Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 8

25 October 2017

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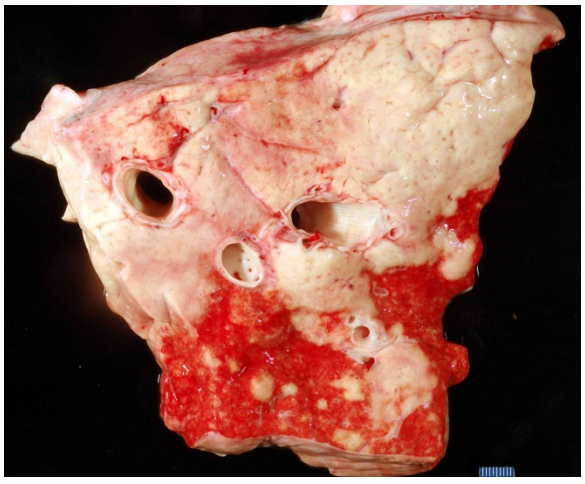
CASE I: 11111437 (JPC 4019838).

Signalment: 10-year-old, gelding, Thoroughbred, *Equus caballus*, equine.

History: Horse presented to the Veterinary Teaching Hospital with a two-month history of anorexia, weight loss and multiple oral ulcers. He was treated by the referring veterinarian with antibiotics, anti-inflammatory drugs and immune stimulants (dosages and types not provided by owner). On ultrasound, multiple pulmonary masses were noted distributed throughout all lung fields. Differential diagnoses included infection vs. neoplasia. Owner opted for euthanasia over further diagnostics and treatment. Patient was euthanized and submitted for necropsy.

Gross Pathology: The lungs are firm throughout, with prominent pleural vessels on the pleura and sub-pleura. Approximately 80% of the lung tissue is tan-to-white and matte; these regions are large and irregular. The remaining 20% of the lung tissue has multifocal, coalescing lesions, ranging from 1-5mm in diameter, also tan-to-white and matte. Yellow-brown purulent material is occasionally present in the bronchi on the left side. Bronchial lymph nodes are diffusely, severely enlarged and on cut surface are homogeneous and tan.



Lung, horse. Approximately 80% of the lung was replaced by white-tan fibrous tissue; the remaining 20% contain numerous fibrotic nodules. (Photo courtesy of: Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Rm 250 McElroy Hall, Stillwater OK 74078 USA http://www.cvhs.okstate.edu)

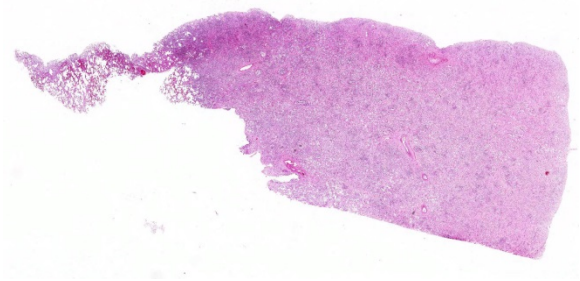
Laboratory results: None performed.

Microscopic Description: Lung: Within severely affected areas of the lung, alveolar septa are widely expanded due to dense mature fibrous connective tissue. There is moderate to marked pneumocyte hyperplasia that partially to completely line alveoli, and most lumens contain foamy macrophages interspersed with cell debris and other inflammatory cells including neutrophils, both viable and degenerate. Bronchiolar epithelium is hyperplastic and lumens contain debris, neutrophils, mucus, and foamy macrophages. Multifocally, within more normal areas, foamy macrophages are present within alveoli and bronchioles. Interalveolar septa are often mildly expanded due to fibrous connective tissue. Hemosiderin-laden macrophages are numerous within alveolar lumens and within septal walls.

The normal architecture of the bronchial lymph node is effaced by thick bands of fibrous connective tissue throughout the node. The cortex and medulla are not distinct, sinusoids are small and cellular, and lymphocytes throughout are mainly small lymphocytes. There is moderate extension of lymphocytes through the capsule.

The spleen (sections not provided) exhibits marked hemosiderosis of the red pulp. In white pulp, the lymphocytes are widely separated. Small lymphocytes predominate and are interspersed with a few large mononuclear cells that contain moderate cytoplasm and large round to oval, dense nuclei. Within some of these cells, there is chromatin margination and equivocal, centrally-located, viral-type inclusion body.

Contributor's Morphologic Diagnosis: Lung: Multinodular, interstitial pneumonia with marked interstitial fibrosis.

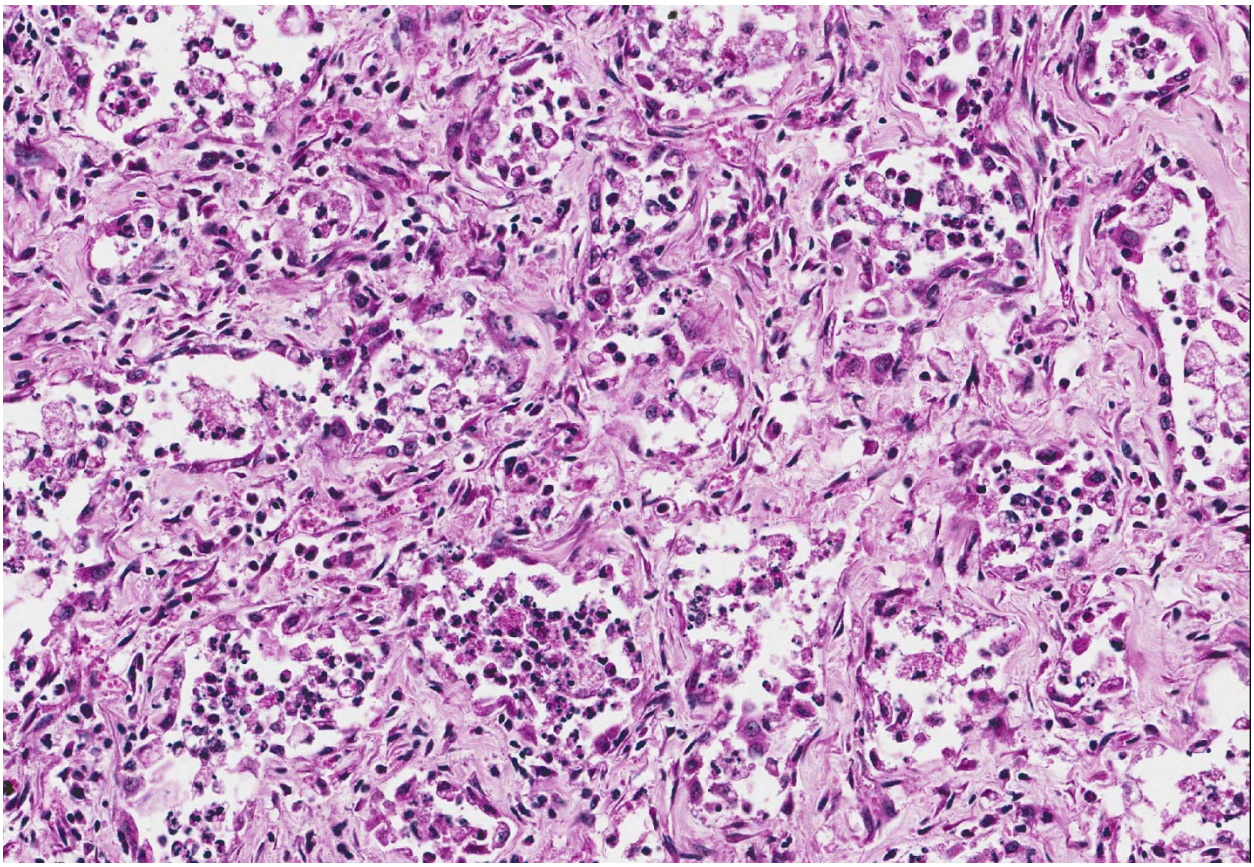


Lung, horse. Approximately 95% of the section is composed of a fibrotic process which effaces normal pulmonary parenchyma. A small area of normal tissue is present at the upper left. (HE, 5X)

Contributor's Comment: The lung lesions are consistent with the equine multinodular pulmonary fibrosis (EMPF) syndrome described by Williams et al. in the United States.[5] The syndrome has also been observed within horses in the UK.[4] This unique disease has been associated with infection by equine herpesvirus-5 (EHV-5).

