Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 16

3 February 2016

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CASE I: 15-0021 (JPC 4065722).

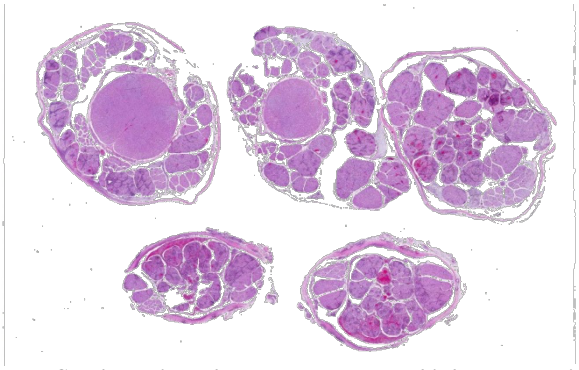
Signalment: 8-year-old male rhesus monkey (*Macaca mulatta)*

History: This SIV-infected monkey had a several day history of reduced cage movement and lower body stiffness. The animal was humanely sacrificed and submitted for necropsy evaluation.

Gross Pathology: The bladder was markedly distended at necropsy. Small areas of superficial congestion were noted from the meningeal surface in the distal lumbar cord and cauda equina region. On transection of fixed cord for trimming, more extensive punctate and focally extensive areas of hemorrhage were seen within the spinal nerves, especially in the more inferior cauda equina areas.

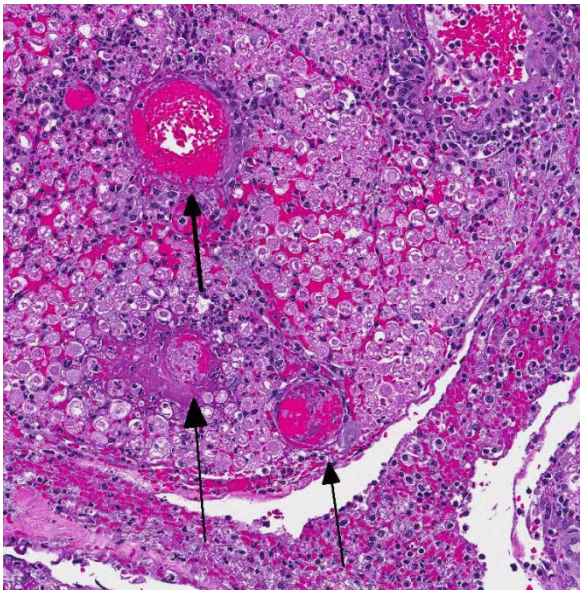
Laboratory Results: None

Histopathologic Description: Sections distributed and corresponding lesions vary somewhat as to the cord level present, with some slides containing only cauda equina tissue without distal lumbosacral spinal cord body. A patchy to focally extensive acute, necrotizing and inflammatory process is noted. Abundant neutrophilic inflammation is present within spinal nerves, admixed with macrophages and globular eosinophilic debris. Within some nerve roots (and some sections of cord), occasional swollen and/or fragmented axons are present. Numerous vessels are infiltrated by dense populations of neutrophils and fibrinoid necrosis and debris are frequently seen within and surrounding vascular walls. Associated with this angiitis are infrequent thrombi.



Cauda equina, rhesus macaque: Multiple cross sections at various levels of the cauda equine demonstrate hemorrhage and a cellular infiltrate within the spinal nerve roots and meningeal space. (HE, 5X)

Scattered throughout the section are large, eosinophilic to amphophilic intranuclear inclusion bodies within enlarged (cytomegalic) Schwann cells and infrequently in endothelium. Also noted in most slides is patchy, necrotizing and fibrinous meningitis. Some sections contain mixed macrophage and granulocytic inflammatory debris within the distal aspect of the central canal.



Cauda equina, rhesus macaque: Within affected spinal roots, inflamed vessels are partially to totally thrombose, and with hemorrhage and fibrin deposition within adjacent tissue. Large numbers of neutrophils, fibrin and hemorrhage are present within the spinal nerve and within the meningeal space (HE, 15X)

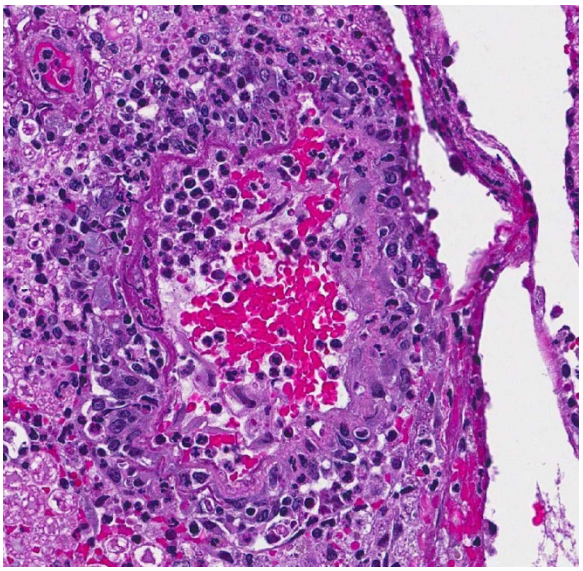
Contributor's Morphologic Diagnosis:

1) Radiculitis, necrotizing and fibrinous, acute, patchy to focally extensive, marked to severe with cytomegaly and intranuclear inclusion bodies, eosinophilic and amphophilic, (Cowdry type A)
2) Angiitis, necrotizing, neutrophil-rich, acute, multifocal with areas of fibrinoid change within and surrounding vessel walls and foci of thrombosis
3) Meningitis, necrotizing and fibrinous, patchy, mild-moderate (most sections)
4) Mixed inflammatory infiltrates, mild-moderate, central canal (some sections)
5) Nerve fiber degeneration, multifocal, mild (some sections)

Contributor's Comment: Immunohistochemical evaluation of sections was strongly positive for rhesus monkey cytomegalovirus (RhCMV) antigen. Rhesus monkey populations are infected with the betaherpesvirus RhCMV at an incidence approaching 100%, with seroconversion generally occurring within the first year of life.[5] Viral shedding in urine and saliva continues throughout life.[2] Once infected, normal host immune response is generally able to control the virus, although not eradicate it.[5] Immunosuppression such as that experienced with SIV infection leads to latent CMV reactivation. The developing rhesus macaque brain is also susceptible to RhCMV infection in the second trimester and intrauterine exposure result in neuropathic outcomes similar to those observed in human congenital CMV infection.[7]

Virus presence has been documented within the gastrointestinal tract, spleen, lung, central nervous system, liver, lymph nodes, testicles, spleen, intestine, nerves and arteries.[4] It is the most frequently identified viral opportunistic pathogen in rhesus macaques.[3] Either multifocal or diffuse interstitial pneumonitis is the most commonly detected lesion seen.[2] Unfortunately in this animal, other tissues were not submitted to determine the extent of organs involved. Infection may also lead to the formation of discrete proliferative masses in the gastrointestinal tract.[3]

From a comparative perspective, human CMV accounts for most HIV-related radiculitis, which in AIDS patients, tends to involve lumbosacral nerve roots, producing

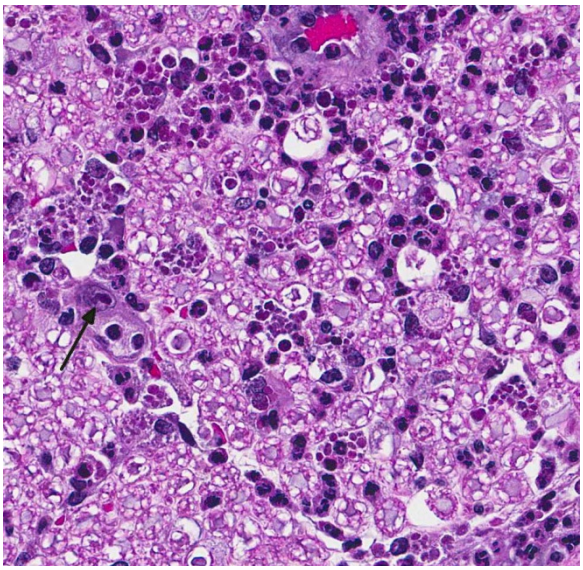


Cauda equina, rhesus macaque: The walls of inflamed vessels contain degenerate neutrophils, extruded fibrin, and moderate amounts of cellular debris, and are surrounded by low to moderate numbers of neutrophils and lymphocytes. (HE, 260X)

a rapidly progressive cauda equine syndrome with severe lower back pain.[8] In humans, highly active antiretroviral therapy (HAART), first introduced in the mid 1990's has dramatically reduced the risk of opportunistic infections and improved the prognosis of patients with HIV infection. CMV disease has become a rare complication in AIDS patients.[6]

JPC Diagnosis: Lumbar spinal roots: Radiculitis, necrotizing and neutrophilic, multifocal to coalescing, with necrotizing vasculitis, fibrinohemorrhagic meningitis, and karyomegalic intranuclear viral inclusions.

