Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

C o n f e r e n c e 17

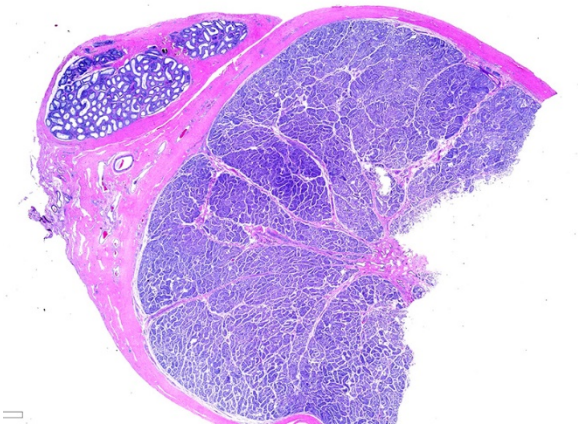
30 January 2019

Conference Moderator:
Patricia Pesavento, DVM, PhD, DACVP
Professor of Pathology, Microbiology & Immunology
UC Davis School of Veterinary Medicine
Davis, CA

CASE I: S1501813 (JPC 4084248).

Signalment: Six-year-old, intact male German shepherd dog (*Canis familiaris*)

History: This animal was born in the East coast of the US and then moved to the West coast, close to the border with Mexico. Up to date with vaccination. Presented to the clinic with signs of lethargy, anorexia and swelling of the right thoracic limb. Treated with antibiotics (Rilexine/cephalexine). The dog showed improvement, but a few days later developed diarrhea and neurological signs. On clinical examination, the dog evidenced disorientation, ataxia, nystagmus, generalized lymphadenomegaly, edema of the limbs and scrotum and peripheral neuropathy. The animal was treated with doxycycline and enrofloxacin intravenously, but died a few hours later.



Testis, dog. There are no visible lesions at subgross. (HE, 5X)

Gross Pathology: The carcass was in good body condition. The subcutis of the distal limbs displayed small amounts of edema. The prescapular, popliteal and submandibular lymph nodes were mildly enlarged and fleshy. Lungs were diffusely red and wet, and the heart appeared rounded due to marked dilation of the right ventricle. The spleen was markedly enlarged with dark red fleshy parenchyma. Mesenteric lymph nodes were minimally enlarged.

Laboratory results: The blood chemistry (Table 1) revealed hypoalbuminemia without alteration of the A/G ratio. Alkaline phosphatase was mildly elevated and hypocalcemia was detected. The CBC (table

2) revealed leukocytosis due to neutrophilia and monocytosis, plus thrombocytopenia. The canine tick borne PCR panel (Table 3) was positive for *Rickettsia rickettsii*.

Table 1: Blood chemistry:

Test	**Result**	**Ref range**	**Units**
Total protein	5.1	5.0-7.4	g/dL
Albumin	**2.1 (LOW)**	**2.7-4.4**	**g/dL**
Globulin	3.0	1.6-3.6	g/dL
A/G ratio	**0.7 (LOW)**	**0.8-2.0**	
AST	58	15-66	IU/L
ALT	38	12-118	IU/L
Alk Phosphatase	**210 (HIGH)**	**5-131**	**IU/L**
GGT	5	1-12	IU/L
Total bilirubin	0.3	0.1-0.3	mg/dl
BUN	15	6-31	mg/dl
Creatinine	0.6	0.5-1.6	mg/dl
BUN/Creatinine ratio	25	4-27	
Phsphorus	4.7	2.5-6.0	mg/dl
Glucose	96	70-138	mg/dl

Calcium	**8.5 (LOW)**	**8.9-11.4**	**mg/dl**
Corrected calcium	9.9		
Magnesium	1.5	1.5-2.5	mEq/L
Sodium	141	139-154	mEq/L
Potassium	4.2	3.6-5.5	mEq/L
NA/K ratio	34	27-38	
Chloride	111	102-120	mEq/L
Cholesterol	277	92-324	mg/dl
Trygliceride	88	29-291	mg/dl
Amylase	723	290-1125	IU/L
Lipase	**65 (LOW)**	**77-695**	**IU/L**
CPK	107	59-895	IU/L

Table 2: CBC:

