Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 12

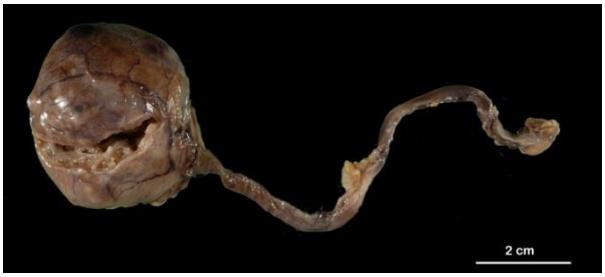
6 January 2016

Moderator:
Tim Cooper, DVM, Ph.D, DACVP
Associate Professor
Department of Comparative Pathology
Penn State Hershey Medical Center
Hershey, PA

CASE I: A10-9691 (JPC 3164430).

Signalment: Adult female guinea pig (*Cavia porcellus*).

History: This underweight adult guinea pig was found abandoned with truncal alopecia and a palpable abdominal mass. Age was estimated at 3 years. Ovariohysterectomy was performed in preparation for adoption.



Ovary, guinea pig. The right ovary was enlarged at 3.5 cm x 4 cm x 3.5 cm. (Photo courtesy of: Purdue University Animal Disease Diagnostic Laboratory: http://www.addl.purdue.edu/ and Department of Comparative Pathobiology: http://www.vet.purdue.)

Gross Pathology: The right ovary was enlarged at 3.5 cm x 4 cm x 3.5 cm. Numerous fluid-filled cysts up to 7-8 mm in diameter were evident on cross-section. Extensive stromal calcification or ossification necessitated decalcification before histologic processing.

Laboratory Results:
No ancillary testing performed.

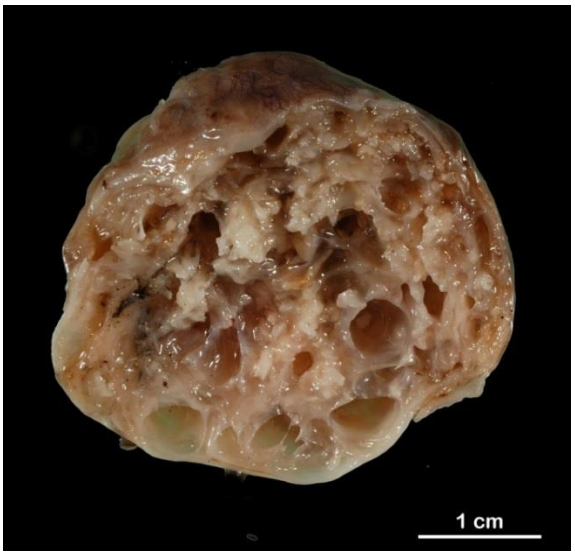
Histopathologic Description: A few viable follicles are in the periphery of the mass, but most of the specimen lacks recognizable ovarian tissue and instead is composed of neoplastic tissue from all 3 germ layers. The endodermal component includes tubuloacinar glands (lobules of serous acini formed by pyramidal cells with bright eosinophilic cytoplasmic granules) and cystic spaces lined by respiratory type epithelium with numerous ciliated cells and mucus-filled goblet cells. The ectodermal component consists of neuroectodermal tissue with axons, glial cells, and neuronal cell bodies; no skin, hair follicles or cutaneous adnexal glands are observed. The mesodermal

component consists mainly of fibrous tissue with scattered plates of well differentiated bone. No lesions were detected in the left ovary or in the uterus.

Contributor's Morphologic Diagnosis: Ovary: Ovarian teratoma

Contributor's Comment: The presence of all 3 germ layers in a neoplastic gonadal mass was the basis for the diagnosis of teratoma. Reproductive tract tumors account for about 25% of neoplasia in the guinea pig. Though not considered common, teratoma is the most frequently reported tumor of the guinea pig ovary, occurring in juveniles and adults, and accounting for all but 6 of 29 ovarian tumors reviewed in a 1976 book chapter.[3] Interestingly, testicular teratomas seem not to have been reported in the guinea pig.

One of two ovarian teratomas reported by Willis[7] spread to peritoneal surfaces. Of 10 cases of ovarian teratoma found at necropsy of about 13,000 guinea pigs over an 8-year period,[6] none had metastasized, though some cases had resulted in abdominal hemorrhage.



Ovary, guinea pig. Numerous fluid-filled cysts up to 7-8 mm in diameter were evident on cross-section (Photo courtesy of: Purdue University Animal Disease Diagnostic Laboratory: http://www.addl.purdue.edu/ and Department of Comparative Pathobiology. http://www.vet.purdue.edu/cpb/)

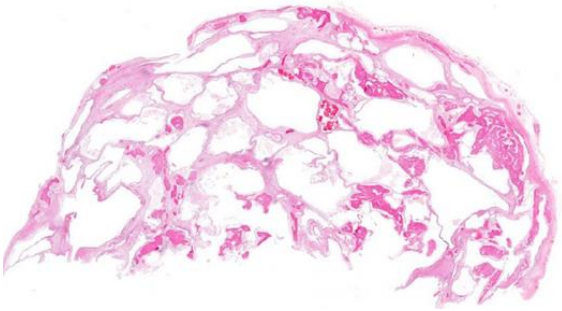
Tissues from at least 2 germ layers were found in all 10 tumors; most tumors had all 3 germ layers, and nervous tissue tended to be the dominant ectodermal component. Nervous tissue also figured prominently as the ectodermal component in another case of ovarian teratoma that had spread to the peritoneal surface of the diaphragm.[1]

Granulosa cell tumor is reported far less commonly than ovarian teratoma in guinea pigs;[3] however, because of its potentially cystic nature, it should be included in the differential diagnosis for an ovarian mass along with cystic rete ovarii.[4] Though distant metastasis has not been recorded in ovarian teratomas in guinea pigs, a few cases have seeded peritoneal surfaces. In this guinea pig, no recurrence or spread of the teratoma was clinically evident at 10 weeks after ovariohysterectomy. The alopecia had resolved, and the guinea pig had gained weight.

JPC Diagnosis: Ovary: Teratoma.

Conference Comment: Conference participants agreed with the contributor that there is a lack of recognizable ovarian tissue on the slide aside from a small section of oviduct at the margin. The neoplasm is large, encapsulated and expansile with haphazardly arranged ectodermal, mesodermal and endodermal elements. Ectodermal elements described and discussed include neurons, neuropil and glia; mesodermal elements include woven bone with osteoclasts, mineralized hyaline cartilage, periosteum and lymphocytes; endodermal elements include ciliated respiratory epithelium with goblet cells, bronchial glands, exocrine pancreatic acini with zymogen granules, and occasional intestinal crypts (not present in all sections). Other features described include hemorrhage, hemosiderin-laden macrophages and large, polyhedral, intensely eosinophilic crystals tentatively identified as hemoglobin crystals.

Alzarin red or Dunn-Thompson stains can be used to confirm the crystals as hemoglobin origin. Other tissue types that can be seen in



Ovary, guinea pig. The subgross view demonstrates numerous cysts as well as large trabeculae of bone (arrows). (HE, 5X)

teratomas which were not present in this example include ectodermal components such as hair, tooth enamel, sebaceous glands and cornified squamous epithelium. Other mesodermal elements can include adipose tissue, bone marrow, skeletal/cardiac/smooth muscle, embryonic mesenchyme and tooth structures including dentin and pulp. Other endodermal components include salivary gland epithelium, renal epithelium and thyroid gland (when thyroid tissue predominates the neoplasm is referred to as "struma ovarii" – literally, "goiter of the ovary").

Other species in which teratoma is common include 129 strain mice where the tumor most commonly occurs in the testis, but can also occur in extragonadal locations such as along the midline. Ovarian teratomas in mice are uncommon, [4] however; malignant teratoma has been documented in the ovary of transgenic mice. Teratomas are also common in cryptorchid testis of male horses. They are uncommon in other domestic/lab animal species, but have been documented in ferrets, dogs, cats, cattle, sheep and domestic poultry.[2] As a general rule, malignant teratomas are far less common in all species than their benign counterparts.

