



WEDNESDAY SLIDE CONFERENCE 2017-2018

Conference 7

18 October 2017

CASE I: F1753191 (JPC 4101076).

Signalment: 9-year-old, female intact, Rock Alpine goat, *Capra aegagrus hircus*, caprine.

History: A 9-year-old, female intact Rock Alpine goat presented to Colorado State University Veterinary Teaching Hospital two months prior to necropsy with a three-day history of hyporexia and lethargy which had progressed to lateral recumbency and complete anorexia. The referring veterinarian had previously diagnosed the doe with louse infestation, endoparasites and a heart murmur. Bloodwork by the referring

veterinarian revealed a regenerative anemia, stress leukogram and hypoproteinemia characterized by hypoalbuminemia and the goat was treated with ivermectin. Bloodwork at CSU revealed hyperglycemia and elevated creatinine, creatine kinase and aspartate aminotransferase levels. A fecal floatation revealed heavy loads of coccidia, strongyles and *Trichuris* spp. During a nine day hospitalization, the doe was treated with intravenous fluids, kapectate, thiamine, fenbendazole, sulfadimethoxine, oxy-tetracycline and multiple blood transfusions. After significant improvement of her clinical signs and bloodwork, including partial resolution of the dermatitis, the doe was



Haired skin goat. The skin was dry, alopecia, and covered with hyperkeratotic crusts and ulcers. (Photo courtesy of: Colorado State University, Microbiology, Immunology, and Pathology Department, College of Veterinary Medicine and Biomedical Sciences, <http://csucvmb.colostate.edu/academics/mip/Pages/default.aspx>)

discharged.

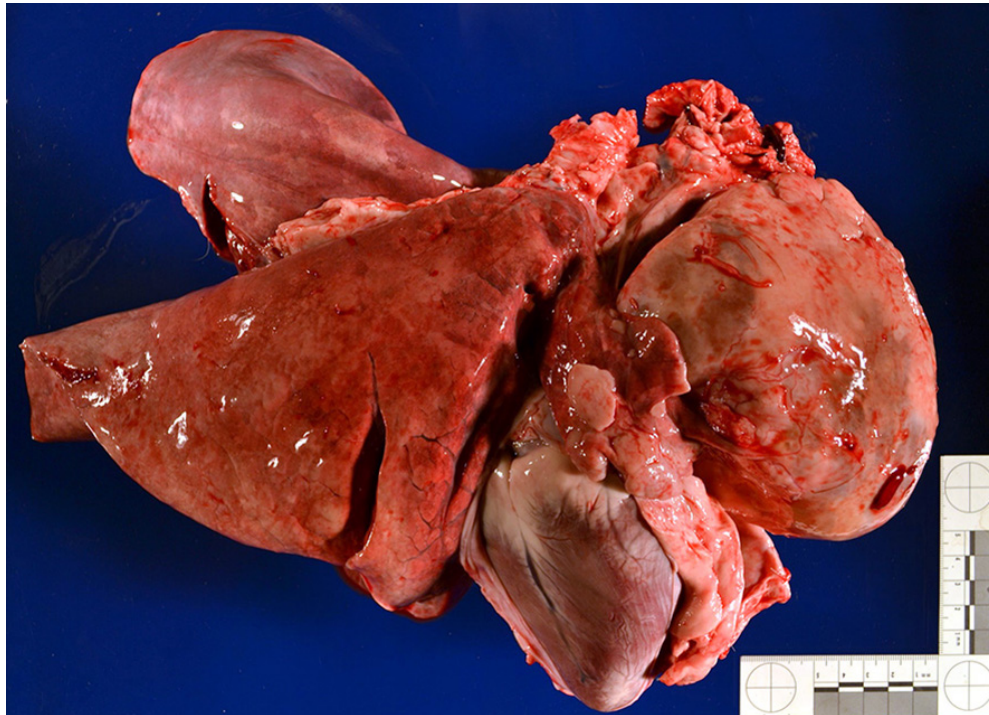
Two months later, the goat presented with a one month history of progressive scaling and ulceration over the withers, dew claws, and coronary bands and acutely progressive lethargy. On physical exam, the doe was febrile, tachypneic and tachycardic. Thoracic radiographs showed a large space-occupying mass which filled the cranial mediastinum and caudally displaced the heart. The doe appeared to be significantly painful, exhibiting shifting leg lameness and refusing to lie down. Humane euthanasia was elected.

Gross Pathology: Presented for postmortem examination was the carcass of a 9-year-old, female intact Alpine goat in good body condition with mild autolysis. Approximately 60% of the skin was markedly dry, thickened and alopecic with

exfoliating epithelial crusts which were often tangled within scant remaining hairs. This lesion most severely affected the skin over the epaxials, the ventral abdomen and teats, coronary bands and dew claws. Lesions were multifocally ulcerated with a reddened hemorrhagic underlying dermis. Occupying approximately 40% of the thoracic cavity was a large 12x8x8cm white, multilobular and cystic mass located cranial to the heart within the mediastinal space. An 8 cm in diameter cystic cavity in the mass was filled with translucent yellow fluid. The heart was caudally displaced and the pericardial sac contained approximately 200 mL of serous fluid. Small strands of fibrin were attached to regions of the pleura and pericardial sac which were in contact with the mass. All other organ systems were grossly within normal limits.

Laboratory results (clinical pathology, microbiology, PCR, ELISA, etc.): Bloodwork was performed at CSU during the doe's second hospitalization.

Initial PCV was 30% with a total protein of 6.5 G/dl. CBC showed neutrophilia (12.2; 2-6 x 10³/ul) and no ongoing evidence of anemia. Chemistry showed hypoalbuminemia (2.2; 3.3-4.2 g/dL), mild

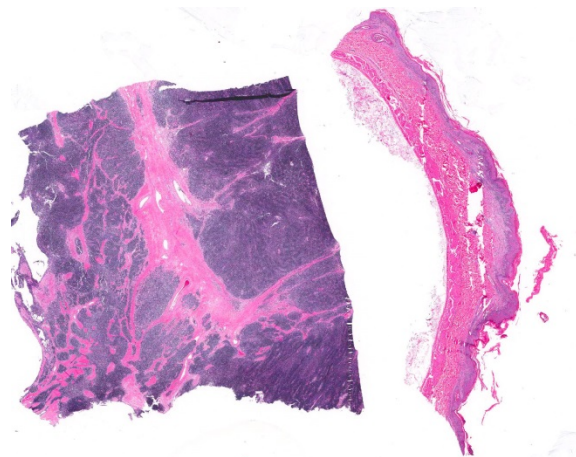


Haired skin goat. An 8cm cystic mass within the cranial mediastinum displaces the heart caudally. (Photo courtesy of: Colorado State University, Microbiology, Immunology, and Pathology Department, College of Veterinary Medicine and Biomedical Sciences, <http://csucvmb.colostate.edu/academics/mip/Pages/default.aspx>)

hyperglobulinemia (5.0; 3.4-4.8 g/dL), hyperglycemia (207; 45-75 mG/dL), hypomagnesemia (1.4; 2.2-2.9 mg/dL), mild hypokalemia (3.67; 3.8-6.3mEQ/L), mild hypochloremia (105.0; 109-117 mEQ/L) and hypoferrremia (51; 110-200 uG/dL)

Microscopic Description: Haired skin: The epidermal-dermal junction and perifollicular interstitium are multifocally infiltrated by moderate numbers of lymphocytes. The superficial dermis is expanded by a moderately dense band of lymphocytes with occasional plasma cells, macrophages and neutrophils. Low numbers of mixed inflammatory cells (lymphocytes, plasma cells, neutrophils and macrophages) are present in the dermis. The epidermis is multifocally acanthotic with orthokeratotic and parakeratotic hyperkeratosis. Folliculosebaceous units are decreased in number (variable in sections). Remaining units are variably atrophied. Individual keratinocytes and basal cells and rare follicular epithelial cells are shrunken with hypereosinophilic cytoplasm and pyknotic or lost nuclei and there is occasional satellitosis. Basal cells are occasionally swollen with abundant vacuolated cytoplasm. There is multifocal ulceration and a thick crust composed of numerous degenerate neutrophils, hair shaft fragments and keratin flakes admixed with abundant eosinophilic debris and occasional serum lakes. Scattered throughout this crust are numerous colonies of 1-3 micron cocci, 2-4 micron long asymmetrically peanut-shaped budding yeasts and rare ruminal contents.