Conference Comment: The most common opportunistic viral infection in SIV-infected rhesus macaques is rhesus cytomegalovirus (RhCMV). While it has been well documented to cause lesions in many organs including the leptomeninges and spinal nerve roots, as seen in this case, its role in peripheral neuropathies is less well-researched. A recent study of RhCMV associated facial neuritis noted a mixed inflammatory population composed of neutrophils and macrophages, with macrophages containing intranuclear inclusion bodies.[1] Lesions were present in nerves of the tongue, lacrimal gland and other facial tissues. Axon loss was proportional to the degree of inflammation, the neuritis associated macrophages were infected with RhCMV and there was absence of evidence to support infection of Schwann cells.[1] Lesions were consistent with demyelination and loss of axons secondary to inflammation, supporting the study's assertion that nerve damage is likely related to inflammation rather than direct viral infection of Schwann cells in RhCMV peripheral neuropathies.[1] Lesions in those cases varied in severity from effacement of nerve architecture to milder lesions localized to the periphery. As mentioned above, human CMV (HCMV) is also associated with radiculoneuritis and lesions include peripheral neuritis, with the facial nerves



Cauda equina, rhesus macaque: Within the adjacent spinal nerve, myelin sheaths are occasionally dilated, often contain granular eosinophilic debris. The endoneurium is infiltrated by moderate numbers of viable neutrophils. Rare Schwann cells are karyomegalic, as a result of a large intranuclear viral inclusion (arrow). (HE, 400X)

being most commonly affected; however, in HCMV associated neuritis there is a demonstrated viral predilection for Schwann cells.[1]

Conference participants noted that the spinal cord was largely unaffected and they described the inflammatory infiltrate and hemorrhage as extending from affected spinal nerves into adjacent perineural connective tissue and meninges. Additionally, the inflammatory infiltrate was identified as primarily neutrophilic. This is attributed to RhCMV induction of a CXC chemokine, which results in neutrophil chemoattraction.[1] Other microscopic features noted by participants included dilated myelin sheaths with numerous spheroids, variable numbers of gitter cells, and low numbers of cyto- and karyomegalic cells (most likely Schwann cells in this case), with large, darkly eosinophilic, intra-nuclear viral inclusion bodies.. Other commonly affected tissues include the lung, gastrointestinal tract and the testes.

Contributing Institution:

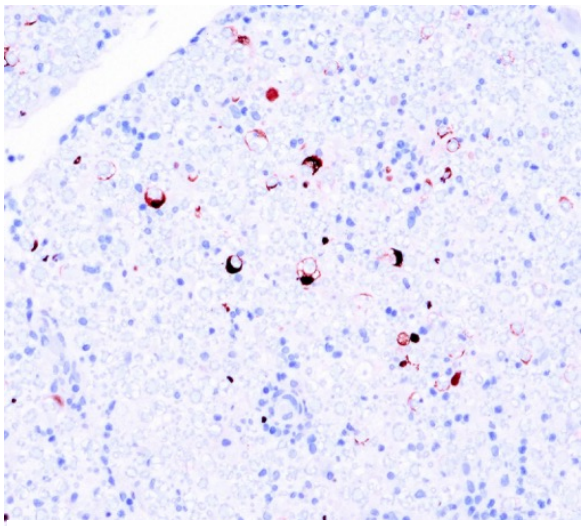
Division of Laboratory Animal Resources, University of Pittsburgh
http://www.dlar.pitt.edu/

References:

1. Assaf BT, Knight HL, Miller AD. Rhesus cytomegalovirus (*Macacine herpesvirus-3*) associated facial neuritis in a simian immunodeficiency virus infected

rhesus macaques. *Vet Pathol*. 2015; 52(1):217-223.

2. Baskin G. Disseminated cytomegalovirus infection in immunodeficient rhesus monkeys. *Am J Pathol*. 1987; 129(2):345-352.



Cauda equina, rhesus macaque: Multifocally, Schwann cells are strongly immunopositive for RhCMV antigen. (antiRhCMV, 100X) (Image courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, Pa 15261 http://www.dlar.pitt.edu/)

3. Hendricks Hutto E, Anderson D, Mansfield K. Cytomegalovirus-associated discrete gastrointestinal masses in macaques infected with simian immunodeficiency virus. *Vet Pathol*. 2004; 41:691-695.

4. Kuhn E, Stolte N, Matz-Rensing K, Stahl-Hennig C. et al. Immuno-histochemical studies of productive rhesus cyto-megalovirus infection in rhesus monkeys (*Macca mulatta*) infected with simian immunodeficiency virus. *Vet Pathol*. 1999; 36:51-56.

5. Powers C, Frick K. Rhesus CMV: An emerging animal model for human CMV. *Med Microbiol Immunol*. 2008; 197:109-115.

6. Salzberger B, Hartmann P, Hanies F, Uyanik B et al. Incidence and prognosis of CMV disease in HIV-infected patients before and after introduction of combination antiretroviral therapy. *Infection*. 2005; 33(5/6):345-349.

7. Tarantal A, Salmat M, Britt W, Lucaw P, et al. Neuropathogenesis induced by rhesus cytomegalovirus in fetal rhesus monkeys

(*Macca mulatta*). *J Infect Dis*. 1998; 177:446-450.

8. Tarulli A, Raynor E. Lumbosacral radiculopathy. *Neurol Clin*. 2007:25:387-405.

CASE II: MK14-5429 (JPC 4070246).

Signalment: 16 year old, intact female, Patas monkey (*Erythrocebus patas*)

History: End of Study. Focal area of consolidation in right caudal lung lobe noted. Submitted for histopathology.

Gross Pathology: Presented is a formalin-fixed pyramidal-shaped piece of lung with a partial cut through the middle. The 3 sides measure 2.5 cm and the base measures 1.5 cm. The tissue is firm and light gray except for one edge that is approximately 2mm x 5 mm that is purplish and somewhat aerated.

Laboratory Results: NA

Histopathologic Description: (slide variation of severity)

