Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 25

5 May, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: RUSVM CASE 1 (JPC 4085383-00)

Signalment:
8 years, spayed female, mixed, *Canis familiaris*, Canine

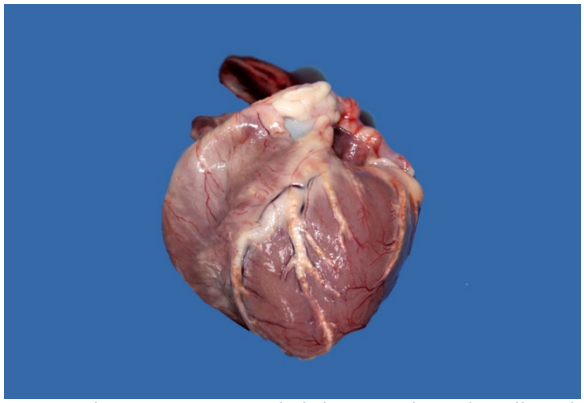
History:
Accidentally run over by owner, presented with multiple trauma, euthanized due to poor prognosis.

Gross Pathology:
Upon internal examination numerous arteries were prominent, irregularly thickened, tortuous, white-yellowish and hard to cut with a gritty consistency (mineralization). Most notably involved were the coronary, dorsal vertebral, gastric, carotid, as well as renal arteries. The descending aorta had similar changes. Some smaller size arteries within the kidneys were also affected. Interestingly, the arteries in the brain presented no gross or microscopic lesions. Marked bilateral symmetrical atrophy of the thyroid glands was observed.

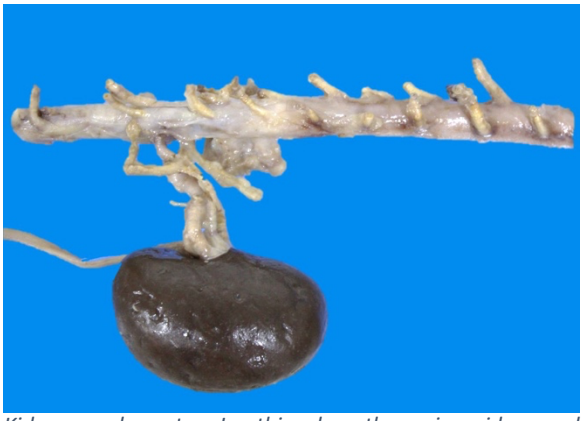
Laboratory results:
N/A

Microscopic description:
The tunica media and intima of most of the medium and large size renal arteries were irregularly infiltrated and expanded by a combination of fibrosis, acicular cholesterol cleft depositions, mineralization and inflammatory cell infiltration mainly by macrophages, lymphocytes and plasma cells. In some areas these changes were transmural and foamy lipid-containing cells (macrophages and/or smooth muscle cells) were commonly observed.

A large re-canalized thrombus partially occluding most of the lumen of a branch of the renal artery was observed in the right kidney (not submitted). The thrombus was formed of laminar areas of fibrin, lipids, cell debris, hemorrhage, viable and degenerate neutrophils, foamy macrophages, lymphocytes and plasma cells.



Heart, dog: Numerous slightly raised and yellowish atheromatous (fibro-fatty) plaques are present along the wall of the coronary arteries. (Photo courtesy of: Ross University School of Veterinary Medicine, Department of Biomedical Sciences, http://veterinary.rossu.edu/)



Kidney and aorta: In this dog there is widespread atherosclerosis within branches of the abdominal aorta, including the renal arteries. (Photo courtesy of: Ross University School of Veterinary Medicine, Department of Biomedical Sciences, http://veterinary.rossu.edu/)

Multifocally within the cortex renal tubules exhibited vacuolated and often attenuated epithelium. Pyknotic nuclei and intratubular cell debris (degeneration and necrosis) were occasionally seen. Evidence of tubular regeneration (basophilic and hypertrophic tubular epithelium) was present in some areas. Multifocally within the tubular epithelium finely granular golden-brown intracytoplasmic pigment was observed; this pigment stained negative with Pearl's stain and was interpreted as bile pigment. Microscopical evidence of chronic liver disease was detected in this dog (no liver section submitted). In addition, intracytoplasmic accumulations of a finely granular eosinophilic proteinaceous material (compatible with hyaline droplets) were present within proximal tubules. Multifocally, tubules were moderately ectatic, lined by attenuated epithelium, and were occasionally filled with varying amounts proteinaceous material (protein casts).

The glomeruli were slightly hypercellular and appeared either expanded by mildly thickened capillary loops or had a mildly thickened Bowman's capsule with hypertrophy of the parietal epithelium and occasional synechia formation. Some glomeruli had ectatic urinary spaces due to the presence of variable amounts of proteinaceous material leading to atrophy, loss or fibrosis of the glomerular tuft.

Some degree of autolysis was also observed.

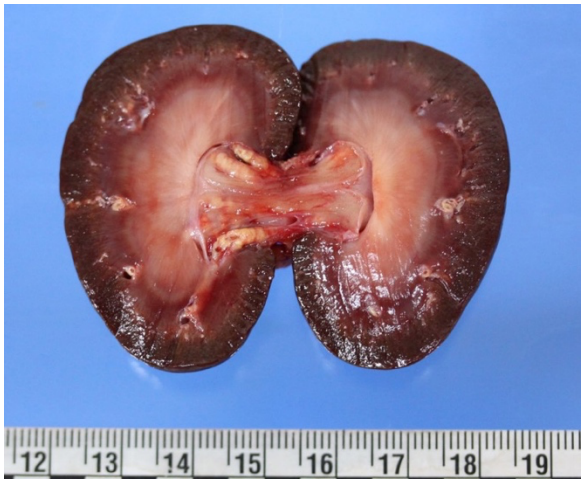
Contributor's morphologic diagnosis:

1. Large and medium size arteries: atherosclerosis, diffuse, severe with cholesterol clefts and mineralization.
2. Nephritis, interstitial, lymphoplasmacytic, chronic, mild, multifocal.
3. Tubular degeneration, necrosis and regeneration with bile pigment and hyalinosis, moderate, multifocal.
4. Membranoproliferative glomerulonephritis, moderate with occasional glomerulosclerosis and atrophy, multifocal.

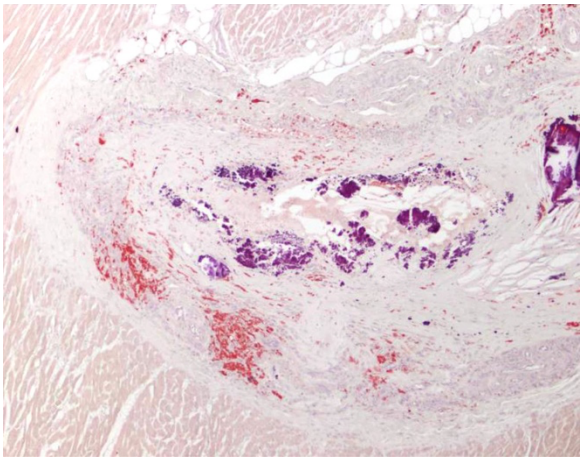
Contributor's comment:

Atherosclerosis has little practical importance in domestic animals, except as a model of the human disease. Animals have different susceptibility to atherosclerosis with rabbits, chickens, and pigs being more sensitive, and dogs, cats, cattle, goats, and rats being traditionally considered atheroresistant. The best large-animal models of human atherosclerosis are swine and nonhuman primates.[3]

Of the domestic animals, the deposition of cholesterol and other lipids in the arteries in more than trace amounts occurs only in dogs and is almost always a consequence of



