Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #5 — 21 September 2022

CASE I:

Signalment:
15-month-old, female, French bulldog, *Canis lupus familiaris*

History:
Fifteen month old French bulldog with chronic diarrhea for months that occasionally contained blood but not always. Some weight loss with a current BCS 4.5/10. Fecal smear was positive for giardia. Treated with fenbendazole / metronidazole. No more blood reported in stools but still chronic diarrhea. The dog was euthanized and the necropsy revealed a very thickened large intestine and distal small intestine. Owner says they have had other dogs with similar symptoms without any successful treatment.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Within the submitted and examined sections of colon the mucosa and underlying submucosa is markedly expanded by a dense infiltrate composed of large numbers of macrophages admixed with lesser numbers of neutrophils, lymphocytes, and plasma cells. These macrophages often contain abundant vacuolated cytoplasm with karyorrhectic debris and rare intracytoplasmic basophilic rod-shaped bacteria. There is also multifocal to coalescing ulceration of the mucosa that varies from superficial to nearly full thickness. The deep mucosa and submucosa underlying the most severely affected areas contains increased numbers of neutrophils. The colonic glands are mildly hyperplastic with evidence of regeneration including piling of glandular epithelium, karyomegaly, increased cyto-

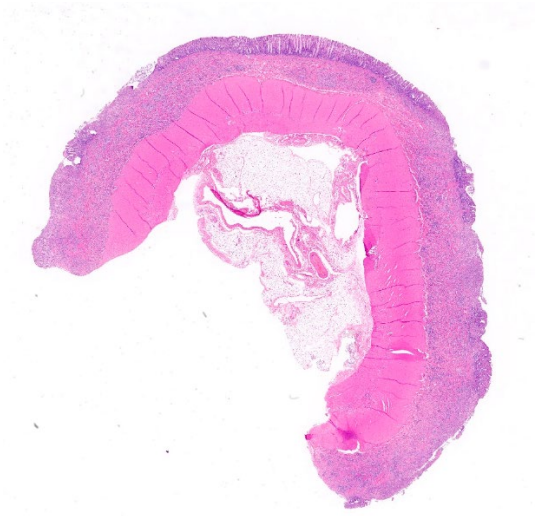

Figure 1-1. Colon, French bulldog. Multifocal ulceration and erosion of the colonic mucosa along with an expanded submucosa with marked increased cellularity. (HE, 5X) (Photo courtesy of: Kansas State Veterinary Diagnostic Laboratory (KSVDL), http://ww.ksvdl.org/)

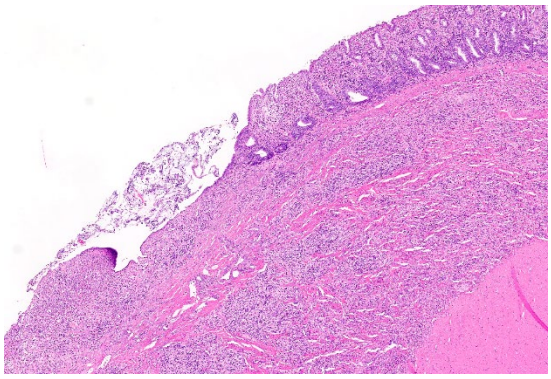

Figure 1-2. Colon, French bulldog. Area of mucosal ulceration with underlying diffuse infiltration of the submucosa. The adjacent intact mucosa is also expanded by a marked cellular infiltrate. (HE, 40X) (Photo courtesy of: Kansas State Veterinary Diagnostic Laboratory (KSVDL), http://ww.ksvdl.org/)

plasmic basophilia, and frequent mitotic figures. Occasionally these glands contain small numbers of intraluminal spirochete-like organisms.

The macrophages present within the mucosa and submucosa contain frequent PAS positive material within their cytoplasm.

Contributor's Morphologic Diagnoses:
Colon: Histiocytic and ulcerative colitis, multifocal to coalescing, severe

Contributor's Comment:
Histiocytic ulcerative colitis (HUC), also known as granulomatous colitis (GC), is a chronic enteropathy of dogs that was first reported in 1965.[15] It is histologically characterized by marked infiltration of the colonic mucosa and submucosa by large, periodic acid-Schiff (PAS)-positive macrophages; these are typically accompanied by mucosal ulceration, loss of goblet cells[8], and infiltrates of lymphocytes, plasma cells, and neutrophils.[1,13,15] Histopathological demonstration of macrophages with intracytoplasmic PAS positive material has been accepted as the pathognomonic feature of HUC[4,10] and the PAS positive material is considered to be glycoprotein from bacterial cell walls.[9]

Although this condition has nonspecific gross lesions, findings in previously reported cases include reduction in the colonic length, eccentric thickening of the intestinal wall, and

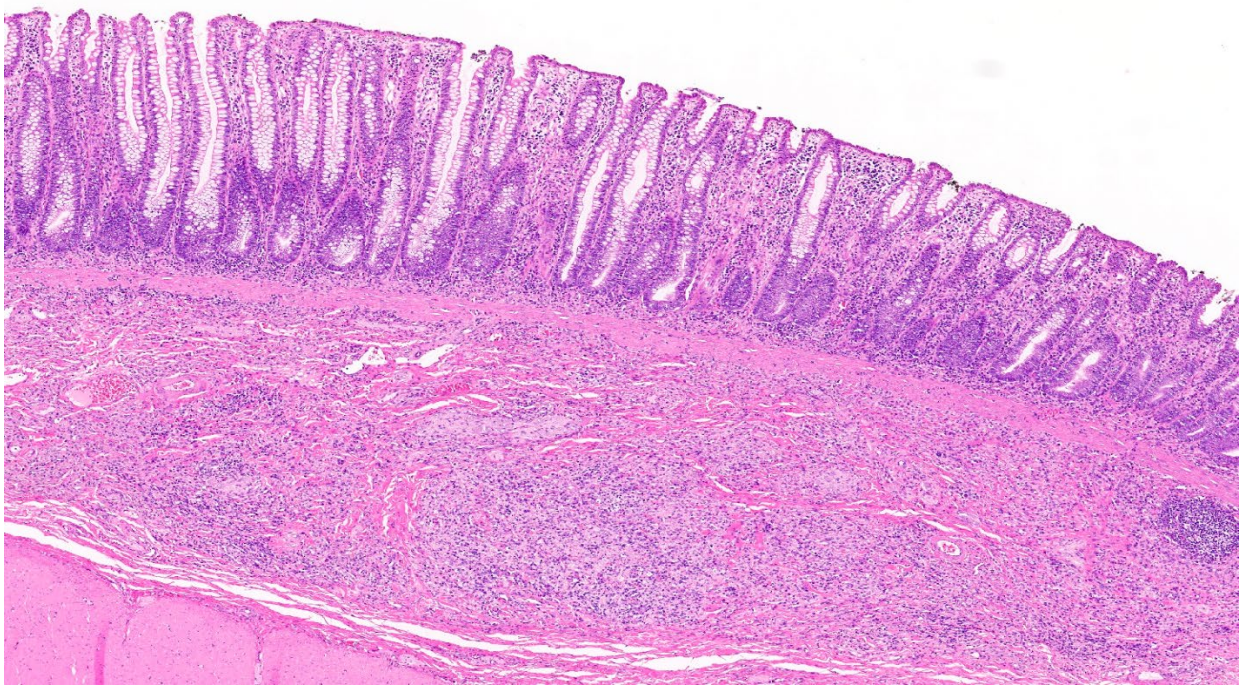

Figure 1-3. Colon, French bulldog. Intact mucosa with glandular hyperplasia and a cellular infiltrate within the lamina propria and submucosa. (HE, 4.5X) (Photo courtesy of: Kansas State Veterinary Diagnostic Laboratory (KSVDL), http://ww.ksvdl.org/)