Cranial mediastinal mass: Examined is an encapsulated, highly cellular neoplasm. The neoplasm is composed of loose cords of large polygonal epithelial cells supported by a delicate fibrovascular stroma. Cells have distinct cell borders and abundant eosinophilic cytoplasm. Cell nuclei are



Thymus, skin, goat. A segment of the mediastinal mass and thickened skin are presented for examination. (HE, 5X)

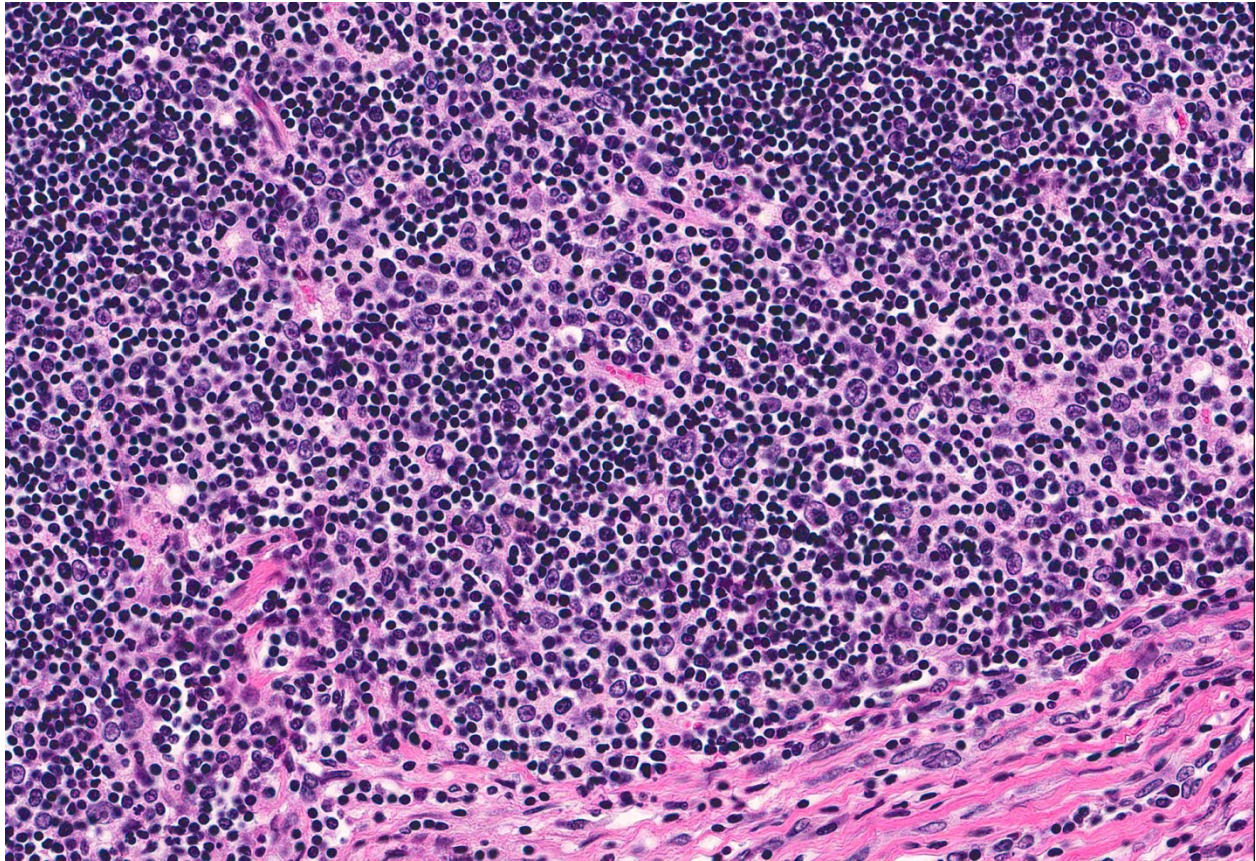
ovoid with finely stippled chromatin and indistinct nucleoli. Anisocytosis and anisokaryosis are marked. Mitoses are rare. The neoplasm is infiltrated and frequently obscured by sheets of numerous small mature lymphocytes.

Immunohistochemistry for cytokeratin, CD3, and CD79a was performed on the cranial mediastinal mass. Neoplastic epithelial cells demonstrated strong, diffuse cytoplasmic immunoreactivity for cytokeratin. Approximately 95% of lymphocytes infiltrating the mass are CD3 immunoreactive. Rare infiltrating lymphocytes are immunoreactive for CD79a within the cytoplasm.

In serial sections of skin, a GMS preparation highlighted superficial argyrophilic asymmetric 2-4 micron long, peanut-shaped yeasts and a Gram stain highlighted 1-3umgram positive cocci.

Contributor's Morphologic Diagnoses:

1. Haired skin: Dermatitis and folliculitis, interface, lymphocytic, chronic active, severe with rare keratinocyte, basal cell and follicular



Thymus, goat. Admixed with numerous lymphocytes is a background of neoplastic epithelial cells with large open-faced nuclei and prominent nucleoli. (HE, 400X)

epithelial cell apoptosis and superficial cocci and yeasts.

2. Cranial mediastinal mass: Thymoma, lymphoepithelial (mixed).

Name of Disease: Thymoma-associated exfoliative dermatitis

Contributor's Comment: Thymomas arise from the epithelial components of the thymus. They are classified as epithelial, lymphocytic or lymphoepithelial (mixed), based on the degree of infiltration by non-neoplastic lymphocytes. In one survey of 102 tumors in goats, thymomas represented the third most common tumor.⁶ Dairy breeds may be predisposed.⁵ These tumors tend to be benign, although there is a report of thymic carcinoma in one goat with metastases to the lung and spleen.⁷

Frequently thymomas are an incidental finding in goats with no associated clinical signs; however, reported sequelae include congestive heart failure and megaesophagus.^{8,9}

Thymoma-associated exfoliative dermatitis is an established paraneoplastic syndrome of cats.^{1, 4, 10, 11} The syndrome has also been reported in rabbits.³ It has been posited that the mechanism of the dermatologic lesion is rooted in the tumor-supported development of a population of autoreactive T cells which target keratinocytes.⁴ The classic feline cutaneous lesion has been previously described as a cell-poor interface dermatitis with telogenization of follicles.⁴ However, in a case series of five cats as well as this goat, the lesion is significantly cell-rich,

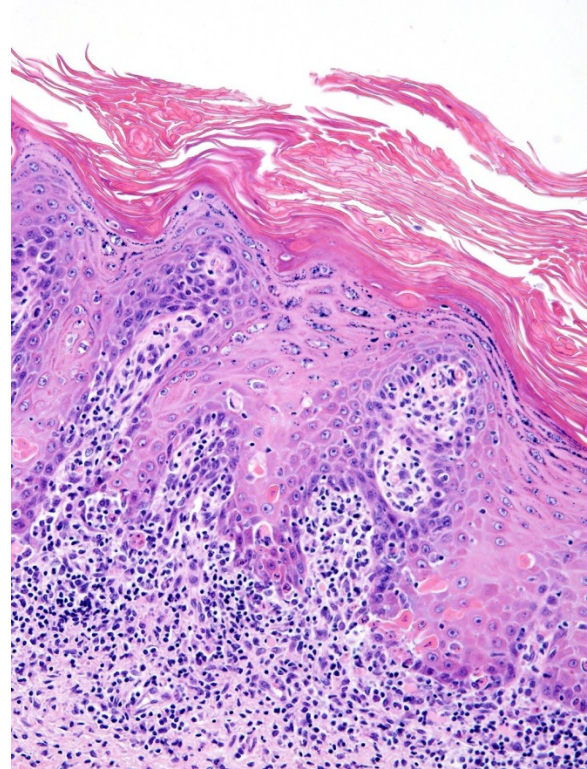
forming large bands of inflammation at the epidermal-dermal interface.¹⁰ Other paraneoplastic syndromes which have been associated with thymomas include myasthenia gravis, polymyositis and granulocytopenia.¹¹

Gross lesions in this case were quite striking. Extensive regions of alopecia, erythema and ulceration with large exfoliative flakes affected the dorsum, ventrum and even the teats and dew claws. Histologically, the lesion is characterized by transepidermal and follicular apoptosis, interface dermatitis and hyperkeratosis. The inflammatory infiltrates are composed of lymphocytes, plasma cells, macrophages and superficially located neutrophils. In some cases, sebaceous glands are lost. The lesion can be quite subtle; however, in this case there are regions which are severely affected. The presence of cocci and yeasts consistent with *Malassezia spp.* varied between sections of the tissue. Secondary infection with bacteria and yeasts can exacerbate the dermatitis and induce pruritus.²

Given the prevalence of thymomas in goats and the frequent lack of directly associated clinical signs, this case is of diagnostic interest for dermatologic lesions in goats. As thymoma-associated exfoliative dermatitis can be histologically similar to erythema multiforme and lupus erythematosus, diagnosis is contingent on the clinical diagnosis of a thymoma. Interestingly, post-thymectomy resolution of the dermatologic lesion has been reported in some cases in cats.^{1,2}

JPC Diagnosis: 1. Thymus: Thymoma (lymphocytic type), Rock Alpine goat (*Capra aegagrus hircus*), caprine.

2. Skin: Dermatitis, lymphocytic, interface, diffuse, mild to moderate with multifocal



Haired skin, goat. There is a prominent lymphocytic infiltrate in the superficial dermis. Shrunken, hypereosinophilic keratinocytes (arrows) are present at all levels of the dermis, and there is a thick layer of orthokeratotic hyperplasia overlying the dermis. (HE, 400X)(Photo courtesy of: Colorado State University, Microbiology, Immunology, and Pathology Department, College of Veterinary Medicine and Biomedical Sciences, <http://csucvmb.colostate.edu/academics/mip/Pages/default.aspx>)

epithelial hyperplasia, keratinocyte apoptosis, intracorneal pustules, and orthokeratotic and parakeratotic hyperkeratosis.

