Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2014-2015

Conference 19

1 April 2015

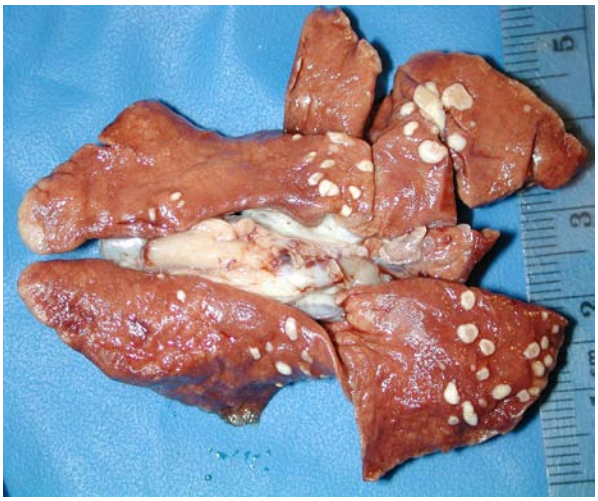
CASE I: PA 4683 (JPC 3136052).

Signalment: Adult (age unspecified) female ferret, *Mustela putorius furo.*

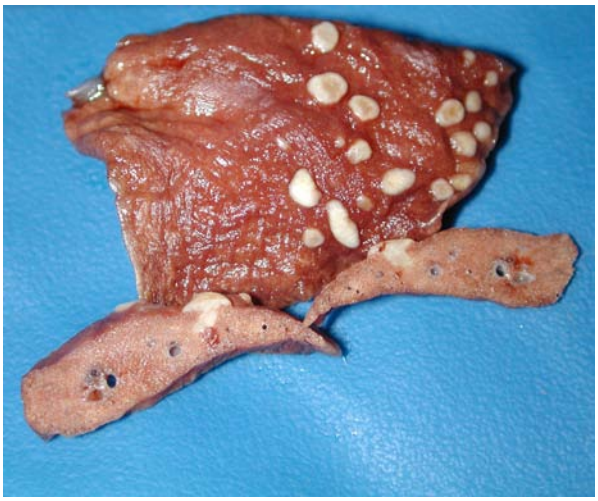
History: This was one of several ferrets in this colony suffering from debilitation due to watery diarrhea. The animal died despite supportive care and a necropsy was performed by the overseeing clinical veterinarian.

Gross Pathologic Findings: No gross lesions were reported in the gastrointestinal tract. Lungs appeared collapsed and atelectatic, with multifocal pale, plaque like areas of discoloration noted throughout all lobes.

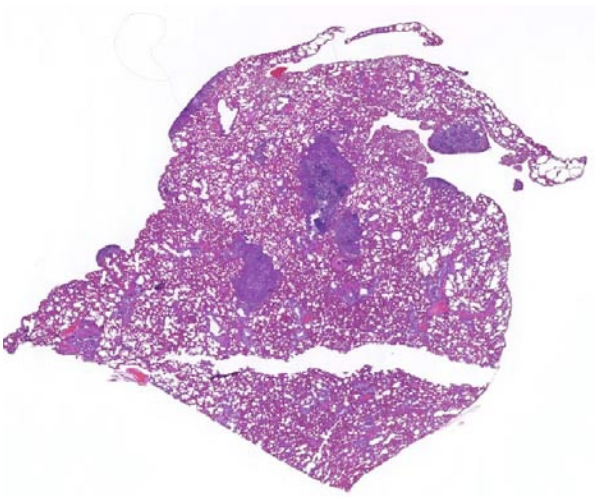
Laboratory Results: PCR evaluation of feces was positive for ferret enteric coronavirus (FECV).



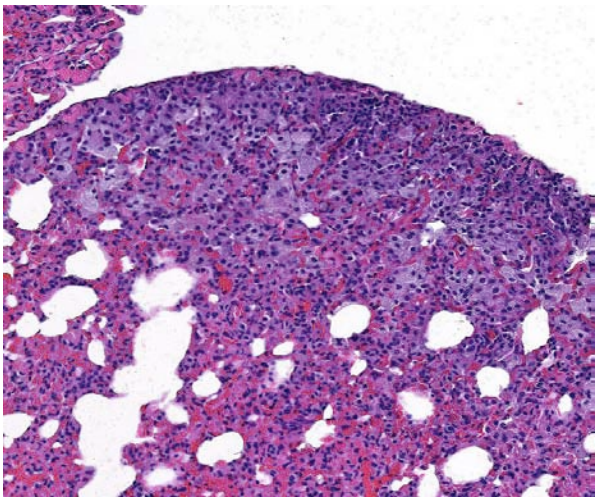
1-1. Lung, ferret: Numerous variably-sized white plaques are present within all lung lobes but are most concentrated in cranial lobes. (Photo courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh. http://www.dlar.pitt.edu/)



1-2. Lung, ferret: Cross sections of lung tissue demonstrate the pleural location of the inflammatory nodules. (Photo courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh. http://www.dlar.pitt.edu/)



1-3. Lung, ferret: Multiple 2-3mm inflammatory nodules are scattered throughout the lung parenchyma. (HE 6X)



1-4. Lung, ferret: Smaller subpleural inflammatory nodules are composed of foamy, lipid laden histiocytes admixed with fewer lymphocytes and plasma cells. (HE 128X)

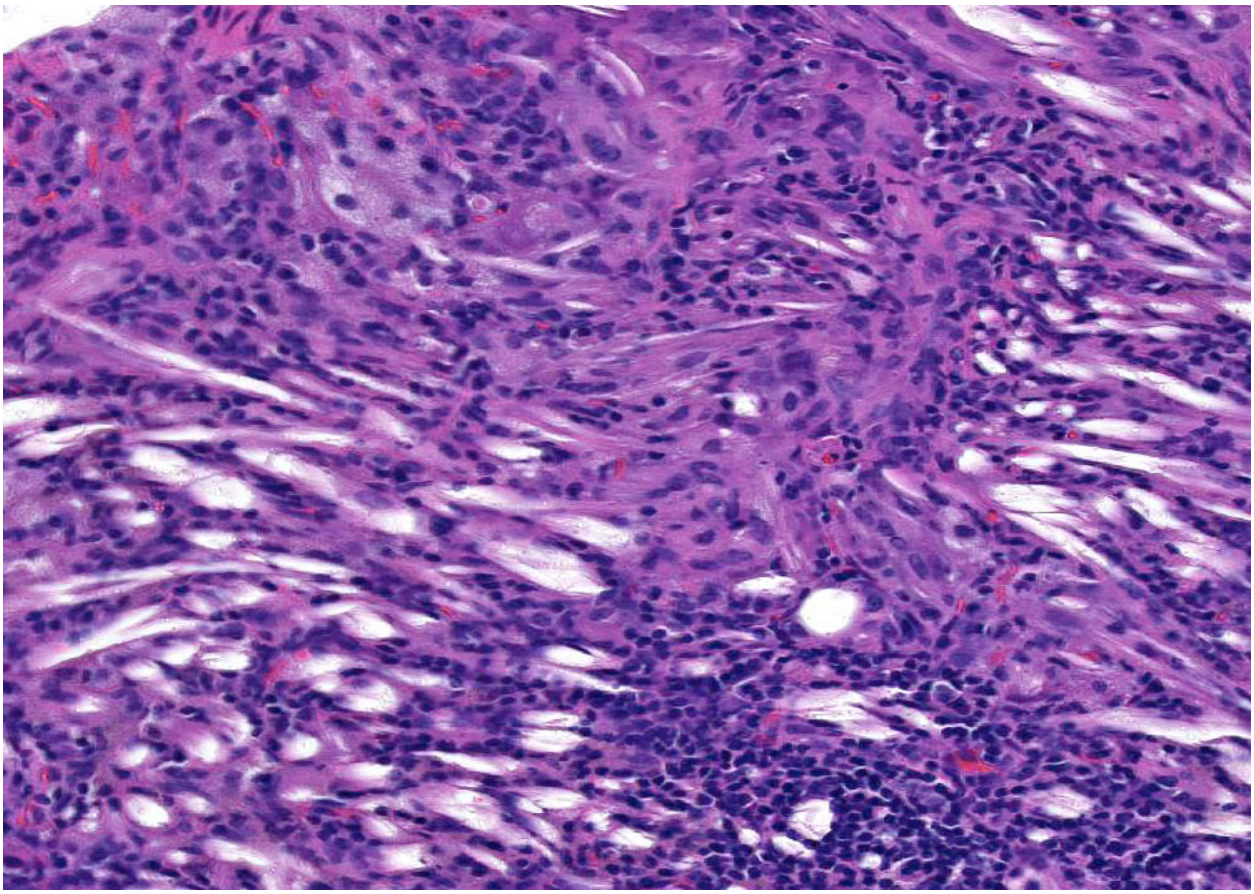
Histopathologic Description: Multiple slides were submitted from different blocks in this case and there is some variation in the appearance and distribution of lesions between cuts.