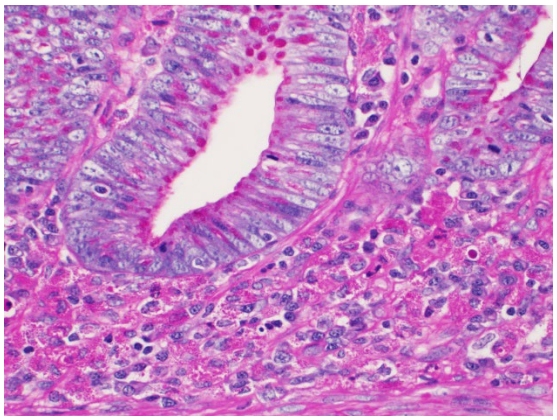

Figure 1-4. Colon, French bulldog. Eosinophilic intracytoplasmic material is contained within histiocytes in the lamina propria. (PAS, 400X)

multifocal to coalescing areas of ulceration that can be present in the colon, cecum and rectum.[2,4,14] Clinical features include chronic diarrhea, increased frequency of defecation, and feces that are soft, tan, granular, glistening, and sometimes or always contain blood. Affected dogs are afebrile and late in disease often develop dehydration with weight loss.[16]

HUC has been described most commonly in young Boxers and French Bulldogs, although it has been sporadically reported in a variety of other breeds; Mastiff, Alaskan Malamute, Doberman Pincher, American Staffordshire Terrier[1], and Beagle.[2,13,14] It has also rarely been reported in cats, including a Persian-crossbred and two domestic shorthaired cats.[6,9,15]

HUC was previously regarded as an idiopathic immune-mediated disease[14], and although the exact cause and pathogenesis are still not well understood, recent reports have identified *Escherichia coli* in both canine[1,2,5,7,8,11] and feline cases.[6,9] In both species, clinical remission as well as histologic resolution has been described after eradication of the invasive *E. coli* by use of antibiotics including Enrofloxicin.[8,9] The response to these treatments varies by case and antibacterial resistance, especially to enrofloxacin, can affect the outcome with nonresponsive or refractory cases.[14] Thus, the regression of clinical and histologic lesions suggest that *E. coli* has a critical role in the development of HUC.[9] The predisposition of Boxers to HUC is thought to be due to a heritable anomaly that confers susceptibility to the invasion and persistence of an adherent and invasive group of *E. coli.*[1] The rarity of disease in cats may suggest variation in hereditary and host susceptibility to *E coli*-induced HUC between dogs and cats.[9]

Contributing Institution:
Kansas State Veterinary Diagnostic Laboratory (KSVDL)
http://ww.ksvdl.org/

JPC Diagnosis:
Colon: Colitis, histiocytic and ulcerative, diffuse, marked, with glandular hyperplasia.

JPC Comment:
While the presence of PAS-positive cytoplasmic material in macrophages on histopathology is virtually pathognomonic for histiocytic ulcerative colitis, fluorescent in-situ hybridization (FISH) is the definitive diagnostic test for identifying *E. coli* in this condition.[3, 8, 12] This technique uses fluorescent probes to specifically target and hybridize with *E. coli*

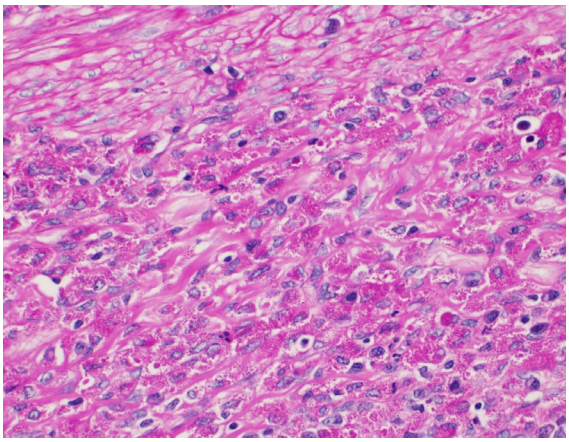

Figure 1-5. Colon, French bulldog. Eosinophilic intracytoplasmic material is contained within histiocytes in the submucosa. (PAS, 40X)

16S ribosomal rDNA, thus enabling identification and localization of the bacteria within HUC lesions.[8] In addition to allowing direct observation of *E. coli* within macrophages, FISH has several methodological advantages: it can be conducted on formalin-fixed paraffin-embedded sections, it is not confounded by normal flora present in the colon, and it can detect multiple bacterial species at once.[12]

Two recent reports have described the correlation of histologic and cytologic findings in two dogs with HUC.[3, 12] Both dogs had a history of chronic diarrhea and hematochezia and were painful with thickened, irregular mucosa on rectal exam.[3, 12] Rectal scrapings in both dogs revealed mixed inflammation and occasionally macrophages contained pink cytoplasmic granules or phagocytosed bacteria.[3, 12] In one case, the pink cytoplasmic material was confirmed to be PAS-positive.[3] In both cases, HUC was confirmed with histopathology and FISH.[3, 12] These are the first published reports of cytologic findings of HUC, so the sensitivity and specificity of rectal scrapings for this condition is unknown.[12] The results suggest, however, that cytologic examination may be helpful as an initial test for guiding further diagnostics, particularly since it is non-invasive, economical for owners, and does not require a specialized laboratory for analysis.

References:

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CASE II:

Signalment:
Two 6-month-old, black, American mink (*Neovison vison*), one male and one female.

History:
A farm of approximately 50,000 all black, American mink experienced increased mortality within two shelters. Approximately 15-20 animals died over the course of two days, with two submitted for necropsy.

Gross Pathology:
Two reportedly six-month-old, black mink weighing 1.9 kg (female) and 2.4 kg (male) were necropsied. The bodies of both were in good condition (BCS 3/5) and postmortem autolysis was moderate. The lungs in both animals were diffusely firm and mottled bright red to purple with areas of paler color. At least one section of lung from both animals sank in formalin; other sections floated.

Laboratory Results:
Black mink, female, lung tissue, Aerobic culture: 4+ *Pseudomonas aeruginosa*.

Black mink, male, lung tissue, Aerobic culture: 4+ *Pseudomonas aeruginosa* - sensitivity performed on female, see Table 2-1.

Microscopic Description:
Lung. Sections of lung in which there is diffuse, coagulative necrosis and suppurative inflammation effacing up to 90% of the pulmonary parenchyma. These areas contain necrotic cell debris, seroproteinaceous fluid, neutrophils and myriad clusters of small, gram-negative bacteria. In nearby alveoli, there is intraalveolar hemorrhage. Several bronchioles are occluded or partially occluded by aggregates of fibrin and neutrophils, and cell debris.

Contributor's Morphologic Diagnoses:
Lung: Bronchopneumonia, severe, acute, diffuse, hemorrhagic, fibrinosuppurative.