Kidney, dog: Atherosclerosis of the renal arteries, mid-sagittal section of kidney. Observe the prominent raised-yellowish fibro-fatty plaques within the vascular wall. (Photo courtesy of: Ross University School of Veterinary Medicine, Department of Biomedical Sciences, http://veterinary.rossu.edu/)



Kidney, dog. Section of the heart with special stain (Oil Red O) to stain lipids (they appear reddish/orange), atherosclerosis of a mid-sized coronary artery. Abundant amount of lipid is present throughout the thickened vascular wall. Note multifocal areas of mineralization (stain purple) within the expanded tunica intima and tunica media. (Photo courtesy of: Ross University School of Veterinary Medicine, Department of Biomedical Sciences, http://veterinary.rossu.edu/)

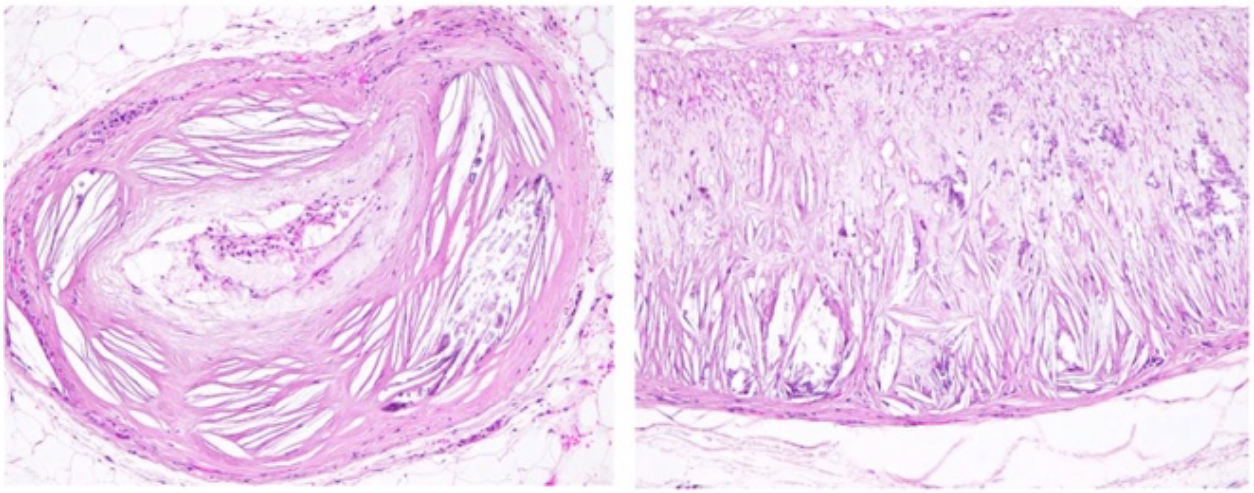
hypercholesterolemia associated with hypothyroidism or diabetes mellitus. Miniature Schnauzers are predisposed to atherosclerosis maybe as a result of idiopathic hyperlipoproteinemia.[3]

The deposition of lipids in dogs begins in the middle and outer layers of the media and occurs more extensively in the small muscular arteries than in the large elastic ones. The media may be greatly increased in thickness by the accumulation of lipid, most of which is in foam cells, but some is present in identifiable smooth muscle cells of the media or free in the interstitium as droplets or crystals. The deposition of lipid in the internal layers of the media leads to disruption of the internal elastic lamina and involvement of the intima. There are no changes associated with the veins.[3]

Even though gross lesions in several major organs such as heart, brain, or kidney can be impressive, clinical consequences of atherosclerosis are infrequent in dogs. Nevertheless, rupture of atheromatous plaques into the vascular lumen with subsequent thrombosis or even widespread lipid embolism have been observed.[3]

There are a few differences in the morphology of atherosclerotic lesions between dogs and in humans; in dogs some degree of lipid deposition is observed in the intima, but the primary location is in the media and adventitia of atherosclerotic arteries, whereas in humans, lipid is present primarily in the intima.[3]

Contributing Institution:
Ross University School of Veterinary Medicine
Department of Biomedical Sciences
http://veterinary.rossu.edu/



Kidney, dog: Cross and longitudinal sections of a peri-renal arteries with severe atherosclerosis. The elongated clear spaces within the wall represent cholesterol deposition (cholesterol crystals or clefts). Note partial obliteration of the vascular lumen in the artery cut on transverse section. (Photo courtesy of: Ross University School of Veterinary Medicine, Department of Biomedical Sciences, http://veterinary.rossu.edu/)

JPC diagnosis:

1. Kidney, renal, arcuate and sublobular arteries: Atherosclerosis, diffuse, severe.
2. Kidney: Nephritis, interstitial, lymphoplasmacytic, diffuse, mild.

JPC comment:

While atherosclerosis typically results in fewer cases of overt disease in veterinary species, it is widespread in its reported cases. Apes, elephants, perissodactyls, captive and free ranging bottlenose dolphins, emu, penguins, captive and free ranging birds of prey, psittacines, finches, hornbills, rhamphastids, and a variety of lizard species.[5]

The pathogenesis of atherosclerosis is multifactorial, with most research performed in humans. The generation of atherosclerotic plaques involves interaction between endothelium, smooth muscle cells, platelets, T lymphocytes, and monocytes. When low-density lipoprotein cholesterols are oxidized, they may home to and damage endothelium. Monocytes arrive at the site and attempt to phagocytose the cholesterol from the tunica intima, resulting in foam cells. The surplus of oxidize LDL induces additional metabolic changes that create a procoagulant environment. Platelets may aggregate, and form thrombi.[2]

Using information gained from experimental models, it has been shown that CD4+ T_h1 cells and natural killer T cells (NK-T) have pro-atherogenic properties, while T_{reg} cells are anti-atherogenic. While the specific roles of some subtypes have yet to be elucidated (T_h2, T_h9, T_h17, T_h22, T_{FH}, CD8+, γδ T cells), we know the microenvironment can force T_{reg} lymphocytes to convert into proinflammatory subtypes T_h1, T_h17, or T_{FH}. Recruitment of T lymphocytes to the atherosclerotic plaque occurs though the chemokines and chemokine receptors CCR5, CXCR3, and CXCR6. The current body of research suggests atherosclerosis is a chronic inflammatory disease with an autoimmune component, but further research is needed to identify all relevant self-antigens.[4]

The moderator commented on the importance of animal models in human research of atherosclerosis. An ApoE-knockout mouse model has been used to monitor the progression of this disease in the hopes that the research may improve human outcomes. Apo-E knockout mice fed a high fat diet developed characteristic atherosclerotic lesions most suitable for comparison in the brachiocephalic artery, and in the coronary artery to a lesser extent.[1]

References:

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CASE 2: H19-107A (JPC 4141185-00)

Signalment:

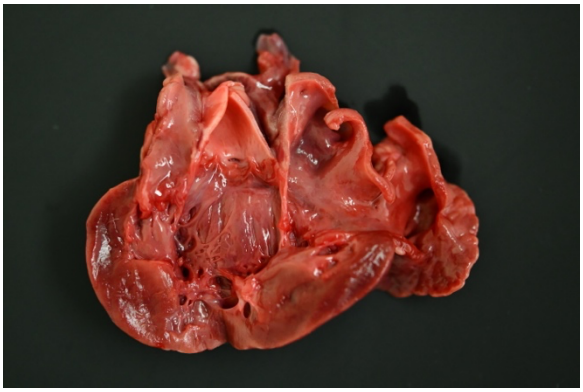
A 4-year-old female entire Bengal cat

History:

The cat was submitted to the anatomic pathology diagnostic service after sudden unexpected death.