Clinically, horses with multinodular pulmonary fibrosis syndrome have a mean age of 13-14 years and there is no sex or breed predilection.[5] The patients present with a variable display of tachypnea, tachycardia, respiratory difficulty, cough, and anorexia and weight loss.[5] Clinical pathology often shows a leukocytosis secondary to a mature neutrophilia and elevations of the acute phase inflammatory protein fibrinogen (hyperfibrinogenemia). Lymphopenia, characteristic of acute viral infection, is found in some EMPF horses.[5]

Grossly, there are two morphological forms of the disease.[3] Most common are large, individual to coalescing nodules of fibrosis that typically leave little to no normal lung left. A less common gross presentation are individual, disseminated nodules of fibrosis separated by normal lung tissue; this presentation can be confused with a



Lung, horse. Alveolar septa are markedly expanded by mature collagen and lined by cuboidal cells which resemble type II pneumocytes. Alveoli are variably filled by moderate numbers of neutrophils and foamy macrophages. (HE, 200X)

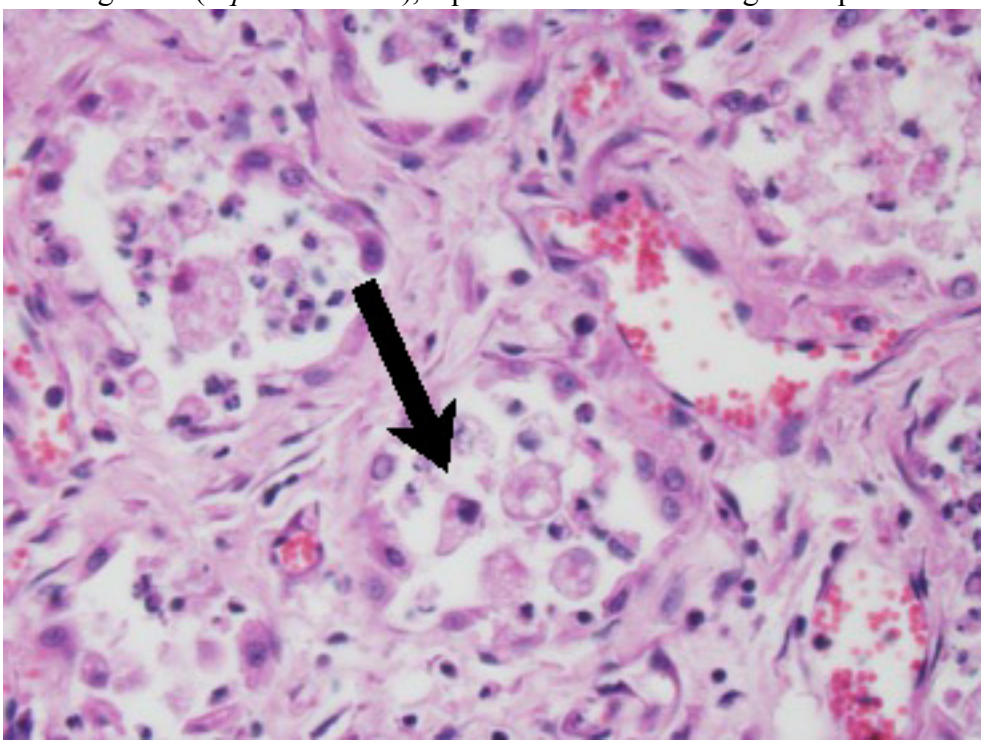
metastatic, neoplastic process, which was a clinical differential in the current case. The current case exhibited more of the "common" gross lesion characterized by near effacement of the lung by coalescing masses of fibrosis.

Histologically, there is marked interstitial fibrosis that can either retain the open alveolar architecture or efface it by less well organized bundles of fibrous connective tissue deposition. Retained airways are often filled with inflammatory cells. Bronchial lymph node lesions recognized by Williams et al.[5] included reactive lymphoid hyperplasia. In the current case, the lymph node was partially effaced by broad sweeping bands of fibrous connective tissue that obscured the corticomedullary junction and otherwise lacked the reactive hyper-plasia of the original report.

EMPF has been associated with EHV-5 infection. Intranuclear viral inclusions consistent with herpesvirus are seen primarily within macrophages located in the lesions.[3] In the present case, definitive inclusion bodies were not seen in any organ; however, equivocal inclusion bodies were seen within macrophages of the spleen. The spleen otherwise had no significant microscopic lesions. Although the patho-genesis of EMPF and relationship with EHV-5 has not yet been elucidated, murine models of pulmonary fibrosis may shed some clues. When pulmonary fibrosis is induced in mice infected with MHV68 (gammaherpesvirus) virally infected mice exhibit exacerbation of pulmonary fibrosis over non-infected controls.[6] This observation is accompanied by increased in the production of CCL-2 and

CCL-12, chemokines important for fibroblast recruitment.

JPC Diagnosis: Lung: Fibrosis, interstitial, focally extensive, severe with marked type 2 pneumocyte hyperplasia and rare intra-nuclear intrahistiocytic viral inclusions with marked intra-alveolar inflammation, Thoroughbred (*Equus caballus*), equine.



Lung, horse. Rare macrophages contain intranuclear viral inclusions consistent with equine herpesvirus-5. (HE, 400X)

Conference Comment: Conference participants discussed the extensive alveolar remodeling and the possibility that the cuboidal cells lining the airspaces were not type II pneumocytes but another cell type altogether. It was decided that type II pneumocyte hyperplasia was the most accurate option for the morphologic diagnosis because type II pneumocytes are the progenitor cells within the lung parenchyma. These cells repair damaged alveolar epithelium by covering the surface, repopulating the epithelium, secreting new basement membrane, and eventually differentiating into type I pneumocytes.[1]

The conference moderator (who discovered this entity) referred to a study by Marenzoni, et al. that identified *Equine herpesvirus 5* (EHV-5) using bronchoalveolar lavage and biopsy specimens antemortem and then during the postmortem examination were able to use quantitative real-time PCR on several tissues to identify the EHV-5 DNA load in those tissues. They concluded that the viral load was greatest in areas of fibrosis of lung and that higher viral burden resulted in more severe lesions.[2]

In this case, although the contributor only identified inclusions in the spleen (not submitted), the moderator and conference attendees were able to identify several convincing intranuclear viral inclusion bodies within swollen alveolar macrophages.

One remarkable aspect of this disease is the lack of temporal heterogeneity of the lesions. There is no progression in maturity of the lesions and they all appear to have begun at the same time. The conference moderator did not have an explanation for this finding and indicated it is an interesting aspect for future investigation.

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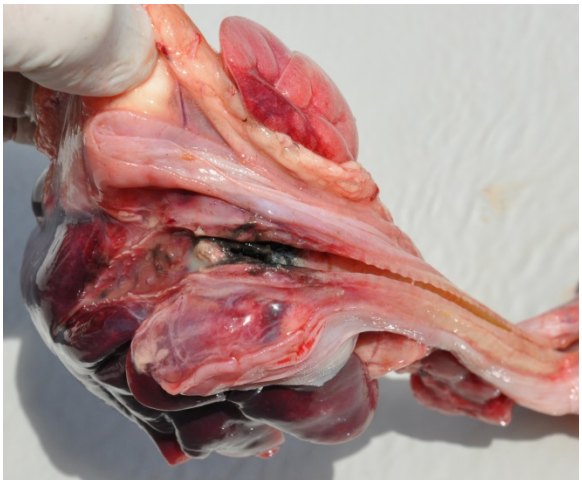
CASE II: N450-14 (JPC 4101759).

Signalment: 16-year-old, female, spayed, domestic crossbred, *Felis catus*, feline.

History: The cat had polyuria, polydipsia, anorexia, severe dyspnea, lethargy and progressive weight loss. In light of the clinical signs, a diagnosis of uncontrolled diabetes mellitus was suspected. The cat was hospitalized and treated to correct the fluid deficit and the increased electrolyte levels and received intramuscular insulin treatment (0.2 IU/ kg q24h). On the 10th day of hospitalization, after the acute onset of severe dyspnea, the cat died.

Gross Pathology: The lungs were swollen and heavy with a firm elastic consistency and were severely congested and slightly hemorrhagic. At the entrance to the right and left main bronchi there was a large quantity of friable, black material, interspersed with yellow foci that obstructed the bronchial lumen.