Multifocal, often coalescing areas of lipid pneumonia are present. These are often (correlative to the gross distribution of lesions seen) present in subpleural locations, although foci deep within pulmonary parenchyma are often noted. Some areas consist solely of homogeneous alveolar aggregates of foamy macrophages. Others are comprised of more dense mixed inflammatory infiltrates including histiocytes, lymphocytes, scattered neutrophils and occasional multinucleated giant cells. Cholesterol clefts and crystals are often seen in association with these latter areas. Infrequent focal areas of alveolar thickening and mild interstitial fibrosis are also noted in some lesions.

In general, most other adjacent lung tissue is atelectatic due to post-mortem collapse, but evidence of other concurrent chronic or active pulmonary disease processes (e.g. foreign body material, observable organisms, neoplasia, etc.) was not noted.

Contributor's Morphologic Diagnosis: Lipid pneumonia (lipogranulomas), subacute-chronic, multifocal and coalescing, mild to moderate (section dependent) – consistent morphologically with endogenous lipid pneumonia of ferrets.

Contributor's Comment: Endogenous lipid pneumonia is seen commonly as an incidental finding in ferrets[9,11] and was not thought to be related to the cause of death or of clinical significance in this animal. Although the specific cause of lipid pneumonia in general is often unknown, the entity has been reported widely over a large spectrum of species, both wild and domestic,[2,3,7,12,14] as well as in humans.[1,13] In general, such disease (i.e. lipid pneumonia) is often categorized as exogenous or endogenous (EnLP). The former is usually associated with aspiration from oral or nasal administration of mineral oil or petroleum-based compounds due to a failure to provoke either airway (glottic closure or cough) or mucociliary transport response mechanisms.[1] EnLP has a variety of both known and suspected causes as well as numerous other observed associations. Despite this, in many cases the exact underlying pathogenesis remains undetermined and is probably multifactorial.[3,7] In general, either bronchial obstruction or direct pneumocyte injury leads to overproduction of surfactant with high cholesterol content. Phagocytosis of this lipid leads to accumulations of foamy macrophages which may subsequently incite other inflammatory responses.[7] Other factors altering lipid mobilization and metabolism may also play a role. For example, the rapid mobilization of body fat by some mustelids has led to speculation that the lung could possibly act as a route for excretion of lipids.[5] A correlation with pulmonary nematode parasitic infestation has been made in several species.[2,7] EnLP has been previously reported in rats, where it was described



1-5. Lung, ferret: Nodules of longer duration are larger, with increased numbers of lymphocytes and abundant cholesterol clefts. (HE 180X)

with high spontaneous frequency under varying descriptive designations, including alveolar lipoproteinosis, multifocal histiocytosis, and alveolar proteinosis.[14] It is also well described in this species as a consequence of oral cadmium exposure (as well as other toxins), with subsequent type II pneumocyte hyperplasia and club (Clara) cell activation.[10]

In most cases when present, EnLP is an incidental finding secondary to some (often prior & undetermined) direct pneumocyte injury or bronchial obstructive process, and effort should be made to detect either resolved or active concurrent pulmonary disease. However, caution should be exercised in ascribing a major primary role in morbidity and mortality to these lesions alone.

The cause of death in this animal was thought to be debilitation and dehydration secondary to ferret enteric coronavirus infection (as suggested by a typical clinical presentation[15] and confirmed by PCR evaluation of feces). Microscopic assessment of the GI tract was of little additional benefit due to the degree of autolytic change present. Although the clinical presentation and other gross and histopathologic findings are distinctly different, the lesions of EnLP should not be mistaken for those associated with multisystemic coronavirus infection (resembling FIP) in ferrets.[4]

JPC Diagnosis: Lung: Pneumonia, granulomatous, subpleural, multifocal, moderate, with intrahistiocytic lipid vacuoles and cholesterol clefts.

Conference Comment: Endogenous lipid pneumonia (EnLP) is a common but obscure entity with an elusive pathogenesis that is diagnosed routinely in ferrets usually as an incidental finding. Its occurrence in cats and occasionally in dogs is less frequent and also of unknown significance; however, it is frequently reported in the vicinity of cancerous lesions in dogs, cats and people, possibly due to the accumulation of lipid breakdown products of

neoplastic cells.[8] This finding, in addition to other reports of EnLP's association with a variety of intrapulmonary and extrapulmonary conditions, indicates it may occur secondarily to underlying disease, equating its discovery as evidence supporting further pursuit of a primary illness.[6]

This case illustrates EnLP's classic histologic presentation as subpleural accumulations of histiocytes, lipid, and cholesterol. This distinctive subpleural location is curious given the proposed pathogenesis of pneumocyte damage and subsequent cholesterol release from disintegrating cell membranes.[7] Conference participants could not determine how such alveolar accumulations routinely end up forming subpleural nodules, and most agree with the contributor's suspicions of a multifactorial pathogenesis in the formation of EnLP.

Contributing Institution: Division of Laboratory Animal Resources, University of Pittsburgh
http://www.dlar.pitt.edu/

References:

1. Banjar H. Lipoid pneumonia: A review. Bahrain Medical Bulletin. 2003;25(1):36-39.
2. Brown CC. Endogenous lipid pneumonia in opossums from Louisiana. *Journal of Wildlife Diseases*. 1988;24(2):214-219.
3. Dungworth D. The respiratory system. In: Jubb K, Kennedy P, Palmer N, eds. *Pathology of Domestic Animals*. 3rd ed. Orlando, FL; Academic Press Inc: 1985;470-474.
4. Garner MM, et al. Clinicopathologic features of a systemic Coronavirus-associated disease resembling feline infectious peritonitis in the domestic (Mustela putorius). *Vet Pathol*. 2008;45:236-246.
5. Guarda F, Bollo E, Macchi E. Lipoid pneumonia in the Red fox (*Vulpes vulpes*). *JME*. 1997;4:13-16.
6. Himsworth CH, Malek S, Saville K, Allen AL. Endogenous lipid pneumonia and what lies beneath. *Can Vet J*. 2008;49(8):813-815.
7. Jones DJ, Norris CR, Samii VF, Griffey SM. Endogenous lipid pneumonia in cats: 24 cases (1985-1998). *JAVMA* 2000;216(9):1437-1440.
8. Lopez A. Respiratory system, mediastinum, and pleurae. In: Zachary JF, McGavin MD, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Elsevier Mosby; 2012:530.
9. Marinez J, Reinacher M, Perpinan D, Ramis A. Identification of group 1 Coronavirus antigen in multisystemic granulomatous lesions in ferrets (mustela putorius furo). *J Comp Path*. 2008;138:54-58.
10. Molina AM, Raya AI, Carrasco L, Blanco A, Monterde JG, Rueda A, Moyano R. Endogenous lipid pneumonia in rats after subacute exposure to CdCl2. *Toxicology and Industrial Health*. 2008;24:677-681.
11. Personal communication, Dr. Bruce Williams
12. Raya AI, Fernandez-de Marco M, Nunez A, Afonso JC, Cortade LE, Carrasco L. Endogenous lipid pneumonia in a dog. *J Comp Path*. 2006;135:153-155.
13. Sharma A, Ohri S, Bambery P, Singh S. Idiopathic endogenous lipoid pneumonia. *Indian J Chest Dis Allied Sci*. 2006;48:143-145.
14. Weller W. Alveolar lipoproteinosis, rat. In: T.C. Jones, U. Mohr and RD Hunt, eds. *Monographs on Pathology of Laboratory Animals; Respiratory System*. Berlin: Springer-Verlag; 1985; 171-176.
15. Williams BH, Kiupel M, West KH, Raymond JT, Grant CK, Glickman LT. Coronavirus-associated epizootic catarrhal enteritis in ferrets. *JAVMA*. 2000;217(4):526-530.