Gross Pathology:

The cat was in heavy body condition (4/5) and there was minimal post-mortem decomposition. Multifocal, haphazardly arranged, approximately 1-3 mm diameter thick, firm to hard bands of smooth tissue were present running between the endocardial surfaces of the left ventricle near the apex (endomyocardial fibrosis). The total weight of the heart was 21.9 g (0.49% of total bodyweight, within normal limits). The left



Heart, cat. Multifocal, haphazardly arranged, approximately 1-3mm diameter thick, firm bands of smooth tissue were present running between the endocardial surfaces of the left ventricle near the apex (endomyocardial fibrosis) (Photo courtesy of: Department of Veterinary Anatomic Pathology, The Animal Hospital at Murdoch University, https://theanimalhospital.com.au/pathology-services)

auricle was approximately 1.5 times the diameter of the right auricle (left auricular dilation). There was a focal, pale, irregularly shaped focus of myocardium measuring approximately 0.5 cm x 0.5 cm x 0.5 cm within the apex of the left ventricle (myocardial necrosis, presumptive). Approximately 1 ml of dark, red-tinged fluid was present within the pericardial sac (post-mortem fluid accumulation vs. pericardial effusion). Approximately 15 ml of red-tinged fluid was present within the thoracic cavity (hydrothorax). The lungs were reduced in size, occupying approximately 60-70% of the thoracic cavity (atelectasis). The lungs were diffusely mottled, dark red to tan (post-mortem autolysis vs. atelectasis). A small amount of frothy material was found within the entire length of the trachea, and the pulmonary parenchyma oozed a small amount of clear, dark red tinged fluid on transection (pulmonary edema).

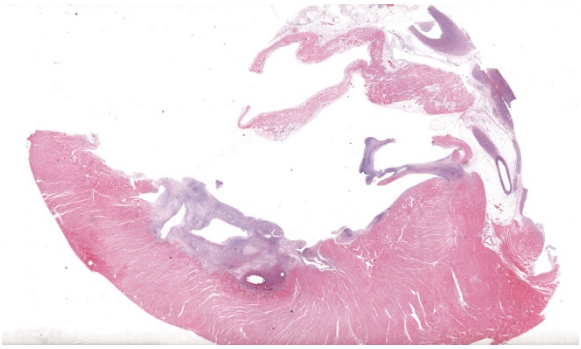
Laboratory results:
None performed.

Microscopic description:
A single section of the left ventricle and atrium are examined. Diffusely the endocardium of the left ventricle and mitral valve is moderately to markedly expanded by moderate amounts of variably dense fibrous connective tissue with multifocal mineralization and chondroid differentiation. This fibrous connective tissue also multifocally infiltrates into the underlying myocardium, occasionally surrounding and isolating individual and small groups of cardiac myocytes. Multifocally scattered throughout the expanded endocardium there are variably sized roughly circular occasionally cavitated formations of swirling spindle cells with associated collagen (recanalized blood vessels vs. atypical endocardial formations). Small amounts of pale eosinophilic fibrillar material, which stains bright red on M.S.B. histochemistry (fibrin) is present either within the walls or lumens of these structures (thrombosis). Low numbers of lymphocytes, plasma cells, histiocytes and occasional neutrophils are multifocally scattered throughout the epicardial adipose.

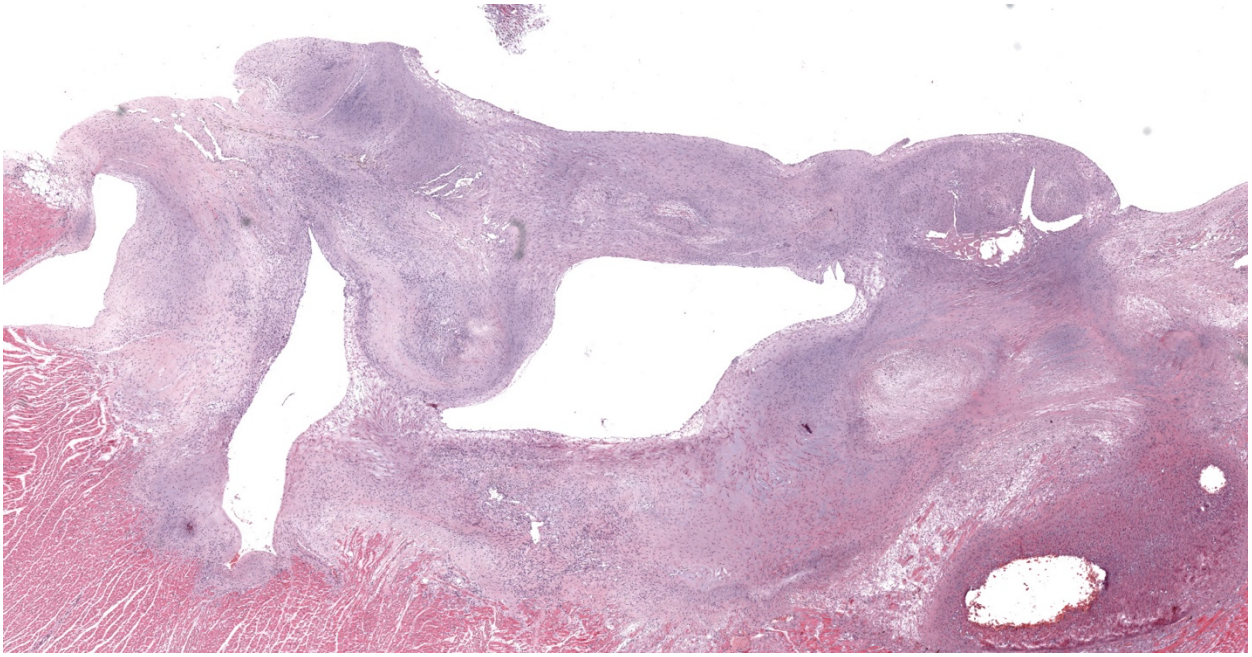
Contributor's morphologic diagnosis:
Heart: Severe, multifocal, chronic, left ventricular and mitral valvular endocardial to subendocardial fibrosis with chondroid metaplasia and multifocal thrombosis

Contributor's comment:
Histopathology is consistent with a diagnosis of left ventricular and mitral valvular fibrosis, also known as restrictive cardiomyopathy. Secondary changes including left atrial dilation, pulmonary edema, hydropericardium and hydrothorax indicate that the patient was in congestive heart failure at the time of death. There was no gross evidence of aortic or iliac bifurcation thromboembolism in this case.