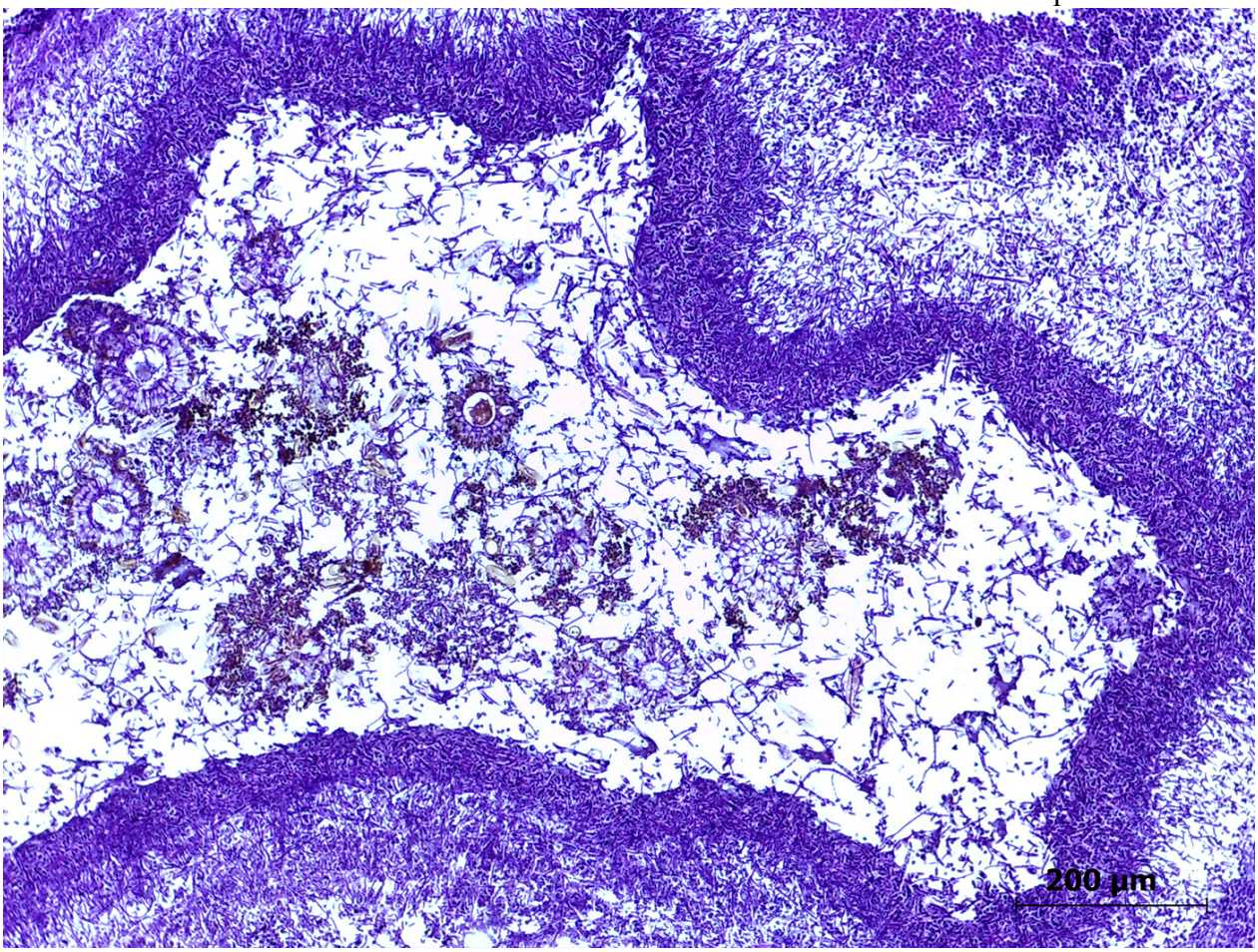
Laboratory results: Anaemia (hematocrit 21%, reference range [RR] 24-45%), leucopenia (4.0 x 10^9 /l; RR 5.0-19.5 109 /l),



Bronchus, cat. The tracheal bifurcation is occluded by a friable aggregate of black and yellow material. (Photo courtesy of: Setor de Patologia Veterinária, Universidade Federal do Rio Grande do Sul, Brazil, http://www.ufrgs.br/patologia/).

neutropenia (1.07x109 /l; RR 2.5-12.5 109 /l) and increased fructosamine (533.7 µmol/l; RR 219-247 µmol/l) and glucose (46.0 mmol/l; RR 4.05-7.43 mmol/l) levels were found. Urinalysis showed a moderate level of protein and a high level of glucose, with a specific gravity of 1.018 (RR >1.035). An ultrasound examination revealed a diffuse increase in the size and echogenicity of the liver and pancreas, likely resulting from hepatic lipidosis secondary to diabetes mellitus and pancreatitis. Sections of the lymph nodes and bone marrow underwent immunohistochemical evaluation for detection of feline immunodeficiency virus, feline calicivirus, and feline leukemia virus (FeLV). No viral antigen was observed in these tissues.

Microscopic Description: Lung: The pulmonary parenchyma and bronchial lumen revealed numerous septated, acute-angled or dichotomous branching hyphae (3-4 mm diameter). In addition, biseriate conidial heads with brown phialides and metulae, encompassing the entire surface, as well as smooth-walled, hyaline or darkened conidiophores, were found. The metulae developed in a double series and produced dark brown to black globose to subglobose conidia with rough walls (3-4 mm diameter). Septate hyphae occupied numerous air spaces and infiltrated the alveolar septa. An intense

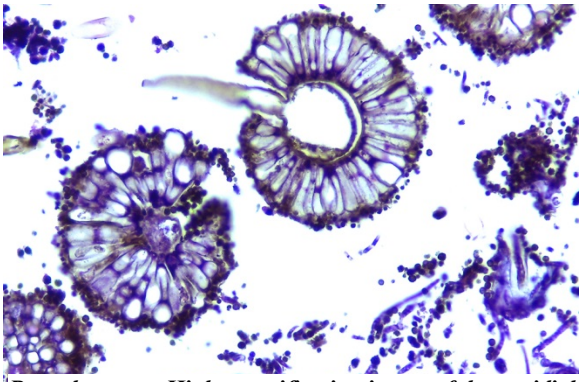


Bronchus, cat. The obstructive material was composed of a thick multilayered mat of fungal hyphae surrounding a moderate number of fruiting bodies (conidia). (Photo courtesy of: Setor de Patologia Veterinária, Universidade Federal do Rio Grande do Sul, Brazil, http://www.ufrgs.br/patologia/). (HE, 100X)

infiltrate that consisted predominantly of neutrophils and macrophages with fibrin deposition, associated with necrosis of the bronchial epithelium, and thrombosis was also observed. A methenamine silver stain was performed on the lung sections to highlight the fungi. Sections of the pulmonary parenchyma and bronchi were viewed under polarized light and revealed a high number of birefringent crystals with radiating spokes, consistent with calcium oxalate. No other tissue had evidence of fungal infection.

Contributor's Morphologic Diagnosis: Lung: Bronchopneumonia, pyogranulomatous necrotizing, chronic-active, multifocal and extensive, severe, with intralesional fungi showing conidial heads and dichotomous branching hyphae of *Aspergillus* section *Nigri* and oxalate crystals.

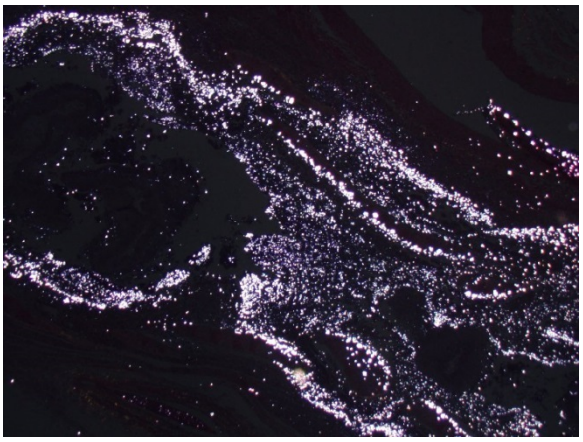
Contributor's Comment: There was a second cat with similar signs which also had diabetes and that had been sent, after its death, to necropsy. Fungal isolation was performed (only in the second case) by seeding samples and arranging tissue fragments in Sabouraud's agar and malt agar. Fungal disease was not suspected in the first cat and unfortunately, no samples were collected for culture. The cultures from the second cat were incubated with chloramphenicol at 37ºC for 7 days for the isolation of *Aspergillus* spp. Identification of the fungal genus and section was performed by observing both the gross and the microscopical aspects of the colonies, which showed a fungus with an aerial black-stained mycelium and colorless reverse, identified as *Aspergillus* section *Nigri*.