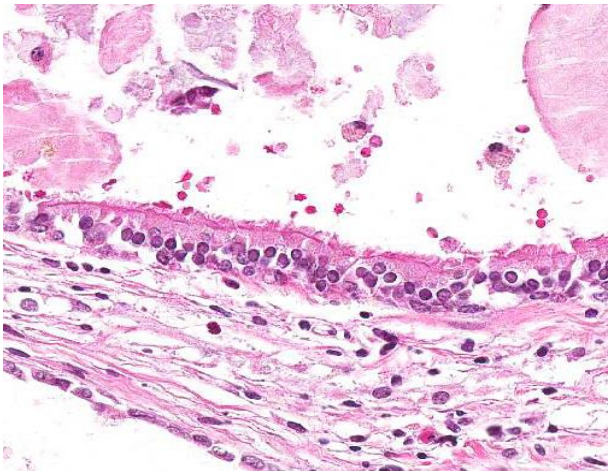
Ovarian neoplasms can be broadly divided into three groups: a) sex-cord stromal tumors which include granulosa-theca cell tumors, and thecoma/luteoma; b) tumors of the epithelial surface which include papillary adenoma/carcinoma and cystic adenoma; and c) germ cell tumors which include dysgerminoma and teratoma. Teratomas arise from totipotential germ cells that have differentiated along two or more somatic lines. Dysgerminomas, in contrast have not undergone somatic differentiation and still resemble germ cells, similar to their testicular counterpart, the seminoma. Other rare germ cell tumors include yolk sac carcinoma, choriocarcinoma and embryonal carcinoma.[5]

Contributing Institution:

Purdue University
Animal Disease Diagnostic Laboratory: http://www.addl.purdue.edu/
Department of Comparative Pathobiology: http://www.vet.purdue.edu/cpb/

References:

1. Frisk CS, Wagner JE, Doyle RE. An ovarian teratoma in a guinea pig. *Lab Anim Sci.* 1978; 28:199-201.



Ovary, guinea pig. Cysts are primarily lined by a single layer of tall columnar ciliated epithelium and contain a moderate amount of eosinophilic secretory material and sloughed cells in their lumina. (HE, 200X)

2. Leylek RA, Blankenship-Paris TL, Boyle MC. Pathology in practice. Malignant teratoma of the left ovary of a mouse. *J Am Vet Med Assoc*. 2014; 245(2):191-193.

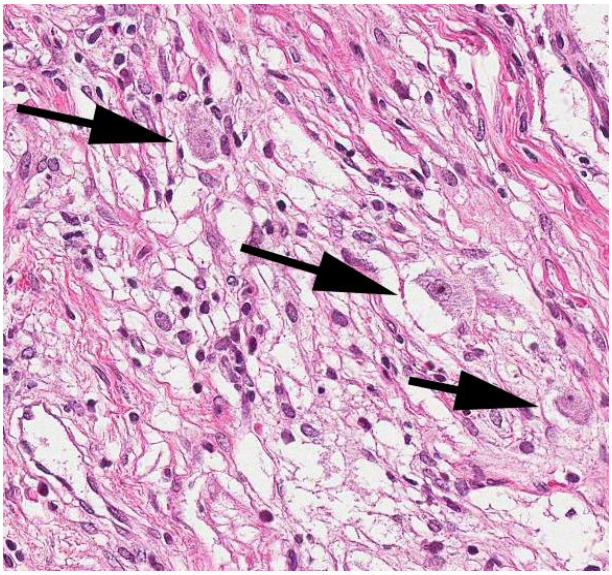
3. Manning PJ. Neoplastic diseases. In: Wagner JE, Manning PJ, Eds. *The Biology of the Guinea Pig*. New York: Academic Press; 1976:211-225.

4. Percy DH, Barthold SW. *Pathology of Laboratory Rodents and Rabbits*. 3rd ed. Ames, IA: Blackwell; 2007:121, 248-251.

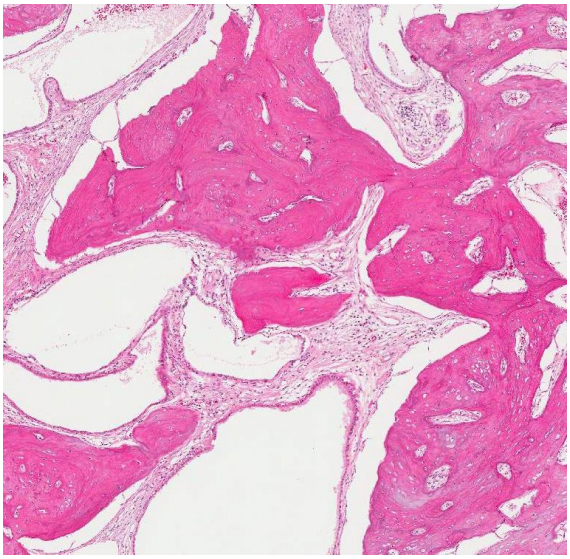
5. Schlafer DH, Foster RA. Female genital system. In: Maxie GM, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals.* Vol 3. 6th ed. St. Louis, MO: Elsevier; 2016:375-377.

6. Vink HH. Ovarian teratomas in guinea-pigs: A report of ten cases. *J Pathol*. 1970; 102:180-182.

7. Willis RA. Ovarian teratomas in guinea-pigs. *J Pathol Bacteriol*. 1962; 84:237-239.



Ovary, guinea pig. The stroma between cysts contains large areas of neural tissue with occasional neurons (arrows). (HE, 200X)



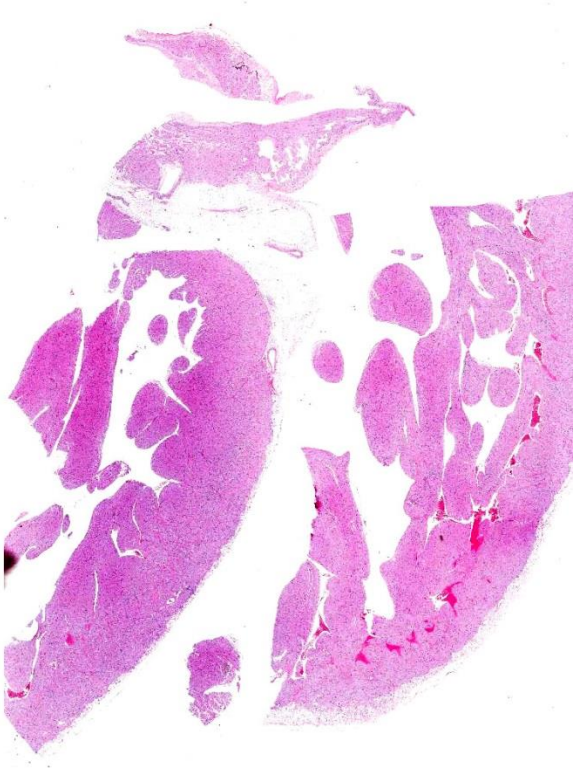
Ovary, guinea pig. Trabeculae of well-differentiated bone are scattered throughout the neoplasm. (HE, 36X)

CASE II: AFIP2 (JPC 4001216).

Signalment: Four-year-old, male cynomolgus macaque (*Macaca fasciculata)*

History: Ten months before this animal was euthanized, it showed depression and loss of appetite. After a complete clinical examination, the electrocardiogram revealed an inverted QRS complex on lead II, consistent with a Grade III heart block. Thoracic radiographs showed an enlarged heart. During a second electrocardiogram, retrograde polarization and wide QRS were noted. Days before the euthanasia, the animal was markedly depressed. On a third electrocardiogram, heart block was diagnosed.

Gross Pathology: The left ventricle of the heart was dilated and pale. There was



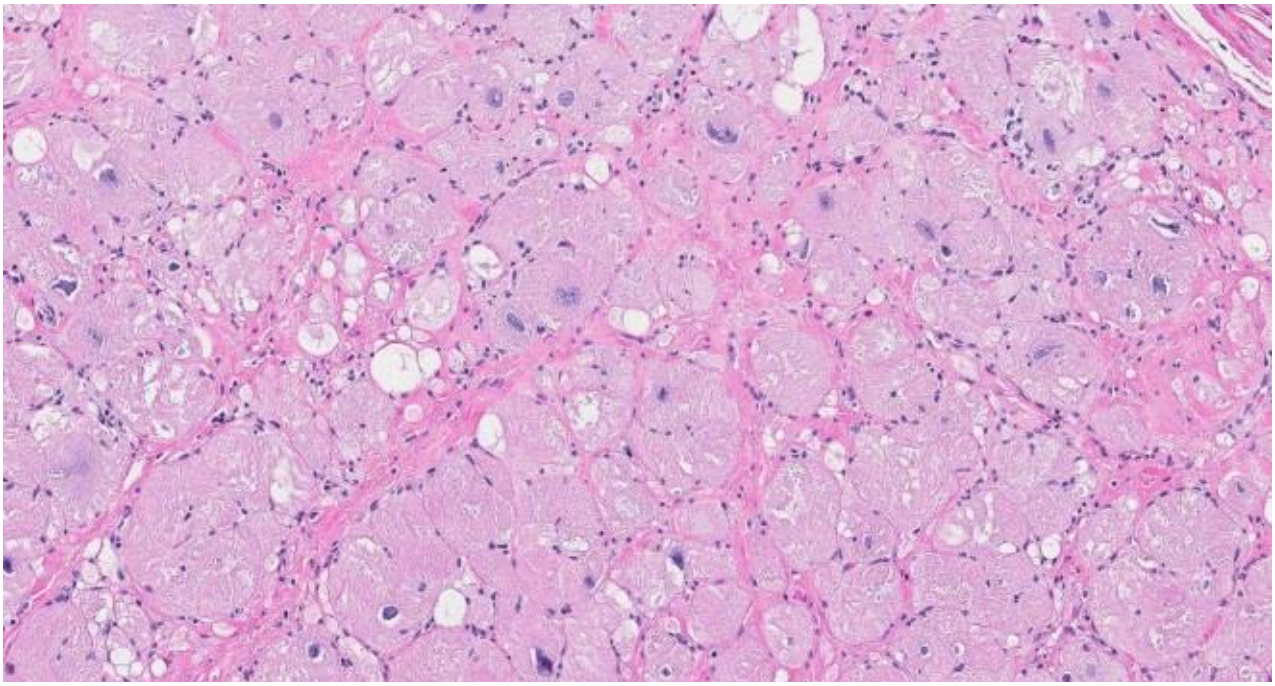
Heart, cynomolgus macaque: Subgross view of the markedly thinned left ventricle. (HE, 5X)