Following hypertrophic cardiomyopathy, restrictive cardiomyopathy is the second most

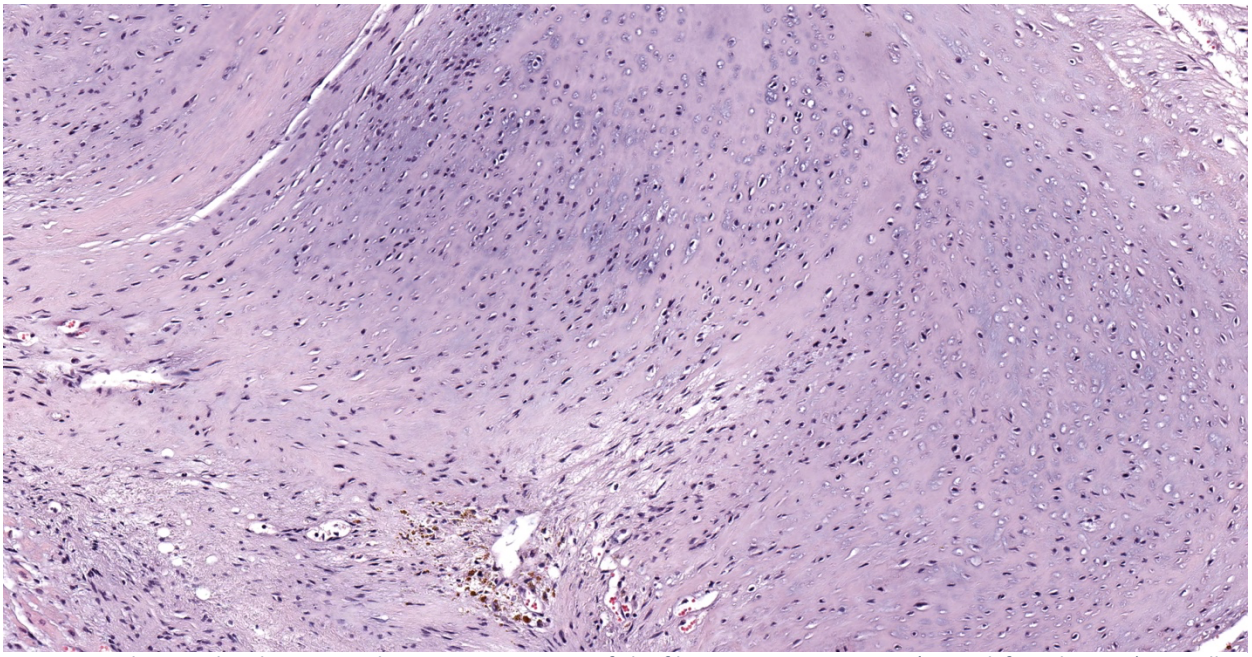


Heart, cat. The endocardium of the left ventricle and root of the mitral valve is markedly expanded by blue-tinged fibrous connective tissue. The walls of epicardial arteries are blue-tinged as well. (HE, 4X)

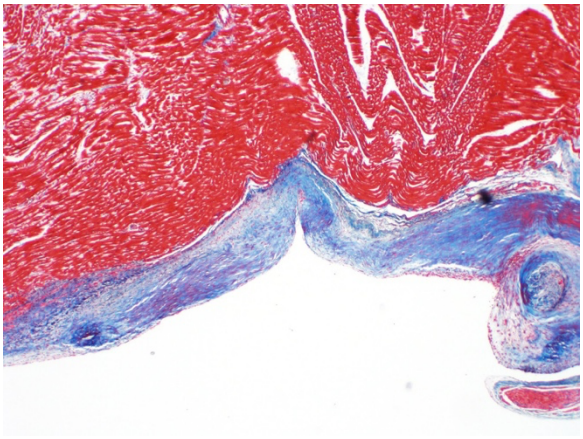


Heart, cat. The fibrous connective tissue forms bands across the lumen, some of which contain polymerized fibrin (right). The fibrous connective tissue also infiltrates the underlying myocardium (HE, 19X)

common form of cardiomyopathy in cats.[3] Restrictive cardiomyopathy refers to any pathologic process which leads to restricted filling and reduced diastolic volume of one or both ventricles.[10] Affected animals will often have normal left ventricular wall thickness and normal or minimally decreased systolic function.[7] Similar to humans, restrictive cardiomyopathy can be categorized either as a myocardial form (in which myocardial involvement predominates) or the more common endomyocardial form (in which endocardial involvement predominates).[6] The most common pathologic processes causing restrictive cardiomyopathy in cats include endomyocardial scar formation and myocardial



Heart, cat. There is chondromatous change in some areas of the fibrous connective tissue (upper left and center) as well as scattered siderophages within the matrix (lower center) (HE, 153X)



Heart, cat. A Masson's trichrome demonstrates the collagen within the endocardium. (Masson's trichrome, 200X)

fibrosis, however infiltrative processes have also been reported.[5]

In left ventricular endomyocardial restrictive cardiomyopathy, the fibrotic scar tissue typically involves the left ventricular apex and outflow tract including both the left ventricular free wall and interventricular septum.[8] Diffuse endomyocardial fibrosis, cardiomyocyte degeneration, infarction, and inflammation are less commonly described.[8] Biventricular involvement is rare in cases of endomyocardial restrictive cardiomyopathy.

The etiopathogenesis of feline restrictive cardiomyopathy remains unknown. A continuum between endomyocarditis and endomyocardial fibrosis has been proposed due to age relationships and common lesion distribution.[4] Whilst canine parvovirus has been associated with myocarditis leading to myocardial fibrosis in dogs, in recent publication McEndaffer et al (2017) demonstrated no association with feline panleukopenia virus and restrictive cardiomyopathy in cats.[8] The endomyocardial form of restrictive cardiomyopathy in cats has been described in numerous breeds, however specific breed predispositions have not been reported.[6]

Prognosis for cats with endomyocardial restrictive cardiomyopathy is poor with many affected cats dying from either congestive heart failure or aortic thromboembolism.[6] A retrospective study of feline endomyocarditis and left ventricular endocardial fibrosis in one university pathology service reported these pathologies as the cause of death in >4% of cats compared to 2.3% of cats having died from hypertrophic cardiomyopathy and 2.2% of cats within the service having died from dilated cardiomyopathy.[12]

Contributing Institution:
Department of Veterinary Anatomic Pathology
The Animal Hospital at Murdoch University
https://theanimalhospital.com.au/pathology-services

JPC diagnosis:
Heart, left ventricle, endocardium: Fibrosis, diffuse, severe with chondroid metaplasia.

JPC comment:
Feline restrictive cardiomyopathy (RCM), like hypertrophic cardiomyopathy (HCM), is a disease of diastolic dysfunction. In human medicine, the endomyocardial form of RCM is subclassified into two forms, Loeffler endomyocarditis, and endomyocardial fibrosis. Both have similar and histologically indistinguishable endpoints, but different pathogeneses. Interestingly, there is also a geographic distinction between these two processes, with the Loeffler endomyocarditis most common in temperate climates, and endomyocardial fibrosis most common in equatorial Africa. In Loeffler endomyocarditis, fibrosis is the result following release of granules from infiltrating eosinophils in early stages of the disease, and is associated with hypereosinophilia, thromboembolism, and arteritis. Endomyocardial fibrosis usually affects younger patients and is not associated with eosinophilia.[11]

Research in human restrictive cardiomyopathy has suggested a number of different genetic mutations that can lead to disease. One revealed mutation (p.Y122H) occurs in the *DES* gene, a highly conserved coil-1 subdomain of desmin, and results in defective filament production by myocytes.[1] Other implicated mutations occur in the *MYH7*, *ABCC9*,[9] and *MYL2*.[13] Therapies addressing these mutations are in active development. The relevance to feline RCM remains to be determined.

RCM may be secondary to certain infiltrative conditions, such as amyloidosis or sarcoidosis. However, in the race to address SARS-CoV-2 with medical treatments, hydroxychloroquine and chloroquine were suggested and touted as effective treatments. These two drugs were originally developed as anti-malarial medications but are now used for the treatment of rheumatoid arthritis and systemic lupus erythematosus. One noted adverse effect from these medications includes cardiotoxicity, and restrictive cardiomyopathy, dilated cardiomyopathy, and conduction abnormalities have been recorded. In these cases, presenting clinical signs may be non-specific, so endomyocardial biopsy is the key diagnostic step to perform.[2]

The conference moderator shared an immunohistochemical stain of the tissue, highlighting most of the proliferative tissue as smooth muscle actin positive. Conference participants and the moderator, in conjunction with an outside consultation, suggest the cells may be reparative cardiac myofibroblasts, and are hyperplastic in the face of a chronic stimulus. Conditions considered potentially likely included hypertension, or turbulence due to remodeling. Alternatively, this cat may have had a genetic connective tissue anomaly that contributed to this lesion.