Conference Comment: Paraneoplastic syndromes are systemic conditions caused most commonly by excessive production of a “normal” hormone by neoplastic cells. The clinical signs they produce are often quite remarkable and are usually the reason the animal is brought to the veterinarian. Clinically, they serve as convenient markers for diagnosticians, and may serve to indicate neoplastic response to treatment. One or more of the following criteria must be met in

order to classify a clinical syndrome as paraneoplastic: (1) when the neoplasm is removed or treated, the concentration of the hormone decreases, (2) after removal of the normal gland producing the hormone, the concentration remains the same or increases, (3) there is a positive arteriovenous concentration of the hormone across the tumor, (4) there is production and secretion of the hormone product *in vitro*. In veterinary literature, the first criterion is most common.

There are several proposed pathogenic mechanisms of paraneoplastic syndromes including: (1) gene de-repression, resulting in the production of active hormones that are usually repressed; (2) ectopic receptor production by a tumor, resulting in displaced hormonal activity (an example is acetylcholine receptors produced by

thymomas resulting in anti-acetylcholine receptor antibodies produced by the host immune system, leading to muscle weakness and megaesophagus that characterizes myasthenia gravis); (3) exposure to normally “hidden” substances which the immune system perceives as foreign and mounts a type III hypersensitivity reaction against, with formation of immune complexes. Cachexia is the most common paraneoplastic response and occurs secondary to any neoplastic process because it is caused by rapid tumor growth and utilization of nutrients at the expense of the animal. It is hypothesized to be related the effects of the following pro-inflammatory mediators: tumor necrosis factor (TNF), interleukins 1 and 6 (IL-1 and IL-6) and interferon gamma and alpha (IFN γ and IFN α).²

Table 1: Common paraneoplastic syndromes in veterinary species^{2, 15}

Paraneoplastic syndrome	Associated neoplasm
Endocrine	
Hypercalcemia of malignancy	Lymphoma Apocrine gland carcinoma of the anal sac gland Mammary carcinoma Thymoma
Hypoglycemia	Hepatocellular carcinoma Salivary gland carcinoma Leiomyoma/leiomyosarcoma Plasma cell tumor Lymphoma
Ectopic ACTH	Pulmonary carcinoma
Cutaneous	
Pemphigus	Lymphoma
Alopecia	Pancreatic carcinoma (cat)
Exfoliative dermatitis	Thymoma (cat, rabbit)
Necrolytic migratory erythema	Glucagonoma
Hematologic	
Hypergammaglobulinemia	Multiple myeloma

	Lymphoma
Anemia	Numerous neoplasms
Erythrocytosis	Renal carcinoma
Neurologic	
Myasthenia gravis	Thymoma
Peripheral neuropathy	Insulinoma
Renal	
Glomerulonephritis	Multiple myeloma Polycythemia vera
Gastrointestinal	
Gastroduodenal ulceration	Mast cell tumors Gastrinoma
Miscellaneous	
Hypertrophic osteopathy	Pulmonary carcinoma Other thoracic masses Urinary bladder rhabdomyosarcoma
Cachexia	Numerous neoplasms

Thymomas have been rarely reported in most domestic animal species and present as a lobulated mass in the cranial mediastinum in adult to older animals and commonly replace one lobe with thymic remnant compressed at the periphery. In addition to the classification based on cell type and atypia listed above (epithelial, lymphocytic, or mixed) a more accurate classification has been based on two main benign phenotypes: type A (composed of spindle shaped cells) and type B (composed of epithelioid cells). Type B thymomas are more common in dogs and are further subdivided into three subtypes: (1) type B1 which resembles normal thymus with extensive lymphocyte proliferation (may be mistaken for a lymphoid neoplasm), (2) type B2 with neoplastic epithelial cells that are more plump, with vesiculate nuclei that are more easily discernible on a dense background of lymphocytes, and (3) type B3 which contain large sheets of neoplastic epithelial cells and very few lymphocytes. Type AB thymomas

are plausibly a mix of the two with both epithelial neoplastic cells and lymphocytes mixed together. In goats and sheep, type AB are most common and present as striking space occupying masses in older females which are often incidental findings during necropsy.¹⁶ Clinical signs associated with thymomas include respiratory distress and ventral head and neck edema as well as several paraneoplastic conditions often associated with some form of autoimmunity. The most common is myasthenia gravis which results in generalized muscle weakness and megaesophagus (mechanism described above). Secondary neoplasias such as osteosarcoma and mammary tumors, immune-mediated skin diseases, hypercalcemia, and polymyositis have also been reported.¹⁵ Hypercalcemia is due to production of PTHrp which results in increased osteoclastic activity and increased calcium reabsorption in the proximal and distal convoluted tubules of the kidney.¹¹ In human medicine, the mechanism of

increased autoimmunity has been worked out and is explained in the following sentences. The thymus, under normal conditions, plays a central part in the development of immunity and prevention of autoimmunity. Thymic epithelial cells express MHC I and MHC II antigens that react with circulating T-lymphocytes. Thymomas enhance thymic lymphopoiesis; expression of autoantigens and reduced expression of MHC molecules and autoimmune regulator molecules (AIRE) on neoplastic epithelial cells results in unreliable negative selection and release of autoreactive T lymphocytes.¹⁵

For the lesions in the skin, conference participants also considered erythema multiforme (EM) which is an autoimmune skin disorder that has been anecdotally reported in the goat. EM has been reported in association with a number of disorders, including: adverse drug reactions, infectious diseases (parvovirus infection in dogs, *Equid herpesvirus-5*, feline herpesviral infections), and in cats, thymoma-associated exfoliative dermatitis has been equated to EM in the literature. Grossly, EM presents as erythematous papules and plaques with a central area of clearing. The classic microscopic presentation is cytotoxic (interface) dermatitis with necrotic keratinocytes scattered throughout all layers of the epidermis and follicular epithelium often surrounded by lymphocytes (satellitosis).⁸

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<http://csucvmbs.colostate.edu/academics/mip/Pages/default.aspx>

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CASE II: 17-14227 (JPC 4102437).

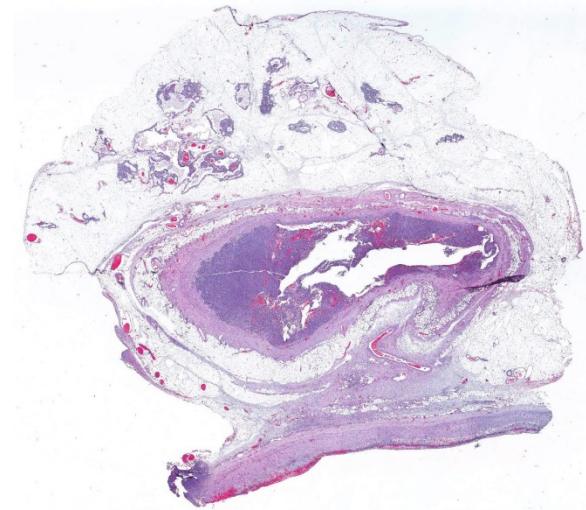
Signalment: 11-year-old female spayed Siberian husky, *Canis familiaris*, canine.

History: Two months prior to surgery, the patient presented to its primary veterinarian for labored breathing after an altercation with another dog. Radiographs at that visit revealed a thoracic mass cranioventral to the heart. The patient was referred to the cardiology service for an echocardiogram which showed mild pericardial effusion and confirmed the presence of an extra-pericardial, thin-walled, fluid-filled structure cranial to the heart with no associated blood flow. A thoracic CT scan found a 5.9 cm x 4.9 cm x 4.2 cm oval, well-defined, thin-walled soft tissue attenuating mass in the

cranial mediastinum with a tubular structure that communicated with the pericardial lumen. Cytology of the fluid within the mass revealed malignant cohesive cells suggestive of carcinoma, but mesothelioma could not be ruled out. The patient presented two months later for tachypnea at which visit moderate pericardial fluid with cardiac tamponade as well as mild pleural effusion was found on cardiac ultrasound. Clinical improvement was short lived (< 5 days) after pericardiocentesis. At that time, a subtotal pericardiectomy was elected to remove the cystic mass.

Gross Pathology: Intra-operative findings: The pericardial cystic mass was connected to the pericardium by a 1.5cm orifice at its craniodorsal aspect. On sectioning of the mass, multifocal nodules with an irregular, granular surface extend from the wall of the cystic mass. The affected and adjacent pericardium was mildly thickened.