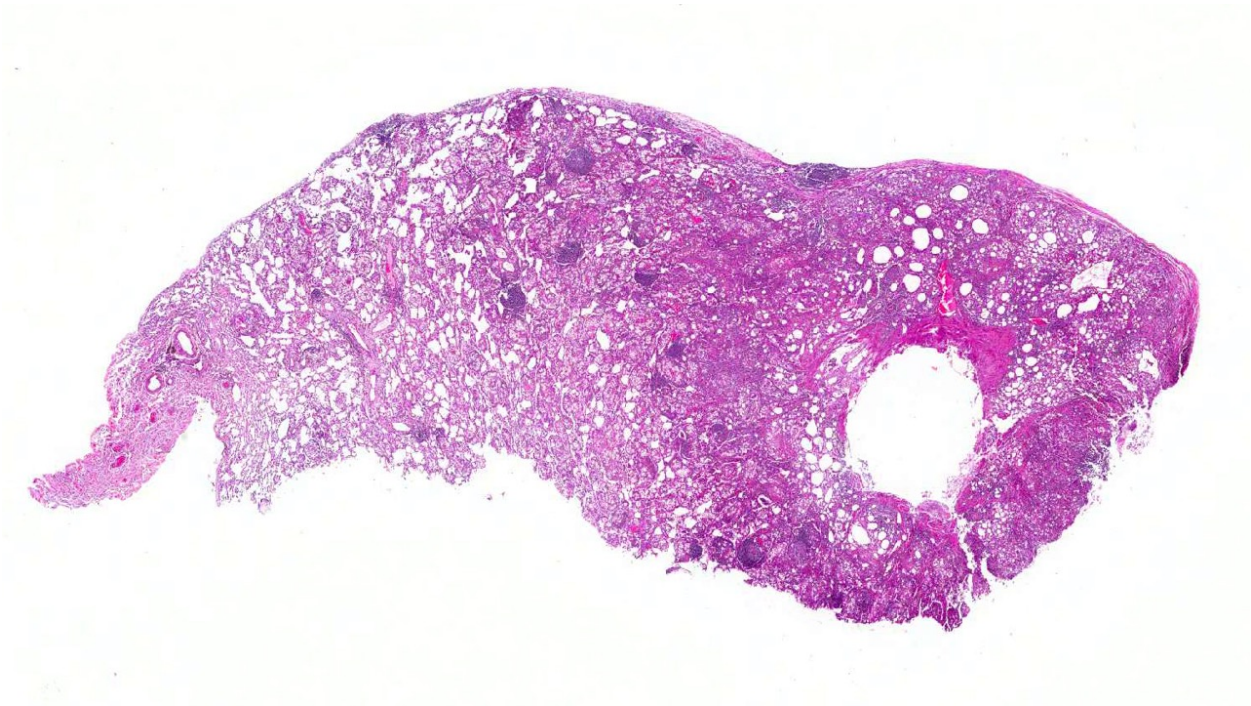
Multifocal to coalescing areas of varying sized infiltrates of macrophages and multinucleated giant cells engulfing and/or surrounding varying sized lipid droplets are observed primarily in alveolar airways. Many of these areas are also partially to completely surrounded by fibrous connective tissue with a mild to moderate lymphocytic and plasmacytic infiltrate. Extensive type II pneumocyte hyperplasia and multiple moderately-sized lymphoid aggregates/nodules are observed. On some slides is an abundance of necrotic mineralizing debris admixed with small amounts of fibrin within a large bronchiole that has denuded mucosal epithelium and focal areas of mild to moderate smooth muscle hypertrophy. Some slides have multifocal areas of pleural fibrosis which extends into the pulmonary parenchyma.

Contributor's Morphologic Diagnosis:

Lung: Pneumonia, granulomatous, multi-focal to coalescing to diffuse, severe, chronic with abundant lipid accumulation and fibrosis

Contributor's Comment: The animal had what is known in the human literature as exogenous lipoid pneumonia. This is a condition where a lipid or fat-like substance is inhaled or aspirated into the lungs eliciting a severe inflammatory response.

There are two types of lipoid pneumonia: endogenous and exogenous. Endogenous lipoid pneumonia has been described in rats [20] dog ,[5, 19] genet,[18] cats ,[11] mongoose,[10] raccoons, [9] llama,[8] African grey parrot,[6] opossums, [3] Siberian tigers.[1] Exogenous lipoid pneumonia has been induced experimentally in mice and rats,[20] and found in two cases of horses[2, 15] and a cow.[21]
Lipoid pneumonia is an uncommon form of pneumonia.[7] It has been reported under different names, such as paraffinoma,[24] cholesterol pneumonia,[22] oil granulomas of the lung,[17] and lipid granulomatosis.



Lung, patas monkey: Subgross magnification reveals patchy consolidation and numerous lymphoid aggregates throughout the section. (HE, 5X)

Exogenous lipoid pneumonia is more commonly reported in human literature[7] whereas it is the least reported in the veterinary literature.[11] It is caused by the inhalation or aspiration of lipid substances: animal fats, vegetable oils or mineral oil. Animal fat/oils elicit a very active inflammatory response. Mineral oils are fairly inert because they have no fatty acids and are rapidly emulsified and consumed by pulmonary macrophages. Vegetable oils are emulsified and not hydrolyzed by the lung lipase.[13] Aspiration may be due to age, psychiatric disorders, loss of consciousness ,[14] abnormality of deglutination (pharyngeal or esophageal) as well as gastro-esophageal reflux[11,22] (oil floats on top of stomach fluids, thus oils may preferentially enter the airways).[14] Most occur when oils are used for medicinal purposes (constipation, oral health, nasal drops, etc).[12, 16, 17] A disproportionate population of fire breathers develop this type of pneumonia because of the liquid paraffin they use.[24]

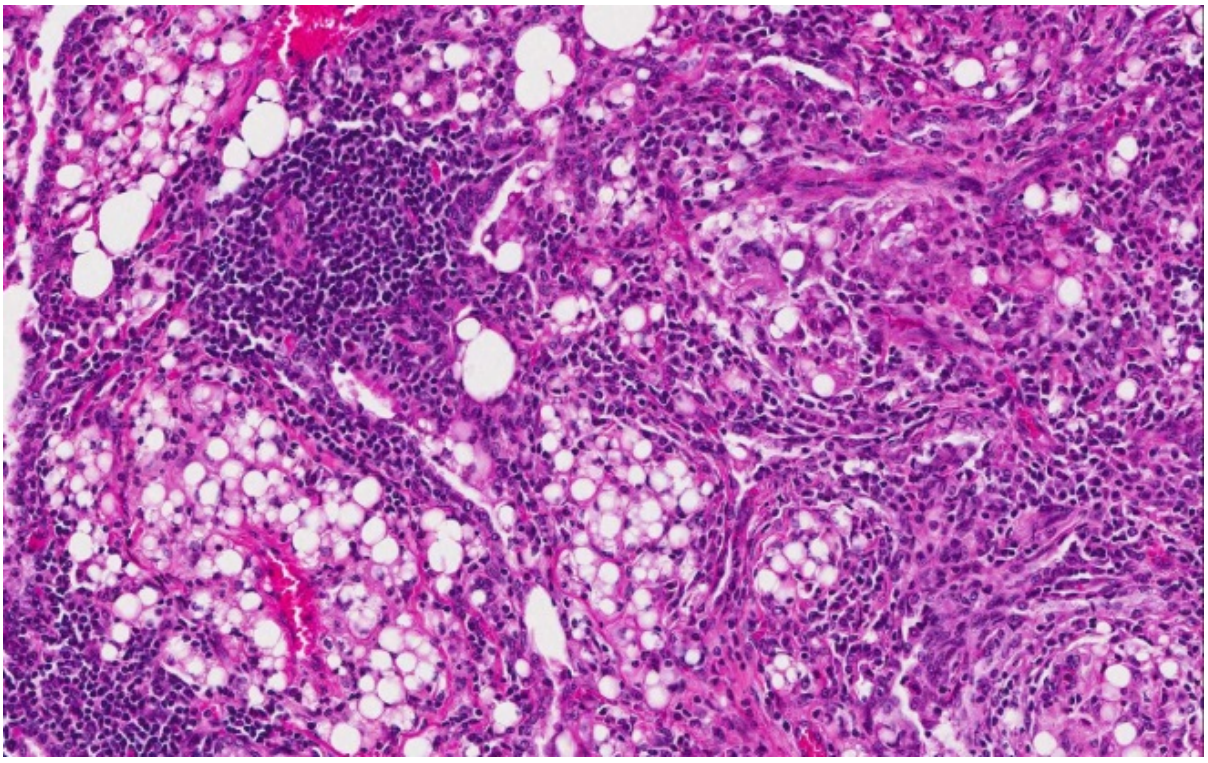
Many lipid substances are non-irritating, can enter the tracheobronchial tree without stimulating a gag or cough reflex, and impair the mucociliary transport system. The pathophysiology is that of a chronic foreign body reaction. Once lipids are in alveoli, they are emulsified and taken up by macrophages. These cells cannot metabolize the fatty substances, so cells die, oil is released and re-phagocytized. Over time, giant cell formation and fibrosis ensue. Diagnosis is made by the presence of large fat globules surrounded by macrophages and multinucleated giant cells as well as lipid-laden macrophages.[23]