Bronchus, cat. High magnification image of the conidial heads of Aspergillus niger. (Photo courtesy of: Setor de Patologia Veterinária, Universidade Federal do Rio Grande do Sul, Brazil, http://www.ufrgs.br/patologia/). (HE, 400X)

Infections caused by *Aspergillus* spp. result in significant mortality in man and animals. Despite their ubiquitous distribution in indoor and outdoor environments, species in the *Nigri* section are not the most frequent cause of aspergillosis.[8] Compared with species in the *Fumigati* section, species in the *Nigri* section have larger conidia, which allow easy uptake by the host mucociliary system and alveolar macrophages. The conidia of *Aspergillus* spp. develop from mycelia under high oxygen tension or severe infection, and they are not usually observed in histological sections.[1] Fungal pneumonia caused by *A. niger* has been identified in horses by fungal culture,[3] in dogs by molecular analysis[6] and in man by fungal culture;[8] however, there have been no reports of *A. niger*-mediated pneumonia in cats.

A. niger is part of a complex that includes many species, and it is difficult, if not impossible, to distinguish other species phenotypically, except using molecular identification by polymerase chain reaction and sequencing.[10] The finding of calcium oxalate crystals in affected tissue is not recognized commonly in domestic animals, although it has been reported in human cases



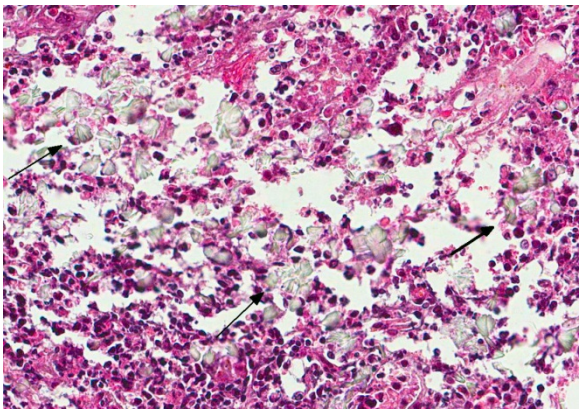
Bronchus, cat. At subgross magnification, there are innumerable birefringent crystals at the periphery of the fungal lesion. (HE, 100X)

and has been considered a classical feature of *A. niger* infection.[7,8] Both calcium oxalate crystals and numerous conidia were observed in the lung tissues of both cats, confirming *A.* section *Nigri* as the etiological agent. Oxalic acid is toxic and can damage tissues and the surrounding blood vessels, which explain the associated inflammatory infiltrate, composed mostly of neutrophils. However, the presence of oxalate crystals is most likely to be compatible with chronic inflammation.

No breed or gender predisposition is apparent for invasive focal or disseminated feline aspergillosis, and cats with sinonasal/sino-orbital aspergillosis can be of any age.[2] Additionally, systemic immuno-compromise due to co-morbidities (e.g. feline parvovirus, FeLV or feline infectious peritonitis virus infection, or prolonged corticosteroid treatment), has been considered supportive evidence of aspergillosis.[2] In this report, diabetes mellitus identified as a risk factor for the development of aspergillosis in cats, may have triggered and/or favoured the development of fungal infection in the lower respiratory tract.[5] Previous reports in man indicate that prolonged diabetes mellitus, prolonged exposure to fungi and old age can predispose patients to mycosis because these patients provide a favourable environment for the growth of *Aspergillus* spp..[11] All of these features are similar to those observed in the present cases.

In man, invasive pulmonary aspergillosis occurs predominantly in immunocompromised hosts; however, the number of cases has increased among immunocompetent patients who have certain pulmonary abnormalities, such as lung neoplasia, as occurred in the first of the present cases. Diabetes mellitus is a metabolic disease that can trigger a series of systemic complications. Furthermore, infections accompanied by high morbidity and mortality are common in diabetic patients. Susceptibility to infection results from immune dysfunction, including reduced cytokine production, immune cell function and migration. Several studies have also indicated an association between secondary toxic metabolites, aspergillosis and immunosuppression of the host.[9] Invasive aspergillosis caused by *A.* section *Nigri* is rare, and the cases described here demonstrate the aggressive nature of species of this section and the potential to cause opportunistic infection in immunocompromised cats. Based on the gross and microscopical findings of fungal hyphae combined with calcium oxalosis and mycological examination, the diagnosis of chronic invasive pulmonary aspergillosis due to *Aspergillus* section *Nigri* was confirmed in two diabetic cats. Not all sections contain conidiophores, however all of them have hypha within bronchial lumina.

JPC Diagnosis: Lung: Bronchitis, pyogranulomatous and necrotizing, severe, with bronchiectasis, numerous fungal hyphae, pigmented conidia, and calcium



Bronchus, cat. Higher magnification demonstrates numerous fan-shaped oxalate crystals at the periphery of the lesion, enmeshed in fibrin and degenerate neutrophils. (HE, 400X)

oxalate crystals, domestic shorthair, (*Felis catus),* feline.

Conference Comment: The conference moderator reviewed the typical gross appearance of these lesions and referred to them as "aspergillomas" or "fungal balls".[4] These lesions occur in areas of the respiratory tract with high oxygen tension (often in the nasal cavity, nasopharynx, or large conducting airways where a high oxygen tension allows for mycelial growth and may result in dyspnea and death. In this case, there were contextual clues indicating chronic disease, specifically, loss of cartilaginous basophilia and collapse of the parenchyma at the periphery of the bronchus.

Aspergillus sp. rarely causes lung disease in domestic animals. Of note, *Aspergillus fumigatus* and *A. flavus* can cause chronic destructive bronchitis in German Shepherd dogs. The lesions generated are similar to this case: erosion of the epithelium, neutrophil infiltration, and granulation tissue formation in ulcerated airways. There are mild microscopic differences in the morphology of the fungus (mentioned above by the contributor). This condition is very severe locally, but there have been no reports of progression to invasive systemic aspergillosis in dogs.[4]

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http://www.ufrgs.br/patologia/

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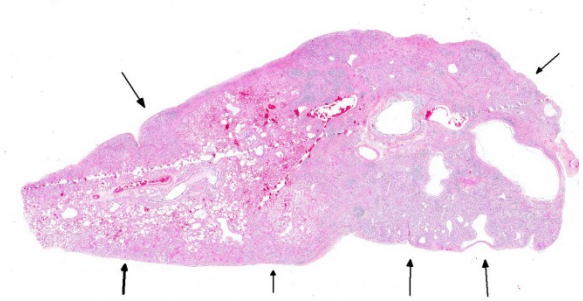
CASE III: P1270-11 (JPC 4018605).

Signalment: 10-year-old, male, neutered, domestic, *Felis catus*, feline.

History: This cat appeared to be in good health at 1 :00pm; the owner reported he had appeared to sleep more than usual for the last few days. At 1:00am that night, the owner found his cat in severe respiratory distress with sialorrhea. The cat died after an hour with no change in his condition, on its way to the veterinarian.

Gross Pathology: The cat was in good body condition. There were numerous firm whitish nodules, 0.5 to 2 mm in diameter, distributed extensively throughout both lungs, with multifocal atelectasis. A mild amount of mucoid material filled the distal trachea.

Laboratory results: Routine bacteriology on the lungs was not significant (rare contaminants), and negative on liver and kidney.