Laboratory results (clinical pathology, microbiology, PCR, ELISA, etc.): Cytology of fluid from the cystic mass was consistent with carcinoma with evidence of hemorrhage; cytomorphic features

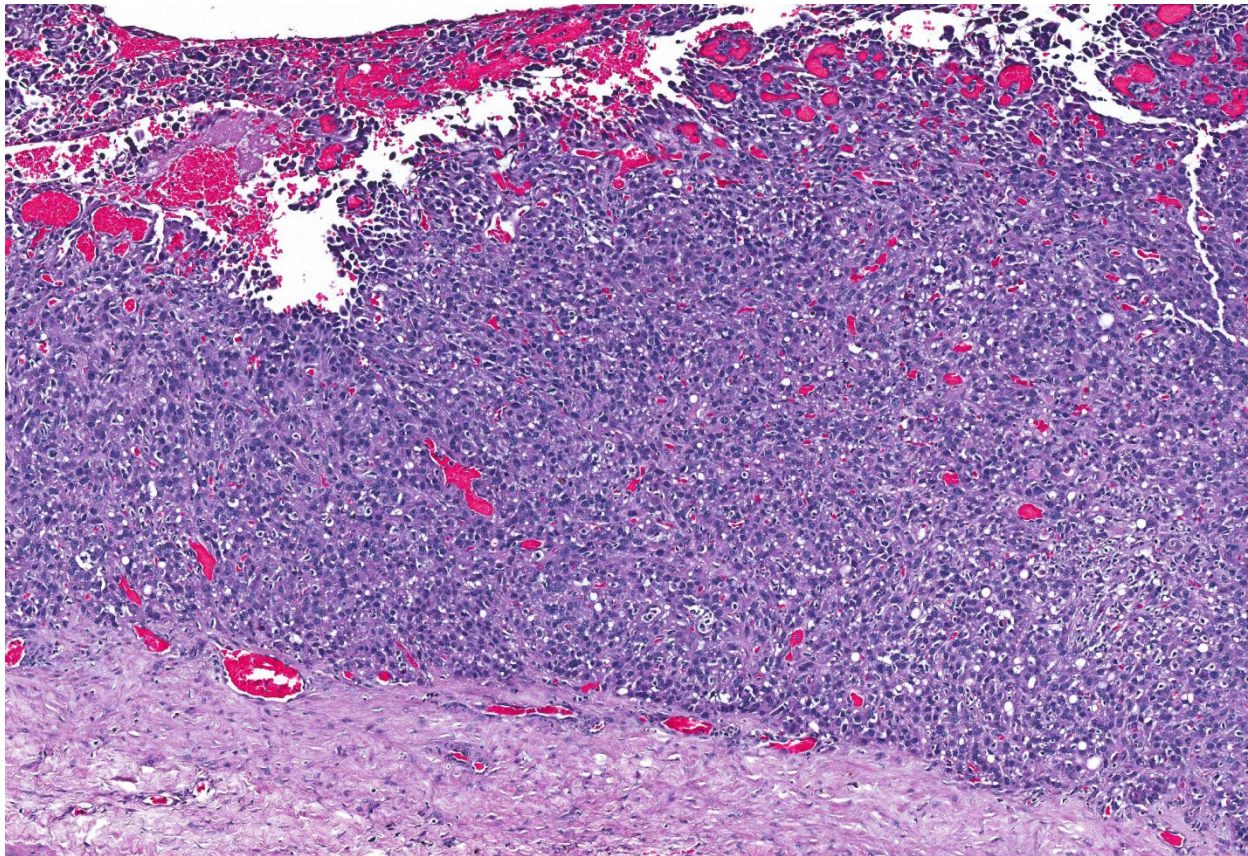


Pericardium, dog. At subgross magnification, there is a densely cellular neoplasm circumferentially lining a sleeve of pericardium. (HE, 5X)

suggest carcinoma (adenocarcinoma) or possible mesothelioma.

Microscopic Description: Pericardial mass: Focally expanding the pericardial adipose tissue is an encapsulated, well-vascularized mass with a large central cavitation. At the periphery, there are multifocal nodules of densely packed sheets of neoplastic polygonal cells with rare papillary formations supported by a small fibrovascular core. Neoplastic cells exhibit marked anisocytosis and anisokaryosis as well as moderate nuclear pleomorphism. Nuclei contain lacy chromatin with distinct, sometimes multiple nucleoli. Cells contain a moderate amount of eosinophilic, variably vacuolated cytoplasm. There are 24 mitotic figures in 10 high power fields with frequent

bizarre mitotic figures. Binucleation and karyomegaly are occasionally present. Intra-capsular (not represented on chosen slides) and lymphatic invasion are present. There is multifocal coagulative necrosis and mild hemorrhage. Within the surrounding adipose tissue there are multiple tubular structures lined by simple, ciliated cuboidal epithelium supported by a small amount of fibrous connective tissue which is associated with numerous lymphocytes and plasma cells. The lumens of these tubules contain amorphous, sometimes beaded, eosinophilic material mixed with foamy macrophages and varying number of lymphocytes and plasma cells. The pericardium is markedly expanded by highly cellular collagenous tissue with abundant neovascularization and hemorrhage mixed with mild



Pericardium, dog. Neoplastic cells arise from the pericardium and are arranged in sheets, forming papillary and micropapillary projections into the lumen. (HE, 112X)

lymphoplasmacytic and histiocytic inflammation. Histiocytes often contain coarse golden-brown pigment (interpreted as hemosiderin).

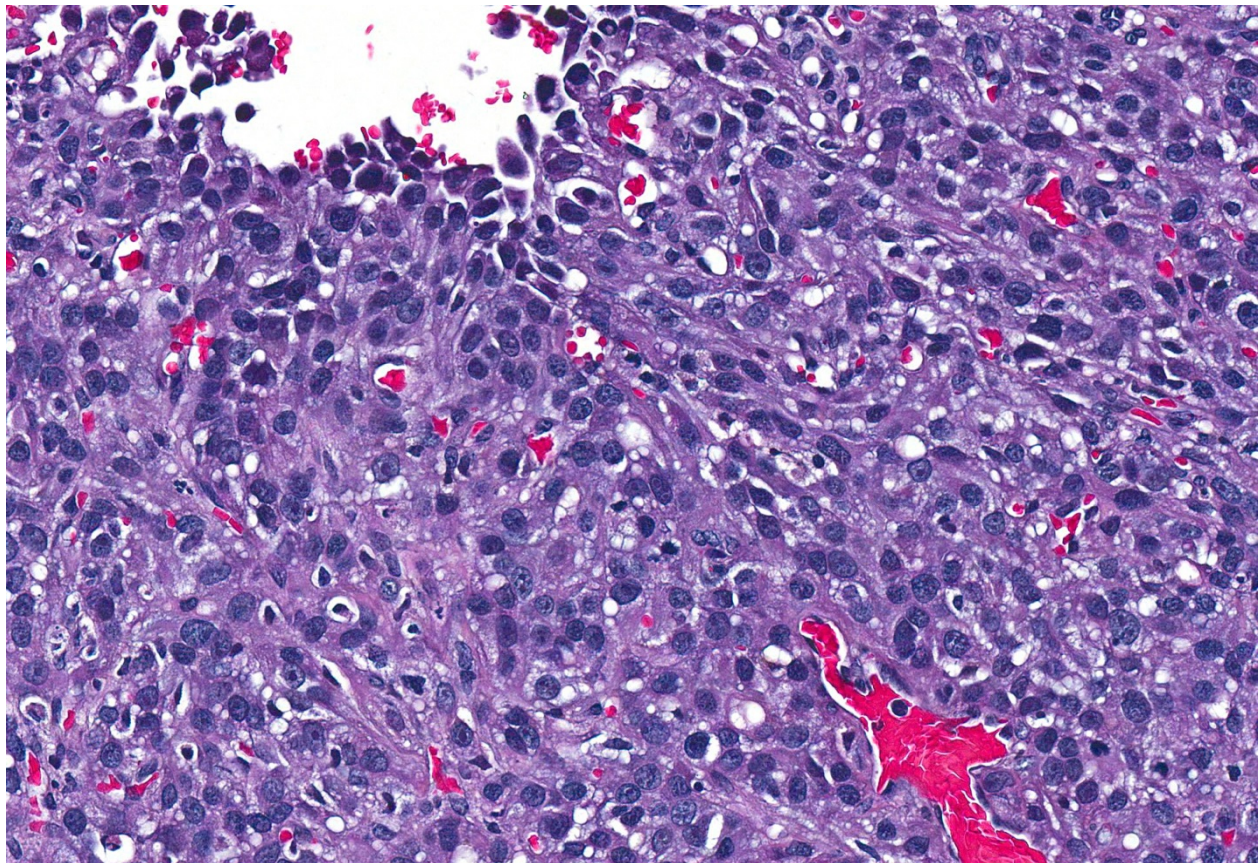
By immunohistochemistry, the neoplastic cells of the pericardial mass exhibit weak diffuse cytoplasmic immunoreactivity for cytokeratin (wide spectrum, WSS) in approximately 5% of tumor cells (mostly cells located centrally and those that are dissociated). Over 80% of cells have moderate to intense diffuse cytoplasmic vimentin staining (staining is most intense centrally within the mass).

Contributor's Morphologic Diagnoses:

1. Mesothelioma, solid variant, with capsular and lymphatic invasion

2. Persistent branchial pouch (embryologic remnants)

Contributor's Comment: Mesothelioma is a rare neoplasm in domestic animal species, which typically arise from serosa of the pleura, peritoneum or tunica vaginalis, though pericardial mesotheliomas have been documented with relative frequency in the dog^{7-9,11,14}. Based on their immunohistochemical profile, mesotheliomas are believed to arise from proliferating multipotent subserosal progenitor cells which are immunopositive for both vimentin and cytokeratin (AE1/AE3 in this study).¹² Their resting counterparts are immunopositive for vimentin but not cytokeratin. Conversely, terminally-differentiated surface mesothelium is immunopositive for cytokeratin but not vimentin.