excessive fluid around the heart (pericardial effusion). There was an abnormal surface to the entire liver and there was an adhesion between the left and right medial lobes. Based on the necropsy findings and clinical observations, a preliminary diagnosis of cardiomyopathy was made and routine histology sections were processed.

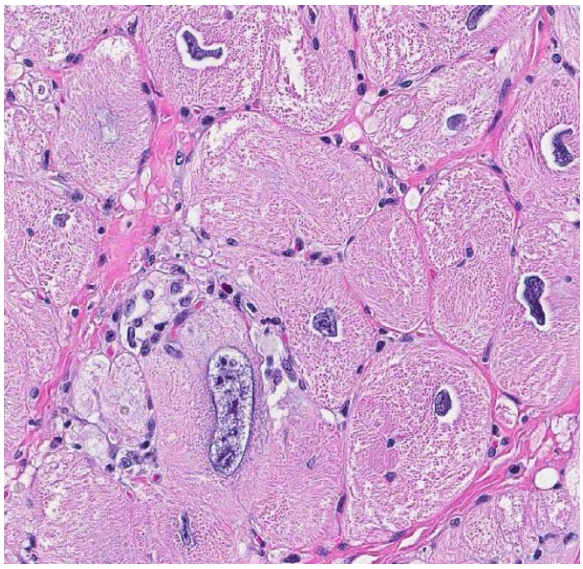
Laboratory Results:

Hemogram and blood chemistry: Unremarkable.

Histopathologic Description: Heart: The left ventricle is markedly dilated and the wall is thin. In the left ventricular wall, myocardial bundles are haphazardly arranged, with multifocal fascicles of dense fibrous connective tissue (myocardial fiber disarray and fibrosis). There is locally extensive enlargement of myocardial cells with coarse, clear cytoplasmic vacuoles and enlarged, bizarrely shaped nuclei. Occasionally, nuclei have central condensation of the chromatin (caterpillar cells). There are multifocal shrunken, hypereosinophilic myocardiocytes with pyknotic nuclei (necrotic cardiomyocytes). Associated with multiple



Heart, cynomolgus macaque: There is massive cyto- and karyomegaly of cardiac myocytes. Wow! (HE, 124X)



Heart, cynomolgus macaque. Nuclei are often markedly enlarged and pleomorphic. (HE, 220X)

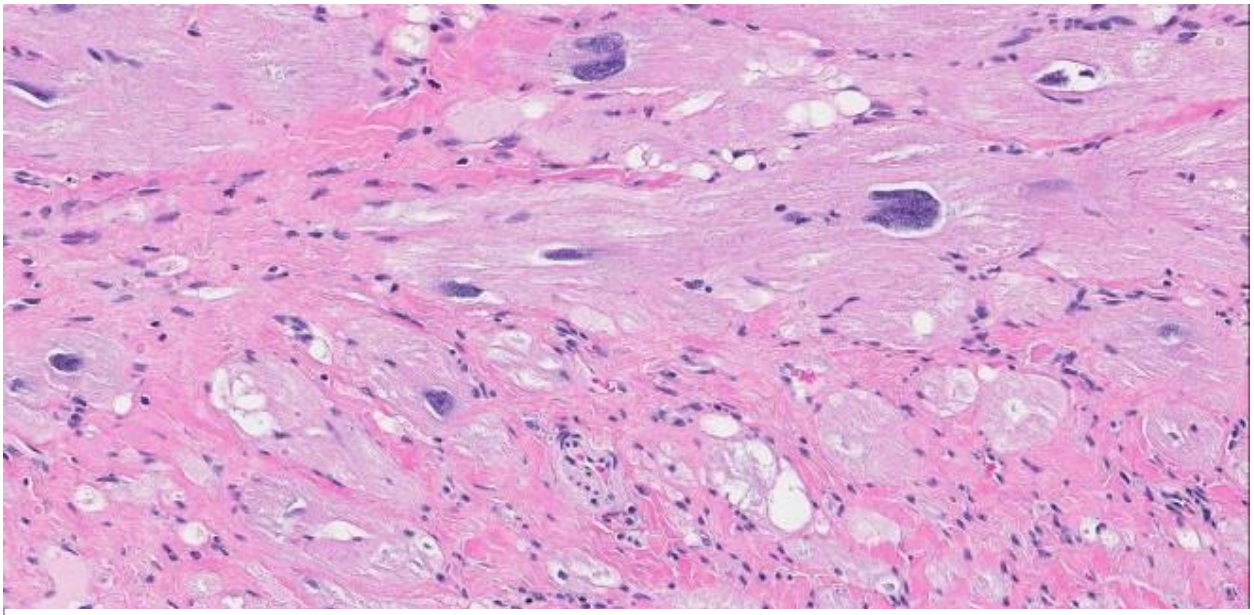
aggregates of mononuclear cells (lymphocytes and macrophages) and lesser numbers of eosinophils. In the interventricular septum, there are similar, but less severe changes as described in the left ventricle. There are discrete segments of endocardial fibrosis with minimal mononuclear infiltrates. In the left auricle, there are focal areas of contraction band necrosis, characterized by the presence of dense hypereosinophilic bands in the sarcoplasm of cardiomyocytes, with pyknotic nuclei. Occasionally, there are focal areas of coagulative necrosis surrounded by inflammatory aggregates.

The right ventricle (not submitted) has minimal aggregates of mononuclear cells in the myocardial interstitium. No histological findings were present in the right auricle.

Other findings include passive congestion of the liver, mild hepatic capsular fibrosis, with subcapsular atrophy and focal hemorrhages.

Desmin immune histochemistry demonstrated altered distribution in enlarged cardiomyocytes of the left ventricle, with focal decreased labeling of intercalated discs and Z bands and pale granular cytoplasmic staining, especially in areas of myocardial disarray.

Contributor's Morphologic Diagnosis: Heart: Myocardial degeneration and disarray, severe, locally extensive, with necrosis, fibrosis, myocardial vacuolation, and karyomegaly, consistent with the dilated

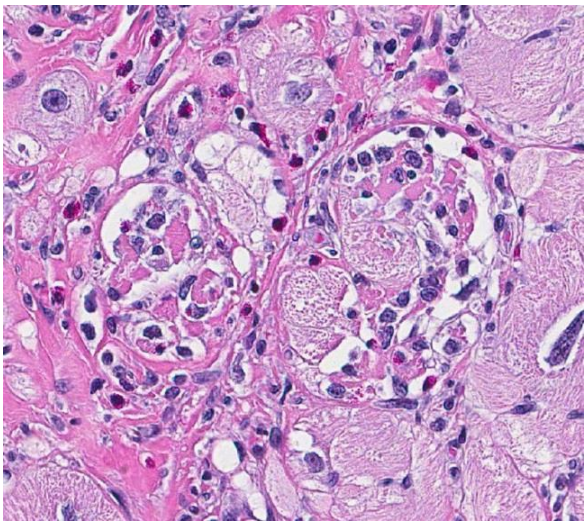


Heart, cynomolgus macaque. There is multifocal to coalescing areas of myofibers loss with replacement with mature collagen. (HE, 164X)

phase of left ventricular hypertrophic cardiomyopathy.