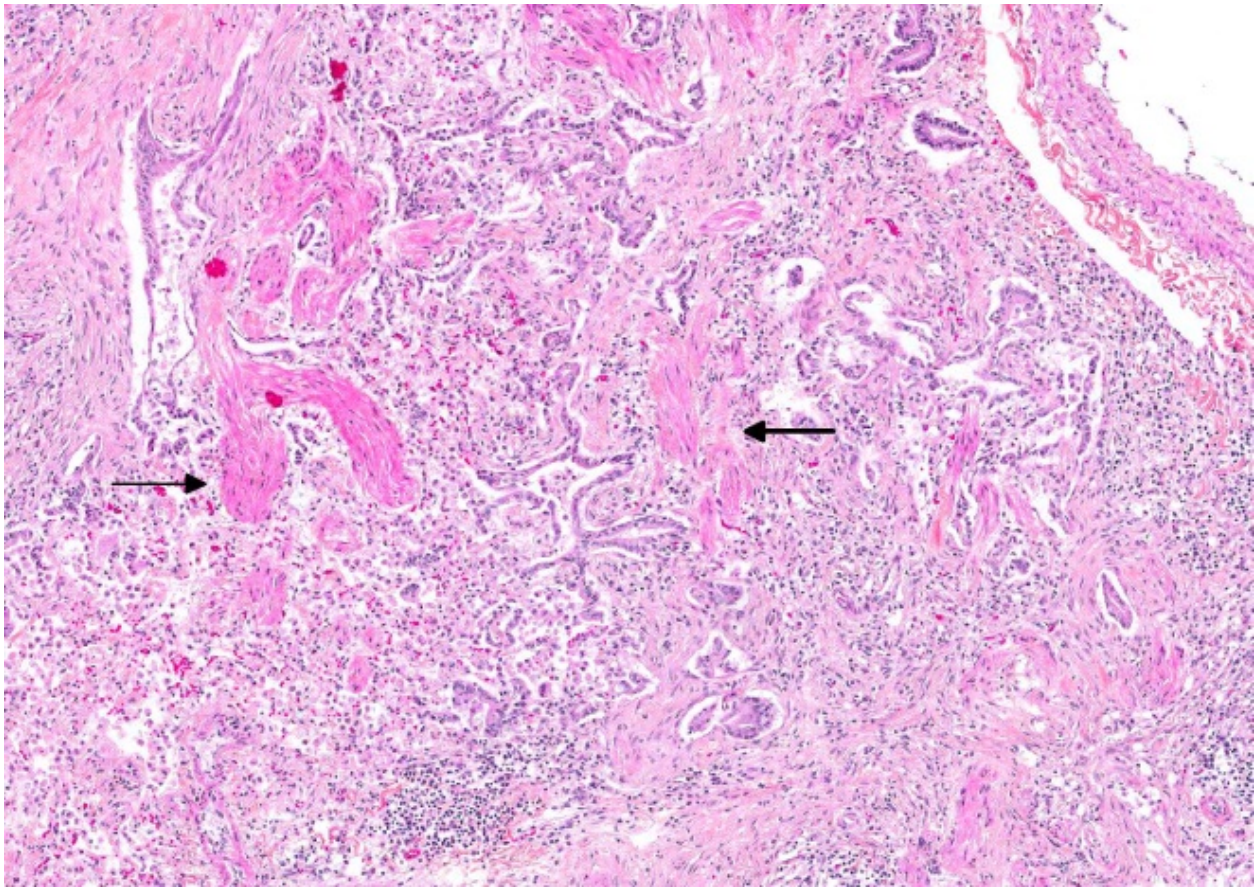


Lung cat. A section of lung with multiple subpleural nodules (arrows) is presented for examination. (HE, 5X)

Microscopic Description: Lung: There is extensive multifocal remodeling of the pulmonary parenchyma, more severe in subpleural areas. The alveolar septa and distal airways are distorted and moderately expanded by dense collagen (fibrosis), smooth muscle bundles and, variably, clusters of mesenchymal cells (fibroblasts/myofibroblasts); they are lined by cuboidal to columnar epithelial cells, and multifocally form larger tubular structures (honeycomb pattern). The alveoli and bronchioles in affected areas are occasionally filled with plump macrophages, and rare to few neutrophils. The epithelium of several, usually larger bronchioles have several goblet cells (mucous metaplasia) and a few foci of squamous metaplasia are visible. There are multifocal moderate lymphoplasmacytic interstitial and subpleural infiltrates. The pleura is multifocally covered by cuboidal to low columnar (hypertrophied) mesothelial cells. There is some variation between the 2 sections/slides submitted. In one of the sections, there is a focal bronchiolo-alveolar proliferation with minimal stroma, suggesting bronchiolo-alveolar adenoma or carcinoma, and focal mucopurulent bronchitis. In the other section, there is multifocal mineralization.

Contributor's Morphologic Diagnosis: Lung: Extensive, multifocal, moderate to marked interstitial pulmonary fibrosis with smooth muscle hyperplasia/metaplasia, epithelial hyperplasia/metaplasia, and fibroblast/myofibroblast foci (probable bronchiolo-alveolar adenoma or carcinoma in one section).

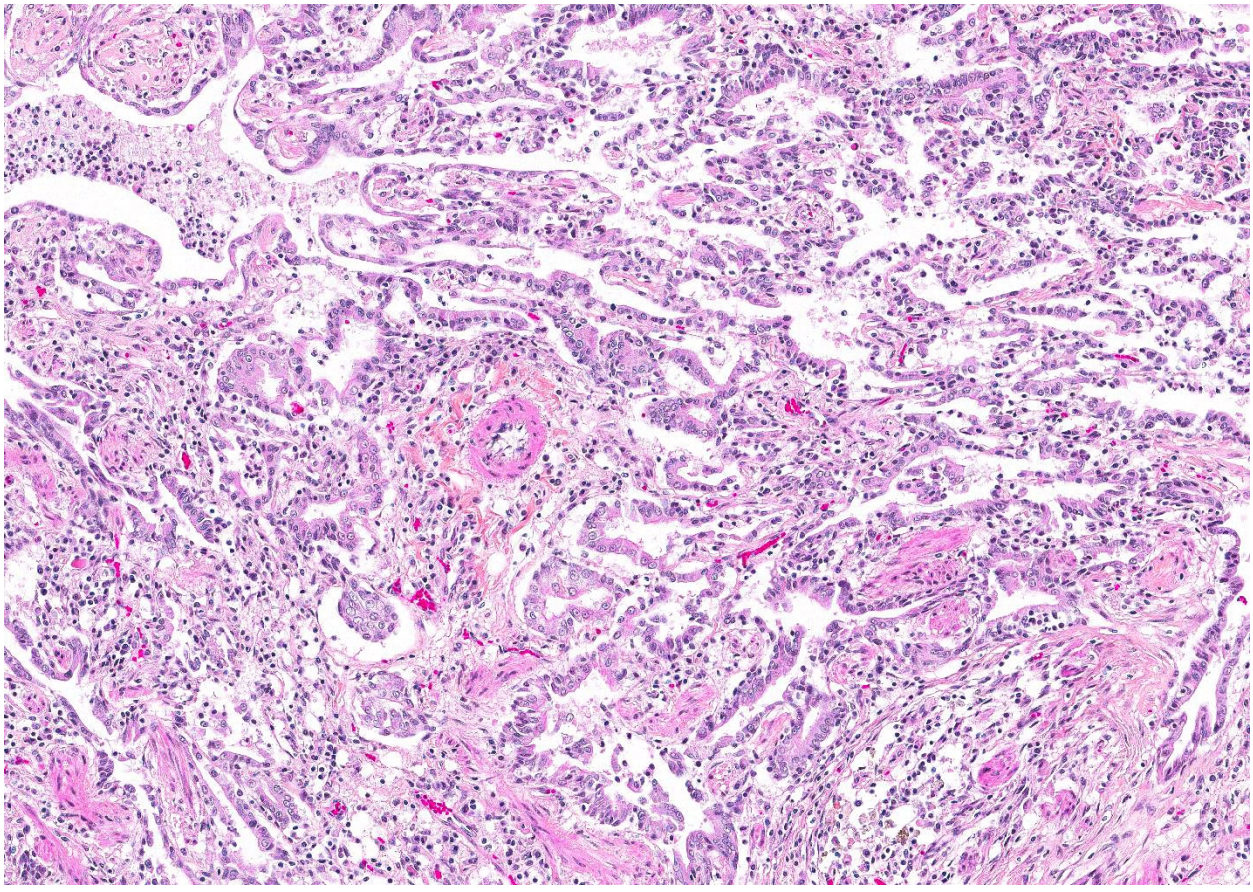
Contributor's Comment: The histological changes in this lung are consistent with feline idiopathic pulmonary fibrosis (FIPF), a previously reported feline interstitial lung disease. Interstitial lung diseases (ILDs) are a heterogeneous group of disorders affecting the pulmonary interstitium, more precisely the alveolar wall, with a variety of etiologies; however, the cause often remains unknown. Most ILDs result from exaggerated inflammatory and reparative responses to an initial insult, which may be infectious, toxic or environmental[2]. Immune-mediated connective tissue disorders result in ILDs in humans and perhaps in dogs; a similar role in feline pulmonary disease has been suggested but has not been proven[2]. In veterinary medicine, lung diseases with a prominent fibrotic component of unknown etiology are often called idiopathic pulmonary fibrosis (IPF). In humans, at least four distinct entities used to be classified as IPF, including usual interstitial pneumonia (UIP)[4]. A consensus statement established by the American Thoracic Society and the European Respiratory Society defined the criteria for the diagnosis of IPF in humans and eliminated all but UIP from the definition of IPF. The histologic features of UIP lungs are



Lung cat. Large areas of fibrosis efface normal pulmonary architecture. Distorted airways and associated smooth muscle (arrows) remain. (HE, 88X)

temporal heterogeneity of lung remodeling, with the primary changes being interstitial fibrosis and ongoing fibroblast/myofibroblast proliferation, metaplasia of the alveolar epithelium (honeycomb change with enlarged airspaces lined by prominent variable epithelium), and scant inflammation[4].