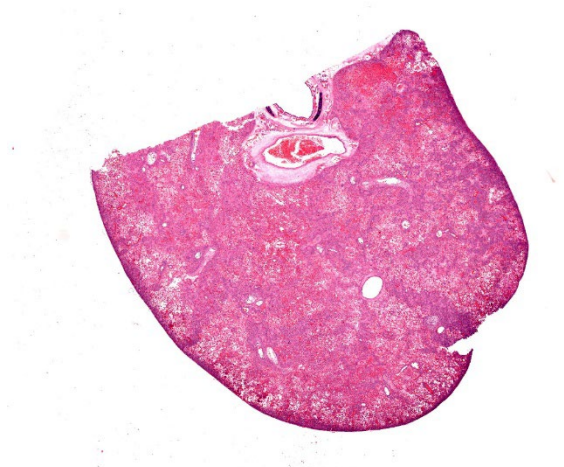

Figure 2-1. Lung, mink. A single, diffusely consolidated section of lung is submitted for examination. (HE, 5X)

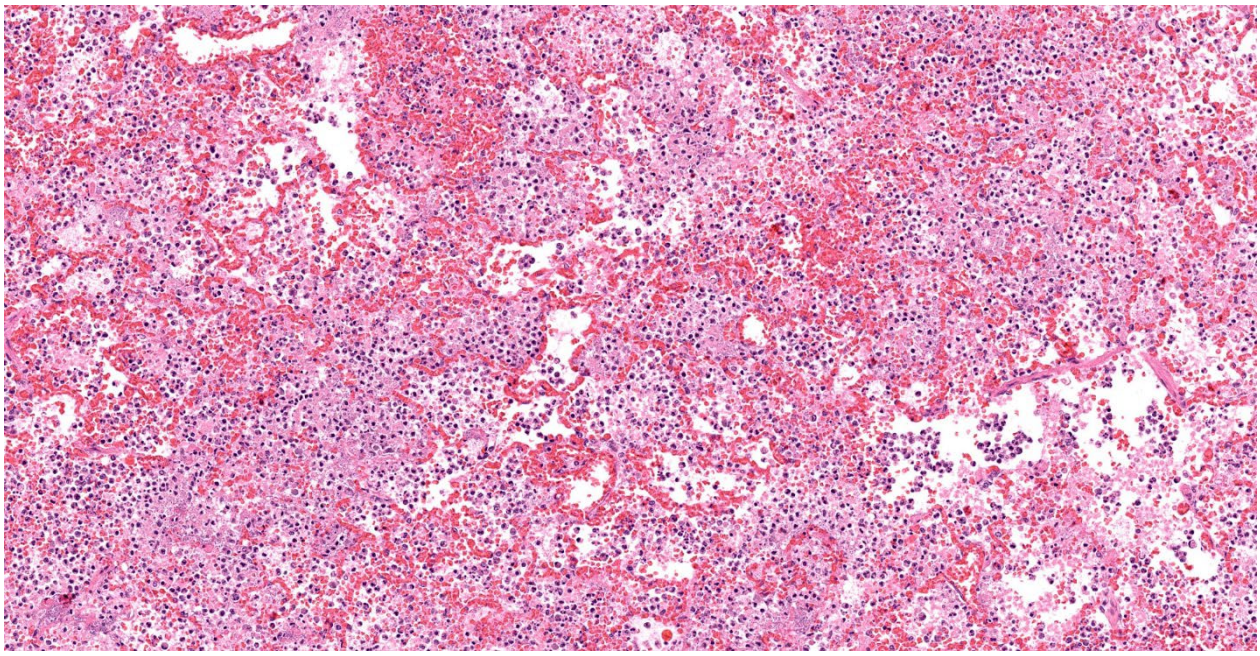

Figure 2-2. Lung, mink. Alveoli are filled with variable combinations and concentrations of viable and necrotic neutrophils, fewer macrophages, cellular debris, fibrin, and edema, and innumerable bacterial rods. (HE, 154X)

Contributor's Comment:

Pseudomonas aeruginosa is a known cause of contagious, hemorrhagic bronchopneumonia in farmed mink, although most mustelids are susceptible.[4] It was first described in 1953.[10] It is a seasonal disease most commonly seen in September to November. It is often acute and fatal and some mink can be found with blood around the nostril and mouth without previous signs of illness.[17,18] Experimentally, the organism can be carried in the nasal cavity without causing disease. There are various serotypes and genotypes and many of these form biofilms.[14,19] Also, numerous vaccines and other therapeutic strategies have been investigated.[8] In one study, Phage YH30 delivered intranasally reduced disease severity.[7] Antibiotic resistance is common in *Pseudomonas* spp. and can vary geographically and temporally; antibiotic resistance and sensitivities seen here are different than those previously reported.[14] Outbreaks of hemorrhagic pneumonia have been associated with hemolytic *Escherichia coli* infection as well.[16]

ANTIBIOTIC	MIC (µG/ML)	INTERPRETATION
AMIKACIN	< 1.000 µg/ml	Sensitive
AMOXICILLIN/CLAVULANIC ACID	>64.000 µg/ml	Resistant
AMPICILLIN	>48.000 µg/ml	Resistant
CEFPODOXIME	>64.000 µg/ml	Resistant
CEPHALOTHIN	>128.000 µg/ml	Resistant
CHLORAMPHENICOL	32.0 µg/ml	Intermediate
CLINDAMYCIN	>12.000 µg/ml	Resistant
ENROFLOXACIN	0.4 µg/ml	Sensitive
GENTAMICIN	1.5 µg/ml	Sensitive
IMIPENEM	< 0.750 µg/ml	Sensitive
MARBOFLOXACIN		Sensitive
ORBIFLOXACIN	2.0 µg/ml	Intermediate
TETRACYCLINE	16.0 µg/ml	Resistant
TMP/SULFA	>12.000 µg/ml	Resistant
TOBRAMYCIN	< 0.380 µg/ml	Sensitive

Table 2-1

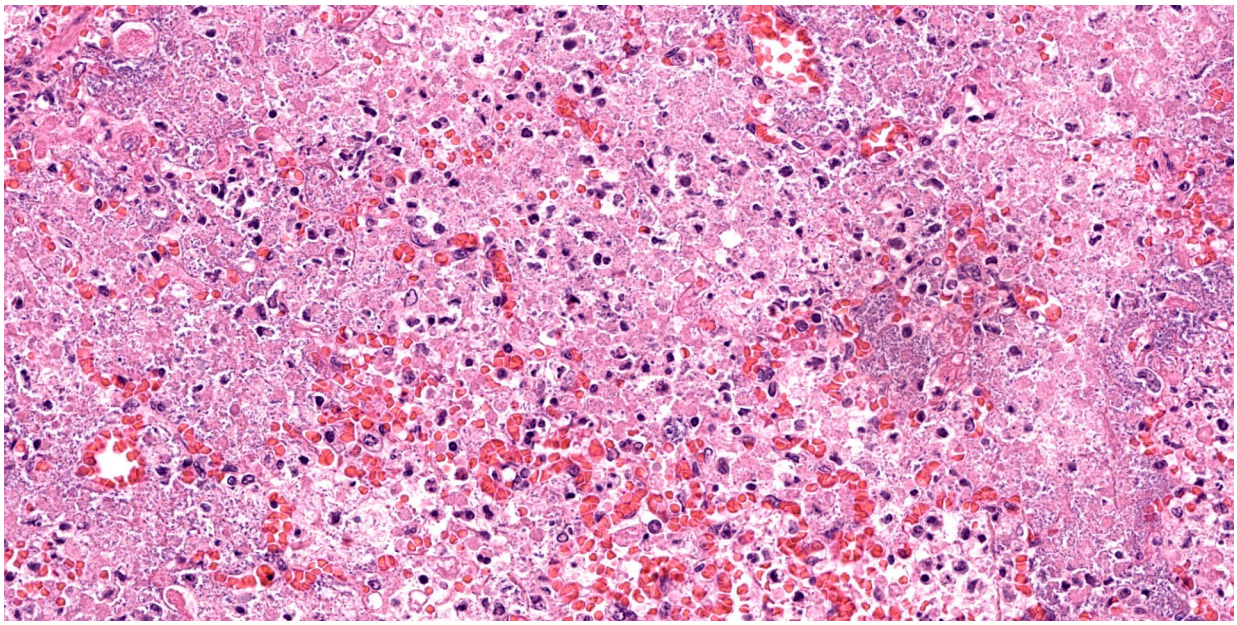

Figure 2-3. Lung, mink. There are areas of septal necrosis scattered throughout the section. Septa are discontinuous with multifocal hemorrhage and exuded fibrin, not to mention the large numbers of bacilli within the expanded alveoli. (HE, 381X)

Contributing Institution:
Oregon State University, Carlson College of Veterinary Medicine, Veterinary Diagnostic Laboratory
https://vetmed.oregonstate.edu/diagnostic

JPC Diagnosis:
Lung: Pneumonia, fibrinosuppurative, diffuse, severe, with septal necrosis, vasculitis and innumerable perivascular and alveolar bacilli.