Test	**Result**	**Ref range**	**Units**
WBC	**15.6 (HIGH)**	**4.0-15.5**	**10^3/uL**
RBC	5.5	4.8-9.3	10^6/uL
HGB	12.5	12.1-20.3	g/dL
HCT	38	36-60	%
MCV	69	58-79	fL

MCH	22.7	19-28	pg
MCHC	33	30-38	g/dL
Blood parasites	None seen		
RBC morphology	Normal		
Platelet count	**75 (LOW)**	**170-400**	**10^3/uL**
Platelet Est	Adequate		
Neutrophils (HIGH)	**13260**	**2060-10600**	**/uL**
Bands	0		
Lymphocytes	1248	690-4500	/uL
Monocytes (HIGH)	**1092**	**0-840**	**/uL**
Eosinophils	0	0-1200	/uL
Basophils	0	0-150	/uL

Table 3. Canine tick borne PCR panel profile:

Anaplasma phagocytophylum	Negative
Anaplasma platys	Negative
Babesia canis	Negative
Babesia spp (non-canis)	Negative
Bartinella henselae	Negative
Bartonella vinsonii	Negative

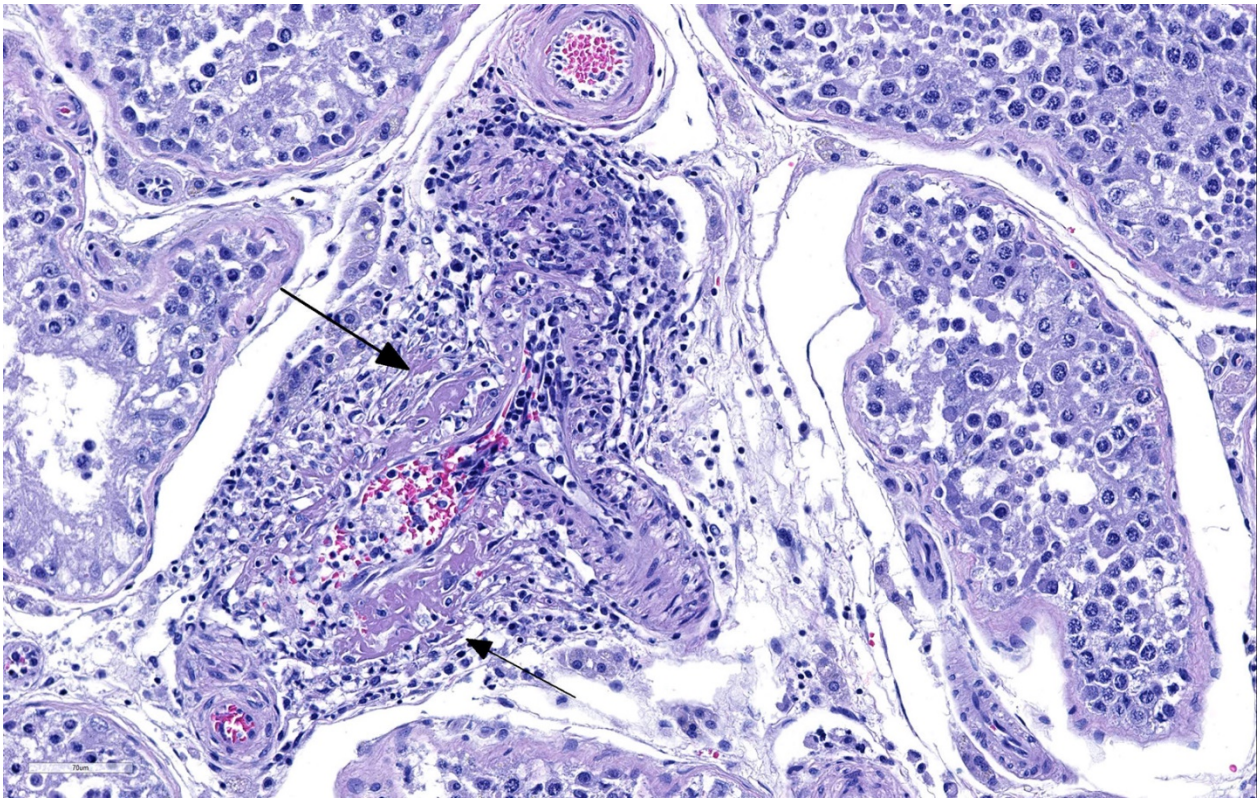
Ehrlichia canis	Negative
M haemocanis/hematoparvum	Negative
Neorickettsia risticii	Negative
Rickettsia rickettsii	**Positive**

