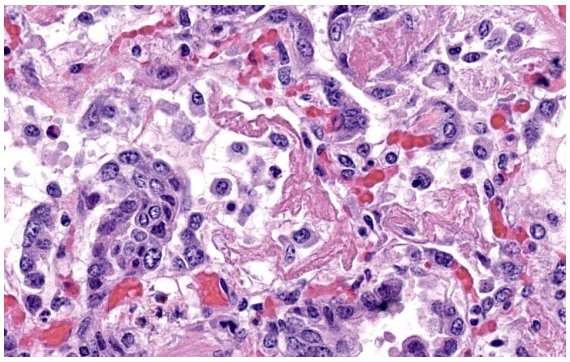

Figure 3-5. Lung, ox. Multifocally, alveoli are filled with polymerized fibrin which occasionally compacts and lines the damaged alveolar septa (hyaline membranes). (HE, 575X)

cies), ingestion of stinkwood (*Zieria arborescens*), extrinsic allergic alveolitis, hypersensitivity to *Dictyocaulus* larvae, and viral pneumonia, particularly that caused by BRSV.

Contributing Institution:
New Mexico Department of Agriculture
Veterinary Diagnostic Services
www.nmda.nmsu.edu/vds

JPC Diagnosis:
Lung: Pneumonia, interstitial, diffuse, severe, with septal necrosis, marked alveolar and interlobular edema, hyaline membranes, and type II pneumocyte hyperplasia.

JPC Comment:
3-methylindole (3-MI) toxicity is a prime example of biotransformation gone bad. Biotransformation refers to metabolic reactions that generally serve to increase the water solubility of compounds and thus enhance their excretion.[4] These reactions transform toxic compounds into benign metabolites and are also critical to the metabolism of endogenous compounds such as cholesterol, steroids, vitamin D, bile acids, and fatty acids.[4] In some instance, however, biotransformation may form reactive intermediates, such as electrophiles and free radicals, that are directly toxic or mutagenic.[4]

Biotransformation is typically described as a phased process, with Phase I reactions consisting primarily of oxidation, reduction, and hydrolytic reactions that cause small changes in the water solubility of the compound.[4] It is the subsequent Phase II reactions where large changes in water solubility are effected, typically via conjugation reactions where a variety of hydrophilic moieties are added to the subject compound.[4] Phase III, the final phase of biotransformation, refers to the transporter-mediated efflux of the metabolite for excretion.

Phase I metabolism is carried out largely through a family of heme-containing enzymes referred to as cytochrome P450s (CYPs). Given its major role in detoxification, the liver expresses high levels of a variety of CYPs located in the smooth endoplasmic reticulum of hepatocytes.[4] In all species, CYPs are more highly expressed in centrilobular zone hepatocytes, leading to centrilobular necrosis when biotransformation creates toxic intermediates. CYPs are not restricted to the liver but are found in a variety of tissues throughout the body. In the lung, CYP2F enzymes are abundant, particularly in the club cells, the nonciliated bronchiolar epithelial cells formerly called Clara cells.[4]

Phase II biotransformation reactions are primarily conjugation reactions that occur in the cytosol. These reactions include glucuronidation, sulfation, and glutathione conjugation, along with less common reactions such as methylation, acetylation, and amino acid conjugation.[4] There are significant species differences in which reactions predominant, particularly with glucuronidation and sulfation reactions. Glucuronidation is the major Phase II metabolic pathway in all mammalian species except for cats, who assert their feline singularity by preferentially using sulfation reactions.[4]

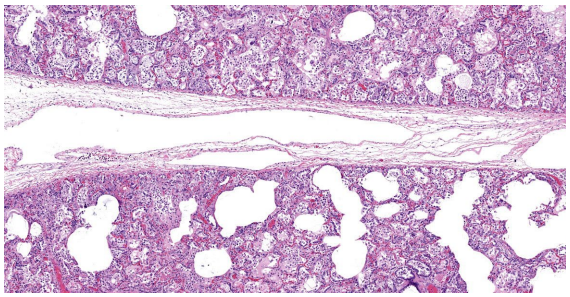

Figure 3-6. Lung, ox. Interlobular septa are markedly expanded by edema. (HE, 47X)

As the contributor notes, the critical step in the pathogenesis of ABPEE is the biotransformation of 3-MI, a relatively inert compound, to a toxic electrophilic intermediate by CYPs in the lung. The resulting toxic intermediate alkylates cellular macromolecules, resulting in lipid peroxidation, membrane damage, and the subsequent necrosis of bronchiolar epithelial cells and type I pneumnocytes.[3] Type II pneumocytes are relatively unaffected despite their high CYP activity due to their high levels of glutathione and phase II enzymes which are thought to protect them from injury.[3] In fact, type II pneumocyte hyperplasia, not necrosis, is a characteristic histologic change of ABPEE as type II pneumocytes proliferate to repair the extensively damage alveolar epithelium.