Feline idiopathic pulmonary fibrosis (FIPF) is an uncommon chronic interstitial lung disease associated with chronic progressive tachypnea, respiratory distress and cough in middle-aged to older cats[2]. Contrary to other ILDs, IPF is believed to be a fibroproliferative disorder. FIPF type II pneumocyte ultrastructure revealed abnormal dense cytoplasmic lamellar body-like inclusions; this finding is similar to a heritable form of human IPF and is possibly related to a defect in type II pneumocyte biology[4]. FIPF is defined by histopathologic characteristics of usual interstitial pneumonia (UIP). Lesions can be diffuse or patchy. Type II pneumocyte and myofibroblasts are important cellular constituents of feline IPF. Williams reported that myofibroblasts were prominent in foci, beneath honeycombing and hyperplastic epithelium, and in alveolar septa away from the remodeling[4]. An association between pulmonary fibrosis and carcinoma is recognized in humans and animals. Cohn described an IPF-like condition with a coincident pulmonary neoplasia in 6 of 23 cats[2]. These pulmonary neoplasms were generally located in areas of



Lung cat. In a focally extensive area of the lung, numerous tortuous airways, lined by hyperplastic epithelium impart a characteristic "honeycomb" appearance to the lung. (HE, 106X)

marked fibrosis but the neoplastic foci were limited in extent. Hypotheses to explain coexistence of lung cancer and fibrotic ILD include progression of epithelial hyperplasia to neoplasia, shared etiologic risk factors, and induction of carcinogenesis by diffuse inflammation.

Another idiopathic ILD, diagnosed as the desquamative form of cryptogenic fibrosing alveolitis has been described in a single cat[3]. Idiopathic fibrotic ILD has been described in the dog (West Highland White Terrier). It is characterized by progressive and poorly responsive interstitial fibrosis[1]. However, the condition in dogs is not histologically equivalent to UIP since it lacks alveolar metaplasia, smooth muscle hyperplasia or metaplasia and foci of ongoing fibrosis[2]. Equine multinodular pulmonary fibrosis is another progressive fibrotic lung diseases that has been associated with an EHV-5 virus[1].

JPC Diagnosis: Lung: Fibrosis, interstitial, subpleural, multinodular, severe with marked alveolar loss, bronchiolarization and bronchiolar epithelial metaplasia, and arteriolar and bronchiolar smooth muscle hyperplasia, domestic (*Felis catus),* feline.

Conference Comment: In contrast with case I (equine multinodular pulmonary fibrosis), this disease entity results in progressive fibrosis with areas of less mature fibrous connective tissue adjacent to mature collagen. The moderator commented that there is very little inflammation in these cases which rules out the possibility of post-inflammatory remodeling. There is also occasional traction bronchiectasis present which is caused by contraction of the fibrous connective tissue pulling apart bronchioles.

There were no pulmonary adenomas or adenocarcinomas in our sections, although it is entirely possible that it was present in the sections viewed by the contributor. Commonly, epithelial hyperplasia associated with this entity is mistaken for a neoplasm; however, IPF can predispose cats to pulmonary adenocarcinoma due to chronic fibrosis and remodeling of the airways.[1]

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College of Veterinary Medicine

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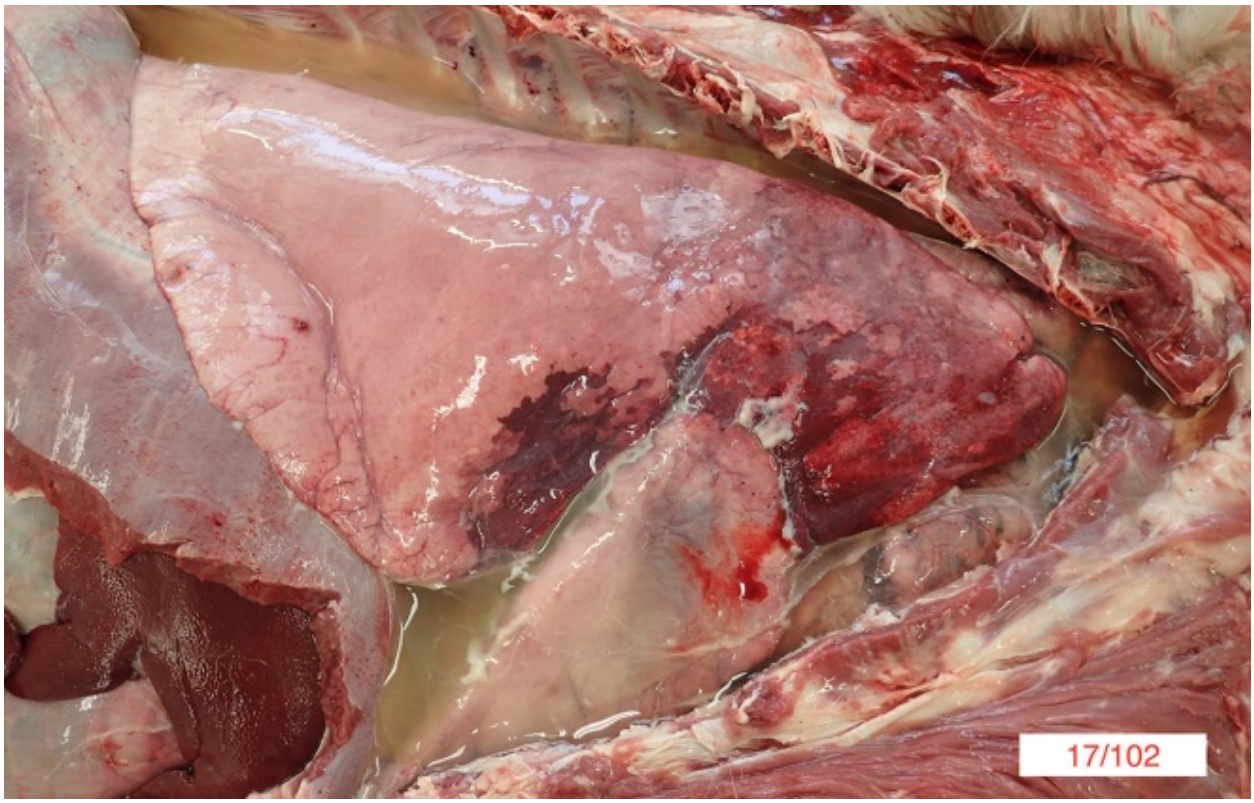
CASE IV: H17-0145J (JPC 4100435).

Signalment: Unknown age, female, *Vicugna pacos*, alpaca.



Lung alpaca. The cranioventral regions of the right lung lobes were dark red and firm and oozed dark watery fluid. (Photo courtesy of: Veterinary Pathology Department, School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, Western Australia. http://www.murdoch.edu.au/School-of-Veterinary-and-Life-Sciences/)

History: A single deceased, female alpaca, which had recently given birth to a premature cria, was submitted to the anatomic pathology service. 23 alpacas had died on the property in the previous week. All ages of animals were affected. Death occurred within approximately 48h after the onset of clinical signs, which included ataxia, respiratory distress, dark brown bloody nasal and oral discharge and abortions. Three deceased alpacas were submitted a day earlier to the Department of Agriculture and Food Western Australia. The farmer reported a history of heavy rainfall 7-10 days prior to the animals becoming ill, and that the property had not had animals on it for 5 years before the alpacas were moved on to the property.

The submitted alpaca was previously treated at The Animal Hospital at Murdoch University with intranasal oxygen and intravenous fluids, however, it was found deceased the next morning and submitted to Murdoch University pathology department.

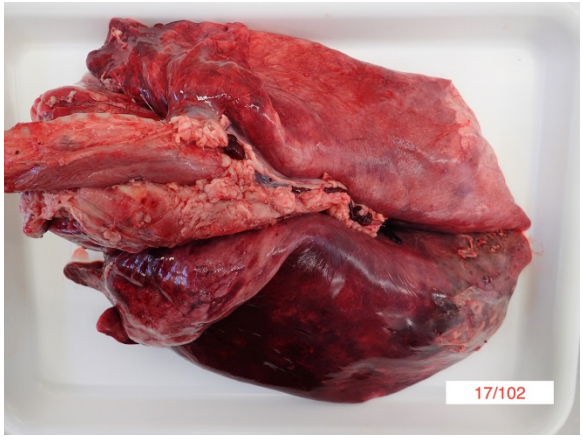
Gross Pathology: On gross necropsy examination the alpaca was in lean body condition (2/5) and had hundreds of approximately 1-3mm diameter dark red foci throughout its oral and conjunctival mucosae, subcutaneous tissues and thoracic pleura.