Endogenous lipoid pneumonia, also called cholesterol or golden (accumulation of lipid in alveoli causing a yellow discoloration to the lungs) pneumonia, usually develop when lipids that normally reside in the lung tissue, most commonly cholesterol and its esters, escape from destroyed alveolar cell

membranes (surfactant) distal to an obstructing airway lesion or damaged by an inflammatory process. It can also occur with fat emboli to the lung, pulmonary alveolar proteinosis, and lipid storage disorders (Niemann-Pick disease).[7] The pathogenesis is complex and may be related to retained epithelial secretions, cell breakdown, leakage from vessels, prolonged hypoxia, oxygen and carbon dioxide tension. It may be the result of transbronchial dissemination of breakdown products of cancer cells and secretions including mucin. Another though involves anoxic tissue injury stimulating phospholipases and mono-oxygenases, which in turn cause modification of low-density lipoprotein cholesterol. This cholesterol enhances lipid uptake by alveolar macrophages. Definitive diagnosis is demonstrating lipid-laden macrophages and cholesterol crystals.[7]

JPC Diagnosis: Lung: Pneumonia, interstitial, granulomatous, multifocal to coalescing, chronic, severe, with abundant intracytoplasmic lipid.

Conference Comment: Endogenous lipid pneumonia is described as an alveolar filling disorder, encompassing several conditions in which abnormal material accumulates within alveoli. This pulmonary lesion can be an incidental finding or responsible for clinical disease. Other alveolar filling disorders include alveolar proteinosis, alveolar histiocytosis, alveolar phospholipidosis, pulmonary hyalinosis and alveolar microlithiasis. Mild forms of alveolar histiocytosis are commonly seen in the dog and alveolar phospholipidosis occurs in rodents secondary to administration of certain types of drugs as well as in conditions where there is a mutation of surfactant protein D. Conditions which result in the accumulation of lipid-laden



Lung, patas monkey: At higher magnification, alveoli are filled with numerous macrophages and few multinucleated macrophages whose cytoplasm is markedly expanded by numerous, variably-sized lipid vacuoles. Affected alveoli are separated by large perivascular aggregates of lymphocytes. (HE, 200X)

foamy macrophages must be distinguished from other conditions which result in accumulation of macrophages with "foamy" appearing cytoplasm such as pneumocystis and histoplasmosis. In alveolar proteinosis, acellular eosinophilic or amphophilic material composed of surfactant proteins and phospholipids accumulates, but inflammation and fibrosis are not prominent features. Pulmonary hyalinosis, an incidental finding the lungs of aged dogs, is characterized by the accumulation of amorphous, laminated or hyaline material in macrophages and giant cells. In pulmonary alveolar microlithiasis, the accumulated material is extracellular and consists of laminated concretions in alveoli and can result in clinical disease depending on distribution of deposits.[5]

Conference participants described the lung as approximately 75% affected by a predominantly granulomatous infiltrate focused on variably sized lipid vacuoles within alveolar lumina. Other prominent features include extensive type II pneumocyte hyperplasia, fibrosis, and multifocal hyaline membrane formation. Conference participants also noted the multifocal nodular lymphoid aggregates which led to a brief discussion on their histiogenesis (i.e. a chronic inflammatory response versus hyperplasia of preexisting bronchus/bronchiolar associated lymphoid tissue (BALT). The lipid expands the interstitium and prominently fills alveoli depending on location; in some areas, the precise location of the lipid, is difficult to determine due to fibrosis and inflammation. Additional histochemical stains used to highlight lipid include Oil Red O and Sudan black.

Contributing Institution:

Division of Veterinary Resources, National Institutes of Health
http://www.ors.od.nih.gov/sr/dvr/Pages/default.aspx

References:

1. Bolo E, Scaglione FE, Chiappino L, etal. Endogenous lipid (cholesterol) pneumonia in three captive Siberian tigers (*Panthera tigris altaica*). *J Vet Diag Invest.* 2012; 24:618-620.

2. Bos M, deBosschere H, Deprez P, etal. Chemical identification of the (causative) lipids in a case of exogenous lipoid pneumonia in a horse. *Eq Vet J*. 2002; 34:744-747.

3. Brown CC. Endogenous lipid pneumonia in opossums from Louisiana. *J Wildl Dis*. 1998; 24:214-219.

4. Carminato A, Vascellari M, Zotti A, et al. Imaging of exogenous lipoid pneumonia simulating lung malignancy in a dog. *Can Vet J*. 2011; 52:310-312.

5. Caswell JL, Williams KJ. Respiratory System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Vol 2. St. Louis, MO: Elsevier; 2015:517.

6. Costa T, Grifols J, Perpinan D. Endogenous lipid pneumonia in an African Grey Parrot (*Psittacus erithacus erithacus*). *J Comp Pathol*. 2013; 149:381-383.

7. Hadda V, Khilnani GC. Lipoid pneumonia: an overview. *Expert Rev resp Med*. 2010; 4:799-807.

8. Hamir AN, Andreasen CB, Pearson EG. Endogenous lipid pneumonia (alveolar histiocytosis) and hydrocephalus in an adult llama (*Llama glama*). *Vet Rec*. 1997; 141:474-475.

9. Hamir AN, Hanlon CA, Rupprecht CE. Endogenous lipid pneumonia (multifocal

alveolar histiocytosis) in raccoons (*Procyon lotor*). *J Vet Diagn Invest*. 1996; 8:267-269.

10. Hayashi T, Stemmermann GN. Lipd pneumonia in the Hawaiian feral mongoose. *J Pathol*. 1972; 108:205-210.

11. Jones DJ, Norris CR, Samii VF, Griffey SM. Endogenous lipid pneumonia in cats: 24 cases (1985-1998). *JAVMA*; 216:1437-1440.

12. Kim JY, Jung JW, Choi JC, et al. Recurrent lipoid pneumonia associated with oil pulling. *Int J Tuberculosis Lung Dis*. 2014; 18:251-252.

13. Laurent F, Philippe JC, Vergier B, et al. Exogenous lipoid pneumonia: HRCT, MR, and pathologic findings. *Eur Radiol*. 1999; 9:1190-1196.

14. Marchiori E, Zanetti G, Mano CM, Hochhegger B. Exogenous lipoid pneumonia. Clinical and radiological manifestations. *Resp Med*. 2011; 105:659-666.

15. Metcalfe L, Cummins C, Maischberger E, Katz L. Iatrogenic lipoid pneumonia in an adult horse. *Ir Vet J*. 2010; 63:303-306.

16. Moreau E, Rerolle C, Deveaux M, et al. Exogenous lipoid pneumonia as a contributory factor in a drug related death. *J Forensic Sci*. 2015; 60:514-517.

17. Papla B, Urbanczyk K, Gil T, et al. Exogenous lipoid pneumonia (oil granulomas of the lung). *Pol J Pathol*. 2011; 4:269-273.

18. Perpinan D, Steffen D, Napier JE. Pulmonary adenocarcinoma and endogenous lipid pneumonia in a common genet (*Genetta genetta*). *J Zoo Wildl Med*. 2010; 41:710-712.

19. Raya AI, Marco F-D, Nunez A, Afonso JC, et al. Endogenous lipid pneumonia in a dog. *J Comp Pathol*. 2006; 135:153-155.

20. Romero F, Shah D, Duong M, et al. Chronic alcohol ingestion in rats alters lung metabolism, promotes lipid accumulation, and impairs alveolar macrophage functions. Am J Respir Cell Mol Biol. 2014; 51:840-849

21. Smith BL, Alley MR, McPherson WB. Lipid pneumonia in a cow. *New Zeal Vet J*. 1969; 17:65-67.

22. Spickard A, Hirschmann JV. Exogenous lipoid pneumonia. *Arch Intern Med*. 1994; 154:686-692.

23. Wang C-W, Colby TV. Histiocytic lesions and proliferations in the lung. *Semin Diagn Pathol*. 2007; 24:162-182.

24. Weinberg I, Fridlender ZG. Exogenous lipoid pneumonia caused by paraffin in an amateur fire breather. *Occupational Med*. 2010; 60:234-235.