CASE II: H10-131A; H10-126A (JPC 4049564).

Signalment: 12-week-old male Sprague-Dawley rat, *Rattus norvegicus* (09-1220); 12-week-old female Sprague-Dawley rat, *Rattus norvegicus* (09-1215).

History: These two rats were part of a larger study investigating the prevalence of lung lesions in Sprague-Dawley and athymic nude rats between 3 and 24 weeks of age, housed in either barrier or isolator facilities. The two rats submitted were both housed in a barrier facility from birth and were sacrificed at 12 weeks of age to look for gross and microscopic evidence of lung pathology.

Gross Pathology: No gross lesions were observed at routine post mortem examination; however, after 10% formalin infusion via the trachea and a minimum of 24 hours immersion fixation, multifocal (4-20), 0.5-1 mm white-grey foci were evident on all lung lobes (09-1215), or just the caudal lung lobes (09-1220).

Laboratory Results:

1. Aerobic and anaerobic culture of lung tissue did not culture any bacteria or fungi.

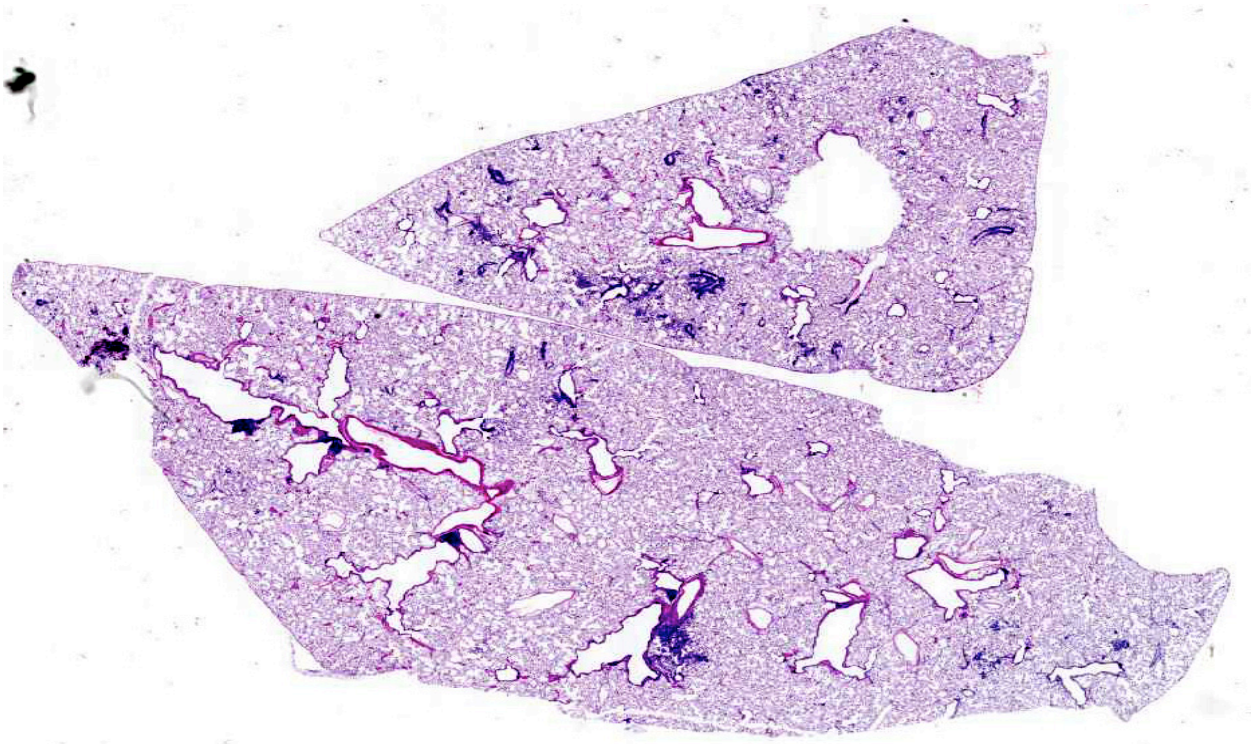
2. Surveillance of sentinel rats during this investigation was negative for pneumonia virus of mice, Theiler's encephalomyelitis virus (GD VII), Hantaan virus (Korean hemorrhagic fever), lymphocytic choriomeningitis virus, Sendai virus, reovirus-3, sialodacryoadenitis (rat coronavirus), rat parvoviruses (rat virus, rat parvovirus-1, Toolan H1 virus), *Corynebacterium kutscheri, Bordatella bronchiseptica, Pasteurella multocida, Streptococcus pneumonia, Streptococcus moniliformis, Staphylococcus aureus, Mycoplasma pulmonis, CAR bacillus, Clostridium piliforme, Salmonella enteriditis*, *Helicobacter* sp., nematodes, cestodes, intestinal protozoa (pathogenic species), *Encephalitozoon cuniculi* and arthropods.

3. PCR testing for *Pneumocystis carinii* was positive in lung tissue harvested from both rats at post mortem.

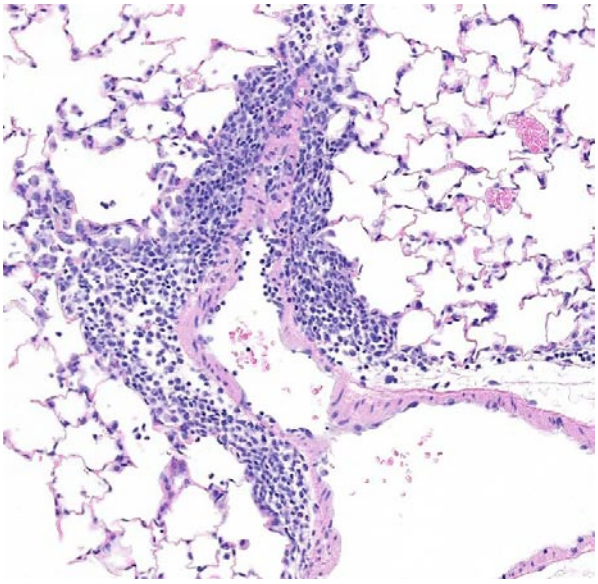
Histopathologic Description:

09-1215 - H10-0126A
09-1220 – H10-0131A

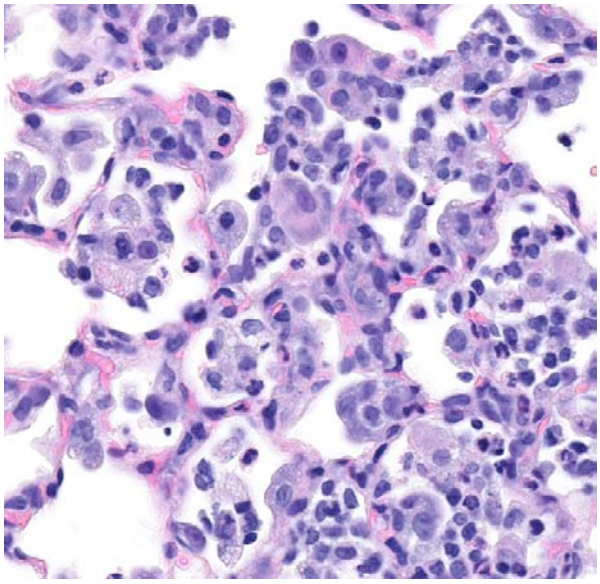
Lung: Multifocally, there are moderate amounts of perivascular, peri-bronchiolar, interstitial and



2-1. Lung, rat: Multiple small foci of inflammation are scattered throughout the lung. (HE 6X)



2-2. Lung, rat: Multifocally, pulmonary arterioles are surrounded by moderate numbers of lymphocytes and histiocytes. (HE 96X)



2-3. Lung, rat: Multifocally, and randomly, alveoli contain low to moderate numbers of foamy macrophages and fewer neutrophils and are lined by type II pneumocytes. (HE 328X)

alveolar hypercellularity. The majority of cells are pleomorphic lymphocytes and large foamy macrophages with fewer neutrophils and occasional plasma cells and eosinophils (mixed inflammatory cell interstitial pneumonia). In some slides the pleura is multifocally mildly expanded by a similar cellular infiltrate (pleuritis). Multifocally, the alveoli are filled with variable numbers of large foamy alveolar macrophages, occasional multinucleate giant cells, moderate amounts of cellular debris and are occasionally lined by plump cuboidal epithelium (mild type II pneumocyte hyperplasia). The perivascular lymphatics are occasionally moderately ectatic (perivascular oedema).