JPC Comment:
Jubb, Kennedy, and Palmer's Pathology of Domestic Animals delineates four main morphologic types of pneumonia: bronchopneumonia, interstitial pneumonia, bronchointerstitial pneumonia, and embolic pneumonia.[2]

Bronchopneumonia is caused by aerogenous delivery of bacteria to the terminal bronchioles, causing the hallmark lesion of an exudate at the broncho-alveolar junction.[2] Exudates can be found in the bronchi, bronchioles, and alveoli, but the bronchiolar epithelium is typically normal.[2] The distribution is typically cranioventral, and affected lobes are firm.[2] Bronchopneumonia may be caused by opportunistic pathogens during times of immunosuppression, impaired pulmonary defenses (i.e. viral or *Mycoplasma* infection), or exposure to overwhelming numbers of bacteria. [2]

Interstitial pneumonia is characterized by inflammation and damage within the alveolar septae and the adjacent terminal bronchioles.[2] Interstitial pneumonia commonly features diffuse alveolar damage, or damage to pneumocytes and/or alveolar capillary endothelial cells.[2] Acutely, this leads to exudation and hyaline membrane formation followed by type II pneumocyte hyperplasia, all of which interfere with gas exchange.[2] Pulmonary fibrosis follows in the chronic phase of interstitial pneumonia.[2] Grossly, affected lungs will fail to deflate and may have rib imprints and a rubbery texture.[2] There are many subtypes and causes interstitial pneumonia, which include viral infections, inhalation of toxic gases or fumes, ingestion of certain toxins, ascarid larval migration, sepsis, and disseminated intravascular coagulation.[2] In bronchinterstitial pneumonia, there is bronchiolar damage and necrosis in addition to the

diffuse alveolar damage characteristic of interstitial pneumonia.[2]

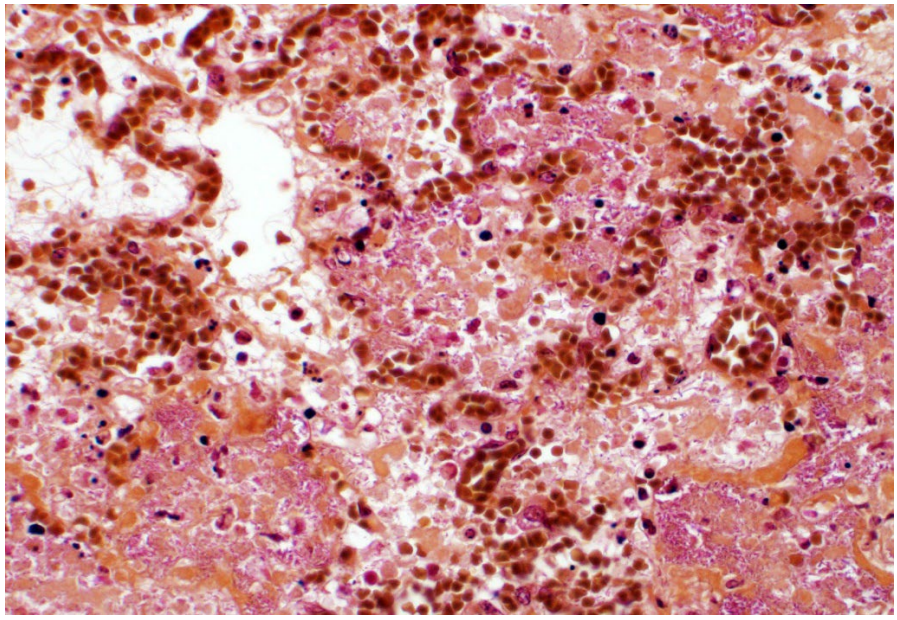

Figure 2-4. Lung, mink. The large numbers of bacilli within the alveoli stain positively as gram negative on a tissue Gram stain. (Brown and Hopps, 400X)

Embolic pneumonias are caused by hematogenous showering of bacterial (or inflammatory) emboli from a nidus of infection resulting in multifocal lesions throughout all lung lobes.[2,12] Embolic pneumonia frequently features pulmonary abscess formation.[2] While abscesses can also occur in bronchopneumonia, the cranioventral distribution distinguishes it from embolic pneumonia.[2] Possible sources of emboli include hepatic abscesses, omphalophlebitis, or endocarditis, and embolic pneumonia has been described secondary to *Pseudomonas aeruginosa* in a puppy with necrotizing enteritis.[12]

The distribution of histologic lesions led to spirited discussion among conference participants regarding the correct morphologic classification of this pneumonia. The pathogenesis of *Pseudomonas* pneumonia in mink is supports a diagnosis of bronchopneumonia, with aerogenous delivery of bacteria to the lower airways.[12] In this case, however, much of the necrosis and bacterial colonies are centered on blood vessels, leading a few participants to consider embolic pneumonia as a morphologic pattern. The contributor did not describe any possible nidus of infection in this case, and perivascular localization of bacteria is characteristic in *Pseudomonas* pneumonias of both mink and rats.[16] Since the pathogenesis and histologic appearance do not fit into one precise category, participants decided not to further subtype the morphologic diagnosis beyond pneumonia.

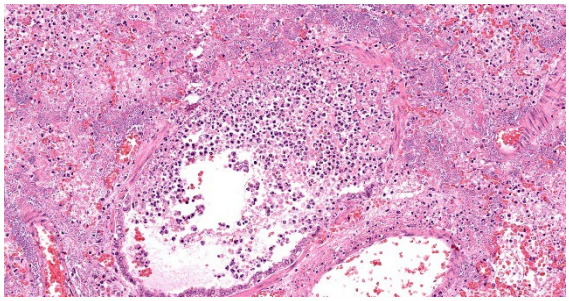

Figure 2-5. Lung, mink. Airways contain aggregates of neutrophils which infiltrate segmental areas of mural necrosis and loss of epithelium. (HE, 385X)

Pseudomonas aeruginosa is typically an opportunistic pathogen which causes disease in immunocompromised animals or animals with surgical implants.[11] The bacteria thrives in moist environments, such as water bottles in laboratory animal enclosures, and gains entry through the gastrointestinal tract or by penetrating inflamed or injured oronasal mucosa or intertriginous skin.[1,11] In immunocompromised mice and rats, infection can lead to bacteremia and gram-negative septicemia with vasculitis, thrombosis, and necrosis in multiple organs, including the lung, liver, spleen, and kidneys.[1] The bacteria can also lead to deep pyoderma, septic arthritis,

discospondylitis, otitis, and a number of other conditions.[1,6,11] Due to the bacteria's propensity for moist environments, improperly chlorinated pools and hot tubs can harbor *P. aeruginosa*, and human skin can be inoculated by abrasive surfaces, resulting in two self-limiting but painful conditions: acute folliculitis and pseudomonas hot-foot syndrome (nodules of suppurative inflammation on the hands and feet).[5]

The pathogenicity of *P. aeruginosa* is determined by a number of virulence factors, including exotoxins which are toxic to macrophages and epithelial cells, a type III secretion system for injecting proteins directly into cells, elastase which destroys pulmonary microbe clearance mechanisms like mucin, and immunomodulatory alkaline protease A.[11,15] *P. aeruginosa* also produces two relatively unique virulence factors: pyocyanin, a redox metabolite, and pyoverdine, a siderophore.[9] These virulence factors are pigments that can impart a blue-green discoloration to superficial infections.[9] Rabbits with moist dermatitis and *P. aeruginosa* develop blue-green tinged fur.[12] In humans, *Pseudomonas* can cause green nail and green foot syndromes, and, when associated with intertrigo, causes blue-green staining of undergarments.[9] Indeed, when *P. aeruginosa* was first cultured from blue-green pus in superficial wounds in the 1880's, it was aptly dubbed *Bacillus pyocyaneus*, or "bacteria of blue pus".[19] The current name also references this distinctive hue: aerugo is Latin for copper rust, which is characteristically green.[19]