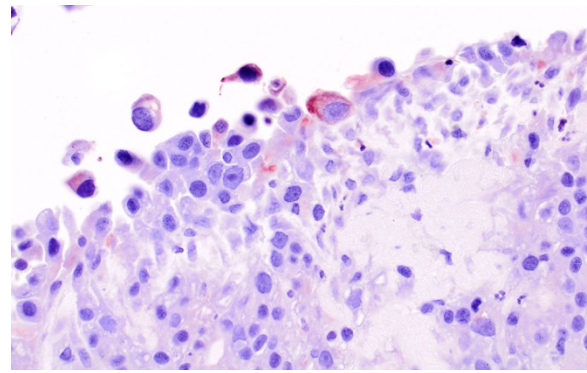
Pericardium dog. Neoplastic cells have indistinct cell borders with moderate amounts of vacuolated eosinophilic cytoplasm. There is moderate anisokaryosis and prominent nucleoli. Lymphocytes are scattered throughout the neoplasm. (HE, 400X)

Several histological subtypes have been categorized: epithelial, spindle and mixed.¹¹ Further classification of the epithelial subtype has been described by Harbison and Godleski in which there are: 1) papillary form supported by abundant fibrous stroma, 2) less organized papillary form with scant stroma and 3) anaplastic form which makes solid sheets.¹⁰ Based on these findings, we have subclassified this particular neoplasm as a solid or anaplastic variant. To our knowledge, histologic subtype has not been found to be prognostically significant.

In addition to being both cytokeratin- and vimentin-positive, mesothelial cells stain with Alcian blue due to their production of hyaluronic acid, a mucopolysaccharide.¹⁵ Common ultrastructural features of mesotheliomas include the presence of long microvilli at their surface, desmosomes and tonofilaments.¹⁰

Biological behavior of pericardial mesotheliomas is similar to mesotheliomas that arise at other sites in that they spread via transplantation, local extension and regional metastasis, with distant metastases occurring rarely.¹⁰ The presence of clusters of atypical mesothelial cells in lymph nodes of patients with pericardial effusion should be interpreted with caution, as embolized mesothelial cells have been found in regional lymph nodes in dogs with non-neoplastic pericardial effusion.^{15,19} It is purported that the local inflammation results in widening of intercellular stomata within the mesothelium, through which desquamated reactive mesothelial cells can enter the subserosal stroma and subsequently enter regional lymph nodes by way of subserosal lymphatics.¹⁹

An underlying cause for the development of mesothelioma in dogs remains to be



Pericardium, dog. Scattered neoplastic cells exhibit moderate cytoplasmic immunoreactivity for cytokeratin. (anti-AE1/AE3, 400X) (Photo courtesy of: Oregon Veterinary Diagnostic Laboratory, Oregon State University College of Veterinary Medicine, <http://vetmed.oregonstate.edu/diagnostic>)

elucidated. Spontaneous mesothelioma of the tunica vaginalis has been well-characterized in male Fisher 344/N rats.⁴ In humans, inhalation of asbestos and exposure to Simian virus 40 (SV40) have been linked to mesothelioma.^{6,10} With regard to pericardial mesothelioma in dogs, asbestos might be a potential contributing factor, particularly for those living in urban settings.¹⁰ A causal relationship between asbestos and mesothelioma has not been established, however, and many cases of canine mesothelioma do not have any known exposure to asbestos. In addition, there is a case series of golden retrievers who developed pericardial mesotheliomas after protracted histories of idiopathic hemorrhagic pericardial effusion, suggestive of a chronic inflammatory pathogenesis.¹¹

Based on the unusual clinical description of this lesion, another one interpretation might be malignant transformation of a pericardial cyst. Sisson et al. describes intrapericardial cysts as unilocular or multilocular cysts lined by reactive mesothelium supported by a small amount of fibrous stroma.¹⁷

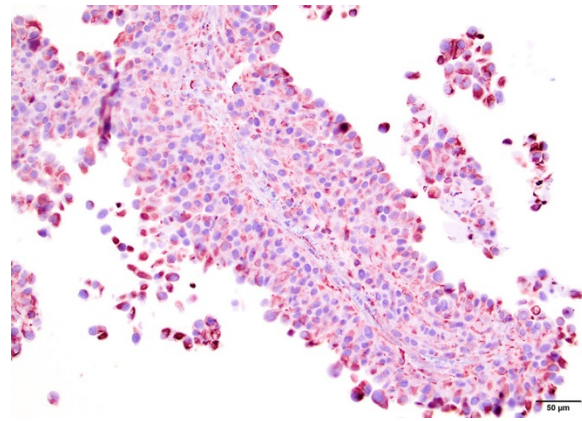
Another differential for mediastinal neoplasia is ectopic thyroid carcinoma.

Investigation into intrapericardial neoplasia in dogs found a single case of ectopic thyroid carcinoma as well as a single case of mesothelioma.⁸ Development of a papillary carcinoma from ectopic thyroid tissue within a branchial cyst has been documented in humans.¹³ Branchial pouches are embryologic structures that develop into various parts of the head and neck, including medullary C-cells of the thyroid gland.¹ Medullary and follicular thyroid carcinomas have been found to be both cytokeratin and vimentin positive using immunohistochemistry.^{4,5,16} Expression of vimentin in carcinomas is believed to facilitate in tumor progression, namely the phenomenon of epithelial-mesenchymal transformation (EMT). Immunohistochemistry for thyroglobulin and calcitonin was not performed on this mass.

JPC Diagnosis: 1. Pericardium: Mesothelioma, Siberian husky (*Canis familiaris*), canine.
2. Pericardial fat: Branchial cysts, multiple.

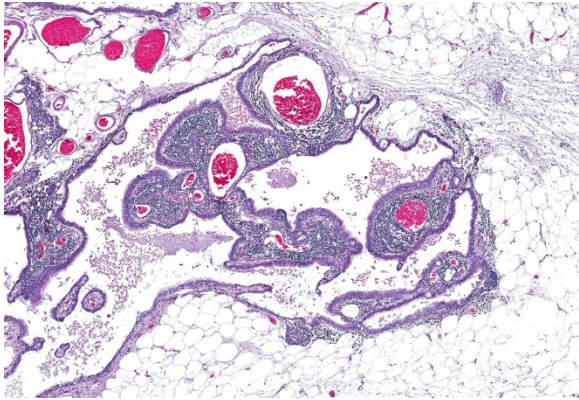
Conference Comment: Mesotheliomas have been reported arising from the pleura, pericardium, and peritoneum, as well as the mesothelial lining of the visceral vaginal tunic (an invagination of the peritoneum) of the testis.^{2, 14, 19} Of the four locations, mesotheliomas of the peritoneum are most common in domestic animals. Affected animals typically present for peritoneal fluid (ascites), which is characterized cytologically by reactive epithelial-like or mesenchymal-like cells. Cytologic distinction between reactive and neoplastic mesothelial cells is notoriously difficult.

As mentioned by the contributor above there are three recognized histologic subtypes of mesothelioma: epithelioid, spindlioid (sarcomatous), and mixed. As with cytology,



Pericardium, dog. Scattered neoplastic cells exhibit strong cytoplasmic immunoreactivity for vimentin. (anti-vimentin, 400X) (Photo courtesy of: Oregon Veterinary Diagnostic Laboratory, Oregon State University College of Veterinary Medicine, <http://vetmed.oregonstate.edu/diagnostic>)

histologic differentiation between reactive mesothelium and neoplasia is difficult and is dependent on four factors: (1) invasion of the underlying tissue, (2) the presence of neoplastic mesothelial cells in draining lymph nodes or distant organs, and (3) multiple masses within a body cavity. In addition, mesothelial neoplasia appears much thicker and irregularly arranged, whereas reactive mesothelium generally appears as a single layer of regularly arranged cells. Cellular morphology is generally not helpful because both reactive and neoplastic mesothelial cells can appear either well-differentiated or anaplastic. While many specific immunohistochemical (IHC) stains, including epithelial membrane antigen, desmin, glucose transporter protein-1, p53, and Ki67 have proven diagnostically unreliable, mesothelial cells are somewhat unique in that they exhibit dual expression of vimentin and cytokeratin. Thus the combination of histologic pattern and IHC features are necessary for diagnosis of mesothelioma.¹⁴ Testicular mesotheliomas are quite rare in most domestic species but are described in dogs, bulls, and rats (Fisher 344 strain).³ Once diagnosed, a presumed



Heart base, dog. The adjacent pericardial fat contains a large multilocular brachial cyst. These cysts are lined by tall columnar ciliated epithelium. (HE, 400X)

primary mesothelioma of the testis must be differentiated from a metastatic mesothelioma that started in one of the other primary locations.²

Conference participants discussed the difficult tissue identification in this case, but most noted that the presence of branchial pouch cysts was beneficial. Branchial pouch cysts are congenital and though rare, are usually found in brachycephalic dog breeds. These cysts are often found in the cranial mediastinum (as in this case), close to thymic tissues, and are lined by a single layer of cuboidal epithelial cells.⁷

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CASE III: S9004764 (JPC 4019378).

Signalment: 7-week-old, male, Holstein calf, *Bos taurus*, bovine.

History: A live, 7-week-old, male calf with history of diarrhea, knuckling of the fetlocks, incoordination and stiffness was submitted for necropsy. Some animals in the group showed dyspnea and coughing. Animals were treated with a broad spectrum antibiotic for 4 days.