Dr. Williams felt that this case was a great, straightforward example of atypical interstitial pneumonia in cattle. He noted areas within the lung where the alveolar type I pneumocytes appeared to be replaced with bronchiolar epithelium, a metaplastic change referred to as bronchiolarization or Lambertosis. This change occurs when bronchiolar epithelium attempts to repair alveolar damage by growing out from the bronchioles and into the alveolar sacs and occurs with severe diffuse alveolar damage.

Dr. Williams also noted what appear as undulating nodules of smooth muscle in the wall of the pulmonary vein. This represents a normal spiral of smooth muscle present normally in the pulmonary veins of bovids and should not be confused with hypertensive or neoplastic change. Finally, Dr. Williams noted that edema fluid can drain through lymphatic slits present around the bronchovascular bundle, making this a good location to find siderophages if you suspect heart failure.

Dr. Williams discussed the edema and emphysema noted by the contributor. While the interlobular septa are markedly expanded by clear space, on the examined slide, these spaces appeared largely to be lymphatic channels filled with edema fluid. Dr.Williams interpreted the remaining clear spaces within the pulmonary parenchyma to be alveolar ducts rather than discontinuous alveolar septa. The JPC morphologic diagnosis reflects these interpretations, arrived at after much discussion.

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CASE IV:

Signalment:
10-month-old, male Dorper sheep, ovine (*Ovis aries*)

History:
The farm has a Dorper sheep herd comprising 1500 animals. Four sheep showed clinical respiratory signs characterized by cough and tachypnea. They were administered antibiotics and nonsteroidal anti-inflammatory drugs, but no clinical improvement was noted. Fifteen animals died within 10 days. One sheep was euthanized owing to poor quality of life and underwent necropsy.

Gross Pathology:
Lung: Gross examination revealed bilaterally consolidated areas that were focally extensive, dark red, and firm on palpation, affecting the cranioventral region of the right (40%) and left (10%) lobes. On cut surface, the parenchyma was light brown to dark red and consolidated. A large amount of translucent seromucous fluid was seen at the opening of the bronchi.

Laboratory Results:
Bacteriology: *Pasteurella multocida* and *Mannheimia haemolytica* PCR were negative (lung).

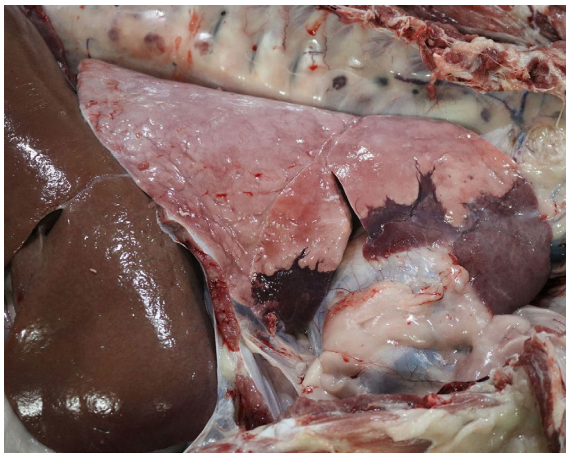

Figure 4-1. Lung, sheep. There is marked consolidation and atelectasis of the ventral aspects of the right cranial, middle and caudal lung lobes. (*Photo courtesy of:* Laboratório de Patologia Veterinária, Faculdade de Medicina Veterinária, Universidade Federal de Mato Grosso, Brazil)

Virology: PCR of the lung tissue was positive for *Mycoplasma* spp. and lentivirus of small ruminants, which were confirmed as *Mycoplasma ovipneumoniae* and Maedi visna using genetic sequencing.