Contributor's Comment: The term cardiomyopathy originated from a group of myocardial diseases in humans with unknown origin or pathogenesis. Generally, cardiomyopathy is characterized by cardiomegaly, mural thrombosis and fibrosis of the myocardium and can be subdivided in a) dilated; b) hypertrophic; and c) restrictive.[8]

The primary differential diagnoses for this case are dilated cardiomyopathy and the dilated phase of hypertrophic cardiomyopathy. In dogs and cats, dilated cardiomyopathy frequently affects all chambers of the heart, with thickened, fibrotic endocardium, myocardial hypertrophy and focal areas of myocardial fibrosis and necrosis. In Holstein cattle, dilated cardiomyopathy most often affects both the right and left ventricles, with similar histological findings as dilated cardio-



Heart, cynomolgus macaque. Scattered throughout the myocardium, there are fragmented and necrotic myofibers undergoing phagocytosis. (HE, 324)

myopathy in dog and cats.[8,11]

Hypertrophic cardiomyopathy can affect all chambers or be restricted to the left ventricle. Common histologic features include myocardial disarray, myocardial hypertrophy and vesicular nuclei, as well as diffuse interstitial fibrosis.[8,11] In general, domestic species have dilated cardiomyopathy and the dilated phase of hypertrophic cardiomyopathy have similar histologic features, which do not indicate etiology or the underlying mechanism of disease process. Histopathology by itself is not a definitive method of differentiation between these two diagnoses. In humans, hypertrophic cardiomyopathy is characterized by similar histological changes as described above in domestic animals. Myocardial disarray is considered a cardinal diagnostic feature of hypertrophic cardiomyopathy in humans when it occupies 20% of one or more tissues blocks.[4] The dilated phase of hypertrophic cardiomyopathy is usually associated with left ventricular myocardial fibrosis and remodeling, thinning of the ventricular wall and dilatation of the chamber, resembling macroscopic morphologic features of dilated cardiomyopathy.[5] Fibrosis is often associated with dysplastic changes in the media of small intramyocardial arteries.[2]

An important feature in human cases of hypertrophic cardiomyopathy is the immunohistochemical expression of desmin. Desmin is an intermediate filament found in muscle tissue, which forms a network around Z-bands, intercalated discs and myofibrils in cardiomyocytes.[3,4] In the case of this nonhuman primate, desmin immunostain displayed altered distribution in enlarged cardiomyocytes of the left ventricle, with granular cytoplasmic staining and decreased labeling of intercalated discs and Z bands, especially in the disarrayed myocardial fibers compared to unaffected areas in the same section. It was proposed that the lack of proper binding between defective myosin and other sarcomeric filaments causes defective sarcomere formation and myofibrillar disorganization, resulting in disarray and compensatory hypertrophy of cardiac

myocytes in cats.[6] Based on this postulate, this author and others conclude that altered expression of desmin might serve as a possible immunohistochemical marker for hypertrophic cardiomyopathy.[3,4]

The electrocardiogram for this nonhuman primate revealed an inverted QRS complex on lead II, diagnostic of a Grade III heart block. Heart block can be the result of several disease states, including myocardial degeneration, necrosis and inflammation, when it occurs in close proximity of the conduction system (J&K). In humans, high grade ventricular block is a rare complication of hypertrophic cardiomyopathy although mild alterations of electrical conduction are commonly observed.[10] In non-human primates, cardiomyopathy has a fairly infrequent occurrence and there is a lack of literature regarding common ECG findings during the clinical course of disease.

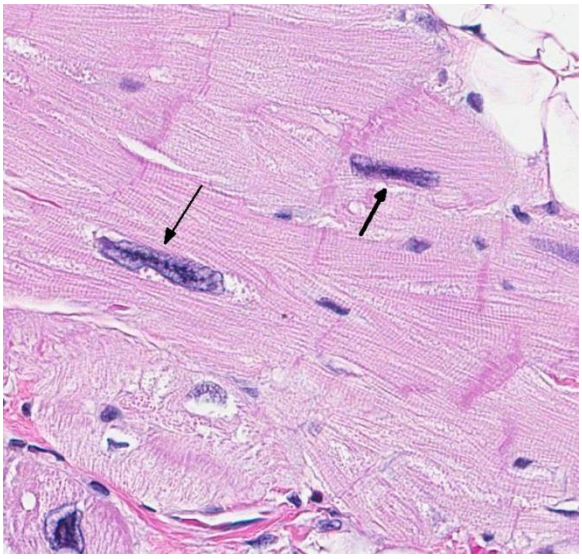
Interestingly, in both human and animals, histological changes of myocardial fibrosis and cardiomyocyte disruption have been reported secondary to stress cardiomyopathy, myocardial infarction, hypertension, cardiac hypertrophy and heart failure. Particularly in stress-induced cardiomyopathy (also known as Takotsubo cardiomyopathy), the literature includes descriptions of contraction band necrosis, leukocyte infiltrates and edema. In this condition, left ventricular dysfunction and wall abnormalities are described in the apical and midventricular myocardium, but the basal myocardium is spared.[7]

In cynomolgus monkeys, the most common spontaneous finding in the heart is focal mononuclear infiltrates in the interstitium or in perivascular regions, often from the subendocardium, with or without minimal myocardial degeneration and necrosis. This histologic change is not associated with any clinical condition and is considered incidental. Other less common spontaneous findings in macaques include mineralization of myocardium and/or blood vessels, endocarditis, pericarditis, eosinophilic infiltrates and fibrosis.[1]

Spontaneous cardiomyopathy was recently described in cynomolgus monkeys.[12] In this case series, there were no clinical or gross abnormalities. Findings were mainly histological, with extensive areas of myocardial disarray, interstitial fibrosis, vacuolation of perimyseal connective tissue, and disrupted myocytes containing vacuolated sarcoplasm and enlarged nuclei (karyomegaly). Despite the appearance of altered cardiomyocytes, this condition was not associated with active cardiomyocyte damage. In the present case, occasional infiltrates of mononuclear cells occurred in the right ventricle and were considered separate from the cardiomyopathy. In the left ventricle, inflammatory infiltrates (containing lymphocytes, macrophages and eosinophils) were the result of myocardial degeneration and necrosis with chronic remodeling, myocardial disarray and fibrosis. We believe that the case presented here is a completely separate entity that shares some of the histological features described by Zabka et al (2009)[12], but has a different clinical outcome and pathophysiology.

The main findings in this case are: a) electrocardiographic evidence of grade III heart block; b) the presence of a dilated and thin left ventricular free wall; c) myocardial disarray in histopathology, and d) decreased and granular sarcoplasmic desmin expression demonstrated by immunohistochemistry. Altogether, these findings support the diagnosis of dilated phase of hypertrophic cardiomypathy in this cynomolgus macaque.

JPC Diagnosis: Heart, left ventricle: Myocyte cytomegaly and karyomegaly, diffuse, severe with myocyte vacuolation, degeneration, necrosis and loss, and diffuse, severe myocardial fibrosis.

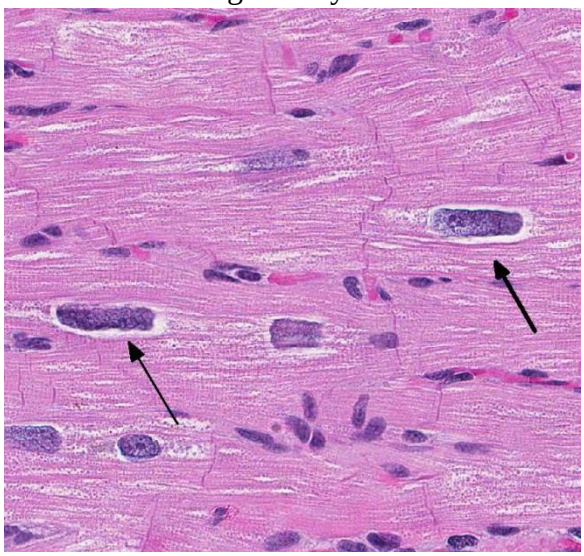


Heart, cynomolgus macaque. Occasionally, myocardial nuclei have linear chromatin condensations "caterpillar cells". (HE, 320X)

Conference Comment: Participants agreed that this is an excellent illustration of this condition, and the moderator commented on the elevated level of difficulty in describing and interpreting the slide in the absence of experience reviewing macaque hearts.

Approximately 60% of the myocardium is affected in the section. The most obvious change is the markedly cytomegalic and karyomegalic cardiac myocytes, varying in size from 10-100 times the normal macaque cardiomyocyte, with evidence of both hypertrophy and degeneration. A Masson's trichrome stain highlights the severe and diffuse (rather than subendocardial) myocardial fibrosis, and the moderator discussed the fibrosis as being a separate distinctive process. In the moderator's opinion, transverse sections of myocardium are best to evaluate for non-parallel orientation of cardiomyocytes (myofiber disarray); in this case he would grade the degree of disorganization as mild to moderate. The moderator led a discussion comparing hypertrophic cardiomyopathy (HCM) with cardiac hypertrophy, and in this case many participants were not able to reach a definitive diagnosis of HCM based on the histologic sections and information provided, although it was certainly considered with the differential diagnosis.