Diffusely the left lung lobes were mottled dark red to black, firm and oozed a moderate amount of dark red, watery, turbid fluid. Multifocally scattered throughout the left lung lobes there were fewer than ten, multifocal to coalescing, approximately 1-

10cm diameter, firm, irregularly shaped nodules, which ranged from pale tan to black. Approximately 10-20% of the cranioventral region of the right lung lobes was discoloured dark red and firm and oozed a small amount of dark red watery fluid. Approximately 600mL of pale yellow, clear fluid, which contained moderate amounts of pale tan, easily broken down, stringy material was present within the thoracic cavity.

Approximately 500mL of pale yellow, clear fluid, which contained moderate amounts of pale tan, easily broken down, stringy material was present within the abdomen. Approximately 20mL of a similar fluid was present within the pericardial space.

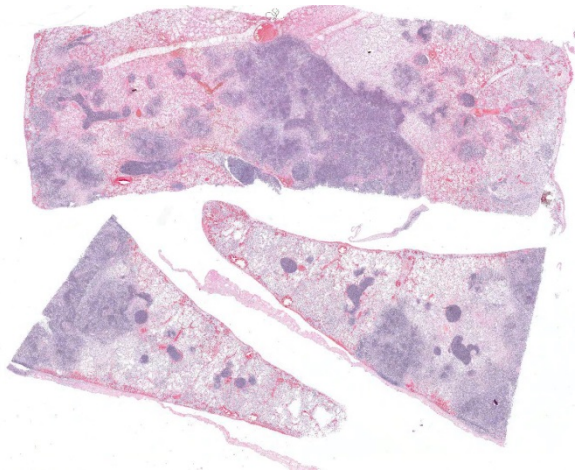
The duodenal mesenteric lymph nodes measured approximately 3cm x 4cm x 5cm to 4cm x 4cm x 7cm respectively with replacement of the normal architecture by moderate amounts of pale tan, thick, turbid fluid and pale tan, firm to crumbly material.



Lung alpaca. Scattered through the left lung lobe are >10 1-10cm tan to black nodules. (Photo courtesy of: Veterinary Pathology Department, School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, Western Australia. http://www.murdoch.edu.au/School-of-Veterinary-and-Life-Sciences/)

Laboratory results: *Burkholderia pseudomallei* was isolated from aseptically collected samples of both the lung and mesenteric lymph node.

Microscopic Description: Lung: Multifocally disrupting and infiltrating the pulmonary architecture are variably dense aggregates of large numbers of frequently degenerate neutrophils, low numbers of



Lung alpaca. Three sections of lung are submitted for examination. (HE, 5X)

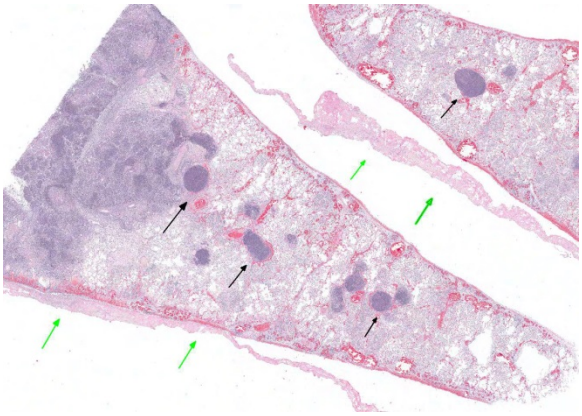
foamy macrophages and large amounts of pale eosinophilic fibrillar material (fibrin) intermixed with moderate amounts of amorphous eosinophilic and karyorrhectic debris (necrosis). Multifocally present within the cytoplasm of occasional macrophages and occasionally free within the tissue there are low numbers of approximately 2µm in length, 1µm in diameter, gram-negative bacilli. The pulmonary pleura is multifocally expanded and infiltrated by large amounts of pale eosinophilic fibrillar material (fibrin), intermixed with moderate numbers of degenerate neutrophils and fewer lymphocytes and macrophages. Within the remaining pulmonary parenchyma,alveoli contain and are occasionally filled by moderate amounts of a similar inflammatory infiltrate intermixed with moderate numbers

of extravasated erythrocytes (haemorrhage) and moderate numbers of macrophages, which frequently contain one to two intracytoplasmic erythrocytes.

Contributor's Morphologic Diagnosis: Lung: Severe, acute, multifocal to coalescing, necrosuppurative and fibrinous bronchopneumonia with haemorrhage, fibrinous pleuritis and intra- and extracellular gram-negative bacilli.

Contributor's Comment: This case represents a case of fatal melioidosis, caused by the bacterium *Burkholderia pseudo-mallei*, in an unusual geographic location within Australia. In addition to the alpaca submitted to our diagnostic necropsy service, three additional alpacas from the same property were also involved in the initial outbreak and confirmed to have similar gross and histopathologic changes with a positive culture of *B. pseudomallei*. Furthermore, a single macaw from a neighboring property, which was co-infected with *Chlamydia psittaci*, was also found dead with similar gross necropsy changes and positive *B. pseudomallei* culture.

Differentials for pneumonia reported in alpacas, particularly neonatal alpacas, include bovine respiratory syncytial virus, Parainfluenza type 3 virus, *Pasteurella multocida*, and *Mannheimia haemolytica*. The aforementioned infectious agents have been described in crias to elicit variable gross and histologic pneumonias ranging in severity (mild to severe), distribution (focal to diffuse) and pathologic processes (e.g. necrotizing, fibrinous, suppurative).[14]Additionally bovine diarrhea virus, bovine herpesvirus-1, influenza virus A and *Mycoplasma spp*. have been shown to be associated with lower respiratory diseases in alpacas.[14]



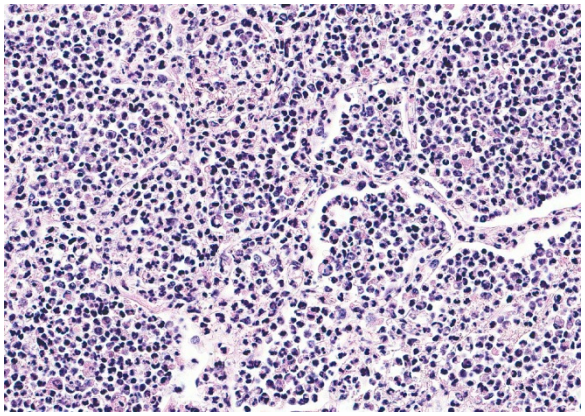
Lung, alpaca. At higher magnification, bronchioles are outlined by a dense cellular exudate (black arrows). Alveoli at left are filled with a similar exudate in a geographic pattern at left. The pelura is covered by a thick mat of fibrin. (HE, 10X)

Melioidosis is typically considered endemic to northern Australia and Southeast Asia with endemic and sporadic cases also reported in various countries within South and North America, Africa, the Middle East and Oceania in addition to China, India Puerto Rico, Haiti, Guadeloupe, Haiti and Martinique.[6,15] The location of the outbreak reported in this case is unusual, considering its geographic occurrence within the southwestern wheatbelt region of Western Australia. Further investigation revealed historical evidence of previous outbreaks of melioidosis on the same property. It is thought that the reported heavy rainfall, atypical for the season, immediately prior to this outbreak lead to a re-emergence of the bacterium.

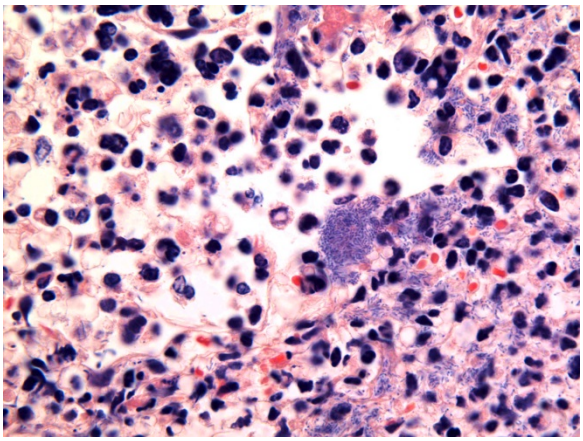
B. pseudomallei is a saprophytic gram-negative motile non-spore forming bacillus which is able to survive in soil and water for many years and in adverse environmental conditions including low pH and high temperatures.[7,10] Infection typically occurs from inhalation of contaminated dust, ingestion of contaminated water or introduction of contaminated soil or water

into skin wounds.[10] Horizontal transmission between infected animals and people has not been reported; however, vertical transmission through the placenta has been reported.[9] Whilst no evidence of zoonotic transmission for *B. pseudomallei* has been demonstrated, indirect transmission from animals and animal products has been argued to pose a possible risk to human health.[8]