Contributor's Morphologic Diagnosis: Moderate, chronic, multifocal lymphoplasmacytic, neutrophilic and histiocytic interstitial pneumonia with occasional, mild type II pneumocyte hyperplasia.

Contributor's Comment: In the late 1990's distinctive and apparently novel pulmonary inflammatory lesions were identified and described in the lungs of laboratory rats from multiple institutions in the USA[2] and Europe.[7] These lesions included "pale or tan" macroscopic foci in the lungs and perivascular inflammatory cell cuffing throughout the lungs, accompanied by alveolar infiltrates of macrophages, neutrophils and lymphocytes, variably increased amounts of BALT and type II pneumocyte hyperplasia. Extensive serological testing, bacterial culture and protozoal identification testing failed to identify a causative agent. Electron microscopy failed to demonstrate the presence of viral-particles, although structures interpreted as bacterial bacilli were observed in the majority of rats with lung lesions.[2]

Further research undertaken in an attempt to isolate and transmit an infectious cause for the lung lesions led to the description of a novel enveloped virus and the disease was given the working name "rat respiratory virus" (RRV).[5,6] However, despite these early findings, other attempts at virus isolation from infected lungs were unsuccessful and the etiology of the pulmonary inflammatory lesions also known as "RRV" was undetermined and histopathology remained the only method of diagnosis (Albers, Simon et al. 2009).

In 2011 and 2012, two independent research groups, released publications containing convincing evidence that the fungal agent *Pneumocystis carinii* was the cause of the distinctive lung lesions known as RRV. Techniques used to demonstrate the correlation between the presence of *P. carinii* and the presence of the characteristic lung lesions included PCR, serology and experimental

infection studies.(Livingston, Besch-Williford et al. 2011, Henderson, Dole et al. 2012).

The gross and histopathological changes in the rat lungs submitted, combined with the negative microbial culture and serological testing results and positive PCR results for *Pneumocystis carinii*, led to the diagnosis of *Pneumocystis* pneumonia in these rats.

JPC Diagnosis: Lung: Pneumonia, interstitial, histiocytic, multifocal, mild, with multifocal lymphoplasmacytic perivascular inflammation.

Conference Comment: This case provides a detailed look at the rapid evolution of this mild but historically important pulmonary condition in rats. The contributor adequately outlined the historical aspects surrounding this lesion, and describes the current consensus identifying *Pneumocystis carinii* as the inciting agent. We performed Grocott's methenamine silver stain in an effort to identify an organism, but were only successful in observing one questionable fungal cyst.

P. carinii typically requires immune suppression for infection to cause clinical disease. In humans the organism is hypothesized to be continually present within the lungs from the first few years of life, often in the absence of overt pathology.[8] In certain rat colonies, *P. carinii* is apparently common and has been documented in the lungs of clinically healthy, immunocompetent rats; research suggests neonatal rats acquire it within hours after birth.[4] A survey of research institutions revealed 6% of rats had the characteristic perivascular lymphocyte accumulation associated with that described in the 1990's for RRV.[4] These lesions were reproduced experimentally in immunocompetent rats by inoculation of *P. carinii* and its transmissible potential was documented in independent studies.[3,4] Further, organism identification by PCR was highly correlated with lung pathology, and the organism was never identified in cases lacking the characteristic lung lesions, allowing characteristic microscopic changes to be an accurate predictor of *P. carinii* infection.[3] Despite the evidence, definitive causation can still not be assigned as the inoculum utilized in both studies was derived from infected rat lungs, thus the possibility of a second or primary agent remains, though that possibility is argued as highly improbable.[4] Regardless, both experimental and natural infections spontaneously resolve with time.[1,3]

Contributing Institution: Pathology Section; College of Veterinary Medicine; School of Veterinary and Life Sciences; Murdoch University http://www.murdoch.edu.au/School-of-Veterinary-and-Life-Sciences/Facilities-and-labs/College-of-Veterinary-Medicine/

References:

1. Albers TM, Simon MA, Clifford CB. Histopathology of naturally transmitted "rat respiratory virus": progression of lesions and proposed diagnostic criteria. *Vet Pathol*. 2009;46(5):992-999.
2. Elwell MR, Mahler JF, Rao GN. Have you seen this? Inflammatory lesions in the lungs of rats. *Toxicol Pathol.* 1997;25:529-531.
3. Henderson KS, Dole V, Parker NJ, Momtsios P, Banu L, Brouillette R, et al. Pneumocystis carinii causes a distinctive interstitial pneumonia in immunocompetent laboratory rats that had been attributed to "rat respiratory virus. *Vet Pathol*. 2012;49(3):440-52.
4. Livingston RS, Besch-Williford CL, Myles MH, Franklin CL, Crim MJ, Riley LK. Pneumocystis carinii infection causes lung lesions historically attributed to rat respiratory virus. *Comp Med*. 2011;61(1):45-59.
5. Riley L, Purdy G, Dodds J, et al. Idiopathic lung lesions in rats: search for a etiologic agent. *Contemp Top Lab Anim Sci.* 1997;36:46.
6. Riley L, Simmons J, Purdy G, et al. Research Update: idiopathic lung lesions in rats. *ACLAD News*. 1999;20:9-11.
7. Slaoui M, Dreef HC, van Esch. Inflammatory lesions in the lungs of Wistar rats. *Toxicol Pathol.* 1998;26:712-713, discussion 714.
8. Swain SD, Meissner N, Han S, Harmsen A. Pneumocystis infection in an immunocompetent host can promote collateral sensitization to respiratory antigens. *Infect Immun.* 2011;79(5): 1905-1914.

CASE III: MS14-887 (JPC 4052867).

Signalment: Adult female Adrenal Specific Prkar1a knockout (ADKO) mouse, *Mus musculus*.

History: Euthanized because of a large subcutaneous mass and abdominal distension.

Gross Pathology: A 15 mm diameter abscess is present in the subcutaneous layer of the dorsal left abdominal wall. This abscess extended into the abdomen and is contiguous with the left uterine horn. The subcutaneous portion measures approximately 15 mm in diameter. The abdominal aspect of the mass measures 31 x 12 x 12 mm. It is filled with thick yellow material, a swab of which is taken for bacterial culture. Abscesses are present within lumbar aortic lymph nodes. The mesenteric lymph node is markedly enlarged, swollen and diffusely pink. The liver and kidneys are pale and swollen. The spleen is approximately five times normal in size. It is adhered to the abdominal mass. The left lung is tan. The brain is not examined.