Microscopic Description:
Lung: Alveolar lumina are variably filled by large numbers of neutrophils and macrophages admixed with mild edema affecting 30-40% of the parenchyma within submitted sections. Multifocally, moderate numbers of affected alveoli are lined with plump and cuboidal epithelial cells (type II pneumocyte hyperplasia). Multifocally, the lumens of the bronchi and bronchioles are also filled with variable numbers of neutrophils admixed with fewer macrophages. The submucosa of the bronchi and bronchioles, as well as the adventitia of the blood vessels, are frequently expanded by cuffs of lymphocytes and plasma cells arranged in follicular aggregates.

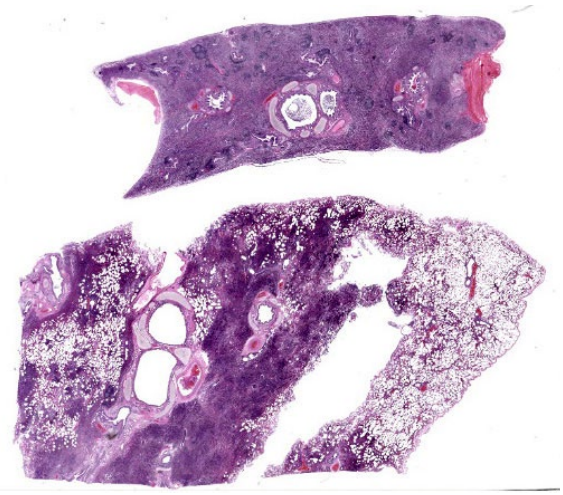

Figure 4-2. Lung, sheep. Two sections of lung are submitted for examination. One section exhibits total consolidation. A second section demonstrates profound peribronchiolar and peribronchial lymphoid hyperplasia. (HE. 6X)

Contributor's Morphologic Diagnosis:
Lung: Bronchopneumonia, suppurative and histiocytic, chronic-active, diffuse, severe, with bronchial epithelial and type II pneumocyte hyperplasia and peribronchiolar lymphofollicular proliferation.

Contributor's Comment:
Maedi-visna (MV), also known as ovine progressive pneumonia (OPP), is an incurable viral disease in sheep with a prolonged incubation period that develops into a life-long infection.[3] This disease is caused by non-oncogenic exogenous retroviruses, namely maedi-visna virus (MVV) and caprine arthritis-encephalitis virus (CAEV), both of which belong to a subgroup of viruses known as small ruminant lentiviruses (SRLVs).[10] For years, lentiviruses isolated in ovines were considered MVV and ones in caprines were considered CAEV, and the two were considered to be species-specific; however, phylogenetic analyses and findings of cross-infection have demonstrated differences in genotypes and lentiviral subtypes that can infect both goats and sheep.[1,5-7,12,14,16,17]

SRLVs exhibit tropism for the mononuclear-phagocyte system and induce slow, chronic, and persistent inflammation mainly in four target organs (the lung, joints, nervous system, and mammary gland), resulting in different clinical phenotypes (i.e., pulmonary, articular, nervous, and mammary, respectively). Interestingly, the occurrence of each clinical form and lesion severity depends on viral factors, as well as the host immune response.[2,4,9,11] In the present case, the lung lesions were typical of SRLVs, characterized by lymphoplasmacytic infiltration in septa with type II pneumocyte hyperplasia and infiltration of lymphocytes in the bronchi and bronchioles, and eosinophilic exudates in alveoli.[3,17]

The farm where the outbreak occurred had a system of extensive breeding without control measures. Ewes and breeding males were acquired from another Brazilian state, Bahia, where seroprevalence studies have demonstrated the presence of SRLV.[15] Thus, it is probable that the purchase of these animals introduced the disease into the herd via asymptomatic animals.

In the present case, *M. ovipneumoniae* was detected by PCR from pulmonary tissue. *M. pneumoniae* is a respiratory bacterium commonly detected in both healthy and diseased lambs.[8] Although speculation, it is possible that Maedi, a retrovirus, may cause immunosuppression, thereby contributing to the establishment of subsequent *ovipneumoniae* colonization.

A definitive diagnosis of maedi was made based on the characteristic lesions identified grossly and histopathologically, along with a supportive clinical history of the disease. The diagnosis was confirmed by molecular techniques.