References:

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CASE 3: AB968/9 (JPC 4121062-00)

Signalment:
1-month old, male, German Shepherd, *Canis lupus familiaris*, dog.

History:
This animal belongs to a litter of 12 puppies. Eight of them unexpectedly died with no evident clinical signs. The owner submitted only one of them for necropsy.

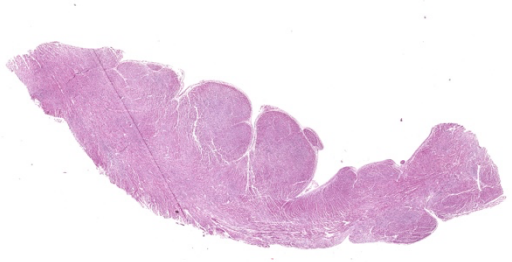
Gross Pathology:
The animal was in moderate body condition and good preservation status. Oral and ocular mucosae were moderately pale and there were multifocal areas of subcutaneous edema in the thoracic and abdominal regions. Mild amount of foam was present within the trachea and major bronchi. Within the thoracic cavity, there was a mild amount of serous fluid. White-grey, irregularly shaped, multifocal to coalescing areas were present on the epicardium, most prominent in the ventricles. On cut surface, many of these foci extended and affected the myocardium. Multifocal reddish areas were present on the mucosa of esophagus, stomach, and intestine. The stomach was full of digested milk. The liver was moderately and diffusely congested.

Laboratory results:
None.

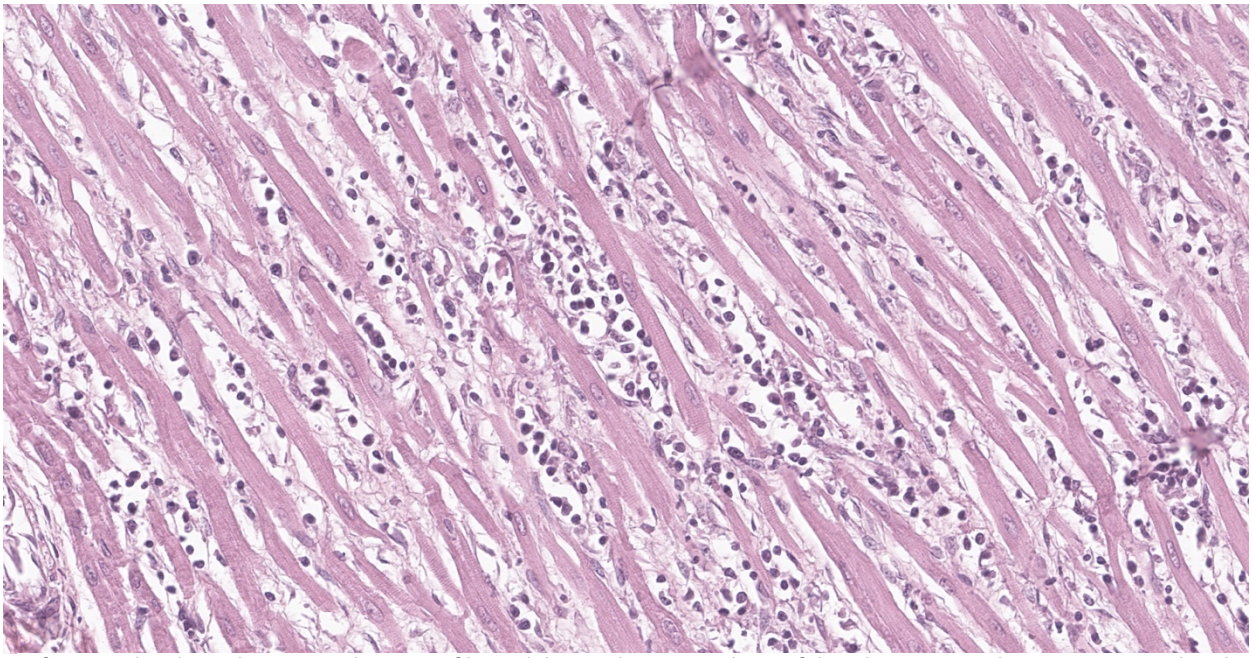
Microscopic description:
Heart: multifocal to coalescing areas of the myocardium, affecting more than 50% of the tissue, are characterized by moderate to severe fiber loss. In these areas, at transverse section, myocytes are often atrophic showing rounding – up, irregular size (up to less than 3-4micron), and loose arrangement with peripheral large empty spaces. Occasionally they show also sarcoplasm fragmentation, rare splitting and rare sarcoplasmic vacuolation. At longitudinal section, they manifest abrupt interruption and fragmentation of sarcoplasm (disruption of microfilaments). In the affected areas, within the space between the myocytes there is moderate interstitial slightly eosinophilic and fibrillar material (loosely arranged collagen - fibrosis) with minimal amorphous mildly eosinophilic material (proteinaceous fluid, edema) associated with the presence of numerous mostly individually disseminated lymphocytes and plasma cells and rare neutrophils and macrophages. In the affected areas, frequently to occasionally (depending on the section) intranuclear amphophilic-basophilic oval sometime with blunted ends inclusion bodies entirely filling the nucleus are present within the myocytes. The lymphoplasmacytic infiltrate is mildly and multifocally extending into the epicardium and endocardium.

Contributor's morphologic diagnosis:
Heart: Myocarditis, multifocal to coalescing, lymphoplasmacytic, moderate to severe, with fiber loss, fibrosis and edema, and with intramyocytes intranuclear amphophilic-basophilic viral inclusion bodies (*Parvovirus* spp.), *Canis lupus familiaris*, dog.

Contributor's comment:
Canine myocarditis is associated with disastrous effects that lead the animal either to death or to permanent cardiac damage.[9] Typically, lymphoplasmacytic myocarditis is a lesion of viral infections.[10] Canine parvovirus (CPV) is a well-known cause of myocarditis in young puppies.[5] CPV is a member of the *Parvoviridae* family, whose viruses are small (23-26 nm in diameter), non-enveloped, and icosahedral with linear single-stranded DNA, that infect rapidly dividing cells, such as hematopoietic precursors in bone marrow and the gastrointestinal crypt cells.[2] Parvovirus-1 (CPV-1), the first subtype of Parvovirus affecting dogs, was identified in 1967 as a cause of gastrointestinal and respiratory tract diseases in dogs.[1] A few years later, in 1978, a new strain of this virus, Parvovirus-2 (CPV-2),