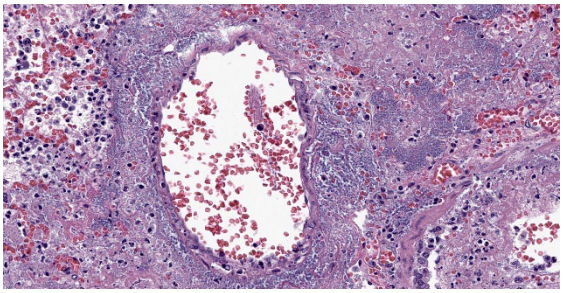

Figure 2-6. Lung, mink. Vessel adventitia is markedly expanded by bacilli which fill adjacent alveoli as well. (HE, 375X)

References:

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CASE III:

Signalment:
Five year old intact male ferret (*Mustela putorius furo*), mustelid.

History:
This is the second ferret from a rescue facility to present to the referring veterinarian in a severely debilitated state, emaciated, dehydrated and hypothermic. The ferret was euthanized.

Gross Pathology:
This male ferret was in emaciated body condition, with a poor, sparse hair coat and hair loss of the distal tail. Internally, there were still some subcutaneous adipose stores in the inguinal fatpad, around the kidneys and in the mesentery, however there was generalized

muscle wasting. The heart was globose. The adrenal glands were small and symmetrical. Small intestinal content was scant, and there were soft dark brown feces in the colon.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Small intestine: Diffusely throughout the section, there is villus blunting and fusion with loss of goblet cells and marked cryptal epithelial hyperplasia, characterized by elongated and tortuous crypts lined by tall columnar epithelial cells with abundant lightly eosinophilic cytoplasm and elongate vesicular nuclei. The crypt epithelium is disorganized and occasionally piles up to five cells thick at the base of the crypts. There is frequent single cell death within the mucosal epithelium, characterized by individualized and hypereosinophilic enterocytes with fragmented or condensed nuclei; mitotic figures are also frequent. Occasional crypt lumens are dilated, lined by attenuated low columnar to cuboidal epithelium, and contain necrotic cellular debris or mucinous fluid. The lamina propria and submucosa are variably expanded by clusters of lymphocytes, plasma cells and macrophages. The villus epithelium is predominantly intact, multifocally attenuated at the surface, and contains scattered apicomplexan organisms of various life stages, including meronts, macrogametocytes, microgametocytes and occasional oocysts visible within the luminal debris.

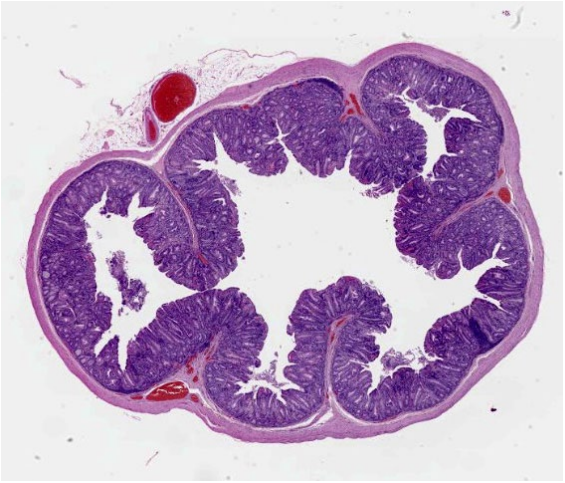

Figure 3-1. Colon, ferret. One section of colon with marked thickened and rugose mucosa is submitted for examination. (HE, 7X)

Warthin Starry silver stains of the intestine reveal numerous argyrophilic curved small (approximately 5 x 1 micron) bacilli concentrated in the apical cytoplasm of hyperplastic mucosal epithelial cells. Immunohistochemistry was performed using a polyclonal antibody to *Lawsonia intracellularis*, confirming the identity of the bacteria.

DNA was extracted from the frozen intestine and from formalin-fixed paraffin-embedded tissue scrolls. PCR was conducted to amplify short regions of the nuclear 18S rDNA (231 bp) and mitochondrial cytochrome c oxidase subunit I (COI) (512 bp). PCR and sequencing of the resulting amplicons identified the coccidia present as *Eimeria furonis*. In addition, measurement of 6 oocysts observed in the intestinal content averaged 12.1 x 10.6 µm with a shape index of 1.14. These measurements are also consistent with the dimensions of oocysts of *E. furonis*.

Contributor's Morphologic Diagnoses:
Enteritis, proliferative, chronic, moderate with villus atrophy, crypt abscesses and numerous argyrophilic intraepithelial bacilli (*Lawsonia intracellularis*), and intralesional coccidia (*Eimeria furonis)*

Contributor's Comment:
The debilitated state of this adult ferret is associated with concurrent infection by *Lawsonia intracellularis* and the coccidian parasite, *Eimeria furonis*.

The histologic lesions in this case are dominated by changes due to infection by *Lawsonia intracellularis*. *L.* intracellularis is a

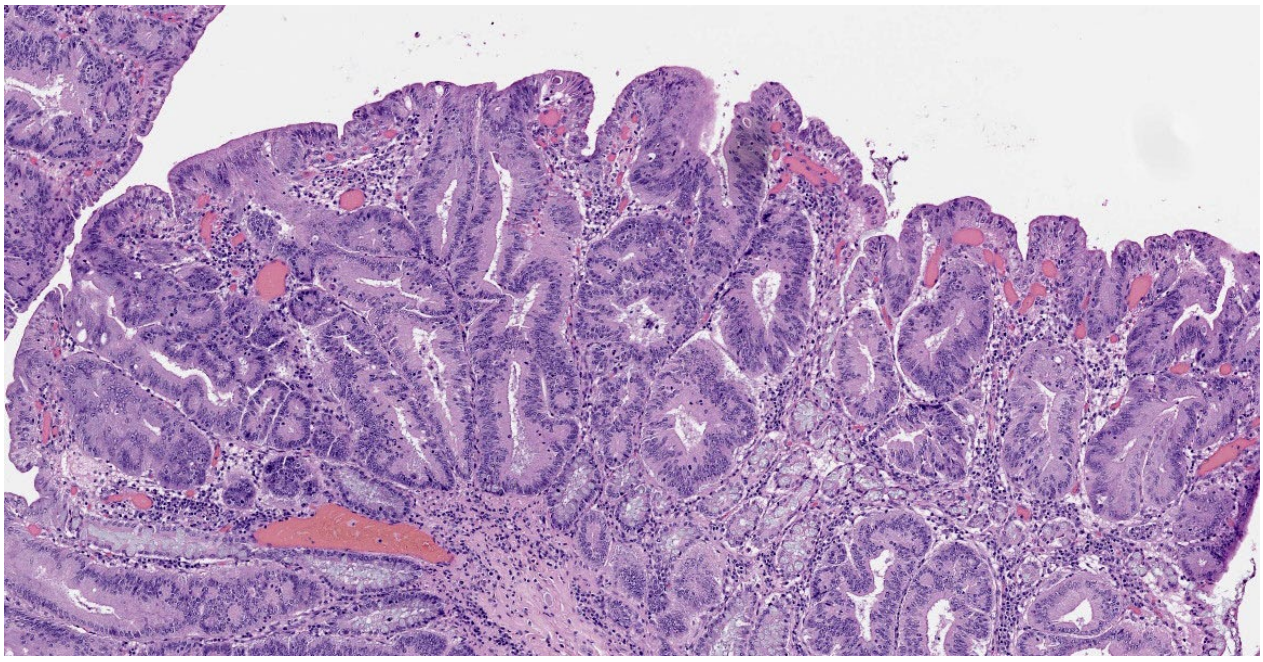

Figure 3-2. Colon, ferret. Colonic glands are hyperplastic and lack goblet cells. Hypercellularity is evident in the lamina propria. (HE, 60X)