Gross Pathology: The calf was in good nutritional condition. The abdomen was moderately distended with gas. Tissues were in good state of postmortem decomposition. The forestomachs and abomasum contained a mixture of ingested feed (grain and green forage). There was about 3 liter of straw-colored fluid with fibrin strands in thoracic and abdominal cavities; thick loosely-adherent sheets of yellow fibrin on serous surfaces; and copious amounts of yellowish fibrinous exudates in multiple joints (polyarthritis). Three other in-contact calves had similar necropsy findings

Laboratory results (clinical pathology, microbiology, PCR, ELISA, etc.):



Brainstem, calf: A section of brainstem with 4th ventricle (at right) is presented for examination. (HE, 5X)

Aerobic culture from liver yielded mixed coliforms

Salmonella culture on the colon and liver was negative

Chlamydia culture on tissue pool was negative (guinea pig inoculation, chicken embryos and cell culture).

Virus isolation on tissue pool (lung, brain, spleen) was negative

Immunofluorescent test for *Chlamydia* on the liver and spleen was negative

Immunofluorescent test for bovine corona virus, IBR, BVD was negative

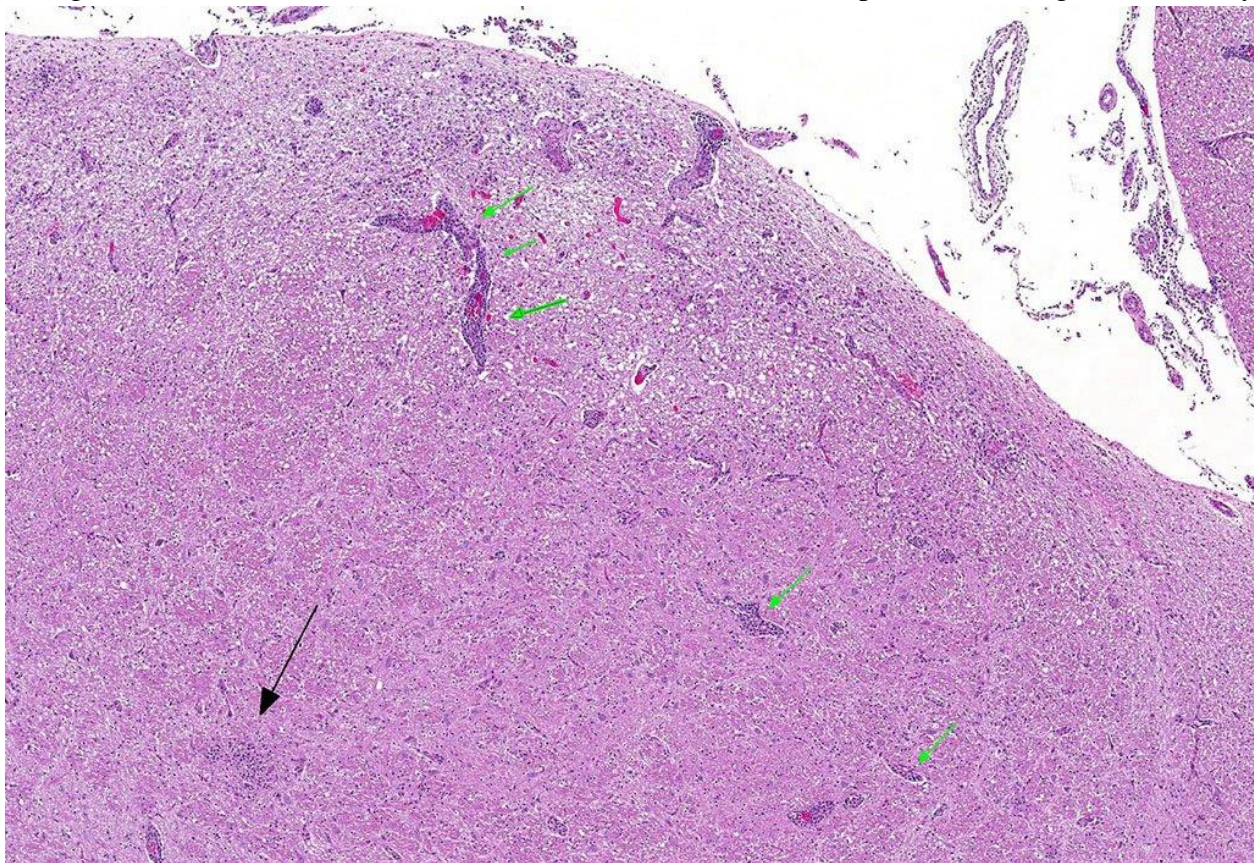
Immunology for IBR was negative (1:4); and BVD was 1:32.

Heavy metal screen, selenium (liver) and cholinesterase (brain) had normal background levels.

Immunohistochemistry using genus-specific *Chlamydia* antiserum on brain and liver sections from parafinized tissue blocks (archived for 22 years): Positive for *Chlamydia* (new name, *Chlamydophila*) elementary bodies.

Microscopic Description: Brain: Thromboembolic malacia, multifocal, with proliferation of glial cells, vasculitis, perivascular cuffing, axonal degeneration, meningitis; lymphocytic, moderate to severe.

Immunohistochemistry (IHC) staining of the brain is positive for elementary bodies (EB) of *Chlamydophila*; presumptive, *Cp. pecorum*. Photomicrograph of brain IHC is included (see positive staining of elementary



Brainstem, calf: Throughout the section, there is prominent cellular expansion of Virchow-Robin's space (green arrows), and there is pallor of the submeningeal parenchyma. Glial nodules are scattered randomly throughout the section (black arrows) (HE, 40X)

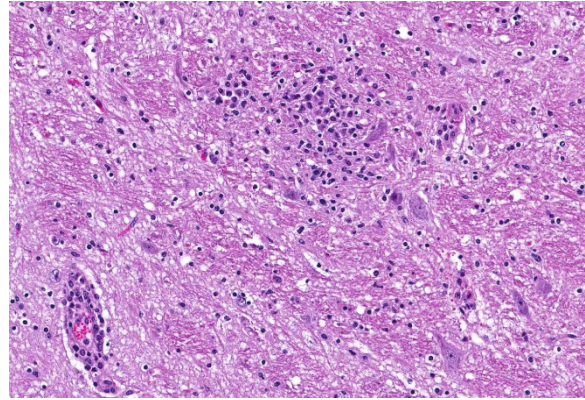
bodies in macrophages and endothelial cells).

Contributor's Morphologic Diagnoses:

Brain: Encephalitis, lymphocytic, multifocal with thromboembolic malacia, vasculitis, perivascular cuffing and meningitis; moderate to severe.

Contributor's Comment: When this case was submitted about 22 years ago, a presumptive diagnosis of sporadic bovine encephalomyelitis was made based on clinicopathological findings. The attempt to isolate chlamydial agent was negative, perhaps because of medication with broad-spectrum antibiotics. Serum sample from another sick calf in the group with similar clinical signs and lesions was positive by complement fixation test for *Chlamydia* antibodies. Recently, because of the availability of IHC, the paraffin block of the brain was retrieved from our repository and tested. We detected positive staining elementary bodies of *Chlamydophila* (new name for *Chlamydia*) using genus-specific *Chlamydia trochomatis* (MONOTOPE™) antiserum.⁹ According to the manufacturer, the antiserum has cross reactivity with *C. pneumoniae* and *C. psittaci*.

Intracellular bacteria of the order *Chlamydiales* were first associated with diseases of cattle (*Bos taurus*) when McNutt isolated such organisms from feedlot cattle with sporadic bovine encephalomyelitis.⁶ Menges⁷ and Wenner¹⁰ in 1953 studied the disease further and proved it was due to an agent belonging to the psittacosis-lymphogranuloma group of viruses. There are currently four families, six genera and 13 species within the order *Chlamydiales* that have valid published names.⁴ Two species of the genus *Chlamydophila* cause disease in ruminants: *Cp. abortus* (formerly *Chlamydia psittaci* serotype 1) and *Cp. pecorum*



Brainstem, calf: Higher magnification of glial nodule. (HE, 175X).

(formerly *Chlamydia pecorum*).⁸ Jee et al reported that the prevalence of *C. pecorum* in calves to be approximately five times as high as that of *C. abortus*, with the highest detection rate being with vaginal swabs, compared to rectal or nasal swabs.³ The intestinal chlamydial infection may represent the initial event in the pathogenesis of such disease syndrome as polyarthritis, pneumonia, and encephalomyelitis and probably also transplacental infection and abortion.²

Chlamydophila pecorum is a small, obligate intracellular gram-negative bacterium that grows in eukaryotic cells. It has many characteristics of a gram-negative bacterium but it differs in that it lacks peptidoglycan. *C. pecorum* infects certain mammalian hosts like goats, koalas, sheep, swine and cattle. Because *C. pecorum* grows slowly within the natural host, actual disease syndromes are not as evident until later in the infectious process. *C. pecorum* is found mostly in mammals like cattle, sheep, goats, koalas and swine. In koalas, it causes urinary tract disease, infertility, and reproductive diseases. In other mammals, it is associated with abortion, conjunctivitis, encephalomyelitis, pneumonia, polyarthritis and enteritis.⁵

JPC Diagnosis: Brainstem and cerebellum (sections varied): Meningoencephalitis, lymphohistiocytic and neutrophilic, multifocal, moderate with necrotizing vasculitis, Holstein (*Bos taurus*), bovine.