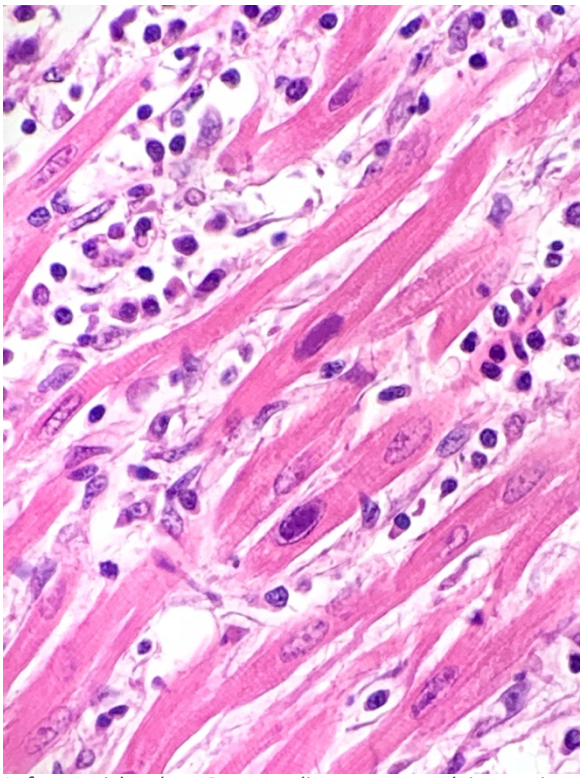
Left ventricle, dog. A section of left ventricle is presented for examination. (HE, 5X)



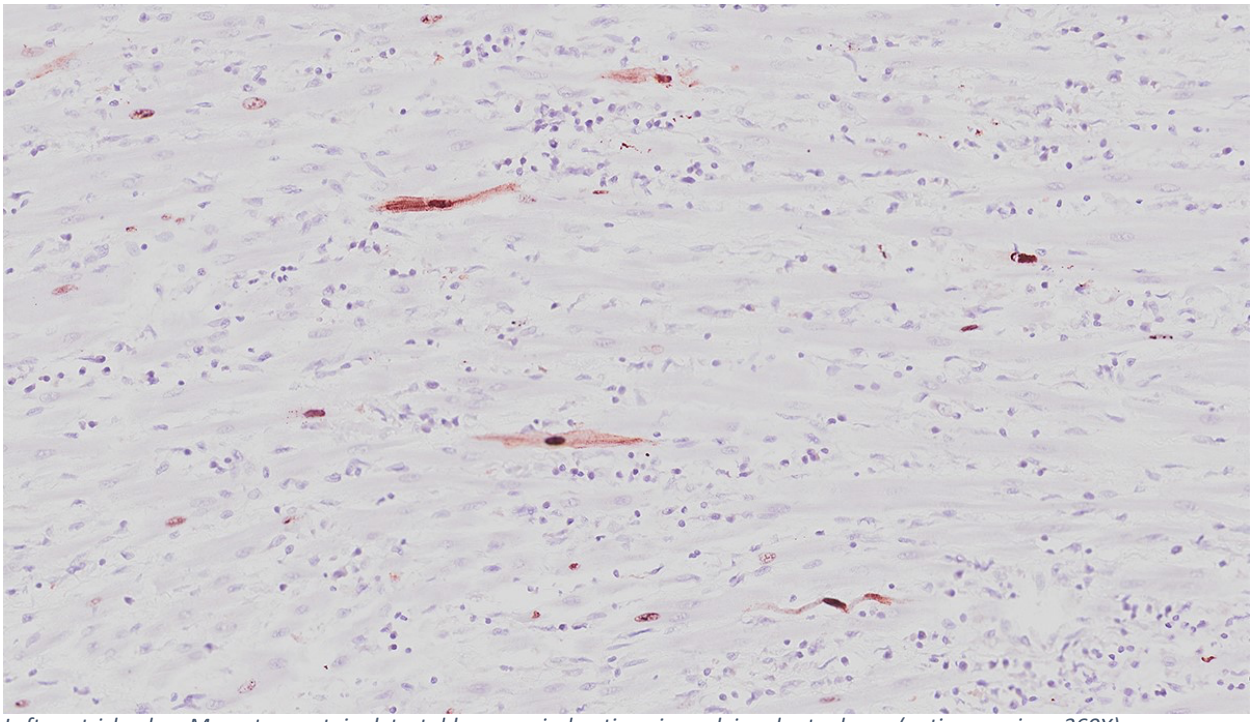
Left ventricle, dog. The myocardium is infiltrated by moderate numbers of lymphocytes. Cardiomyocytes are shrunken, fragmented and occasionally replaced by the infiltrating cells and separated by small amounts of wispy collagen and edema (HE, 358X)

was discovered. After infection through fecal-oral route, the virus replicates primarily in the lymphoid tissue of the oropharynx.[9] The virus spreads hematogenously to infect epithelial cells present in the gastrointestinal crypts, 3-to-4 days after infection.[5] This tropism is called *radiomimetic.*[10] Because the villous basement membrane is exposed, villous fusion occurs, resulting in lack of scaffold for enterocytes replacement once the crypts recover. As a result, ulceration, permanent atrophy and, in severe cases, necrosis of the crypt epithelial cells cause an effusive and malabsorptive diarrhea that leads to severe dehydration and often death.[9]

A less common consequence of parvoviral infection in dogs is myocarditis often associated with necrosis of myocytes. The myocardial cells are susceptible in puppies until 15 days of age, when they stop dividing.[5] A peracute and acute forms of myocarditis are described. The peracute form affects puppies at 3 to 8 weeks of age or, in case of in utero infection, even younger with sudden death a few days after parturition. The acute form typically develops in puppies ≥ 8 weeks of age and causes severe dyspnea, signs of depression, eventually leading the animal to death. For both forms, myocarditis is associated with necrosis of myocytes. In the peracute form, sudden death is caused by severe necrosis, whereas in the acute form the affected areas are replaced by fibrosis. In the latter case, death is



Left ventricle, dog. Rare cardiomyocyte nuclei contain a deeply basophilic intranuclear viral inclusion that fills the nucleus. (HE, 518X)



Left ventricle, dog. Myocytes contain detectable parvoviral antigen in nuclei and cytoplasm. (anti-parvovirus, 360X)

caused by the severe damage. As a result of both forms, circulatory changes affect lungs and liver causing edema and congestion.[5,6] Unfortunately, no treatments are available, and prognosis is poor.[9]

Macroscopically, mottled pale patches and bands in the left ventricular wall are evident. If the dogs have also the enteric signs, other findings include hemorrhagic enteritis of the small intestine, enlargement of enteric lymph nodes, and reddening of Peyer patches.[6] Microscopically, lymphoplasmacytic myocarditis often associated with necrosis and subsequent fibrosis, with amphophilic-basophilic intranuclear inclusion bodies are the most characteristic findings of this disease. Macrophages and fibroblasts might be present within the interstitium.[6]

The most effective way to control Parvovirus infection is vaccination and environmental control. Proper vaccination of female dogs prior to parturition provides passive immunity to puppies through transfer of maternal antibodies.[9]

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JPC diagnosis:
Heart: Myocarditis, lymphohistiocytic, diffuse, marked, with cardiomyocyte necrosis, loss, and atrophy, interstitial fibrosis, and cardiomyocyte intranuclear inclusion bodies.

JPC comment:
The contributor provides a succinct summary of canine parvoviral myocarditis. A number of other viruses, in addition to other causes, are implicated in myocarditis in domestic species, including canine distemper virus, canine herpesvirus, and pseudorabies, porcine circovirus 2, porcine reproductive and respiratory syndrome (PRRS) virus, encephalomyocarditis virus, West Nile Virus, and the novel Bungowannah pestivirus.[7] In humans, enteroviruses, including coxsackievirus, parvovirus B-19, and human herpesvirus 6 are frequent causes of myocarditis.[3]

The parvovirus genome encodes a major nonstructural protein 1 (NS1), and two structural proteins, VP1 and VP2. NS1 is responsible for

most steps of viral replication, while the VP2 protein is the primary component of the viral capsid. VP1 is similar to VP2 but differs by having an additional 226 amino acids at its amino terminal end.[4]

Viral myocarditis has received additional attention recently, as there have been numerous reports of COVID-19 associated myocarditis in humans and has even been recognized as the cause of death in some patients. The lesions of COVID-19 myocarditis are usually focal, but some patients progress to arrhythmia, fulminant heart failure, and cardiogenic shock. This disease manifestation occurs as a result of both direct cell injury, as well as T-cell mediated cytotoxicity; both mechanisms can be exacerbated by an associated cytokine storm. IL-6 is currently identified as the key mediator of the cytokine storm, which mediates the pro-inflammatory responses from immune cells.[8]

The moderator was able to demonstrate cardiomyocytes with and without intranuclear inclusions were positive for anti-CPV2 antibodies.