CASE III: S 1215/09 (JPC 3167218).

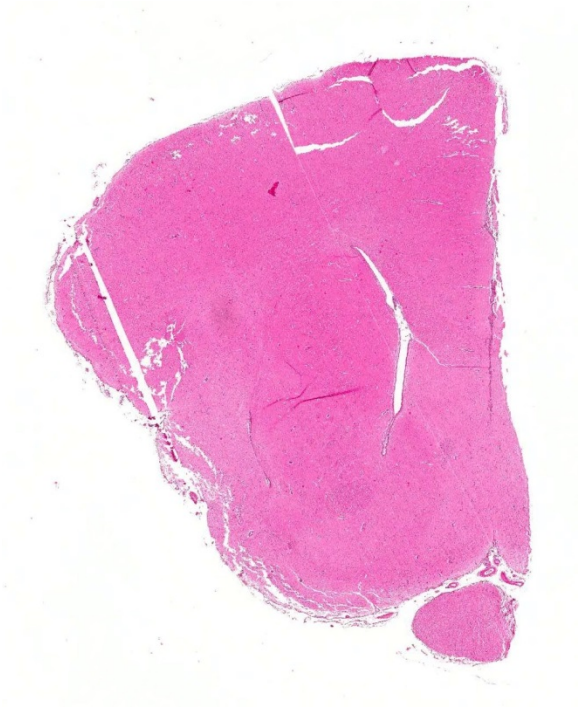
Signalment: 1 ½ years, female, rabbit (*Oryctolagus cuniculus)*

History: The rabbit was presented with symptoms of salivation, seizures, somnolence and fever. Meningitis was suspected.

Gross Pathology: Yellowish covering of the eye´s proximity; ulceration of the right cornea; edema of the mucosal-cutaneous intersections; slight enlargement and blood-reabsorption of the lymph nodes at the head; little yellowish dry mass in the outer ear canals; no abnormalities at the inner ears; slight lipidosis of the liver; dilated uterus, filled with white mucous.

Laboratory Results: Herpes simplex virus (HSV) was verified by polymerase chain reaction of paraffin-embedded, formalin-fixed brain material. Specificity of HSV-1, was confirmed by restriction enzyme digestion with BamHl and Sma-digestion (Institute of virology, TU Munich, Germany).

Histopathologic Description: Brain: multifocal moderate to severe perivascular accumulation of lymphocytes, plasma cells and histiocytes at meningeal and cortical blood vessels; multifocal extensive neuronal and glial necrosis at the cerebral cortex; detection of intranuclear eosinophilic to amphophilic inclusion bodies in cortical pyramidal neurons and glial cells, often filling the entire nucleus; electron micro-scopy showed high numbers of intra-nuclear particles of icosahedral nucleocapsids, consistent with the morphology of herpesvirus.



Cerebrum, rabbit: The submitted specimen is a transverse section of the frontal cortex, without visible lesion. (HE, 5X)

Eye (not on the slide): ulceration of the cornea; small foci of infiltrating lymphocytes and plasma cells at the limbus.

Contributor's Morphologic Diagnosis:

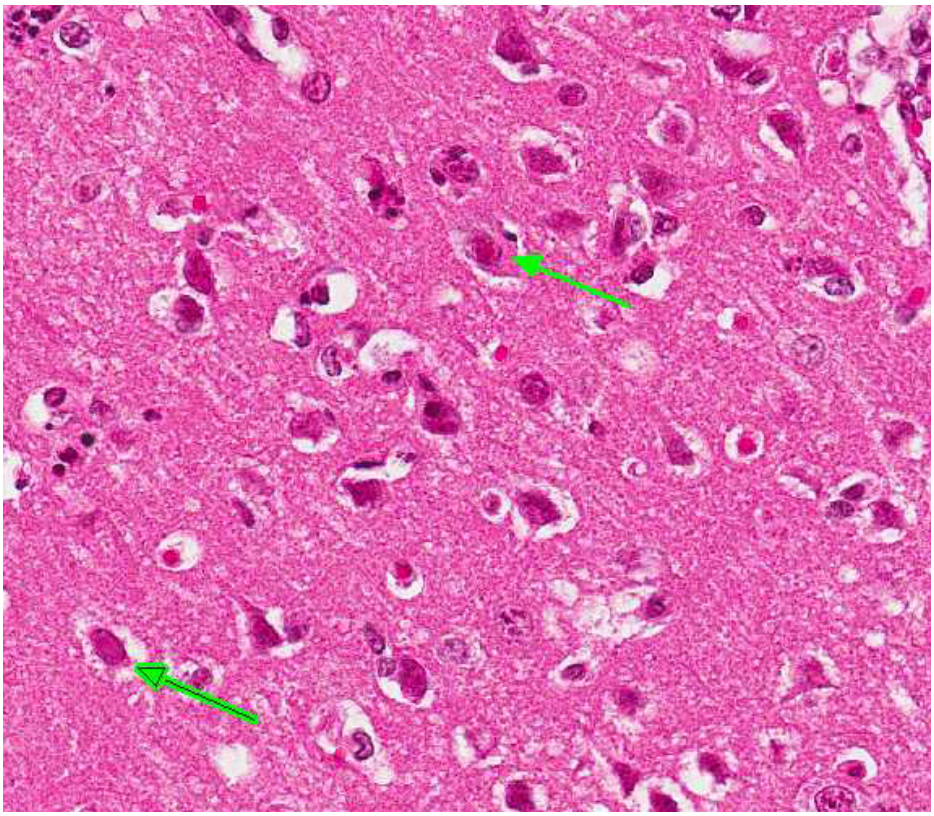
Brain: Meningoencephalitis, severe, multifocal, non-suppurative, with detection of numerous intranuclear inclusion bodies in neurons and glial cells.

Contributor's Comment: Herpesvirus infection is an uncommon disease in domestic rabbits. *Herpesvirus sylvilagus* and *cuniculi* (formerly known as virus III or herpes-like virus) are known to be either only pathogenic for individual breeds or only slightly pathogenic for the domestic rabbit.[5,2] A third, unclassified herpesvirus causes haemorrhagic dermatitis, pneumonia and necrotizing splenitis in rabbits.[6]
Naturally occurring encephalitis in domestic rabbits by herpes simplex virus infection has only been reported twice.[9,1] In both reported cases a person with herpetic infection and close contact to the rabbit was suspected to be the source of infection for the animal.

Rabbits can be easily infected experiment-tally and act as an animal model for human herpes simplex infection.[7] Trigeminally inn-ervated areas, as for example the cornea, serve as the portal of entry for the human herpes simplex virus.[7] Furthermore, nasal infection is known to lead to focal lesions in the brain.[8]

JPC Diagnosis: Cerebrum: Neuronal necrosis, multifocal, with intranuclear inclusion bodies and mild lymphocytic meningitis.