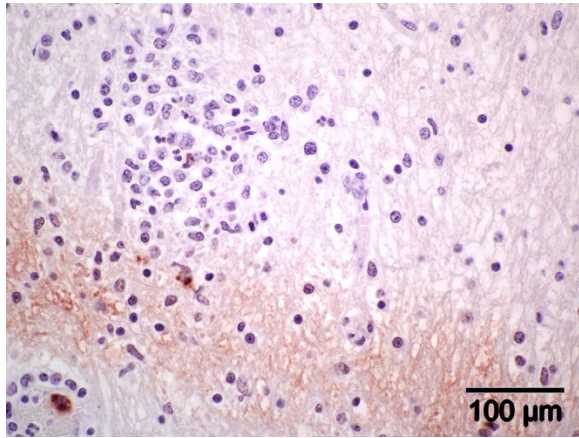
Conference Comment: Sporadic bovine encephalomyelitis (SBE) is caused primarily by *Chlamydophila pecorum* (although *C. psittaci* has also been reported and causes identical disease) which induces severe, diffuse meningoencephalomyelitis in young calves (cattle and buffalo) less than six months old. *C. pecorum* is an obligate intracellular gram-negative bacterium that has a tropism for blood vessels, mesenchymal tissue, and serous membranes which explains its range of clinical syndromes: encephalomyelitis, polyarthritis, metritis, conjunctivitis, and pneumonia. Microscopically, vasculitis and polyserositis are hallmark lesions. Grossly, serofibrinous inflammation of serosal membranes (most commonly the peritoneum) and synovia are characteristic.

Gross lesions in the brain are typically sparse with possible fibrin tags and mild congestion of the meninges, which contrasts the remarkable microscopic findings of diffuse meningoencephalomyelitis, particularly severe around the base of the brain. Meningeal inflammation is composed predominately of histiocytes and plasma cells which expand Virchow-Robbins spaces, resulting in marked perivascular cuffing. Prolonged inflammation results in endothelial damage and encephalitis. Small numbers of chlamydiae can be found as elementary bodies within the cytoplasm of mononuclear cells. ¹

Microscopic differentials in this case are: *Histophilus somni* (thrombotic meningoencephalitis), *Listeria monocytogenes*, and bovine herpesvirus-5.

Histophilus somni, the only member of the genus *Histophilus* in the family *Pasteurellaceae*, is a facultative anaerobic gram-negative coccobacillus which is normal flora of the male and female genital tracts and nasal cavity. *H. somni* initially begins as a septicemic process which may result in acute death, but if the animal lives, it can spread throughout the body causing widespread petechiation and necrosis. Images and literature often focus on the cerebral lesions because they are the most intense; however, microscopically, the hallmark of this disease is vasculitis with secondary thrombosis. Grossly, there is multifocal hemorrhage and necrosis throughout the brain and spinal cord. The pathogenesis is largely unknown, but there are several known important virulence factors: lipo-oligosaccharide (LOS), immunoglobulin Fc-binding proteins, inhibition of oxygen radicals, and intracellular survival. There are two proposed mechanisms of the vasculitis characteristic of this disease: (1) LOS induces activation of caspase-3 and subsequent apoptosis of endothelial cells and (2) LOS activates host platelets which initiate endothelial cell apoptosis by direct activation of caspase-8 and 9. Apoptosis is further boosted by endothelial cell cytokine production, adhesion molecule expression, and production of reactive oxygen species. Additional lesions include: synovitis with petechiae (atlanto-occipital common), pneumonia (when associated with the bovine respiratory complex), ulcerative laryngitis, , retinal hemorrhages, abscesses in the papillary muscles of the left ventricular free wall of the heart, and, less often, otitis externa/media, mastitis, and abortion.¹

Listeria monocytogenes is a facultative anaerobic gram-positive bacillus that is



Brainstem, calf: Elementary bodies of chlamydiae are labeled using genus-specific antibodies. (anti-C. trachomatis, 400X)

ubiquitous and able to withstand extreme environmental conditions. *L. monocytogenes* is an intracellular bacteria that lives in macrophages, neutrophils, and epithelial cells, and has several important virulence factors: internalin (allows it to overcome intestinal, placental, and blood-brain barriers by internalizing with E-cadherin) and cholesterol-binding hemolysin (lysing phagosomes). Subsequently, the bacterium replicates in the cytoplasm of the host cell, and uniquely utilizes host cell actin to transfer from one cell to another. There are three distinct syndromes associated with listeriosis: (1) abortion from infection of the pregnant uterus, (2) septicemia and (miliary????) visceral abscesses, and (3) encephalitis. The septicemic form occurs in neonates and aborted fetuses due to multisystemic bacterial colonization. The encephalitic form occurs in the form of outbreaks of adult animals that have eaten partially fermented silage with a pH of 5.5 or above. The bacteria enter through wounds in the mucosa (from rough feed) and travels via axons to the trigeminal nerve to the brain, with an unusual affinity for the brainstem. Gross lesions are rarely observed; however, microscopically, there are numerous microabscesses composed of

viable and degenerate neutrophils, glial nodules, and marked lymphohistiocytic perivascular cuffing.¹

Finally, bovine herpesvirus-5 (BoHV-5), the causative agent of bovine necrotizing meningoencephalitis, occurs as a sporadic disease in young calves. Bovine herpesvirus-5 is antigenically related to bovine herpesvirus-1 (the causative agent of infectious bovine rhinotracheitis), an alphaherpesvirus; both share a tropism for nervous tissue, are latent in the trigeminal ganglion, and exhibit viral recrudescence. Once the organism is introduced nasally or reactivated, it travels via olfactory nerves to the grey matter of the rostral cerebrum (including the olfactory bulb) where it causes severe necrotizing nonsuppurative meningoencephalitis, with marked gliosis. Gross lesions are rare; however, microscopically there is marked lymphoplasmacytic perivascular cuffs, typically more than 6 cell layers thick. There is neuronal necrosis with occasional intranuclear viral inclusions within neurons and astrocytes.¹

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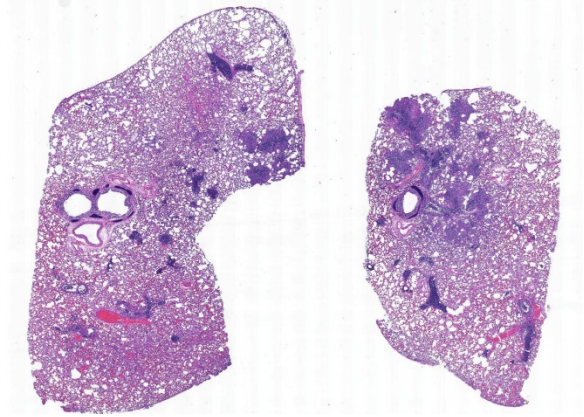
CASE IV: L17-994 (no label) (JPC 4100736).

Signalment: 4-month-old, female spayed, Domestic shorthair, *Felis catus*, feline.

History: The kitten presented weak and dehydrated with a packed cell volume (PCV) of 12%. Several other kittens had already died over the past 3-4 months.

Gross Pathology: A spayed female Domestic shorthair kitten presented for postmortem examination in fair body condition, weighing 1.55 Kg, with minimal postmortem autolysis. Small amount (1 ml) of blood-tinged, yellow, clear fluid was present within the thoracic cavity and moderate amount (approx. 35 ml) of similar fluid mixed with some strands of fibrin was present within the abdominal cavity. Dark brown fluid was present within the upper and lower respiratory tract (interpreted as terminal aspiration). The lungs were mottled pink and red. Minimal amount of dark brown fluid was also present throughout most of the digestive tract, extending from the oral cavity to the small intestines. The colon was distended with abundant dark red to black, soft feces. The gastrointestinal mucosa was unremarkable. The mesenteric lymph nodes were moderately enlarged. The liver had an accentuated lobular pattern and multiple, up to 3 mm diameter, tan to white, smooth foci that were best apparent on the capsular surface. The omentum, ileum, and colon had multiple, 1-2 mm diameter, white, slightly raised nodules on the serosal surface.

Laboratory results (clinical pathology, microbiology, PCR, ELISA, etc.):



Lung, kitten. Bronchioles multifocally contain a dense cellular infiltrate which extends into surrounding alveolar spaces. (HE, 6X)

Bacterial culture (aerobic): Lung: *Bordetella* sp. (heavy growth)

Special stain (Gram stain): Lung: Moderate numbers of cilia-associated or free gram-negative coccobacilli are within the bronchial and bronchiolar lumen.

Fluorescent antibody test: Lung, liver, intestine, and mesenteric lymph node: Positive for Feline Coronavirus; Intestine, mesenteric lymph node, and spleen: Negative for Feline Parvovirus

Immunohistochemistry for Feline Coronavirus: Lung: Within a focus of peribronchiolar inflammatory cell infiltrate, moderate numbers of macrophages have strong brown (positive) cytoplasmic labeling.