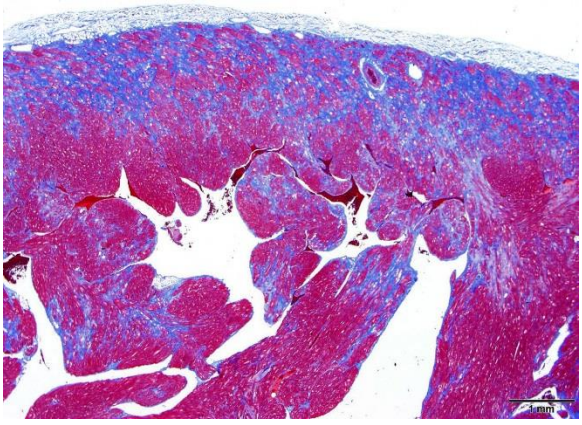
The cytologic appearance of the myocyte in this case is consistent with both HCM and myocardial hypertrophy but other differentiating histologic features were not identified. While myofiber disarray is the most significant diagnostic criteria for HCM, it is a relative rather than absolute change, and minor disarray can be evident with other cardiac diseases and even in normal hearts. Additionally, because there is usually asymmetric hypertrophy of the interventricular septum in HCM, concurrent evaluation of the IVS (not submitted) would aid in diagnosis. Fibrointimal dysplasia of small and medium mural coronary arteries is often a feature of HCM (particularly in the IVS), but was not evident in the examined section. HCM is generally considered to be a



Heart, cynomolgus macaque. Myocardial nuclei often display an enlarged rectangular profile ("boxcar" nuclei") (HE, 320X)

makes diagnosis more challenging. Contraction band necrosis was described by participants in the atrial myocardium, but was not a prominent feature, and is distinguished from hypercontraction artifact.

A subtle feature pointed out by the moderator is small tags of fibrous tissue extending from the pericardium, which provides evidence of pericardial effusion. Pericardial effusion and passive congestion in the liver are both features of right sided heart failure, and in this case the right ventricle (not submitted) was described by the contributor as having minimal changes. It is postulated that the marked degree of left ventricular hypertrophy likely compressed the right ventricular lumen, impairing function and resulting in pericardial effusion and passive congestion.



Heart, cynomolgus macaque. A Masson's trichrome stain demonstrates the amount of fibrosis within the left ventricle, primarily in the subepicardial region. (Masson's, 40X)

increases in both pressure and volume. Volume overload may also result in dilation of the ventricle lumen. Both mechanical stimuli and trophic stimuli can initiate pathologic hypertrophy, and result in changes such as increased protein synthesis and myocyte hypertrophy, which result from changes in gene expression. However, myocardial changes that occur in pathologic hypertrophy, including impaired contractility and relaxation, eventually limit the benefit of the compensatory hypertrophic response. Once hypertrophy is no longer able to compensate
for the increased workload, degenerative changes occur in the myocardium secondary to factors such as poor vascular supply. Interstitial fibrosis may also result, leading to deterioration of function and eventual failure. Concentric hypertrophy typically results from increases in afterload and eccentric hypertrophy (dilated chamber) results from increases in preload.[9] Myofiber disarray is generally not a prominent feature of cardiac hypertrophy.

Contributing Institution:

Pfizer Inc.
Global Research and Development
Groton/New London Laboratories
Eastern Point Road MS 8274-1330
Groton, CT.
Phone: 860-441-4498
www.pfizer.com

References:

1. Chamanza R, Parry NMA, Rogerson P, Nicol JR, Bradley AE. Spontaneous lesions of the cardiovascular system in purpose-bred laboratory nun-human primates. *Toxicol Pathol*. 2006; 34:357-363.

2. Davies MJ, McKenna WJ. Hypertrophic cardiomyopathy. Pathology and pathogenesis. *Histopathology*. 1995; 26:493-500.

3. Francalanci P, Gallo P, Bernucci P, Silver MD, d'Amati G. The pattern of desmin filaments in myocardial disarray. *Human Pathol*. 1995; 26:262-266.

4. Gallo P, d'Amati G. Cardiomyopathies. In: Silver MD, Gotlieb AI and Schoen FJ, eds. *Cardiovascular pathology*. 3rd ed. pp. 285-

325. Philadelphia, PA: Churchill Livingstone; 2001:285-325.

5. Hamada T, Kubo T, Kitaoka H, Hirota T, et al. Clinical features of the dilated phase of hypertrophic cardiomyopathy in comparison with those of dilated cardiomyopathy. *Clin Cardiol*. 2010; 33:E24-E28.

6. Hayman R, Une Y, Nomura Y. Desmin as possible immunohistochemical finding for feline hypertrophic cardiomyopathy. *J Vet Med Sci*. 2000; 62:343-346.

7. Lyon AR, Rees PSC, Prasad S, Poole-Wilson PA, Harding SE. Stress (Takotsubo) cardiomyopathy – a novel pathophysiological hypothesis to explain catecholamine-induced acute myocardial stunning. *Nat Clin Pract Cardiovasc Med*. 2008; 5:22-29.

8. Maxie MG, Robinson WF. Cardiovascular system. In: Maxie MG, ed. Jubb, Kennedy and Palmer's Pathology of Domestic Animals. Vol 3. 5th ed. Philadelphia, PA: Elsevier; 2007: 44-50.

9. Robinson WF, Robinson NA. Cardiovascular System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 3. 6th ed. St. Louis, MO: Elsevier; 2015:7-10, 45-47.

10. Tamura M, Harada K, Ito T, Enoki M, Takada G. Abrupt aggravation of atrioventricular block and syncope in hypertrophic cardiomyopathy. *Arch Dis Child*. 1995; 73:536-537.

11. Van Vleet JF, Ferrans VJ. Myocardial diseases of animals. *Am J Pathol*. 1986; 124:98-178.

12. Zabka TS, Irwin M, Albassam MA. Spontaneous cardiomyopathy in Cynomolgus monkeys (Macaca fascicularis). *Toxicol Pathol*. 2009; 37:814-818.

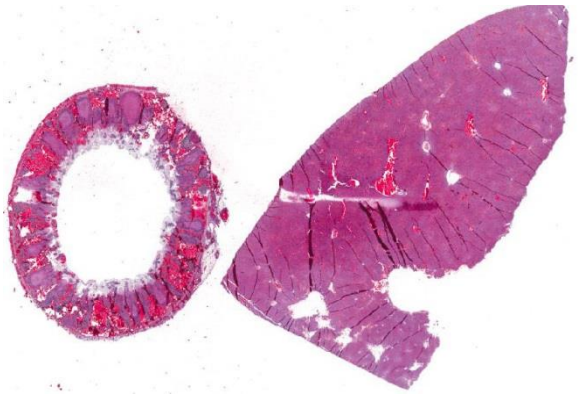
CASE III: 15-0816 (JPC 4067883).

Signalment: Juvenile (< 1yr), male, European brown hare (*Lepus europaeus)*

History: Found dead in a forest in January

Gross Pathology: The hare was in a good body condition. The spleen was moderately enlarged, swollen, dark (weight

3.4 g). The distal part of the caecum (vermiform appendix) was distended; the wall was swollen and hemorrhagic. On cross section, multiple white foci could be seen in the wall. Similar foci were seen in intestinal lymph nodes. The lungs had large hemorrhages. No gross lesions were observed in the liver.

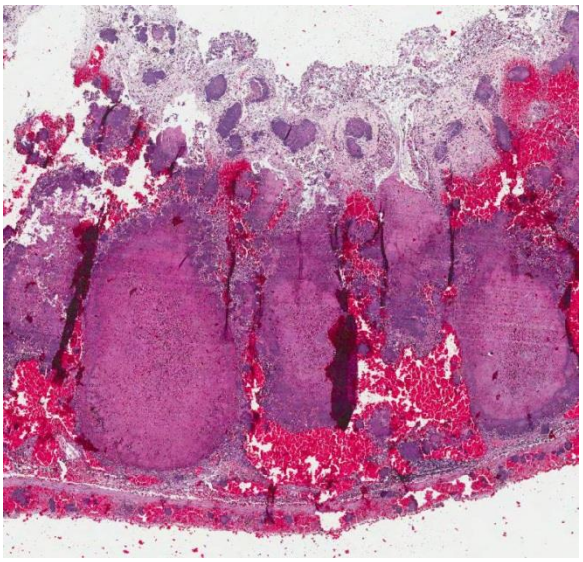


Appendix and liver, hare. Subgross view. (HE, 5X)

Laboratory Results:

Bacteriology: aerobic culture on blood agar: *Yersinia pseudotuberculosis* in lung, liver, spleen, intestinal lymph node and caecum.