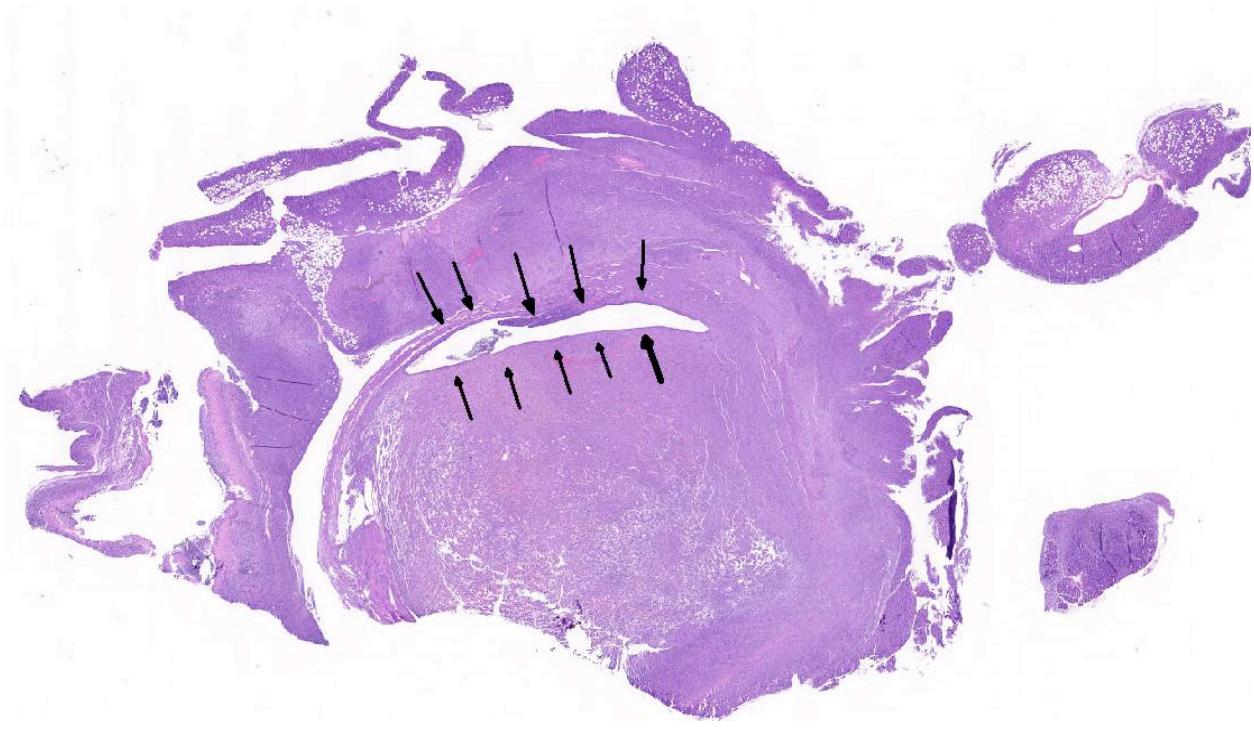
Laboratory Results: Bacteriology: Aerobic: *Pasteurella pneumotropica* present; Strict Anaerobe: *Peptostreptococcus anaerobius* present.

Histopathologic Description: The left uterine horn has severe necrosuppurative inflammation in the lumen and extending through the muscularis. Colonies of small bacterial rods are present.

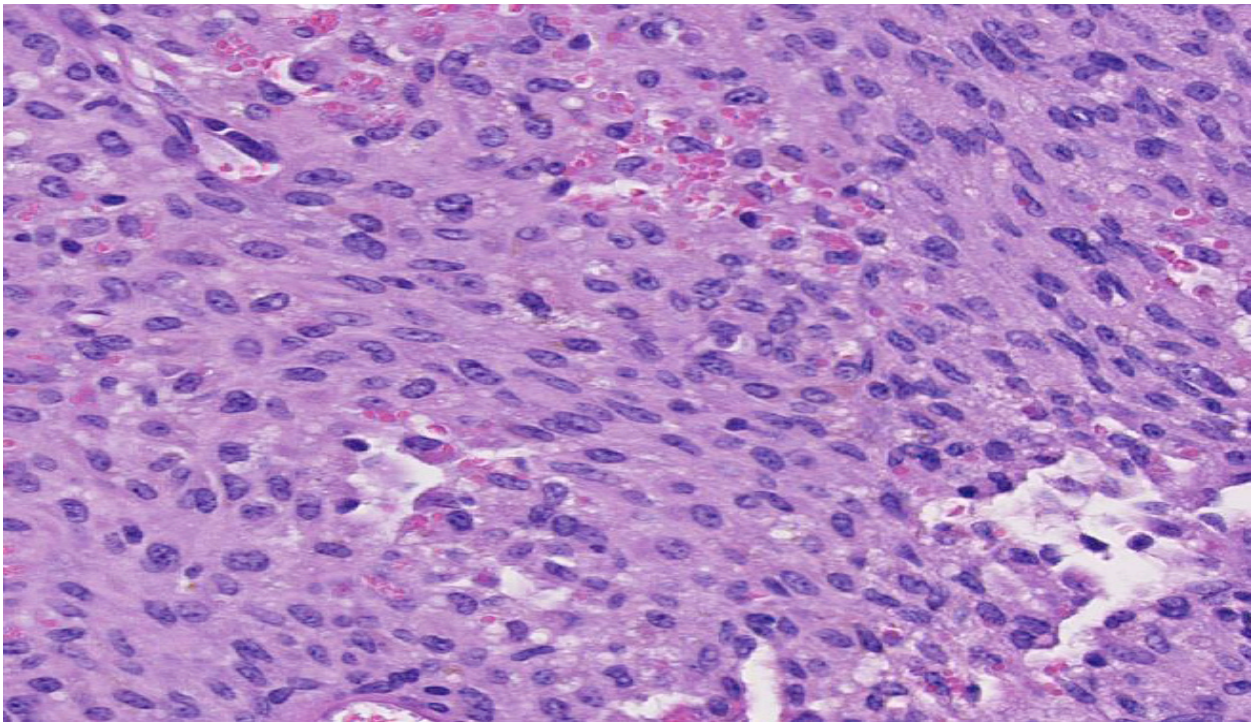
The uterus also has a neoplastic cell population in the endometrium, myometrium and surrounding soft tissue. In some areas the neoplasm has sheets of oval to round cells with a moderate amount of amphophilic cytoplasm and eccentric nuclei. In the uterine wall the cells have a fusiform to spindle morphology. Nuclei are elongated to reniform with marginated chromatin. There are approximately 8 mitotic figures per ten 400X field. Similar neoplastic cells efface the pancreas and are present in the mesometrium, ovary, gall bladder, in abdominal lymph nodes and the spleen. Kidneys have cytoplasmic eosinophilic droplets in the proximal tubular epithelium.

Immunohistochemistry results: Performed on neoplastic aggregates present in the mesovarium and ovary: F4/80: Positive. Smooth muscle actin and desmin: Negative.

Gram stain: Uterus: The short bacterial rods are gram negative; occasional gram positive cocci are present.



3-1. Uterus, mouse: The wall of the uterus is markedly expanded by an infiltrative neoplasm which infiltrates the adjacent mesometrium, mesentery, and in this section, pancreas. Arrows delineate the compressed lumen. (HE 7X)

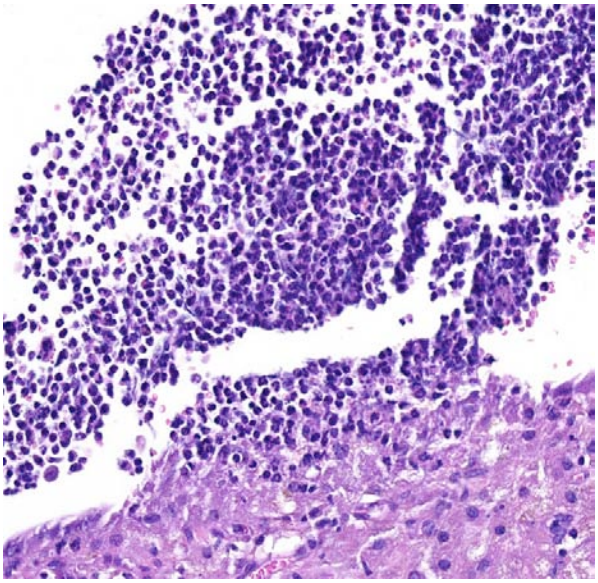


3-2. Uterus, mouse: The neoplasm is composed of histiocytic round cells which often spindle. This morphology, coupled with an intrauterine location, is strongly suggestive of histiocytic sarcoma. (HE 120X)

Contributor's Morphologic Diagnosis: Uterus: Histiocytic sarcoma; pyometra (Pasteurella).