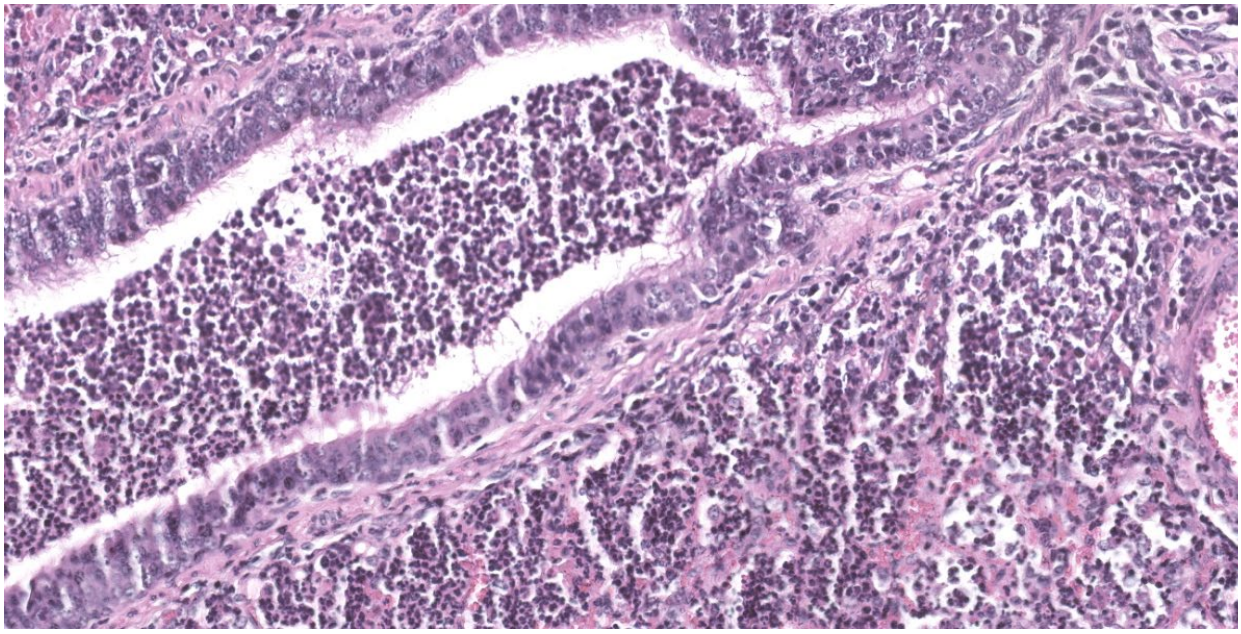

Figure 4-3. Lung, sheep. Airways and surrounding alveoli contain innumerable neutrophils. (HE, 166X)

Contributing Institution:
Laboratório de Patologia Veterinária
Faculdade de Medicina Veterinária
Universidade Federal de Mato Grosso
Brazil.

JPC Diagnosis:
1. Lung: Bronchopneumonia, neutrophilic, diffuse, moderate, with bronchiolar epithelial hyperplasia, alveolar collapse, and peribronchiolar and perivascular lymphoid hyperplasia.
2. Lung: Pneumonia, interstitial, lymphohistiocytic, multifocal, mild.

JPC Comment:
Maedi-Visna virus (MVV) was first described in Iceland in 1954 by Bjorn Sigurdsson, who made history three years later by isolating MVV and thereby becoming the first scientist to isolate a lentivirus. Maedi-visna and Iceland have history that began in 1933 when the country imported several Karakul sheep tasked with improving the native Icelandic sheep breed. Unfortunately, Icelanders had the wool pulled over their eyes as the apparently healthy Karakul managed to introduce ovine pulmonary adenomatosis, pseudotuberculosis, and MVV to the native herds.[18] The long incubation period characteristic of MVV delayed the first clinically apparent epidemic until six years after importation of the sheep, allowing the virus to spread throughout the country undetected and unimpeded for years.[18]

What followed the discovery of MVV was a concerted effort to understand, contain, and eradicate the disease. The disease name originates in the Icelandic words for dyspnea (maedi) and wasting (visna), and the virus was officially designated Maedi-Visna Virus to honor the outstanding efforts of Icelandic scientists.[18] Though it persists throughout most countries in the world today, the disease was eradicated from the Icelandic islands during the 1960s through mass slaughter of sheep on affected farms.