Conference Comment: Rabbits serve as experimental models for herpes simplex virus (HSV) type 1 (*human herpesvirus 1*)



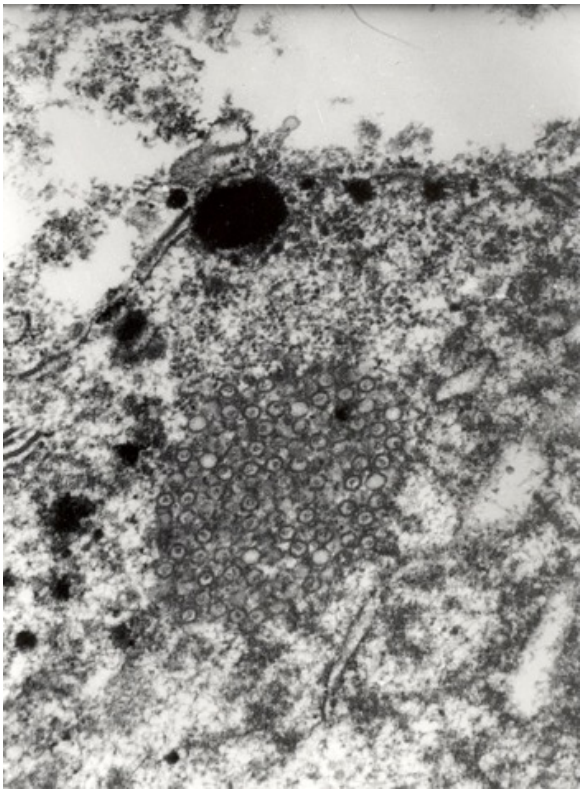
Cerebrum, rabbit: There is extensive neuronal degeneration and necrosis within the superficial gray matter. Necrotic neurons are shrunken and angular, with occasionally pyknosis karyorrhexis; and occasionally abutted by one or more glial nuclei (satellitosis). Occasionally degenerating neurons (green arrows) contain eosinophilic intranuclear viral inclusions. (HE, 260X)

encephalitis in humans, and in rabbits, the infection is exclusively neurotropic. In humans, the intranasal route of infection is most important in development of encephalitis. Experimental intranasal inoculation in rabbits leads to migration via the olfactory nerves, leading into the frontal and temporal lobes. Intraocular inoculation of the virus in rabbits has also been used to study cell spread of HSV, and rabbits can present with neurologic signs as early as 2 days post infection; the disease is also known to progress quickly in spontaneous cases.[3] Following intraocular inoculation, the virus travels through the optic nerve to the corpus geniculatum.[1,3] Both ocular and nasal routes of inoculation can lead to seizures and death but the intranasal route has a higher mortality rate. Histologic lesions described in other cases of both natural and experimental infection are similar to those seen in this case.[1,3,9] Gross CNS lesions in rabbit HSV infections are uncommon which may be due in part to the rapid course of disease. In some reported cases of natural HSV infection in rabbits, humans in close contact were reported to have cold sores prior to the onset of clinical signs in the rabbit. Nonetheless, it is unclear if spontaneous HSV encephalitis in rabbits can arise from reactivation of latent infection or if disease only occurs shortly after exposure.[3]

HSV can also result in fatal disease in nonhuman primates. In Old World primates, the course of HSV infection is comparable to humans with localization to the mucocutaneous tissues and relatively mild disease. However, New World primates (NWP) are considered highly susceptible and infection often leads to severe systemic disease and death with a rapid clinical course in many cases. In some NWPs such as owl monkeys and marmosets, they also develop ulceration of the oral mucous membranes but it is acc-ompanied by hemorrhage and necrosis in the cerebral cortex as well as many other organs. In most of the reported cases in non-human primates, close contact with an infected human was found to

be the source of infection. The most characteristic lesions in HSV infection in NWPs are oral ulcerations and lesions at the mucocutaneous junction, which cannot be grossly differ-entiated from lesions caused by herpesvirus T (*Herpes tamarinus*) infection. Molecular methods are needed to differentiate infections caused by the two viruses due to similarity of lesions.[4]

The conference description included multifocal, random areas of neuronal necrosis within the superficial gray matter accompanied by gliosis, satellitosis and low numbers of infiltrating heterophils. Peri-vascular cuffing by mononuclear cells is present multifocally within the gray matter and a similar mononuclear infiltrate as well as edema expand the meninges. The lack of neuronal degenerative changes such as swelling was noted by some participants; however, in some viral infections, neuronal necrosis (even in acute infection) may be the defining lesion.



Neuron, rabbit. Ultrastructural evaluation demonstrates an aggregate of targetoid icosahedral nucleocapsids consistent with herpesviral particles within degenerating neurons. (Photo courtesy of Institute of Veterinary Pathology, Ludwig-Maximilians-University Munich, Veterinaerstrasse 13; D-80539 Munich, Germany, www.patho.vetmed.uni-muenchen.de)

Contributing Institution:

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www.patho.vetmed.uni-muenchen.de

References:

1. Grest P, Albicker P, Hoelzle L, Wild P, Pospischil A. Herpes simplex encephalitis in a domestic rabbit (Oryctolagus cuniculus). *J Comp Pathol*. 2002; 126: 308-11.

2. Hinze HC. New Member of the Herpesvirus Group Isolated from Wild Cottontail Rabbits. *Infect Immun*. 1971; 3: 350-354.

3. Nesburn AB. Isolation and characterization of a herpes-like virus from New Zealand albino rabbit kidney cell cultures: a probable reisolation of virus 3 of Rivers. *J Virol*. 1969; 3: 59-69.

4. Matos R, Russell D, Van Alstine W, Miller A. Spontaneous fatal Human herpesvirus 1 encephalitis in two domestic rabbits (*Oryctolagus cuniculus*). *J Vet Diag Invest*. 2014; 26(5):689-694.

5. Mätz-Rensing K, Jentsch KD, Rensing S, Langenhuyzen S, et al. Fatal Herpes simplex infection in a group of common marmosets (*Callithrix jacchus*). *Vet Pathol*. 2003; 40:405-411.

6. Onderka DK, Papp-Vid G and Perry AW: Fatal herpesvirus infection in

commercial rabbits. *Can Vet J*. 1992; 33:539-543.

7. Paeivaerinta MA, Roeyttae M, Hukkanen V, Marttila RJ. Nervous system inflammatory lesions and viral nucleic acids in rabbits with herpes simplex virus encephalitis-induced rotational behaviour. *Acta Neuropathol*. 1994; 87: 259-68.

8. Stroop WG and Schaefer DC. Production of encephalitis restricted to the temporal lobes by experimental reactivation of herpes simplex virus. *J Infect Dis*. 1986; 153: 721-731.

9. Weissenboeck H, Hainfellner A, Berger J, Kasper I and Budka H. Naturally occurring herpes simplex encephalitis in a domestic rabbit (*Oryctolagus cuniculus*). *Vet Pathol.* 1997; 34: 44-7.

CASE IV: 14-062 (JPC 4068766).

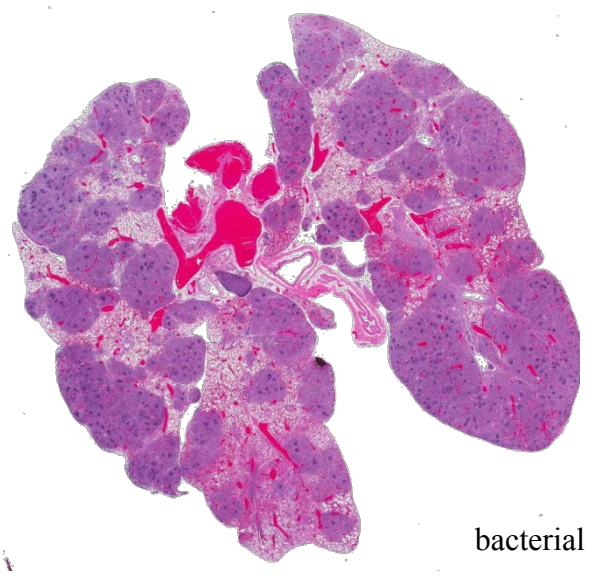
Signalment: 7 month-old, male, Cybb transgenic mouse, (*Mus musculus*)

History: Found dead in cage.

Gross Pathology: The lungs were described by the submitter as diffusely "mottled with multifocal masses throughout"; *Staphylococcus xylosus* infection was suspected.