gram-negative, non-spore-forming, microaerophilic, curved rod, and is an obligate intracellular pathogen. It is the etiologic agent of proliferative enteropathy (PE), characterized clinically by diarrhea associated with loss of functional mucosal surface area, as well as wasting due to protein-losing enteropathy. Histologically, PE is characterized by thickening of the distal small and/or large intestinal mucosa due to crypt enterocyte infection and proliferation. PE is an important endemic disease in swine herds and is also becoming an important disease of horses, predominantly weanling foals, worldwide. However, the bacterium can cause disease in a broad host range including donkeys, hamsters, rabbits, ferrets, foxes, dogs, rats, sheep, deer, emus, ostriches, nonhuman primates and guinea pigs.[6] There are some differences in clinical and pathologic presentation of disease among affected species. Mucosal hyperplasia is characteristic of PE in all species, however the type and extent of associated inflammatory reactions vary and may depend on host-specific differences.[6] The bacterium infects intestinal crypt cells, causing alterations in host gene expression resulting in induction of cell proliferation and mucosal thickening, as well as inhibition of secretory goblet cell and absorptive enterocyte differentiation leading to altered mucosal integrity.[2] These changes are associated with simultaneous induction of Notch-1 signalling and attenuation of B-catenin/Wnt pathways.[2]

Several species of coccidia have been reported to infect the intestinal tract of the ferret, including *Isospora laidlawi*, *I. eversmanni*, *Eimeria ictidea*, *E. furonis,* and *E. vision*. *Emeria hiepei* also has been reported to infect the biliary epithelium. *E. furonis* is the most commonly identified species, and is generally associated with subclinical infections. *E. furonis* infects the small intestinal and rectal epithelium although there is a single case report of infection of the epithelium of hepatic bile ducts and gallbladder.[5] Speciation of *Eimeria* is based on differentiating characteristics including host affected, specific location of the parasite within the host, and organism size and shape. This *Eimeria* species has approximately 12.8 x 12.0 µm diameter, roughly spherical oocysts containing

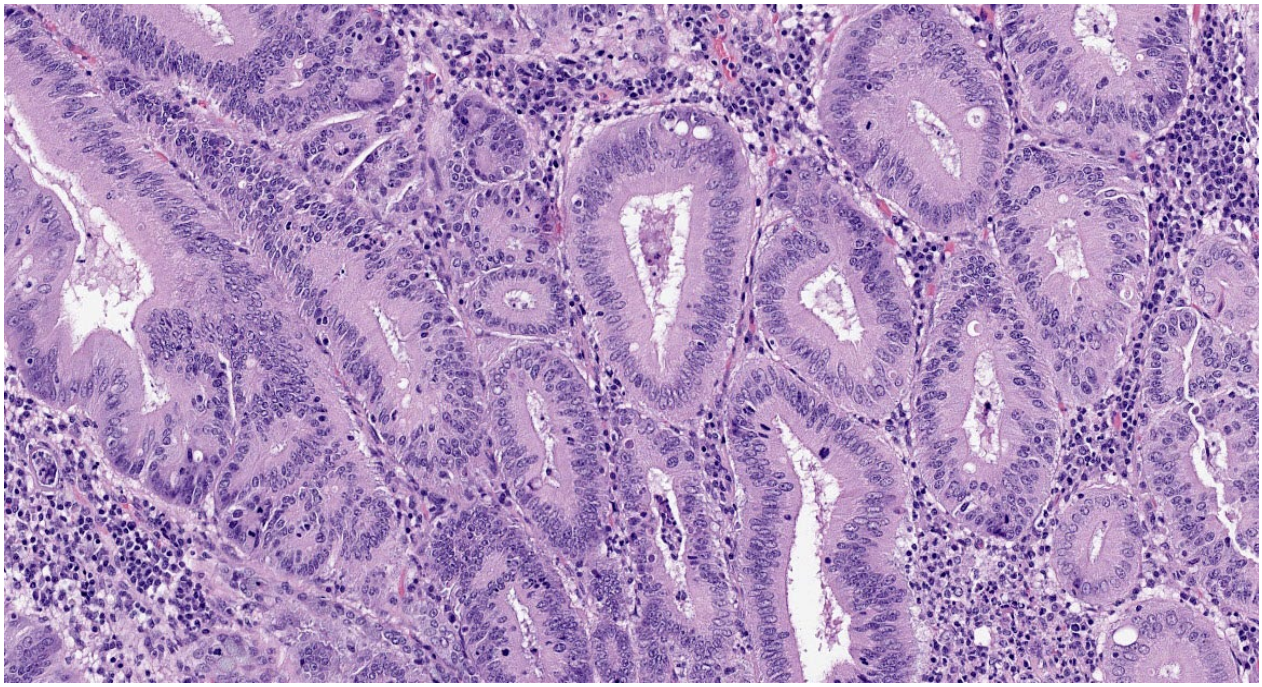

Figure 3-3. Colon, ferret. Colonic glands are tortuous with cellular basophilia, nuclear crowding, and numerous mitotic figures. Goblet cells are absent. (HE, 194X)

4 sporocysts each with 2 sporozoites. By comparison, *E. ictidea*, a closely related species, has oocysts measuring 23 x 17.5 µm. Laboratory identification can be assisted by sporulating viable oocysts from fresh feces in the laboratory, or using molecular techniques.[1]

Although historically rarely associated with clinical disease in ferrets, a recent report by Sledge et al. documents severe outbreaks of enteric coccidiosis due to *E. furonis* in three ferret rescue shelters, a situation similar to the case described here.[5] Clinical signs in these groups of affected animals included foul-smelling diarrheic feces, melena, lethargy, weight loss, dehydration, anorexia and weakness. In one of the reported outbreaks in a shelter population of 42 ferrets, greater than half were clinically affected and 7 died, while in a second group of 63 ferrets, 13 died and at least 21 additional animals displayed clinical signs but recovered. No other co-pathogens were discovered in these groups in this study. The authors suggest that high population density and the dynamic population structure of these groups, with regular introductions of newly rescued or adopted naïve animals may have predisposed to outbreak situations. Diagnosis can be challenging, as shedding of oocysts may be intermittent and may occur in low enough numbers to be not easily detected by routine fecal examination.