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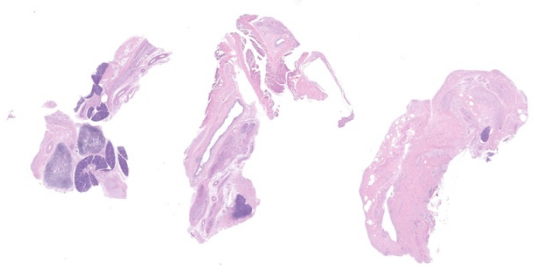
CASE 4: 19A087 (JPC 4135350-00)

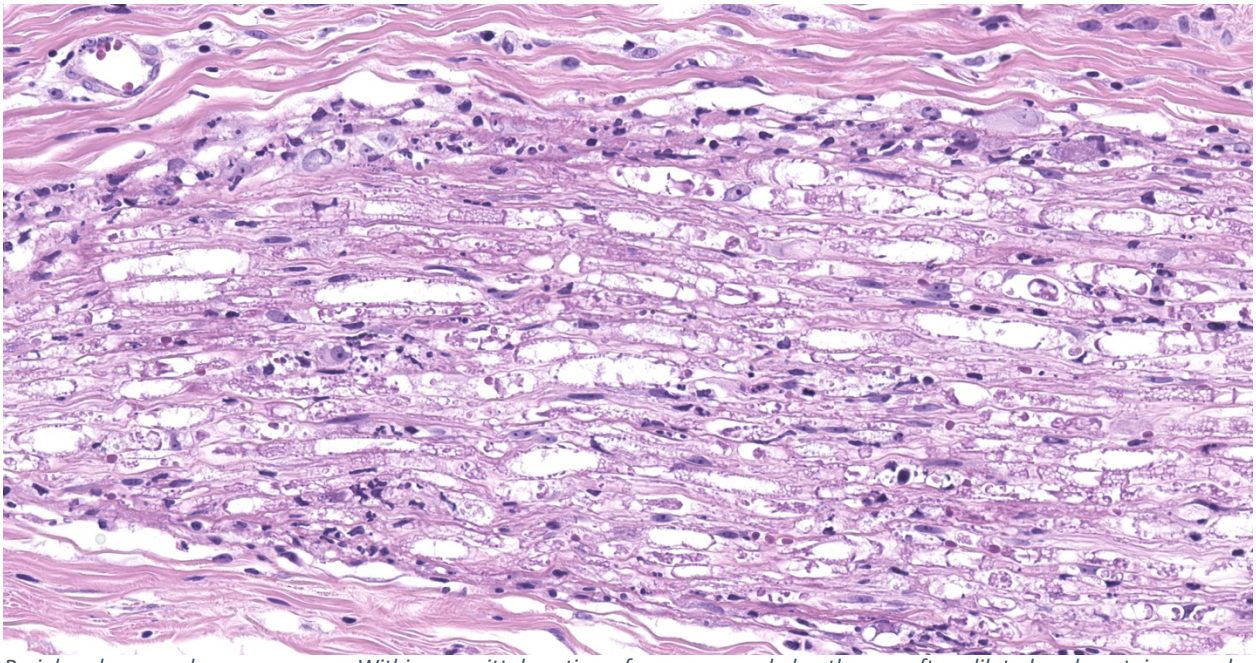
Signalment:
2.8 year-old, male, Indian-origin rhesus macaque (*Macaca mulatta*)

History:
This animal was part of an SIV vaccine trial and had received multiple immunizations prior to being challenged with pathogenic SIV. Physical exam findings revealed mild dehydration, facial swelling, left-sided facial paralysis and diarrhea. After 3 weeks with no response to treatment humane euthanasia was elected.

Gross Pathology:
Presented for postmortem examination is a 2.8 year-old, male, Indian-origin rhesus macaque. There is moderate, left-sided fascial swelling. On



Cervical soft tissue, rhesus macaque. Multiple sections of soft tissue from the anterior neck are submitted containing salivary gland and duct, lymph node, and multiple sections of peripheral nerve. (HE, 5X)



Peripheral nerve, rhesus macaque. Within a sagittal section of nerve, axonal sheaths are often dilated and contain granular eosinophilic axonal debris. (HE, 308X)

cut surface the subcutis is expanded by moderate edema. The left trigeminal ganglia are enlarged and reddened compared to the right. The mucosa of the gastric antrum is diffusely reddened. The duodenum, jejunum, and ileocecal junction are similarly reddened. The colon contains copious, liquid, fetid feces.

Laboratory results:

Complete Blood Count		
	Value	Reference
Wbc	18.3	6.6-15.5
Rbc	5.9	4.1-7.8
Hgb	12.8	10.1-15.9
Hct	40.9	34.8-55.2
Mcv	69.9	63.7-86.9
Mch	21.9	19.1-27.7
Mchc	31.3	28.9-35.4
Rdw	13.8	10.9-15.3
Plt	476000	193-676
Mpv	10.7	
%Neu	89.9	20.6-46.9
%Lym	5.2	35.2-84.1
%Mon	4.4	1.8-9.9
%Eos	0.2	
%Bas	0	
#Neu	16.5	
#Lym	1	
#Mon	0.8	
#Eos	0.2	
#Bas	0	

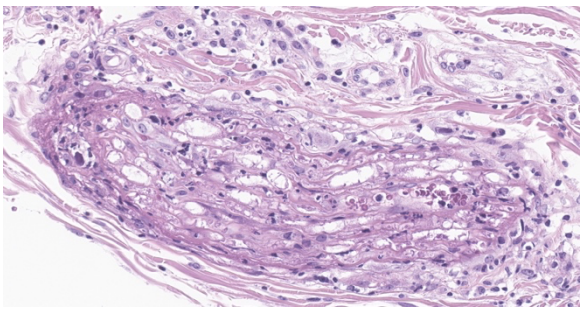
Chemistry Panel		
	Value	Reference
Na	145	136-145
K	4.1	3.5-5.1
Cl	3.6	98-107
Pro	5.2	6.4-8.9
Alb	3.1	3.5-5.7
Glob	2.1	1.9-3.9
A/G	1.5	0.5-3.5
BUN	33	25-Jul

Glu	5	70-105
Crt	0.2	0.6-1.3
Ast	116	13-39
Alt	76	
Bn/Cr	220	
Csf pro	132	

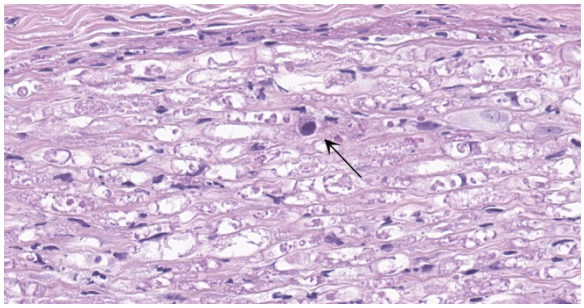
Microscopic description:
Salivary gland and soft tissues of the face – Infiltrating the perineurium of a facial nerve are moderate numbers of neutrophils with lesser numbers of macrophages and lymphocytes. The perineurium is mild to moderately expanded by edema. Inflammatory cells (presumed macrophages) multifocally exhibit karyomegaly with intranuclear 1x3 µm, eosinophilic, hyaline inclusions that peripherally marginate the chromatin. There is marked axonal degeneration characterized by dilation of myelin sheaths with spheroid formation. Occasionally dilated axon sheaths are infiltrated by macrophages (digestion chamber formation). The surrounding fascia is similarly expanded by inflammatory cells and edema.