Contributor's Comment: Histiocytic sarcoma is a neoplasm of monocyte origin. Incidence of histiocytic sarcoma in mice varies with strain, for example being rarely reported in 129/sSvJae and FVB/N mice to 15% in C57Bl in a study on comparing mortality between mouse strains.[1] Some histiocytic neoplasms have large cells with abundant cytoplasm and multinucleated cells; others have smaller oval to spindle cells.[4,8] Hyaline droplet accumulations in the kidney are commonly associated with histiocytic sarcoma. The droplets are caused by accumulations of lysozyme.[8] However, lymphomas and myelogenous leukemia in mice have been associated with hyaline droplets in the kidney.[3] In this case, spindle to round cell morphology, the presence of hyaline droplets in the kidney and strong positivity of neoplastic cells for F4/80 is consistent with a neoplasm of histiocytic in origin. A list of histiocyte markers is listed in the accompanying table.

Differential diagnoses for histiocytic sarcoma include stromal tumors like leiomyoma and Schwannoma, and other hematopoietic cell tumors such as histiocyte-associated lymphomas (HAL) and myelogenous leukemia. Significant populations of histiocytic cells can be present in lymphomas; the macrophages associated with HAL tend to be large and round with abundant cytoplasm. Histiocyte rich lymphomas can be distinguished by clonal rearrangements of Ig heavy chain (B-cell) or T-cell receptor loci in



3-3. Uterus, mouse: The lumen of the uterus contains numerous degenerate neutrophils and cellular debris, unassociated with the neoplasm, suggesting a concomitant uterine infection. (HE 340X)

Mouse Histiocyte markers[9]

Marker	Function	Cell location	Macrophage expression	Other tissues
Arginase 1	Arginine metabolism	Cytostolic	M2* macrophage	Other tissues
CD68	Lysosome/ phagosome A glycoprotein	Cytostolic	Tissue macrophages, thymus, lymph node, Kupffer cells, alveolar macrophages	Also other cells with lysozomes/ phagosomes
CD163	Scavenger receptor cysteine rich superfamily	Membrane	Monocytes and tissue macrophages except tingible body macrophages in germinal centers	
F4/80	Immune regulation[8]	Membrane	Tissue macrophage; not splenic white pulp	
IAB1	Actin/calcium binding protein	Cytoplasm and nucleus	All but alveolar macrophage and lymph node dendritic cells	
iNO_2	Nitric oxide expression	cytoplasmic	Activated macrophages via CD_4TH1 pathway	neutrophils
Lysozyme	Innate immune system	Cytoplasmic	Alveolar macrophage, Kupffer cells, Lymph node sinuses	Granulocytes, monocytes
MAC 2	Adhesion molecule	Cytoplasmic and membrane	All	Also various epithelial cells
Mac3	Glycoprotein	cytoplasm	Macrophages and dendritic cells	Epithelial, megakaryocytes, endothelial cells, granulocytes
YM1	Expression associated with inflammation	Cytoplasm	M2 macrophage, Alveolar macrophages	M2

*M2 macrophage: Macrophages involved in the $CD4TH_2$ pathway.

lymphocyte populations versus having IgH and TcR receptor genes in germline (non-rearranged) configuration. This is done by Southern blot analysis. If neoplastic cells lack IgH gene rearrangements, neoplasms of B-cell origin are excluded; however, rarely cells with histiocytic cell morphology have displayed IgH rearrangements. Some histiocyte-rich neoplasms have nodular proliferations of more pleomorphic macrophages increased numbers of mitotic figures and lymphocytes with clonal immune receptor and rearrangements; these may represent tumors with both neoplastic histiocytes and lymphocytes.[5] Immunohistochemistry for T-cell and B-cells were not performed in the submitted case.

Pasteurella is a common pathogen in laboratory mice that has been associated with uterine infections as well as male reproductive, ocular, ear, nasal, skin and mammary gland infections.[8] *Peptostreptococcus* has not been reported as a cause of spontaneous disease in mice but has been associated with genitourinary tract infections in people.[7]

JPC Diagnosis: 1. Uterus, mesometrium, pancreas, mesentery: Histiocytic sarcoma.
2. Uterus: Endometritis, necrotizing, diffuse, severe, with intra- and extracellular bacilli.

Conference Comment: Sections from three different blocks were provided for this case and equally distributed among conference participants and contributors; there is significant variation between different sections. Among them, histiocytic sarcoma is present within the pancreas, uterus and mesentery, inciting an array of extensive secondary pathologic changes in the affected organs, distorting tissue architecture and in some cases, rendering tissue identification impossible. The spleen is present in some sections although we did not observe the neoplasm within it. The neoplasm and secondary changes provide for a descriptively challenging case.

The contributor provides an excellent overview of histiocytic sarcoma in rodents and its immunohistochemical attributes. Immunohistochemistry has afforded greater clarity in teasing out cellular origin of various histiocytic diseases, effectively eliminating commonly used diagnoses such as malignant fibrous histiocytoma and splenic fibrohistiocytic nodules while identifying the majority of histiocytic proliferations in dogs and cats as Langerhans cell or interstitial dendritic cell origin.[6,11] Only hemophagocytic sarcoma is still recognized as originating from macrophages.[6] Histiocytic proliferative diseases may occur as neoplastic processes or dysregulated inflammatory processes in dogs, while only neoplastic processes are identified in cats.[6]

Histiocytic sarcoma is one of the few neoplasms known to cross the joint space and is often confused with synovial cell sarcoma when present at an articular surface. Synovial cell sarcomas are derived from type B synovial cells, which are specialized fibroblasts that readily attract large numbers of histiocytes. The two are differentiated by the dominant cell population and its CD18 expressivity.[6] Histiocytic sarcoma is one of three typically disseminated neoplasms in rodents, to also include hemangiosarcoma and lymphoma, that are believed to arise synchronously in multiple organs. It is interesting there is no mention of liver involvement in this case, where it occurs so commonly and was often cited as a primary location in older literature.[2]

Pyometra most commonly occurs following estrus in most species, while the uterus is under progesterone influence.[10] In mice, pyometra may also occur secondarily to mucometra, and *Klebsiella* spp. and *Pasteurella* spp. are the most commonly cultured organisms.[2,8]

Contributing Institution: National Institutes of Health, Division of Veterinary Resources
http://www.ors.od.nih.gov

References:

1. Brayton CF, Treuting PM, Ward JM. Pathobiology of aging mice and GEM: Background strains and experimental design. *Vet Path*. 2012;49(1):85-105.
2. Davis BJ, Dixon D, Herbert RA. Ovary, oviduct, uterus, cervix, and vagina. In: Maronpot RR, ed. *Pathology of the Mouse*. Vienna, IL: Cache River Press, Inc.; 1999:438.
3. Decker JH, Dochterman LW, Niquette AL, et al. Association of renal tubular hyaline droplets with lymphoma in CD-1 Mice. *Toxicol Pathol*. 2012;40(4):651-655.
4. Frith CH, Ward JM, Harlmen JH, et al. Hematopoietic system. In: *International Classification of Rodent Tumors: The Mouse*. Berlin, Germany: Springer-Verlag; 2001:430-431.
5. Hao X, Fredrickson TN, Chattopadhyay SK. The histopathologic and molecular basis for the diagnosis of histiocytic sarcoma and histiocyte–associated lymphoma of mice. *Vet Pathol*. 2010;47(3):434-445.
6. Moore PF. A review of histiocytic diseases of dogs and cats. *Vet Pathol*. 2014;51(1):167-184.
7. Murdoch DA. Gram-positive anaerobic cocci. *Clinical Microbiology Reviews*. 1998;11(1): 81-120.
8. Percy DH, Barthold SW. Mouse. In: *Pathology of Laboratory Rodents and Rabbits. 3rd ed*. Oxford, England: Blackwell Publishing Ltd; 2007:3-111.
9. Rehg JE, Bush D, Ward JM. The utility of immunohistochemistry for the identification of hematopoietic and lymphoid cells in normal tissues and interpretation of proliferative and inflammatory lesions of mice and rats. *Toxicol Pathol*. 2012;40(2):345-374.
10. Valli VE, Jacobs RM, Parodi AL, Vernau W, Moore PF. *Histological Classification of Hematopoietic Tumors of Domestic Animals*. 2nd series. Vol VIII. Washington, D.C.: Armed Forces Institute of Pathology/ American Registry of Pathology; 2002:48-49.
11. van den Berg TK, Kraal G. A function for the macrophage F4/80 molecule intolerance induction. *Trends in Immunology*. 2005;26(10): 506-509.