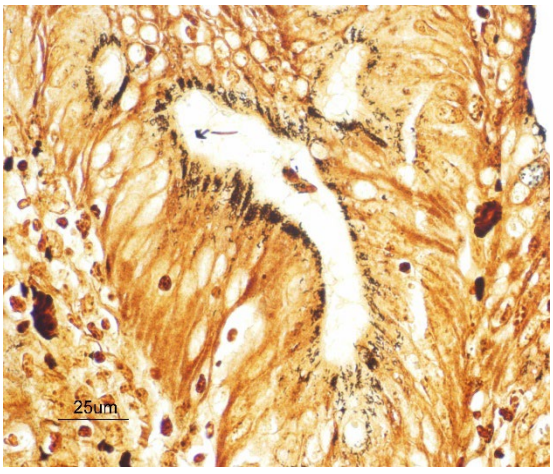

Figure 3-5. Colon, ferret. Warthin Starry silver stains of the intestine reveal numerous argyrophilic curved small bacilli concentrated in the apical cytoplasm of hyperplastic mucosal epithelial cells. (WS, 600X) (Photo courtesy of: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada http://ahl.uoguelph.ca)

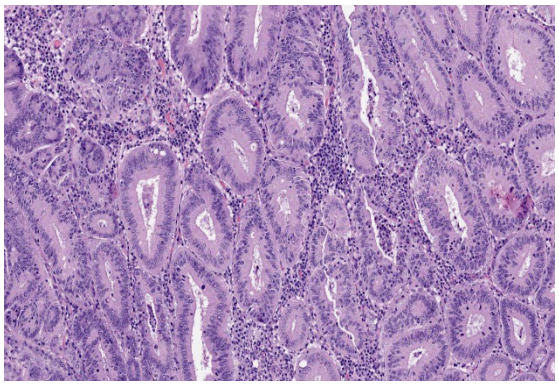

Figure 3-4. Colon ferret. Glands occasionally contain necrotic epithelial cells admixed with few neutrophils and cellular debris (crypt abscesses). The lamina propria is expanded by moderate numbers of neutrophils, lymphocytes and plasma cells. (HE, 194X)

Contributing Institution:
Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada
http://ahl.uoguelph.ca

JPC Diagnosis:
1. Colon: Colitis, proliferative, diffuse, severe with decreased goblet cells.
2. Colon: Intraepithelial apicomplexan schizonts, gamonts, and oocysts, few.

JPC Comment:
Proliferative enteropathy is an economically important disease of swine production, with an estimated 96% of farms affected and a lost production cost of US $1 to $5 for every clinically affected pig.[7] In swine, *Lawsonia intracellularis* infection can cause an acute hemorrhagic enteropathy; in chronic cases, it can cause proliferative intestinal adenomatosis or, in cases of secondary infection, can progress to necrotic enteritis.[7] Pigs are infected through ingestion of the bacteria, which survive the acidic environment of the stomach and, once in the intestine, use polar flagella to reach the enterocytes.[3, 7] The exact mechanism by which the bacteria enters enterocytes is unknown but may be mediated by a type III secretion system (T3SS).[7] The bacteria proliferates by binary fission in the apical cytoplasm of infected cells.[7] Spread of infection between enterocytes occurs mainly when infected progenitor cells giving rise to infected progeny; however, cell-rupture and liberation of bacteria can also lead to infection of neighboring enterocytes.[3, 7]

There are still many unknowns regarding pathogenesis of proliferative enteropathy, as the obligate intracellular and microaerophilic nature of *L. intracellularis* hinders research efforts.[9] A recent study in yeast identified a potential effector protein, named LI1035, injected by the bacteria's T3SS that may target the MAPK system and affect cell growth.[9] Another study demonstrated that *L. intracellularis* can survive and possibly proliferate in porcine macrophages in vitro.[3]

A recent in vivo study in pigs uncovered the possible mechanisms behind mucosal hyperplasia and inhibition of goblet cell differentiation.[2] As the contributor mentions, the cell signaling pathways β-catenin/WNT and Notch are complex intracellular mechanisms which control homeostasis and differentiation of enterocytes. Wnt-1 interaction with

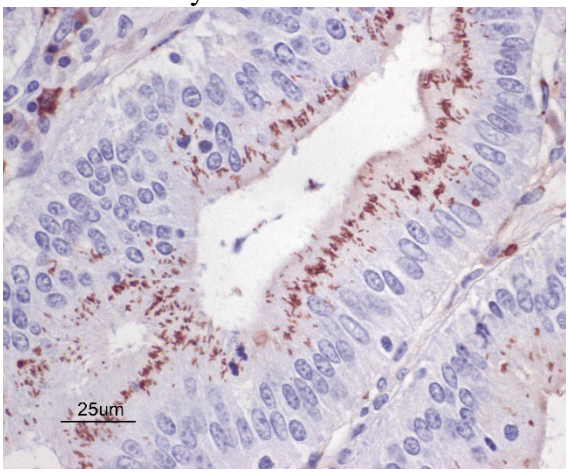

Figure 3-6. Colon, ferret. Curved small bacilli in the apical cytoplasm of enterocytes detected using a polyclonal antibody to Lawsonia intracellularis. (anti-L. intracellulare, 40X) (Photo courtesy of: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada http://ahl.uoguelph.ca)

the membrane-bound Frizzled receptors prevents auto-phosphorylation of cytoplasmic β-catenin, which then migrates to the nucleus and induces gene expression associated with proliferation of intestinal stem cells, the precursor to transit-amplifying progenitor cells.[2, 4] Separately, Notch receptor activation leads to translocation of the Notch receptor intracellular domain (NICD) into the nucleus, which stimulates gene expression that drives progenitor cell differentiation into an absorptive enterocyte and suppresses differentiation into the secretory phenotype (i.e. goblet cell).[2] Huan et. al demonstrated that *L. intracellularis* infection was associated with increased levels of NICD1 and decreased levels of goblet-cell associated *ATOH1* and *MUC2.* Their study also demonstrated increased cytomembranous (and thus decreased nuclear signaling) of β-catenin, which in mice induces proliferation of progenitor cells.[2] This study provides a glimpse into the uncertain yet costly pathogenesis of *L. intracellularis* infections.

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CASE IV:

Signalment:
5 year, 2 month old female Beagle dog (*Canis familiaris*)

History:
A multiparous female from a breeding colony was noted to have bilaterally elevated nictitating membranes and a body temperature of 99.8 °F. No other clinical signs were noted. Two days later the dog was found moribund and was humanely euthanized. Approximately 5 years prior to this episode, the dog had been treated for respiratory disease (pneumonia).

Gross Pathology:
The animal was in good body condition with copious fat deposits. Caudal lung lobes contained multiple, gray brown, 0.5 - 1 cm diameter subpleural nodules. Stomach contents were dark brown with flecks. Small areas of petechiation and yellow chyme-like material were noted in the duodenal mucosa and lumen respectively. No other gross lesions were noted.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Lung: In one or more subpleural or apical foci, alveoli are filled with macrophages, multinucleate giant cells, and amorphous pale gray-brown material; small accumulations of amorphous material are often within multinucleate giant cells. Some alveoli contain only amorphous material (without inflammatory cells). Alveolar septa are thickened by fibrous connective tissue and infiltrated by varying numbers of mononuclear inflammatory cells. Amorphous material is strongly PAS positive, and exhibits birefringent peripheral radial striation under polarized light.

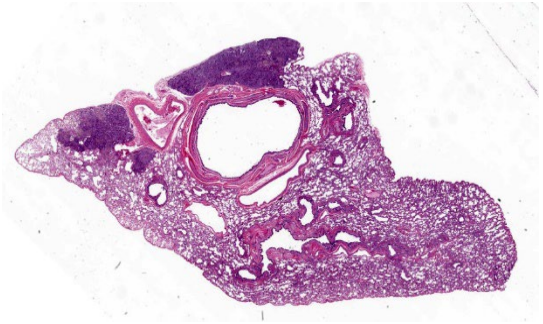

Figure 4-1. Lung, dog. In approximately 15% of the slide, subpleural alveoli are consolidated (HE, 5X)

Contributor's Morphologic Diagnoses:
Pneumonia, granulomatous, multifocal, chronic, with intralesional hyaline material.

Contributor's Comment:
Pulmonary hyalinosis is considered an incidental finding in older dogs characterized by intraalveolar accumulations of intracellular and extracellular amorphous or laminated, amphophilic, PAS-positive material and an accompanying macrophage and multinucleate giant cell response.[2] Scientific literature concerning this condition is sparse. Although the initial report of "pulmonary granulomas associated with PAS-positive bodies" found a greater incidence in brachycephalic dogs,[1] others reported the lesion in laboratory beagles exposed to ionizing radiation.[4]

Contributing Institution:
Covance Laboratories, Inc, Madison, Wisconsin, USA.

http://www.covance.com/industry-solutions/drug-development/services/safety-assessment/nonclinical-pathology-services.html

JPC Diagnosis:
Lung: Pneumonia, granulomatous, multifocal, moderate, with abundant intraalveolar and intrahistiocytic anisotropic hyaline material (Billups bodies).