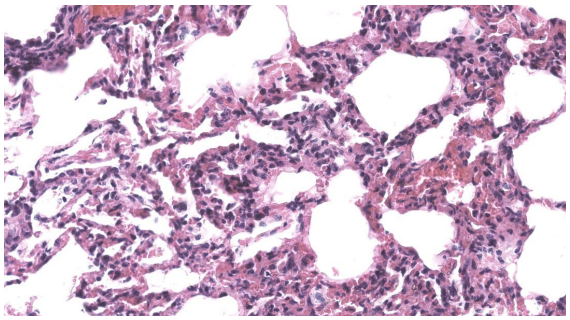

Figure 4-4. Lung, sheep. In areas not totally overrun by the suppurative bronchopneumonia, alveolar septa are expanded by lymphocytes and macrophages. (HE, 166X).

MVV is, in many ways, a typical lentivirus, though it differs from its genus-mates in that it typically does not cause immunosuppression. Lentiviruses are a genus of retroviruses characterized by causing slowly progressing disease. Members of the Retroviridae family are unique in possessing a reverse transcriptase that transcribes the linear, positive-sense single stranded RNA genome into double stranded DNA.[13] This reverse transcriptase is transcribed by one of the three genes that make up the minimum armamentarium of any self-respecting retrovirus: gag, pol, and env. The gag (group-specific antigen) gene encodes structural proteins, the pol (polymerase) gene encodes the reverse transcriptase and integrase enzymes, and env (envelope) encodes the major envelope glycoprotein.[13]

Retroviruses gain entry to host cells through interactions with the envelope glycoprotein and a specific cell receptor that varies depending on the individual virus. Once inside the cell, reverse transcriptase synthesizes double stranded DNA copies of the viral genome in the host which move to the nucleus and are integrated randomly into the host genome via viral integrase.[13] Viral DNA is then transcribed using host cell replication machinery, and mature virions are assembled in the host cytoplasm and acquire an envelope as they bud from the host cell membrane.

Infection with MVV is frequently subclinical, and although virus is widely distributed throughout affected animals, MVV is transmitted mainly in pulmonary exudates, colostrum, and milk.[13] The wide distribution of affected tissues is due to a unique "Trojan horse" pathogenic mechanism where provirus integrated into the genome of monocytes and their precursors is activated only when monocytes develop into macrophages.[13] This restricted viral replication in monocytes permits MVV to transit surreptitiously through the body with minimal immune stimulation.

In conference, Dr. Williams emphasized how much information can be gleaned from subgross examination of this particular slide. The most striking feature is the complete lack of air in the majority of the submitted sections. The affected area is sharply demarcated on the gross aspect (Fig. 4-1) and represents complete alveolar collapse, and Dr. Williams emphasized that these changes should prompt participants to consider small airway disease. Subsequent examination of the bronchioles and surrounding vessels revealed bronchial epithelial hyperplasia and robust smooth muscle within arteriole walls. The arteriolar medial hypertrophy is likely due to the lung's paradoxical response to hypoxia; if the alveoli are not being properly ventilated, endothelin-mediated vasoconstriction attempts to match perfusion with ventilation and shunts the blood away from the ineffectual alveoli. With chronicity, this can result in the arteriolar medial hypertrophy seen throughout this section.

There was robust discussion about ascribing particular histologic lesions to either MVV or *Mycoplasma ovipneumonia* in these particular sections. Generally, MVV should cause BALT hyperplasia and lymphohistiocytic interstitial pneumonia, while *M. ovipneumonia* should cause airway epithelial hypertrophy

and an exudative neutrophilic bronchitis and bronchiolitis. Participants believed that the majority of the histologic lesions were likely attributable to *Mycoplasma ovipneumonia*, as the section was dominated by airway disease and BALT hyperplasia. In less affected areas of the lung, where alveoli were inflated and septa could be properly evaluated, a mild interstitial lymphohistiocytic pneumonia was present, though this was deemed a minor lesion in the evaluated section.

Participants also noted the history, which describes a quickly moving epidemic, and the young age of the animal. Both factors are more consistent with *Mycoplasma ovipneumonia* disease since MVV typically only causes clinical signs in animals many years post-infection. Participants vigorously discussed combining all histologic changes into a single morph; however, a reluctant, sheepish consensus was eventually reached to split the lesions into multiple morphologic diagnoses. The first diagnosis emphasizes participants' assessment that the major histologic lesions were attributable to *Mycoplasma ovipneumonia*, while the second diagnosis emphasizes the mild interstitial disease that is potentially attributable to MVV.

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