Incidence of infections within endemic areas is highest within monsoonal seasons, following high rainfall events. [1,2] The hypothesized cause of this is the contribution of warm, wet conditions to the rapid proliferation of the bacterium within the soil after it has been brought to the surface by a rising water table. [1] Infection has been reported in humans, domestic and non-domestic animal species.[13] Reported cases in domestic species are most prevalent in ruminants and swine, with swine having been reported to be less susceptible to the disease than goats and sheep.[11,13] Additional reports of melioidosis within the literature include horses, cats, dogs, iguanas, rodents, camels, alpacas, horses, deer, tree kangaroos, wallabies, koalas, crocodiles, numerous



Lung, alpaca. Higher magnification of the affected alveoli, which are filled with an exudate of numerous viable and degenerate neutrophils, fewer macrophages and cellular debris. Alveolar septa are likewise expanded by large numbers of similar cells, edema and fibrin. (HE, 280X)



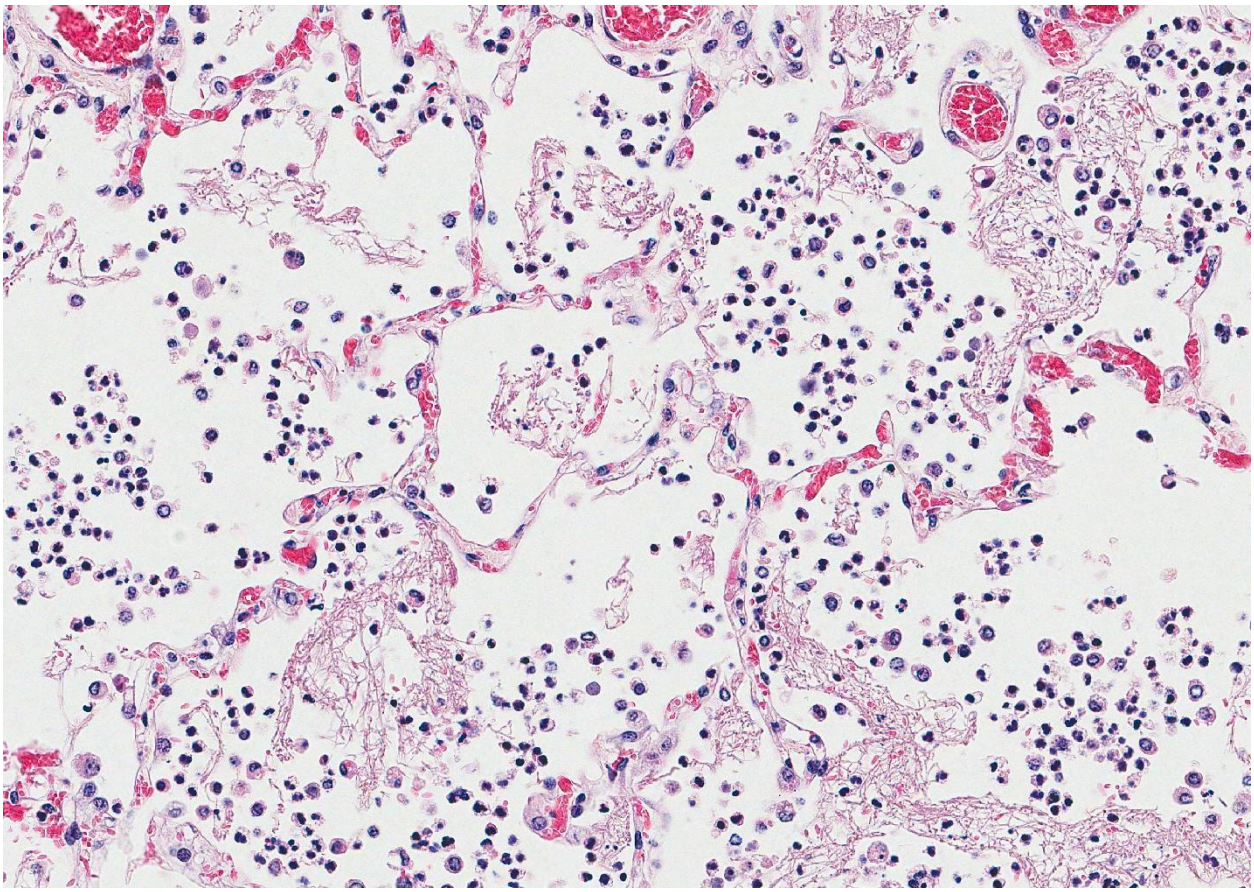
Lung, alpaca. Alveoli contain numerous intra- and extracellular gram-negative bacilli. (Gram, 400X) (Photo courtesy of: Veterinary Pathology Department, School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, Western Australia. http://www.murdoch.edu.au/School-of-Veterinary-and-Life-Sciences/)

avian species, captive marine mammals and a number of non-human primates.[12,13,16,17] Clinical disease and necropsy findings in all species are highly variable, depending on the infection entry site, bacterial strain and immune status of the animal.[15] In pigs, melioidosis is frequently asymptomatic with lesions detected upon routine abattoir inspection of carcasses.[11] In other species, pneumonia and chronic, localized infections are most commonly reported, however bacteremia leading to septic shock, neurologic symptoms and mastitis have also been documented.[15,16] Gross and histopathologic changes can range from acute necro-suppurative foci present within multiple organs to chronic, well-formed granulomas.[16] It is important to note that *B. pseudomallei* can be misidentified as a contaminant, as *Pseudomonas spp*. or other non-pathogenic *Burkholderia spp*. by standard identification methods.[9] Furthermore *B. pseudomallei* do not typically colonize the skin and, thus, identification of the organisms should always be considered as a true infection.[9]

Whilst any animal is potentially susceptible to infection, increased risk of infection, particularly fatal infection, with *B. pseudomallei* is found in people with underlying medical conditions including diabetes mellitus, renal dysfunction, immunosuppression, excessive alcohol intake, pulmonary disease, malnutrition and thalassemia.[9,15] Infection in people can undergo a period of latency, which has been reported to last up to 62 years, with reactivation of latent melioidosis to clinical disease which is well documented in patients with immunosuppression.[3,10] An investigation into the immunologic factors inferring susceptibility to *B. pseudomallei* has been performed using BALB/c-C57BL/6 mouse models. Major findings in this study demonstrated *in vivo* depletion of macrophages rendered C57BL/6 mice highly susceptible to intranasal infection with *B. pseudomallei* and that increased bacterial loads and higher mortality rates were observed for TNF-a, TNFR1 and TNFR2 knockout mice.[4]

Given the above information, the number of alpacas affected the severity of disease and the involvement of a potentially immuno-suppressed macaw in the outbreak has raised a question regarding the susceptibility, including the mechanisms of susceptibility, of alpacas to this disease. This would require further investigation and is a topic for potential future research.

JPC Diagnosis: Lung: Bronchopneumonia, necrosuppurative and fibrinous, diffuse,



Lung, alpaca. Damage to alveolar septa throughout the lung has resulted in extrusion of edema flue and polyermized fibrin both within the septa and into surrounding alveoli, due to the endotoxin release from damage B. pseudomallei. (HE, 224X)

severe, with fibrinous pleuritis, alveolar necrosis, and rare intra-and extracellular bacilli, *Vicugna pacos*, alpaca.

Conference Comment: *Burkholderia pseudomallei* (details described above) result in either acute or chronic disease patterns. In acute disease, which is more common in younger animals, initially the lungs are infected followed by systemic infection. The chronic pattern is more frequent and is characterized by abscesses in multiple organs which are often incidental findings at slaughter. Due to the zoonotic risk, care must be taken at slaughter, as these abscesses are not characteristic and are often mistaken for caseous lymphadenitis (*Corynebacterium pseudotuberculosis*) and glanders (*Burkholderia mallei*). Brain lesions must be differentiated from listeriosis. Besides its endotoxin (typical of a gram-negative bacterium), *B. mallei* has several virulence factors including: malleobactin (an iron-scavenging protein), secreted proteases to degrade tissues, a polysaccharide capsule that protects them from phagocytic killing, and *Burkholderia* lethal factor 1 which inhibits translation and causes death of host cells.

Discussion in this case also covered the two superimposed lesions within the submitted tissue – both a necrotizing broncho-pneumonia as well as a diffuse fibrinous pleuropneumonia, both likely resulting from the presence of *B. pseudomallei*. Protease secretion of the bacterium results in the severe bronchopneumonia; killing of the bacteria results in liberation of endotoxin, diffuse damage to the pulmonary and pleural endothelium, and a fibrinous interstitial pneumonia and pleuritis.

Contributing Institution:
http://www.murdoch.edu.au/School-of-Veterinary-and-Life-Sciences/

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