Contributor's morphologic diagnosis:
Nerve of the face: Neuritis, necrosuppurative, moderate, acute with axonal degeneration and intranuclear inclusions consistent with cytomegalovirus.

Contributor's comment:
Rhesus cytomegalovirus (RhCMV) is ubiquitous with a seroprevalence in rhesus macaques that



Peripheral nerve, rhesus macaque. Affected nerves are infiltrated with low to moderate numbers of neutrophils, and fewer histiocytes. (HE, 400X)



Peripheral nerve, rhesus macaque. Rarely, Schwann cell nuclei are expanded by a darkly basophilic intranuclear inclusion which fills the nucleus (arrow). (HE 570X)

nears 100% by 1 year of age.[11] Experimental infection has shown that immunocompetent hosts clear virus from circulation within two weeks following the production of anti-CMV antibodies.[7] Once cleared, the virus becomes latent within ganglia and animals remain infected for life. Immunosuppression, most often in AIDS and transplant recipients, results in reactivation with multiorgan pathology including interstitial pneumonia, encephalitis, enteritis, lymphadenitis, and orchitis.[3] RhCMV is the most common opportunistic infection of rhesus macaques infected with SIV. Other common opportunistic infections include adenovirus, *Pneumocystis sp.*, and *Cryptosporidia sp*. Facial neuritis, as seen in this case, is an uncommon presentation of RhCMV occurring in <10% of cases.[1]

Contributing Institution:
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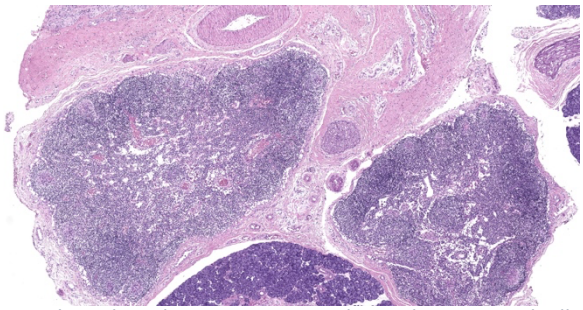
JPC diagnosis:
Peripheral nerves: Neuritis, necrotizing, diffuse, marked with cytomegalic intranuclear inclusions.

JPC comment:
The subfamily *Betaherpesvirinae* encompasses four genera, which include *Cytomegalovirus*, *Muromegalovirus*, *Proboscivirus*, and *Roseolovirus*. These viruses replicate more slowly than alphaherpesviruses, leading to cytomegalic cells. During latency, these viruses are confined to hematopoietic stem cells, and possibly the cells of secretory glands and the kidney. Species within *Betaherpesvirinae* affect

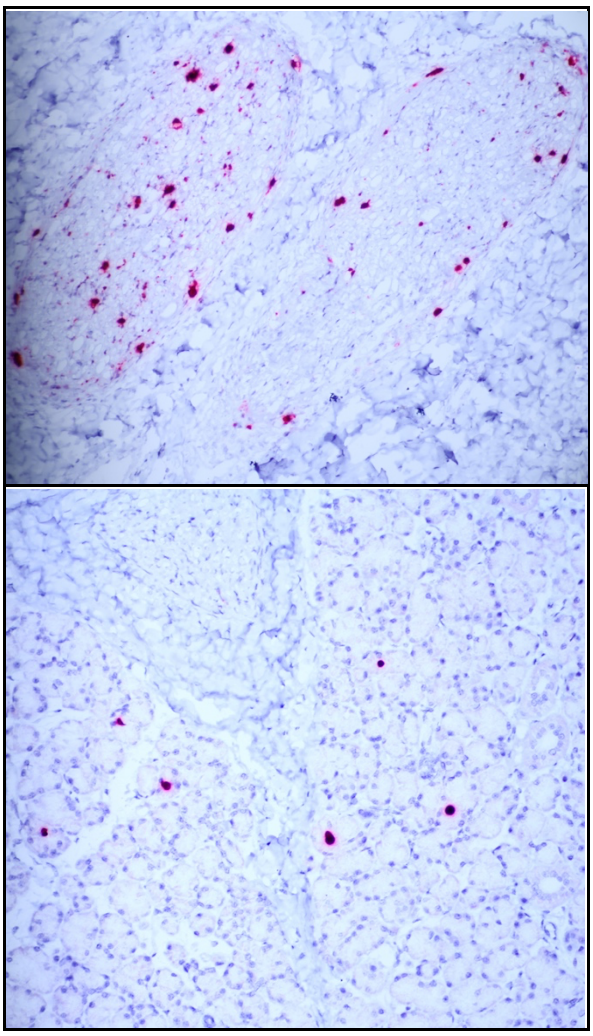
humans and non-human primates (this entity, macacine herpesvirus 3), but also infect mice (murid herpesvirus 1 and 2), rats (murid herpesvirus 8), guinea pigs (caviid herpesvirus 2), elephants (elephantid herpesviruses), and pigs (suid herpesvirus 2).[8] Interestingly, the genome of macacine herpesvirus 3 encodes proteins that help evade the immune system. Peptides are produced that downregulate MHC I expression, and a viral homolog of IL-10 is produced that has anti-inflammatory properties.[12] Human cytomegalovirus also produces proteins with CXC chemokine properties, encoded on viral genes UL146 and UL147. The protein encoded by UL146 is named viral CXC chemokine-1 (vCXCL1) and is at least as potent as innate IL-8 and responsible, in large part, for the neutrophilic response to this virus.[10]

Viral infection continues to be a relevant concern for colonies of macaques used in biomedical research. A seroprevalence study conducted in Germany of 231 macaques found high seroprevalence of cytomegalovirus (98.3%), lymphocryptovirus (89.6%), rhesus rhadinovirus (84.4%), and simian foamy virus (94.8%). These infections appear to be acquired early in life, usually before capture, but researchers should be aware of the background lesions that may be encountered during postmortem and histologic examination.[6]

Recent research has focused on vaccine development using cytomegalovirus as a vector. Promising vaccines have been produced against *Mycobacterium tuberculosis*,[4,5] as well as HIV[2] and Ebolavirus.[9] The cytomegalovirus-vector help producing long lasting memory



Lymph nodes, rhesus macaque. The nodes are markedly depleted as a result of infection by simian lentivirus. (HE, 39X)



Peripheral nerve, salivary gland, rhesus macaque. Top: Probe to CMV:ISH hybridization demonstrates abundant viral nucleic acid within peripheral nerves. Bottom: There is scattered viral nucleic acid within salivary gland epithelial cells. (CMV in situ hybridization)

lymphocytes, contributing significantly to longstanding resistance to infection.

The moderator performed additional diagnostics on this case and demonstrated via in situ hybridization that affected peripheral nerves had large amounts of virus. Additionally, virus was detectable within few salivary gland nuclei, indicating a location for latent viral infection in this animal.

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