Histopathologic Description: Caecum: The mucosa and the normal lymphatic tissue are mostly replaced by large hemorrhages and large focal areas of necrotic debris, mixed inflammatory cells and colonies of rod bacteria. The lesions extend to the submucosa, muscular layer and serosa.



Appendix, hare. There is transmural necrosis of the colonic wall which focuses on lymphoid tissue of the cecal tonsil. There is marked hemorrhage of the intervening lamina propria. Scattered throughout the tissue but most visible in the overlying autolytic mucosa are numerous colonies of large bacilli. (HE, 23X)

Liver: There are multiple, small, often coalescent necrotic foci with colonies of rod bacteria in the middle. Bacterial emboli are also present widely in hepatic sinuses.

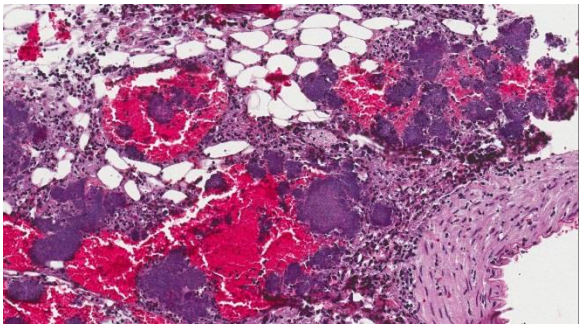
Contributor's Morphologic Diagnosis: Severe, subacute, bacterial typhlitis, hepatitis and splenitis consistent with *Yersinia pseudotuberculosis* septicemia.

Contributor's Comment: *Y. pseudotuberculosis* is a gram-negative rod or coccobacillus closely related to *Y. pestis*, the etiological agent of sylvatic plague.[2] Wild hares (*Lepus* spp.) are known to be susceptible to *Y. pseudotuberculosis* infection.[4] Birds and rodents are considered as reservoirs of the bacterium. Infection is acquired orally by fecally contaminated food or water and bacteria invade the intestinal epithelium in the jejunum or ileum.[2] Bacteria can infiltrate the liver and spleen and less commonly also other organs (lungs, kidneys, bone marrow). Infection can be acute, subacute or chronic. The disease typically occurs in nature in the cold season. In humans, *Y. pseudotuberculosis* has caused outbreaks of food poisoning related to contaminated vegetables.[7]

JPC Diagnosis:

Appendix: Appendicitis, necrotizing, transmural and hemorrhagic, diffuse, severe with marked lymphoid necrosis, vasculitis and numerous large colonies of gram negative bacilli.

Liver: Hepatitis, necrotizing, multifocal to coalescing, severe with numerous large colonies of gram negative bacilli.



Appendiceal serosa and adjacent mesentery, hare. Large colonies of small bacilli are present both within vessels and in the surrounding tissue. (HE, 168X)

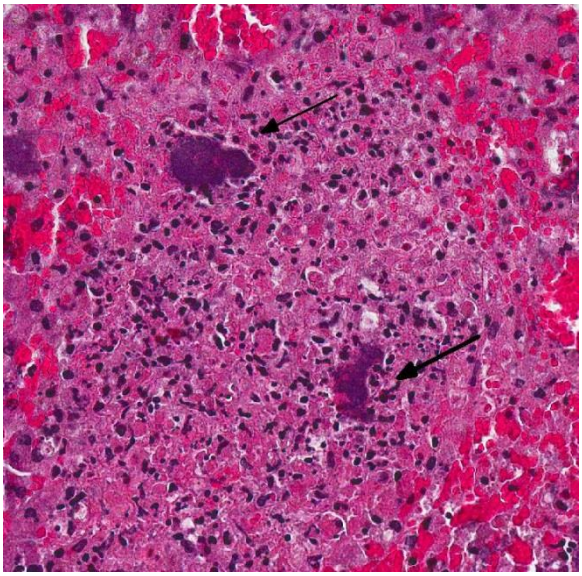
Conference Comment: Enteropathogenic *Yersinia* species bacteria are primarily thought to be contracted via oral infection which is followed by colonization of intestinal lymphoid tissue/Peyer's patches; entry is facilitated through the membrane protein invasin, attaching to β1 integrins expressed on M cells. Bacteria can then spread to regional lymph nodes and to the liver and spleen. Virulence factors include a type III secretion system which plays a role in delivering the Yersinia outer proteins (YOPS) to target cells such as macrophages and neutrophils, which interferes with

signaling pathways, phagocytosis and the inflammatory response.[1]

The bacteria also produce other proteins such as YadA and Ail that protect against phagocytosis and the host immune response. *Yersinia* largely resides extracellularly as small colonies in suppurative foci in the lamina propria of the intestine as well as in lymph nodes, but may also be found intracellularly.[9]

Aside from rabbits, other small mammals such as hamsters and guinea pigs can also become infected with *Y. pseudotuberculosis*. Additionally, virulent strains have been demonstrated experimentally to cause persistent infection in the cecum of asymptomatic mice, and are shed in the feces.[1] Gross lesions in the acute form of *Y. pseudotuberculosis* include pale, yellow-white nodules in the intestinal wall with mucosal ulceration in the distal small intestine and cecum. In subacute and chronic forms of the infection caseous and/or miliary lesions may be seen in the liver, spleen, mesenteric lymph nodes and lungs.[6] *Y. pseudotuberculosis* is often grouped with *Y. enterocolitica* as "Yersiniosis" as infections with these two organisms cannot be reliably distinguished without culture. The disease has also been described in large animal species including sheep, cattle, deer, goats, pigs as well as nonhuman primates. Similar to small mammals, the intestine, mesenteric lymph nodes and liver may be affected and microscopic lesions include abundant colonies of gram negative coccobacilli in the distal small intestine, especially at Peyer's patches, as well as in the large intestine. As infection progresses, suppurative foci eventually replace Peyer's patches and multifocal microabscesses may be seen in the lamina propria of the intestine. Microabscesses or pyogranulomatous foci may also be seen in mesenteric lymph nodes.[9]

Other genera of bacteria to include on the differential diagnosis list for large colonies of bacteria seen microscopically include *Actinomyces, Actinobacillus, Trueperella, Coryneba-cterium, Staphylococcus,* and *Streptococcus*.



Liver, hare. Numerous foci of necrosis throughout the section contain large colonies of bacilli (arrows). (HE, 246 X)

In conference, the section of appendix was described as being diffusely and transmurally effaced by focally extensive areas of lymphoid necrosis centered on lymphoid tissue. Hemorrhage, edema and multifocal venous fibrin thrombi were also described, and the presence of large colonies of short bacterial rods was a key feature in this case. The liver was described as being approximately 15-20% affected by random foci of lytic necrosis. Affected areas contain a mixed inflammatory cell population, predominantly degenerate and viable heterophils, as well as colonies of short bacterial rods and venous fibrin thrombi. The sections of liver and appendix are moderately autolytic, which is not uncommon in rabbits/hares due in part to post mortem production and retention of heat.

Many participants described the section of appendix as cecum or colon, not recognizing the unique appearance of the rabbit appendix. The terminal portion of the cecum is known as the vermiform appendix, a thick-walled blind-ended tube which contains abundant large, expansive, lymphoid follicles which span the depth of the appendix wall, and in this case are characterized by extensive lymphoid necrosis. A morphologically similar structure is present at the entrance to the cecum and is known as the sacculus rotundus.[5,8] These structures are important lymphoid organs in the rabbit and hare, which play a role in the development of B lymphocyte diversification.[3]

Contributing Institution:
Finnish Food Safety Authority Evira
www.evira.fi

References:

1. Fahlgren A, Avican K, Westermark L, Nordfelth R, Fallman M. Colonization of cecum is important for development of persistent infection by *Yersinia pseudotuberculosis*. *Infect Immun*. 2014; 82(8):3471-82.

2. Gasper PW, Watson RP. Plague and Yersiniosis. In: Williams ES, Barker IK, eds. *Infectious diseases of wild mammals*. 3rd ed. London, UK: Manson Publishing Ltd; 2001:313-329.

3. Hanson NB, Lanning DK. Microbial induction of B and T cell areas in the rabbit appendix. *Dev Comp Immunol*. 2008; 32(8):980-991.

4. Mair NS. Yersiniosis in wildlife and its public health implications. *J Wildl Dis.* 1973; 9:64-71.

5. Manning PJ, Ringler DH, Newcomer CE. *The biology of the laboratory rabbit*. 2nd ed. San Diego, CA: Academic Press; 1994:53.