Laboratory Results: None

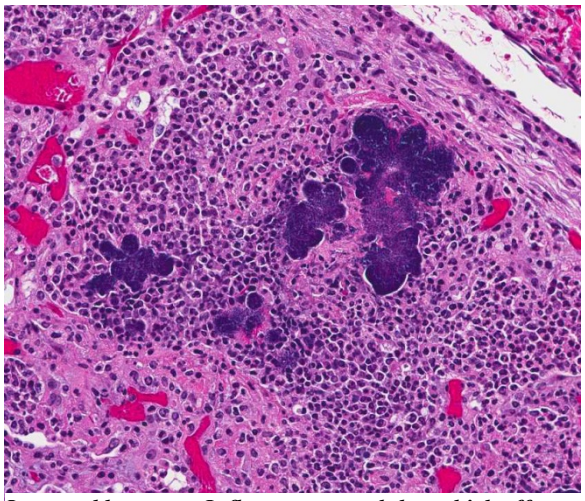
Histopathologic Description: Lungs: Diffusely affecting greater than 90% of the section, multiple, coalescing pyogranulomas are effacing and replacing the normal architecture, compressing the adjacent parenchyma and occasionally distending bronchial and bronchiolar structures. Pyogranulomas are centered on large colonies of bacterial cocci that are often embedded within hyalinized, eosinophilic material that also occasionally contains refractile, acicular crystalline structures. Bacterial colonies are surrounded by large numbers of viable and degenerate neutrophils admixed with variable amounts of amorphous, eosinophilic proteinaceous debris (necrosis) with large numbers of epithelioid macrophages, fewer scattered lymphocytes and plasma cells and rare multinucleated giant cells (Langhans-type). Within the adjacent parenchyma, alveoli are frequently expanded by large amounts of a similar inflammatory infiltrate containing brightly eosinophilic, refractile, needle-like to acicular crystalline material. The crystalline material is typically free within the alveolar spaces but is often found within the cytoplasm of alveolar macrophages and multinucleated giant cells (eosinophilic crystalline pneumonia). There is also diffuse vascular congestion, mild edema and peripheral emphysema.



Lung, cybb mouse: All lung field contain coalescing nodular foci of inflammation. (HE, 6X).

Gram Stain:

Lung: Bacterial cocci are gram positive, consistent with *Staphylococcus sp*.



Lung, cybb mouse. Inflammatory nodules, which efface alveolar parenchyma, are composed of large, poorly formed pyogranulomas centered on large colonies of cocci. (HE, 200X)

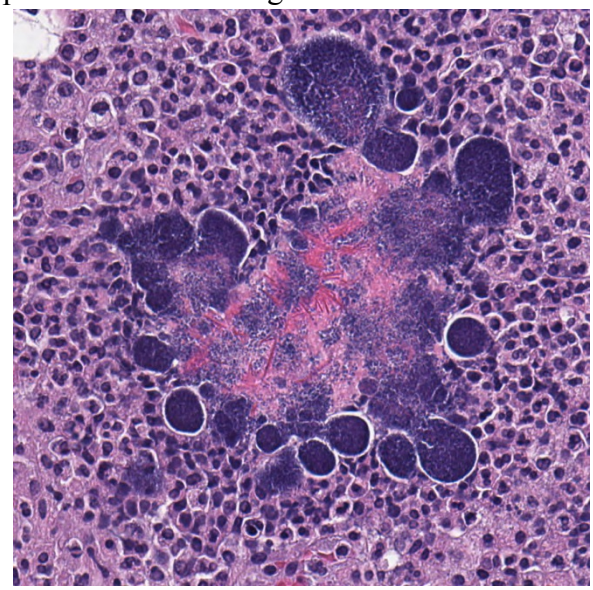
Contributor's Morphologic Diagnosis:

Lung: Diffuse, chronic-active, severe, pyogranulomatous pneumonia with intralesional bacteria (pulmonary botryomycosis).

Lung: Multifocal, subacute to chronic, moderate, eosinophilic crystalline pneumonia.

Contributor's Comment: Botryomycosis is a chronic infection caused by non-filamentous bacteria characterized by pyogranulomas that contain a central necrotic core with colonies of gram-positive cocci embedded within brightly eosinophilic, hyalinized material that occasionally forms club-shaped projections (Splendore-Hoeppli material). The condition is so named due to its resemblance to fungal pyogranulomas, and is not uncommon among domestic and laboratory animal species. Staphylococci are most commonly associated with botryomycosis, however, *Streptococcus sp*., *Pseudomonas sp*., *Actinobacillus sp*., *Pasteurella sp*., *Proteus sp*., and *Escherichia sp*., have also been isolated in reported cases.[3,6,9] In mice, botryomycosis is typically caused by *Staphylococcus aureus* or *S. xylosus*, which are commensals of the skin and mucus membranes. In all species, botryomycosis lesions are typically localized to the skin and subcutis but may extend to the underlying bone and muscle. Pulmonary botryomycosis, as seen in this case, is rare, but has been reported in horses, cattle and guinea pigs.[6]

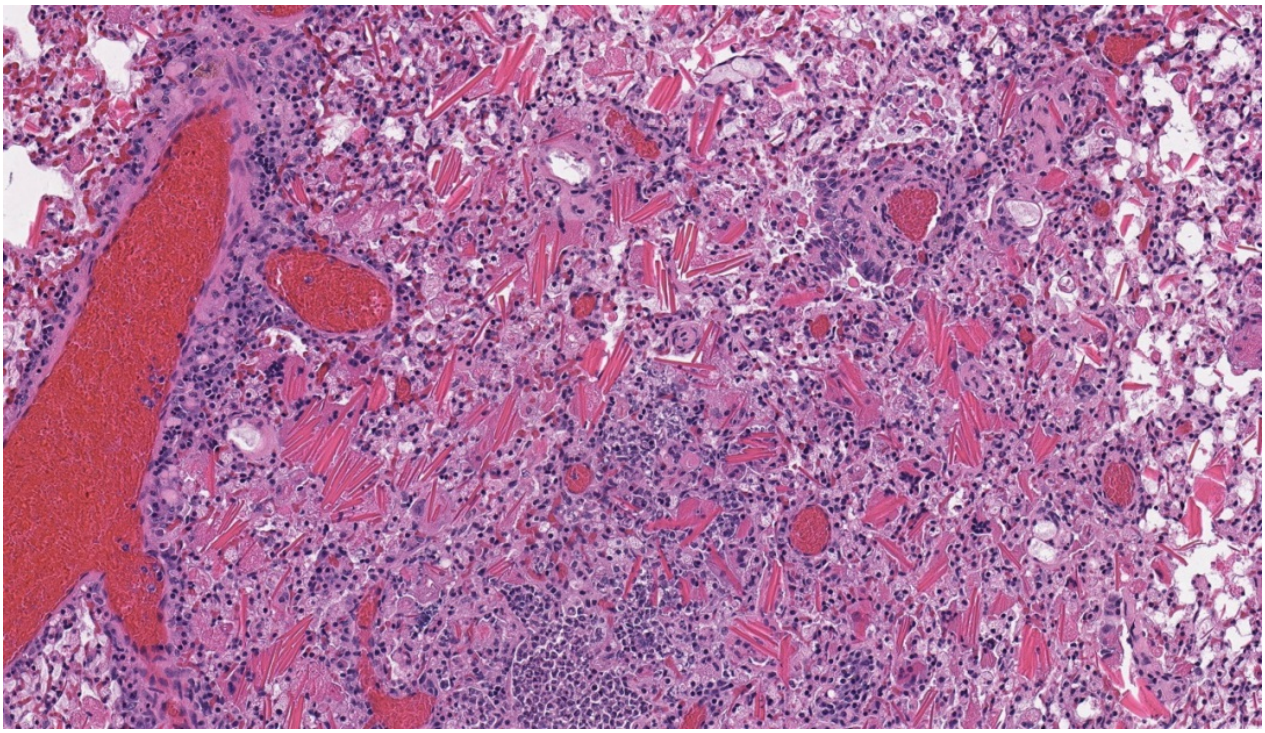
Eosinophilic crystalline pneumonia, sometimes called "acidophilic macrophage pneumonia" or simply "intracellular eosinophilic crystals" is an idiopathic granulomatous pneumonia that occurs spontaneously in many strains of mice, namely the C57BL/6 and strains on a C57BL/6 background.[5,10] This condition is also common among Swiss mice, B6C3F1 mice used by the National Institutes of Health and the National Toxicology Program and 129 strains, particularly the 129S4/SvJae strain in which severe cases resulting in respiratory distress or death have been reported.[1,5,8] Largely considered a background lesion, eosinophilic crystalline pneumonia can range from subclinical to