CASE IV: 09187 WFUHS (JPC 3165083).

Signalment: Adult male vervet monkey, *Chlorocebus aethiops.*

History: After arriving into the CDC quarantine, this animal was noted to have labored breathing and coughing and died on the morning of the next day. There were no experimental procedures performed on this animal.

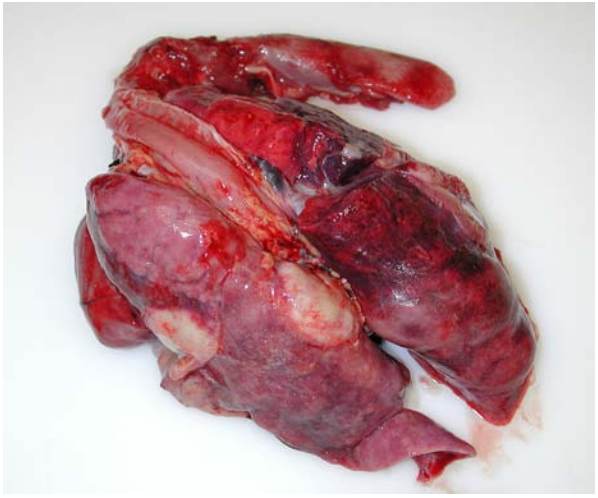
Gross Pathologic Findings: A necropsy was performed as per the CDC quarantine animal necropsy protocol. While opening the diaphragm to enter the thoracic cavity, abundant yellowish-tan, opaque viscous liquid (pus) poured out. The lungs were mottled dark brown to tan to dull red, markedly consolidated, had a consistency similar to that of liver (hepatization of lung), and did not collapse with loss of thoracic cavity negative pressure. Multifocal, tan to white, well demarcated, variably sized pus-filled abscesses reaching up to 2.5 cm in diameter were found on cut sections. Only about 10 percent of apparently normal, remaining pulmonary parenchyma was present. The pleura was thickened and multifocally adhered to the dorsal and lateral thoracic walls. A variably thick layer of pus admixed with dull red to brown fibrillar material (fibrin) was present on the pleural surface and in the thoracic cavity. Mucus admixed with small plugs of pus were present in the distal bronchial lumina. The tracheobronchial lymph nodes were prominent.

Gross Morphologic Diagnoses: Pleuropneumonia, diffuse, chronic, severe, suppurative with abscessation and thoracic adhesions.

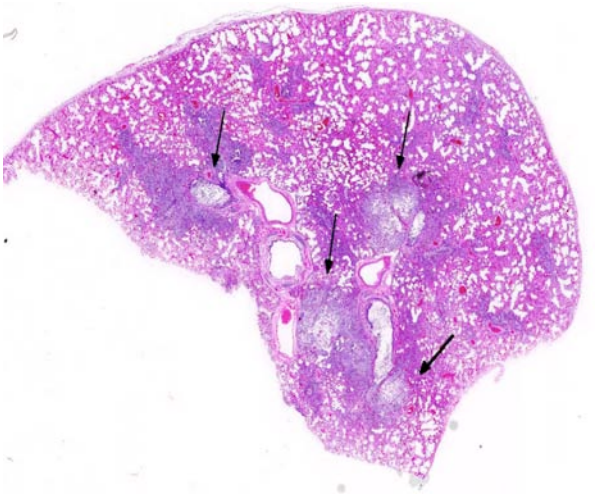
Laboratory Results: Bacterial culture from the lung - *Klebsiella pneumoniae* 3+

Filoviral ELISA – negative.

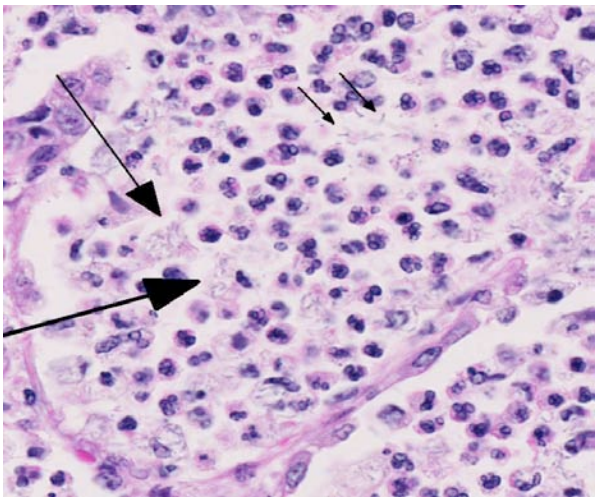
Histopathologic Description: The pulmonary architecture was extensively obscured by abundant suppurative inflammatory cellular infiltrate admixed with edema, necrotic debris and fibrin. Bronchioles and alveolar spaces were filled by numerous neutrophils, many degenerate, which often transmurally infiltrated and effaced alveolar and bronchiolar walls. The affected bronchioles were lined by attenuated epithelial cells lacking cilia and multifocally the epithelial lining was denuded. Most of the blood vessels were surrounded by thick fibrin strands and clear spaces (edema). The alveolar capillaries are congested and small hemorrhages are scattered throughout the pulmonary parenchyma. The pleura was expanded up to 150 microns in thickness by fibrin. Frequently within the airspaces, 1x2-4 micron bacterial rods occasionally arranged in chains were seen extracellularly or within macrophage cytoplasm.



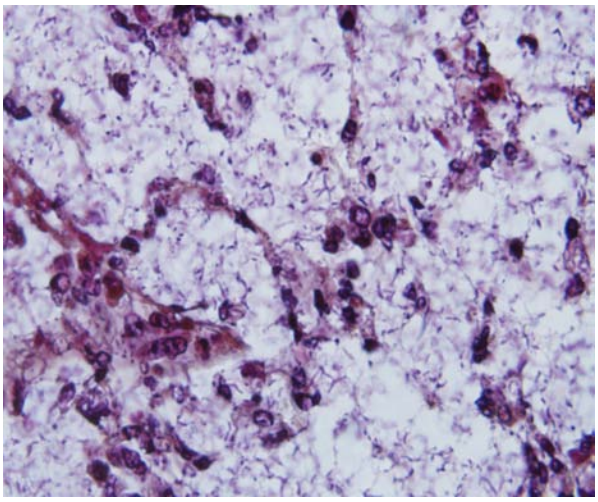
4-1. Lung, vervet monkey: The lungs were mottled dark brown to tan to dull red, markedly consolidated. The lungs contain several well demarcated, variably sized pus-filled abscesses ranging up to 2.5 cm in diameter. (Photo courtesy of: Animal Resources Program, Wake Forest University Health Sciences, Winston-Salem, NC http://www1.wfubmc.edu/pathology/training/index.htm)



4-2. Lung, vervet monkey: Numerous lucent areas are present within the diffusely inflamed parenchyma. (HE 6X)



4-3. Lung, vervet monkey: Lucent areas are composed of ruptured and confluent alveoli which are filled with numerous neutrophils and macrophages which often contain engulfed bacilli. (HE 400X)



4-4. Lung, vervet monkey: Gram stain of bacilli showing the distance between bacilli in areas of inflammation, corresponding to the presence of a capsule. (Brown-Hopps, 600X)

Gram stain: Numerous gram negative 1x2-4 micron single or chains of bacilli were present at the areas of inflammation.

Acid fast stain: The bacterial rods were acid fast negative, but acid fast stain showed a prominent blue stained bacterial capsule.