6. Percy DH, Barthold SW. *Pathology of laboratory rodents and rabbits*. 3rd ed. Ames, IA: Blackwell publishing; 2007:192, 226, 283.

7. Rimhanen-Finne R, Niskanen T, Hallanvuo S, Makary P, et al. Yersinia pseudotuberculosis causing a large outbreak associated with carrots in Finland. *Epidemiol Infect*. 2006; 137(3):342-347.

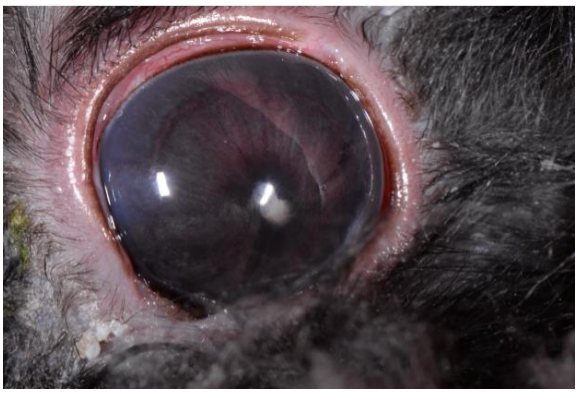
8. Snipes RL. Anatomy of the Rabbit Cecum. *Anat Embryol*. 1978; 155:57-80.

9. Uzal FA, Plattner BL, Hostetter JM. Alimentary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Vol2. St. Louis, MO: Elsevier; 2015:176-177.

CASE IV: F14371301 (JPC 4066314).

Signalment: 6-month-old, male intact, double-maned lionhead rabbit (*Oryctolagus cuniculus*)

History: The 1.49 kg rabbit was seen by the exotics service at the veterinary teaching hospital for evaluation of redness, swelling and excessive watery discharge from the left eye. General physical examination was normal except for the ocular abnormalities. The rabbit was referred for an ophthalmology consult where severe anterior uveitis, iris bombe and a complete cataract (visualized through miotic pupil) were diagnosed. A course of prednisolone acetate, tropicamide (1%) and flurbiprofen (0.03%) eye drops and oral meloxicam (1.5 mg/ml) were prescribed to suppress inflammation and pain. Clinical progression of anterior uveitis, adverse side effects of continued corticosteroid and NSAID therapy, and a high index of suspicion for *Encephalitozoon cuniculi* prompted enucleation of the left eye. The left eye was submitted for histopathological examination.

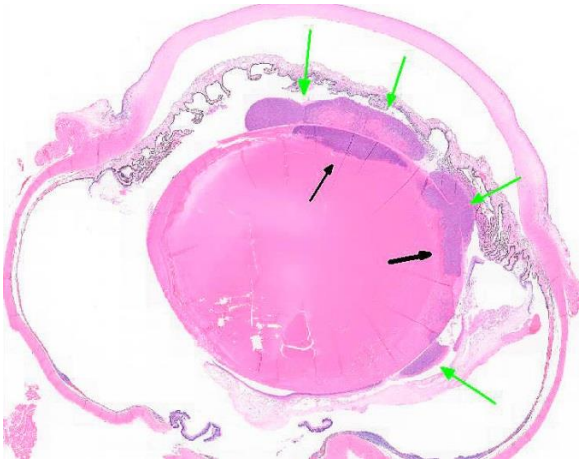


Eye, rabbit. The iris is inflamed and red, and a mature cataract is visible through the contracted pupil. (Photo courtesy of: Department of Microbiology, Immunology, and Pathology, Colorado State University, Ft. Collins, CO. http://csu-cvmbs.colosta

Histopathologic Description: Eye, OS: The equatorial lens capsule is ruptured with edges of the fragmented lens capsule retracted and coiled outward. The anterior segment is flooded with copious foamy macrophages admixed with heterophils, lenticular fragments, and necrotic debris extruding from the lens. There are myriad lenticular spherical microorganisms (2-3 micron). Lenticular architecture is also altered by torn, fibrillated, and denatured lens proteins forming Morgagnian globules and bladder cells. The lens epithelium is undergoing fibrous metaplasia forming a subcapsular fibrous plaque (consistent with cataract). Rafts of inflammatory cells and necrotic debris in the anterior segment are associated with adherence of the iris to the lens (posterior synechiae). The filtration angle is collapsed and the trabecular meshwork is infiltrated by previously described inflammatory cells. Diffusely, the iris is edematous and infiltrated by significant numbers of heterophils and fewer lymphocytes, plasma cells and histiocytes. The anterior face of the iris is covered with mixed inflammatory cells and fibrin. Similarly, the posterior segment is filled with previously described inflammatory cells, necrotic debris, fibrin and extravasated erythrocytes. The choroid is obscured by diffuse lymphoplasmacytic infiltrate. There is diffuse retinal pigment epithelium hypertrophy associated with diffuse retinal detachment, thinning and atrophy of the photoreceptor layer. The conjunctiva is edematous with congested vessels lined by reactive endothelium and cuffed by heterophils and lymphocytes.

Lenticular spherical (2-3 micron) micro-organisms are highlighted with Giemsa stain.

Contributor's Morphologic Diagnosis: Eye, OS: Phacitis, pyogranulomatous with lens capsule rupture, cataract, anterior uveitis, posterior synechiae, lympho-plasmacytic choroiditis, retinal detachment and intralenticular spherical microorganisms, etiology consistent with *Encephalitozoon cuniculi*.



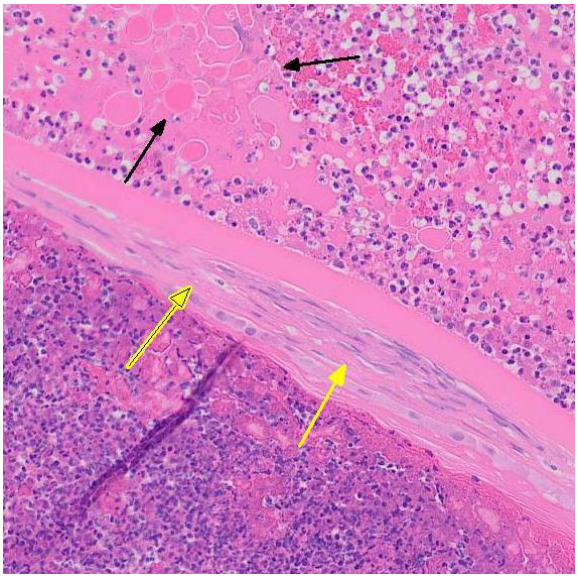
1Eye, rabbit. There is a marked cellular exudate with the posterior chamber and posterior segment (green arrows). There is additionally an infiltrate within the peripheral aspect of the lens itself (black arrows). (HE, 7X)

Contributor's Comment: *Encephalitozoon cuniculi* is a spore-forming microsporidian obligate intracellular parasite with a simple, direct life cycle that infects a wide range of species. The phylum *Microspora* has a unique organelle, the polar filament, which remains coiled inside the spore.[10] Ill-defined environmental cues cause unwinding and extrusion of the polar filament from the spore and injection of infectious sporosplasm into

the host cell.[10] Ingestion or inhalation of spores shed in the urine, feces and mucus secretions are the primary routes of transmission. Once intracellular infection is established, division of the sporoplasm yields proliferative meronts that differentiate and mature (sporogony) within parasitophorous vacuoles.[10] Swollen parasitophorous vacuoles cause the host cell to lyse, releasing spores into the extracellular space and completing the life cycle.[10]

E. cuniculi primarily infects rabbits (Type I genotype), including research colonies used in experimental models of human disease resulting in lesions of *E. cuniculi* being confused and misinterpreted as lesions associated with more serious human conditions.[10] While seroprevalence for the organism is high in rabbits, infection is often subclinical and associated with incidental renal lesions at necropsy.[6] Seropositive rabbits with clinical manifestations of encephalitozoonosis exhibit signs of vestibular disease (head tilt, ataxia), azotemia with nonspecific anorexia, lethargy and weight loss, and white intraocular masses (granulomas), cataracts and uveitis resulting in enucleation.[5] Microscopic lesions include focal to segmental granulomatous interstitial nephritis (early), fibrosing lymphoplasmacytic interstitial nephritis (chronic) and nonsuppurative granulomatous meningoencephalomyelitis.[6] Rabbits with neurological deficits have a more favorable prognosis than those presenting with clinical signs of renal insufficiency.[5]

Dwarf rabbits are especially prone to ocular lesions associated with *E. cuniculi* including white intraocular masses (granulomas), cataracts and phacoclastic uveitis resulting in enucleation.[6]