Lung, cybb mouse: Higher magnification shows that colonies of cocci are enmeshed in a brightly eosinophilic matrix which often contains specular acidophilic crystals. (HE, 400X) (Image courtesy of NIEHS/NTP, 111 T.W. Alexander Drive, Research Triangle Park, NC, 27709 http://ntp.niehs.nih.gov/nnl/)

fatal and is characterized by focal to diffuse accumulation of eosinophilic crystals composed predominantly of Ym1 protein, a chitinase-like protein expressed in neutrophil granule products and active macrophages. As in this case, crystals are readily apparent within macrophages, multi-nucleated giant cells, alveolar spaces and airways.[1,5,8,10] The function of Ym1 protein is not completely understood, but may play a role in host immune defense, tissue repair and hematopoiesis.[5,10]

The Cybb transgenic mouse was designed as a model for developing new treatments for chronic granulomatous disease (CGD); JAX developed this particular transgenic strain targeting the gene involved in X-linked CGD. As a result, male hemizygotes tend to have an increased susceptibility to infection with *S. aureus* due to a lack of phagocyte superoxide production.[7] Given the strain-specific increased susceptibility to bacterial infection and apparent pulmonary botryomycosis, staphylococcal infection is the most likely etiology in this case. Large colonies of Gram-positive cocci are consistent with *Staphylococcus sp.*, most likely *S. aureus* or *S. xylosis*. We were unable to definitively identify the bacterial agent since bacterial culture was not performed. As previously mentioned, eosinophilic crystalline pneumonia is a common spontaneous lesion in the C57BL/6 mouse, which was the background strain used to manufacture this particular transgenic. However, since this condition may also occur in conjunction with other diseases, it is difficult to determine whether the eosinophilic crystalline pneumonia developed prior to the pulmonary botryomycosis, as a spontaneous disease possibly predisposing the animal to infection, or developed secondary to, or in



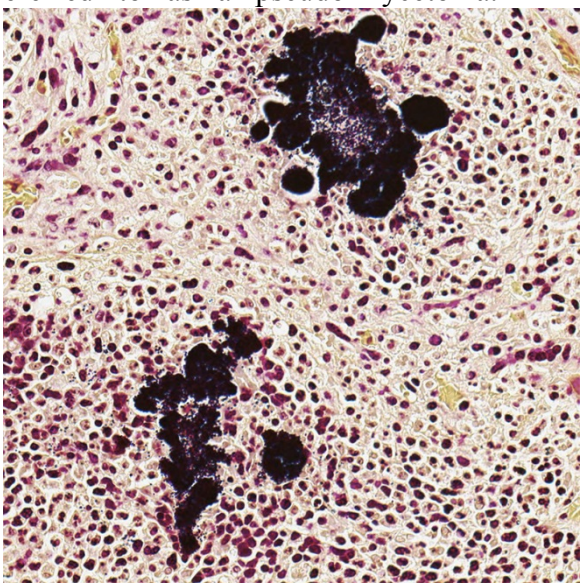
Lung, cybb mouse. Alveoli adjacent to pyogranulomatous nodules contain large numbers of alveolar histiocytes surrounding sheaves of brightly eosinophilic acidophilic crystals.*HE, 20X) (Image courtesy of: NIEHS/NTP, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709 http://ntp.niehs.nih.gov/nnl/)

conjunction with, the pulmonary botryomycosis.

JPC Diagnosis: 1. Lung: Pneumonia, pyogranulomatous, multifocal to coalescing, severe, with Splendore-Hoeppli material and numerous colonies of cocci.

2. Lung: Pneumonia, histiocytic, diffuse, moderate with extracellular and intrahistiocytic eosinophilic protein crystals.

Conference Comment: Botryomycosis also occurs in other species as a result of *Staphylococcus aureus* infection, including horses and pigs, and is most commonly associated with wound infection. The inflammatory reaction and lesions are similar to those seen in this case, consisting of pyogranulomatous inflammation and Splendore-Hoeppli reaction. In the skin and subcutis, it is often associated with nodular masses which progressively enlarge over time. The nodular structure is occasionally referred to as a pseudo-mycetoma. A similar inflammatory reaction is seen in *Actinobacillus lignieresii* (wooden tongue) and *Actinomyces bovis* (lumpy jaw) infections in cattle.[11] In many cases of botryomycosis, the bacteria will form yellow sulfur granules which can be seen grossly. The tissue granules consist of bacterial colonies surrounded by clubs of Splendore-Hoeppli material, which is generally regarded as being composed of antigen-antibody complexes.



Lung, cybb mouse: Clusters of cocci within pyogranulomas are gram-positive. (Gram, 200X). (Image courtesy of: NIEHS/NTP, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709 http://ntp.niehs.nih.gov/nnl/)

The differential diagnosis for a nodular mass composed of pyogranulomatous inflammation includes filamentous bacterial or fungal infections, which result in the formation of a mycetoma.[4] The organisms are not always readily visualized on H&E stained sections and therefore may require special histochemical stains for differentiation. Botryomycosis may also occur in the mammary gland of ruminants associated with staphylococcal mastitis, and in other large animals secondary to post castration staphylococcal infection.[2]

The conference description was very similar to that provided by the contributor. Additional features described and discussed include: multifocal expansion of alveolar septa by fibrous connective tissue; an exudate composed of neutrophils and histiocytes within larger airways, which may represent reflux from alveolar spaces; and the presence of Splendore-Hoeppli phenomenon within the center, rather than the periphery, of the bacterial colonies. Medullary sinuses of a hilar node contain numerous plasma cells, with few neutrophils and macrophages, which attest to the long-standing nature of this infection. There was discussion regarding the route of infection in this case; most participants favored an airway route, as the bronchi and bronchioles are particularly affected by the inflammatory process and the vessels are relatively spared.

Contributing Institution:

NIEHS/NTP
http://ntp.niehs.nih.gov/nnl/

References:

1. Dixon D, Herbert RA, Sills RC, Boorman GA. Lung, Pleura, Mediastinum. In: Maronpot RR, ed. *Pathology of the Mouse*. Vienna, IL:Cache River Press; 1999:318.

2. Foster RA. Male reproductive system. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby Elsevier; 2012:1144.

3. Ginn PE, Mansell JEKL, Rakich PM. Skin and appendages. In: McGavin MD and Zachary JF, eds. *Pathologic Basis of Veterinary Disease. 4th ed*. Mosby Elsevier, St. Louis, MO; 2007:691.

4. Hargis AM, Ginn PE. The Integument. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby Elsevier; 2012:1034.

5. Hoenerhoff MJ, Starost MF, Ward JM. Eosinophilic Crystalline Pneumonia as a Major Cause of Death in 129S4/SvJae Mice. *Vet Pathol.* 2006; 43:682-688.

6. I-B02 - Botryomycosis - fibrovascular tissue – rat; Veterinary Systemic Pathology Online (VSPO); The Joint Pathology Center (JPC); http://www.askjpc.org/vspo/show_page.php?id=285; 2014; accessed: February 6, 2016.

7. Mouse strain datasheet, Strain Name: B6129S-*Cybb*^{tm1Din}/J; JAX Mouse Database. The Jackson Laboratory; http://jaxmice.jax.org/strain/002365.html; 2016; accessed: February 6, 2016.

8. Percy DH and Barthold SW. Mouse: Acidophilic Macrophage Pneumonia/Epithelial Hyalinosis. In: Percy DH and Barthold SW, eds. *Pathology of Laboratory Rodents and Rabbits. 3rd ed*. Ames, IA: Blackwell Publishing; 2007:95.

9. Percy DH and Barthold SW. Mouse: Staphylococcal Infection. In: Percy DH and Barthold SW, eds. *Pathology of Laboratory Rodents and Rabbits.* 3rd ed. Ames, IA: Blackwell Publishing; 2007:74-76.

10. Renne R, Brix A, Harkema J, Herbert R, Kittel B, Lewis D, March T, Nagano K, Pino M, Rittinghausen S, Rosenbruch M, Tellier P, Wohrmann T. Proliferative and Nonproliferative Lesions of the Rat and Mouse Respiratory Tract. *Tox Pathol.* 2009; 37:5S-73S.

11. Valentine BA, McGavin MD. Skeletal muscle. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby Elsevier; 2012:892.