Microscopic Description: Lung: The bronchiolar lumina are filled with abundant viable and degenerate neutrophils interspersed with sloughed epithelial cells. Abundant cilia-associated and free gram-negative coccobacilli are present in the inflamed airways. The alveoli surrounding the inflamed bronchioles are frequently distended by abundant fibrin, neutrophils,

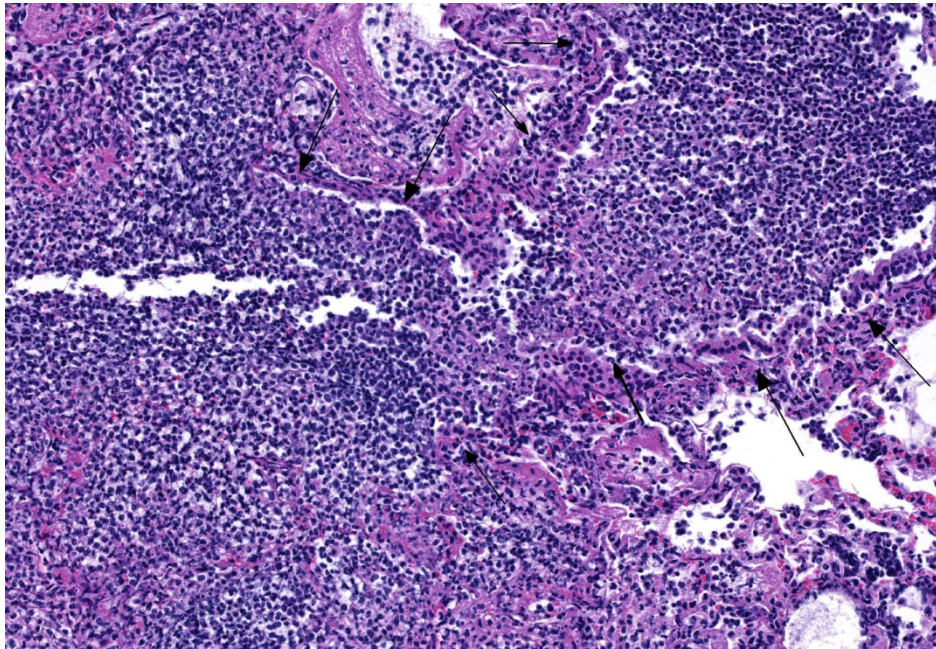
and macrophages. Pulmonary vessels are diffusely acutely congested. There is also mild to moderate, diffuse alveolar edema.

Colon, mesenteric lymph nodes, liver, and spleen (not submitted): In multifocal areas, the serosal surface of the colon, the mesenteric perinodal adipose tissue, and the capsular surface of the liver and spleen are variably expanded by abundant fibrin mixed with numerous viable and degenerate neutrophils and macrophages. In the most severely affected areas, the fibrinous to pyogranulomatous infiltrate extends into the outer longitudinal layer of the tunica muscularis of the colon, the cortical sinuses of the mesenteric lymph nodes, and the hepatic parenchyma.

Contributor's Morphologic Diagnoses:

1. Lung: Bronchopneumonia, suppurative, acute, with gram-negative cilia-associated and free bacteria.
2. Lung: Pneumonia, fibrino-suppurative and histiocytic, multifocal, with intrahistiocytic feline coronaviral antigen.
3. Intestine, mesenteric lymph node, liver, and spleen (not submitted): Inflammation, fibrinosuppurative to pyogranulomatous.

Contributor's Comment: *Bordetella bronchiseptica* pneumonia is particularly important in kittens <12 weeks of age¹ and in puppies between 7-35 weeks of age² due to a greater severity of clinical disease that ensues at this age with a potential of fatal outcome. *B. bronchiseptica* is also important because of its zoonotic potential, particularly for people with immunosuppression.³ A well-recognized virulence attribute of this pathogen is its adherence to cilia.⁴ This



Lung, kitten. The bronchiole (outlined by arrows) is filled with an exudate composed of innumerable degenerate neutrophils admixed with abundant cellular debris. The airway epithelium is multifocally necrotic and lost. (HE, 150X)

adherence is determined by Bvg-regulated expression of filamentous hemagglutinin and pertactin, fimbriae, as well as by adenylate cyclase-hemolysin toxin.⁵ The histologic detection of bronchial and bronchiolar cilia-associated bacteria is a significant feature of the diagnosis of *B. bronchiseptica* as a cause of bronchopneumonia.⁶ Along these lines, we detected cilia-associated and free gram-negative bacteria in the lower airways in the histologic lung sections of this kitten. This, combined with the heavy growth of *Bordetella* sp. from the lung, confirms *B. bronchiseptica* as the primary cause of the respiratory disease in this case.

Although the lung lesions in this case were largely attributed to the *Bordetella* sp. bronchopneumonia, the fibrinosuppurative and histiocytic inflammatory alterations in the surrounding alveoli together with the immunohistochemical detection of FCoV antigen in multiple macrophages in one area suggests that there was at least partial

contribution of this virus to the respiratory disease of this kitten.

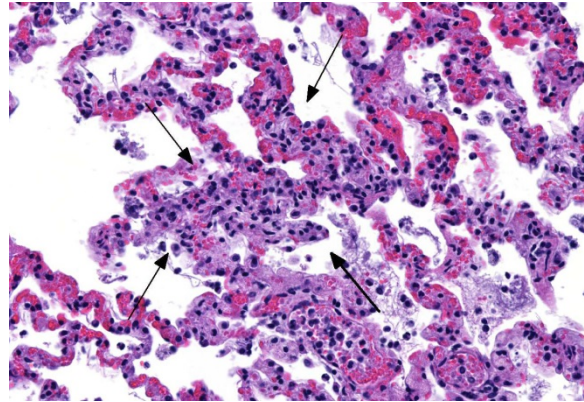
Fibrinosuppurative to pyogranulomatous inflammation associated with feline coronavirus antigen detected by fluorescent antibody testing was also present in several other tissues of this kitten, namely the colon, mesenteric lymph nodes, liver and spleen, consistent with the diagnosis of Feline Infectious Peritonitis (FIP). FIP is a fatal systemic

disease of wild and domestic felids caused by feline coronavirus (FCoV), a member of the family Coronaviridae and genus *Coronavirus*, an enveloped, positive-strand RNA virus that spreads via fecal-oral route. Characteristic lesions of FIP include granulomatous phlebitis, fibrinous to granulomatous serositis, and pyogranulomatous inflammation of multiple organs.⁷ Cats that present with FIP are usually less than 2 years of age and come from a multi-cat environment.⁸ This was true in our case as well, in which the kitten was only 4 months old and came from a multi-cat environment where other kittens had already died. Cats with FIP are lymphopenic,⁷ which may have predisposed this kitten to the respiratory bordetellosis. The clinically noted anemia was attributed to gastric bleeding and melena, although gastric erosions and/or ulcers were not apparent on gross and histologic examination.

JPC Diagnosis: Lung: Bronchopneumonia, necrosuppurative, diffuse, marked, with diffuse fibrinous interstitial pneumonia, vascular thrombosis, and numerous cilia-associated bacteria, Domestic shorthair (*Felis catus*), feline.

Conference Comment: Conference participants agreed with the contributor's presupposition that the majority of the lesions in the lung are caused by *Bordetella bronchiseptica* (which was identified lined up parallel to cilia within large airways using Gram's stain). In the sections distributed to the participants, there are no vascular or other lesions that could be directly attributable to coronavirus infection, but the participants discussed that immunosuppression by FIPV predisposed this kitten to developing a respiratory bacterial infection.

Bordetella bronchiseptica is a normal commensal in the upper respiratory tract of most domestic animals but can act as a primary pathogen resulting in tracheo-bronchitis which can progress to pneumonia in more chronic cases. In pigs, it is the primary cause of nonprogressive atrophic rhinitis resulting in mild nasal discharge and sneezing. Certain strains of *B. bronchiseptica* can adhere to nasal cilia and tonsillar epithelium and produce toxins that cause distortion and loss of cilia, submucosal edema, and resorption of the turbinate bone. This process is completely reversible, but predisposes the animal to developing progressive atrophic rhinitis caused by *Pasteurella multocida* type D. This gram negative bacterium produces a cytotoxin, dermonecrotic toxin (encoded on the *toxA* gene), that results in nonreversible changes to the snout. Similar lesions occur in the epithelium and submucosa with one key difference: this cytotoxin causes proliferation of nearby fibroblasts which



Lung, kitten. Diffusely, alveolar septa are expanded by abundant congestion and fibrin, and there are multifocal areas of septal necrosis (arrows). (HE, 360X)

secrete mediators that induce hyperplasia and increased function of osteoclasts (reabsorbing bone) and decreased function of osteoblasts (reduced formation of bone).²

Expression of virulence factors mediated by the *Bordetella* virulence gene (*bvg*) operon allows for extensive variability among strains of *B. bronchiseptica*. The *bvg* operon encodes the following: (1) proteins necessary for attachment to host cells (adhesive proteins filamentous hemagglutinin (FHA), pertactin, fimbriae which aide in adhesion to ciliated cells) and (2) proteins that allow for bacterial survival (iron scavenging, motility mediation, urease and phosphatase activity). However, the most important contributors to the pathogenicity of *B. bronchiseptica* is the secreted adenylate cyclase toxin (hemolysin) which is of the RTX family related to *Mannheimia haemolytica* leukotoxin and *Actinobacillus pleuropneumoniae* Apx toxin. This potent toxin forms pores in target cells, generally leukocytes, to allow transfer of the adenylate cyclase component, resulting in increased cyclic AMP production, which impairs phagocytosis and oxidative burst and leads to cell death forming the characteristic "oat cells" seen microscopically composed of streaming nuclear debris.²

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<http://www1.vetmed.lsu.edu/laddl/index.htm>
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