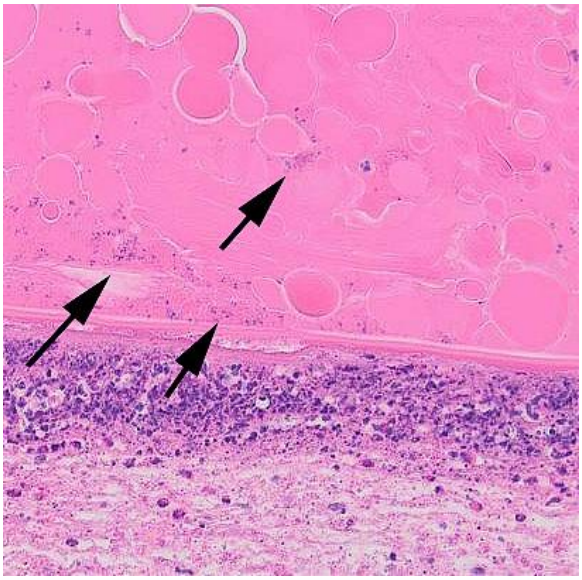
Eye, rabbit. The lens is infiltrated by innumerable degenerate heteroophils and cellular debris (bottom left.) There is fibrous metaplasia of the subcapsular lens fibers (yellow arrows) and numerous viable and degenerate heterophils in the posterior chamber admixed with brightly eosinophilic granular and globular (black arrows) extruded lens material. (HE, 200X)

Ocular lesions are mostly unilateral, and rabbits with ocular disease generally do not suffer extra-ocular clinical abnormalities.[5] Intrauterine infection is the proposed route of transmission with ocular encephalitozoonosis. The organism permeates the developing lens resulting in cataract formation, spontaneous rupture of the lens capsule and extrusion of lens substance inciting phacoclastic uveitis characterized by granulomatous inflammation intimately associated with the lens capsule.[5] Posterior synechia, phthisis bulbi and secondary glaucoma are not uncommon sequelae.[2,4,8] Early in the course of ocular disease associated with the organism, phacoemulsification can be used to treat the condition.[2] The tendency of nearly complete lens regeneration from lens epithelial cells requires large capsulectomy to suppress lens regrowth.[1] The prognosis for treatment of phacoclastic uveitis is poor, and enucleation is almost invariable.

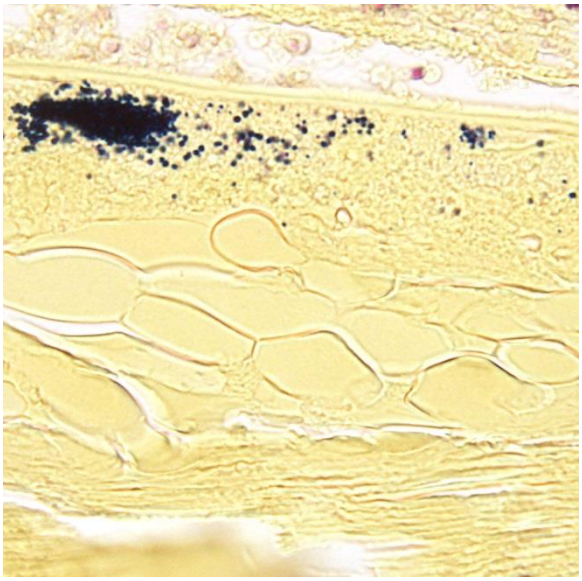
Visualization of small (2-3 micron) spores is challenging with routine H&E stains. Gram, Giemsa, carbon fuschin and Ziehl-Neelsen acid fast stains permit greater visualization of the spores. Immunohistochemical detection of intralenticular *E. cuniculi* organisms and PCR of liquefied lens substance is especially effective in organism detection.[4,5]

Subclinical chronic infections in lagomorphs (Type I genotype) and dogs (Type III genotype) and the risk for zoonoses in immunocompromised individuals require appropriate precautions and biosecurity when working with infected or at risk populations of these species.[3,9] Disseminated encephalitozoonos in the kidney, sinuses, lung, brain and conjunctiva is well-recognized in AIDS patients.

JPC Diagnosis: Eye: Endophthalmitis, fibrinosuppurative, diffuse, severe with posterior synechiae, cataract, lens rupture, retinal detachment and intralenticular microsporidia.



Eye, rabbit. Lens fibers at the posterior face of the lens are degenerate, with loss and liquefaction of lens protein (Morgagnian globules). There are aggregates of microsporidian spores scattered throughout the posterior aspect of the lens (arrows)

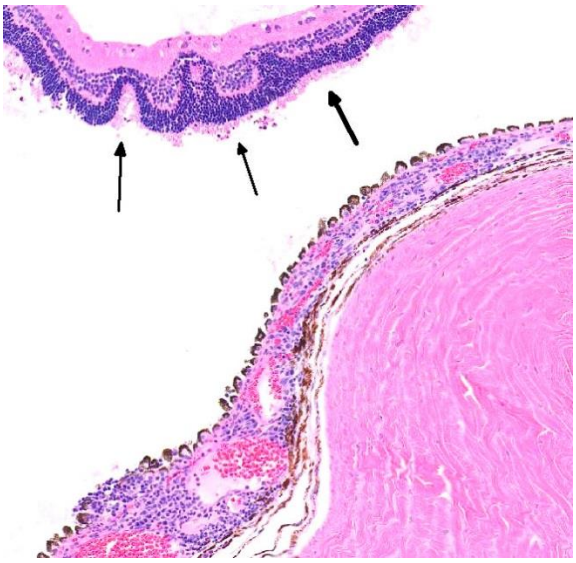


2Eye, rabbit. Moderate numbers of gram-positive microsporidium spores are present within the subcapsular lens. (Brown-Brenn, 1000X)

Conference Comment: Based upon phylogenetic analysis and the presence of chitin within the spore, microsporidia are classified as fungi or at least closely related to fungi. *E. cuniculi* infection has been documented in many mammalian species including rats, mice, squirrel monkeys, horses and foxes.[11]

Although clinical and subclinical *E. cuniculi* infections were once common in laboratory rabbits, the agent is excluded from most modern facilities. Spores are transmitted primarily by ingestion and inhalation (or transplacentally), and they are shed in the urine of infected animals. *E. cuniculi* is capable of causing disease in dogs as discussed above, where lesions are most common in the brain and kidney. The organism targets vascular endothelium resulting in segmental vasculitis. Once infected, animals often remain permanently infected as macrophages are incapable of clearing the organism, although organisms

can be difficult to find in chronic lesions. Within the kidney, they are most common in the renal tubular epithelium and glomerular capillaries. Gross kidney lesions include nonsuppurative interstitial nephritis.[1] In addition to nephritis and meningoencephalitis, *E. cuniculi* infections have also been associated with placentitis in horses, squirrel monkeys, blue foxes and an alpaca.[11] One of the primary differential diagnosis for *E. cuniculi* is toxoplasmosis. The following characteristics can help differentiate the two organisms: *T. gondii* is not birefringent, *E. cuniculi* spores are; *T. gondii* does not stain with gram, acid fast or Luna stains; *T. gondii* stains more readily in H&E sections; *T. gondii* is a larger organism, measuring approximately 3-6um.



Eye, rabbit. The retina (arrows) is detached, with hypertrophy ("tombstoning") of the underlying pigmented retinal epithelium. The underling choroid is congested and moderate expanded by lymphocytes and fewer plasma cells. (HE, 144X)

Conference participants agreed that this is an excellent descriptive slide, and the conference histologic description was aligned closely with the contributor's description above. The inflammation and necrotic debris surrounding the lens was described as containing degenerate eosinophils and heterophils, macrophages and lymphocytes. Within the ruptured lens Morgagnian globules were described as relatively abundant and bladder cells as uncommon. The corneal endothelium is infiltrated with leukocytes, and there is neovascularization and inflammation within the corneal stroma. Microsporidian spores were described as being present extracellularly between lens fibers and rarely within macrophages. Some discussion centered on the nature of the proliferative fibrovascular tissue at the lens margin, i.e. whether the spindled cells represent hyperplastic response or metaplastic lens epithelium. In addition to tissue gram and acid-fast stains, another histochemical stain that can be used to visualize *E. cuniculi* organisms includes the Luna method.[7]

Contributing Institution:
Colorado State University
http://csu-cvmbs.colostate.edu/academics/mip/pages/default.aspx

References:

1. Cantile C, Youssef S. Nervous System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Vol 1. St. Louis, MO: Elsevier; 2015:385.

2. Felchle LM, Sigler RL. Phacoemulsification for the management of *Encephalitozoon cuniculi*-induced phacoclastic uveitis in a rabbit. *Vet Ophthalmol*. 2002; 5:211-215.

3. Fournier S, Liguory O, Sarfati C. Disseminated infection due to *Encephalitozoon cuniculi* in a patient with AIDS: case report and review. *HIV Med*. 2000; 1:155-161.