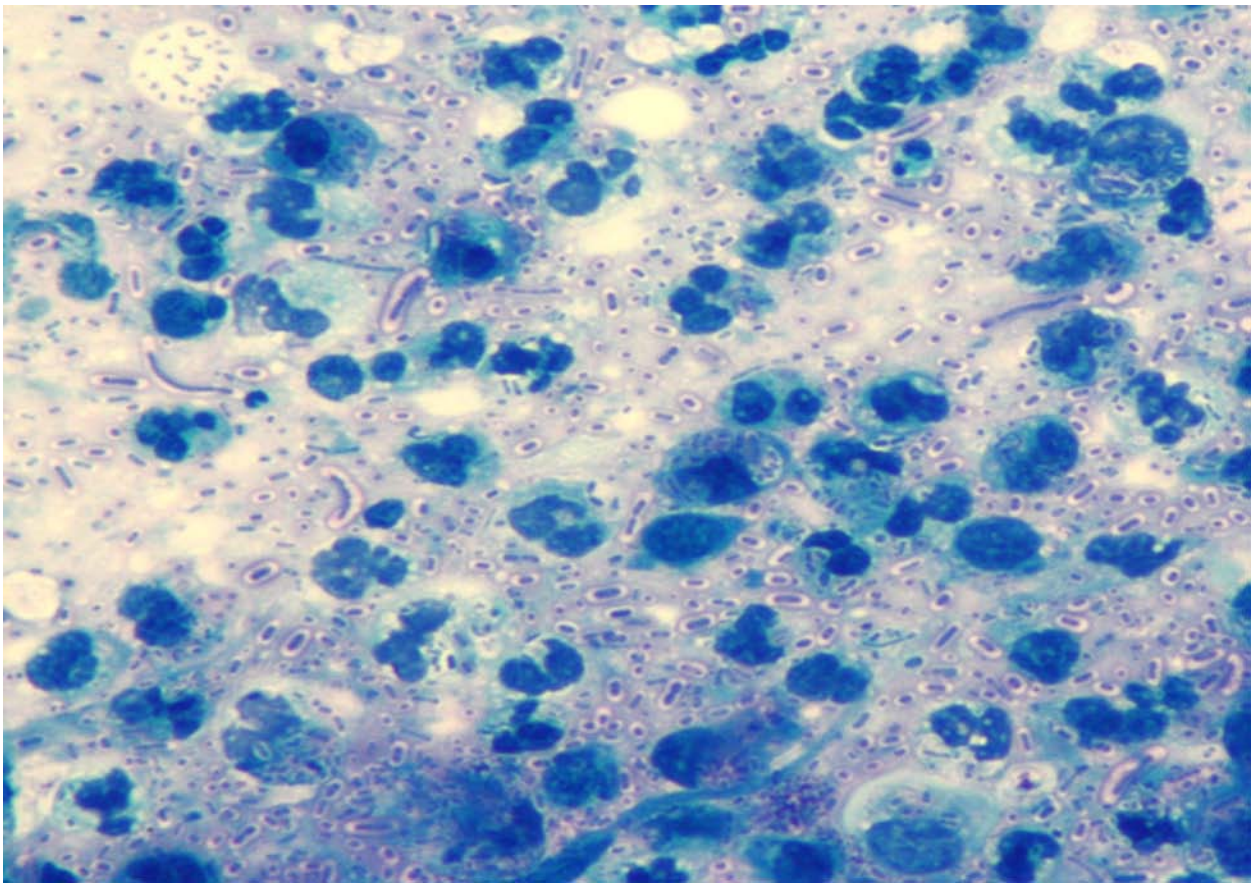
Contributor's Morphologic Diagnosis: Pneumonia, bronchointerstitial, diffuse, severe, subacute, fibrinosuppurative with intralesional gram negative bacteria.

Contributor's Comment: *Klebsiella pneumoniae* is a gram-negative, aerobic, encapsulated, nonmotile rod-shaped bacteria of the family *Enterobacteriaceae*, which are ubiquitous in nature. It is one of the most important nosocomial bacterial infections in humans, which accounts for a significant proportion of urinary tract infections, pneumonia, septicemias, and soft tissue infections.[4] As opportunistic pathogens, *Klebsiella* spp. primarily attack immunocompromised individuals. It is also a frequent cause for pneumonia in nonhuman primates. Differentials for bacterial pneumonia in nonhuman primates include *Mycobacterium tuberculosis*, *Burkholderia* sp., *Klebsiella* sp., *Staphylococcus* sp., *Corynebacterium* sp., *Streptococcus* sp., *Nocardia* sp., *Actinomyces* sp, among others.

Based on the pathological findings, it is presumed that this animal was infected with *K. pneumoniae* and developed pneumonia prior to shipping to Wake Forest, although the transportation-associated stress likely aggravated the condition. Stress associated with shipping and transportation is known to increase the incidence of this infection. Multisystemic abscess formation in African green monkeys caused by invasive, hypermucoviscosity phenotype *Klebsiella pneumonia* has been reported.[6] Serotyping based on the capsular antigen type is the technique for further characterizing *Klebsiella* spp. *K. pneumoniae* characteristically produce vast amounts of capsular polysaccharide covering the bacterial surface.[5] K1 and K2 serotypes are most virulent and they were found to be significantly more resistant to phagocytosis than non-K1/K2 isolates.[1,5] The thick polysaccharide capsule (K antigen) of these bacteria creates a barrier that prevents opsonization and phagocytosis. In this case, capsular serotyping was not performed. This animal also had meningitis, epicarditis and renal arterial thrombosis which suggest bacterial septicemia. Also moderate myeloid hyperplasia in the bone marrow was observed as expected in bacterial infections.

JPC Diagnosis: Lung: Bronchopneumonia, fibrinosuppurative, chronic, multifocal, severe, with numerous intra- and extracellular bacilli.

Conference Comment: This is a classic case of the primate form of shipping fever, a disease affecting both New and Old World primates and can lead to sepsis and rapid death, which likely occurred in this case. The organisms are numerous in this example, and the thick capsule



4-4. Lung, vervet monkey: Gram stain of bacilli showing the distance between bacilli in areas of inflammation, corresponding to the presence of a capsule. (Brown-Hopps, 600X)

so important to its pathogenesis is beautifully depicted in the image of the acid fast stain submitted by the contributor. This capsule also likely plays a role in the increased invasiveness and pathogenicity of the hypermucoviscosity phenotype (HMV), which carries greater resistance to oxidative-mediated killing and imparts a higher level of cytotoxicity upon the host.[2] HMV was first isolated from fatal human infections and was only recently described in nonhuman primates; it characteristically causes rampant abscesses affecting multiple organs.[6]

K. pneumoniae is associated with disease in other lab animal species as well, including enterotyphilitis and bronchopneumonia in rabbits, polyserositis and bronchopneumonia in guinea pigs, and lymph node and renal abscesses in mice and rats.[3] The closely related *K. oxytoca* causes suppurative endometritis and dermatitis readily in mice.[3] Among domestic species, *K. pneumoniae* causes urinary tract infections (dogs), mastitis (ruminants), cerebral abscesses (ruminants) and pneumonia (dogs).[7]

Contributing Institution: Animal Resources Program, Wake Forest University Health Sciences, Winston-Salem, NC
http://www1.wfubmc.edu/pathology/training/index.htm

References:

1. Chuang YP, Fang CT, Lai SY, Chang SC, Wang JT. Genetic determinants of capsular serotype K1 of Klebsiella pneumoniae causing primary pyogenic liver abscess. *J Infect Dis*. 2006;193:645-654.
2. Cox BL, Schiffer H, Dagget G Jr, et. al. Resistance of Klebsiella pneumonia to the innate immune system of African green monkeys. *Vet Microbiol*. 2015;176(1-2):134-142.
3. Percy DH, Barthold SW. *Pathology of Laboratory Rodents*. 3rd ed. Ames, IA: Blackwell Publishing; 2007:64, 152, 222, 275.
4. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev*. 1998;11:589-603.

5. Struve C, Bojer M, Nielsen EM, Hansen DS, Krogfelt KA. Investigation of the putative virulence gene magA in a worldwide collection of 495 Klebsiella isolates: magA is restricted to the gene cluster of Klebsiella pneumoniae capsule serotype K1. *J Med Microbiol*. 2005;54:1111-1113.
6. Twenhafel NA, Whitehouse CA, Stevens EL, Hottel HE, Foster CD, Gamble S, et al. Multisystemic abscesses in African green monkeys (Chlorocebus aethiops) with invasive Klebsiella pneumoniae--identification of the hypermucoviscosity phenotype. *Vet Pathol*. 2008;45:226-231.
7. Zachary JF, McGavin MD. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Elsevier Mosby; 2012:524, 637, 801, 1124.