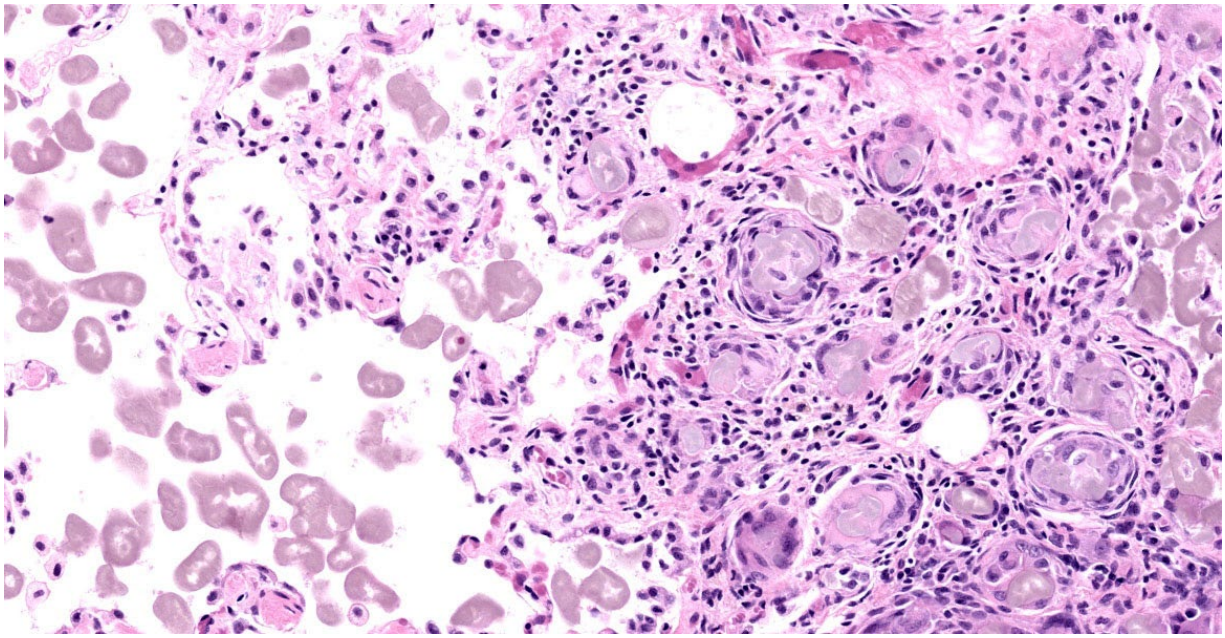

Figure 4-2. Lung dog. Aggregates of amphophilic protein are present within alveoli (left) and in large areas of the lung; they have been surrounded and engulfed by macrophages within affected alveoli. Alveolar septa are expanded by fibrosis and moderate numbers of macrophages, lymphocytes, and plasma cells. (HE, 258X)

JPC Comment:

This disease was first described by Dr. Leonard Billups and Dr. FM Garner in a manuscript published in *Veterinary Pathology* in 1972. Dr. Billups had a long career as an esteemed Army Officer and accomplished veterinary pathologist. He was a Vietnam veteran, completed his residency in veterinary pathology at the Armed Forces Institute of Pathology in 1969, and retired as a colonel (O-6) in 1995. After retiring from active duty, he served as an associate professor of pathology and Dean of Administrative Services at Tuskegee University College of Veterinary Medicine from 1995-2009. Dr. Billups is remembered within our organization as a consummate professional, officer, and academic who ignited a passion for veterinary pathology in many of his students.

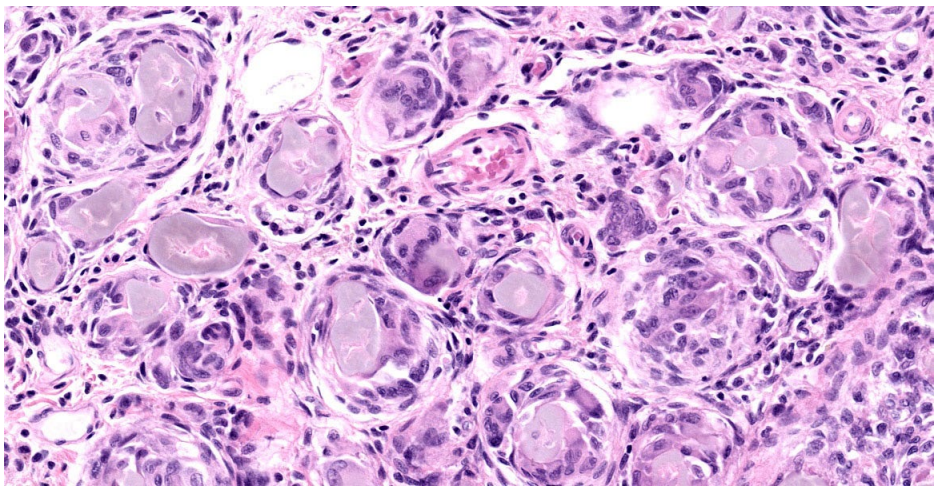

Figure 4-3. Lung dog. Higher magnification of granulomatous inflammation surrounding intra-alveolar protein. (HE, 381X)

As the contributor states, literature on pulmonary hyalinosis is sparse. While not fully understood, this abnormal alveolar material may accumulate due to increased production of an endogenous substance or decreased mucociliary clearance or impaired macrophagic breakdown

of endogenous or exogenous substances.[5] In addition to being PAS positive, the hyaline material has been reported to be diastase resistant, positive for oil red-O, blue when stained with crystal violet, and negative for GMS, Giemsa, Prussian blue, and Congo red.[1, 4, 5]

Pulmonary hyalinosis (PH) has recently been reported in captive sugar gliders and an inbred strain of laboratory rabbits.[3, 5] In a retrospective review of sugar glider necropsies, pulmonary hyalinosis was identified in 6 animals from 18 autopsies with lung tissue available; the animals were of various ages, with two considered young, and PH was considered the cause of death in one animal.[5] In a separate study, the lesion was identified as an incidental finding in 8 of 13 aging audiogenic EIII/JC strain rabbits (which develop seizures after auditory stimuli).[3] In this report, type II pneumocyte hyperplasia was frequently noted.[3] The material was also immunoreactive for surfactant protein-A (SPA) antibody, a finding that had not been evaluated in previous reports on PH.[3] The authors pre ferred the term surfactant pneumonia as it more accurately described the etiology and avoids confusion with the epithelial hyalinosis observed in laboratory mice.[3]

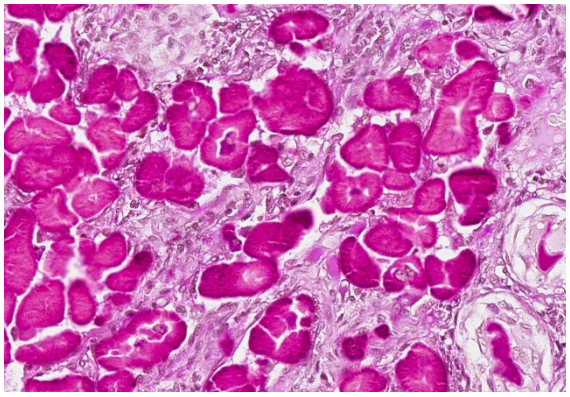

Figure 4-4. Lung, dog. Intra-alveolar protein is PAS-positive. (PAS, 400X) (Photo courtesy of Covance Laboratories, Inc, Madison, Wisconsin, USA, http://www.covance.com/industry-solutions/drug-development/services/safety-assessment/nonclinical-pathology-services.html)

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