Microscopic Description:
Testicle: Section include testicle and tunica albuginea, head of epididymis and vascular plexus. Scattered throughout the section, numerous vessels display segmental inflammatory changes, characterized by fibrinoid degeneration of the vascular media and transmural inflammatory infiltrates, composed mostly of lymphocytes, plasma cells and macrophages. Endothelial cells are plump and frequently detached. In addition to the inflammatory component, necrotic cellular debris are identified within the media and adventitia of the affected vessels. Occasionally, the adventitia is also expanded with fibrin. These findings were more prominent at the testicular vein (pampiniform venous plexus), but could be found in any medium and small caliber vessel. Other findings within the testicle is segmental tubular degeneration, characterized by the presence of multinucleated cells in the lumen of seminiferous tubules.
Similar vascular findings with varying degrees of severity were also identified (but not included in the section) in: brain, subcutis of distal limbs, eye, lung, heart, skeletal muscle, tongue and gastrointestinal wall. Other significant histological findings included meningoencephalitis, mild hepatitis and dermatitis (the latter presumably associated with tick bite site).

Contributor's Morphologic Diagnoses:
Testicle: 1. Vasculitis, lymphohistiocytic to necrotizing, marked, sub-acute, segmental.
2. Testicular degeneration, mild, multifocal.

Contributor's Comment: "Rocky Mountain Spotted fever" (RMSF) is a tick-transmitted zoonotic infectious disease caused by *Rickettsia rickettsii*. Of the tick-borne diseases in America, RMSF is the most severe, and can result in rapid course of disease and high mortality rate. This disease can be transmitted by ticks of the *Dermacentor*, *Rhipicephalus* and *Amblyomma* genera.[7] In the USA, *Dermacentor variabilis* and *Dermacentor andersoni* are most commonly responsible for the transmission of the disease and they act as vectors, reservoirs and naturals hosts for *R. rickettsii*. In its natural cycle, *R. rickettsii* is maintained in persistently infected tick population and small rodent reservoir hosts. Interestingly, transovarial transmission from infected females to eggs and transmission between adult ticks during mating can maintain the infection in a population without additional infectious feedings.[5]

Once a dog is bitten by infected ticks, there is invasion of small vessels and replication, with damage of endothelial cells. Rickettsiae use phospholipase A proteases and free radicals to induce oxidative and peroxidative damage to the host cell membrane, which



Testis dog. Multifocally, the walls of small- and medium caliber arterioles are necrotic and effaced by abundant extruded protein, necrotic smooth muscle, low to moderate numbers of infiltrating neutrophils, macrophages, and lymphocytes and cellular debris (fibrinoid necrosis). (HE, 205X)

will finally result in cell death. In addition, cell mediated immunity induces apoptosis in cells infected with rickettsiae via mechanisms such as the CD8 T lymphocyte cytotoxicity.[1] The net effect of these mechanisms is endothelial cell damage, which is followed by immune and phagocytic cellular responses plus platelet activation and activation of the coagulation and fibrinolytic systems, resulting in thrombosis.[1,7] Platelet consumption is considered as the primary cause of thrombocytopenia in dogs. However, antiplatelet antibodies has also been identified in infected animals.[3] Hypoalbuminemia is often observed and is probably caused by leakage associated with generalized vascular damage.[2]

Because RMSF lesions are characteristically associated with vasculitis throughout the body, it is not surprising that disease can manifest with a wide variety of clinical signs and can be mistaken for an undifferentiated viral illness during the first days of manifestation.[1] Clinical signs includes fever (early manifestation), edema and hyperemia of the lips, penile sheath, scrotum (with abnormal gait), pinna and ventral abdomen. Petechial and ecchymotic hemorrhages may develop subsequent to the acute illness in mucous membranes, skin, pleura and gastric wall, in addition to hemorrhagic colitis and generalized lymphadenopathy.[2,7] Histologically, the most prominent lesion in necrotizing vasculitis of small veins, capillaries and arterioles with perivascular accumulation of mononuclear cells. This lesion is frequently seen in the skin, testicle, alimentary tract, pancreas, kidney, urinary bladder, myocardium, retina and skeletal muscle. In addition, acute meningo-encephalitis, interstitial pneumonia and

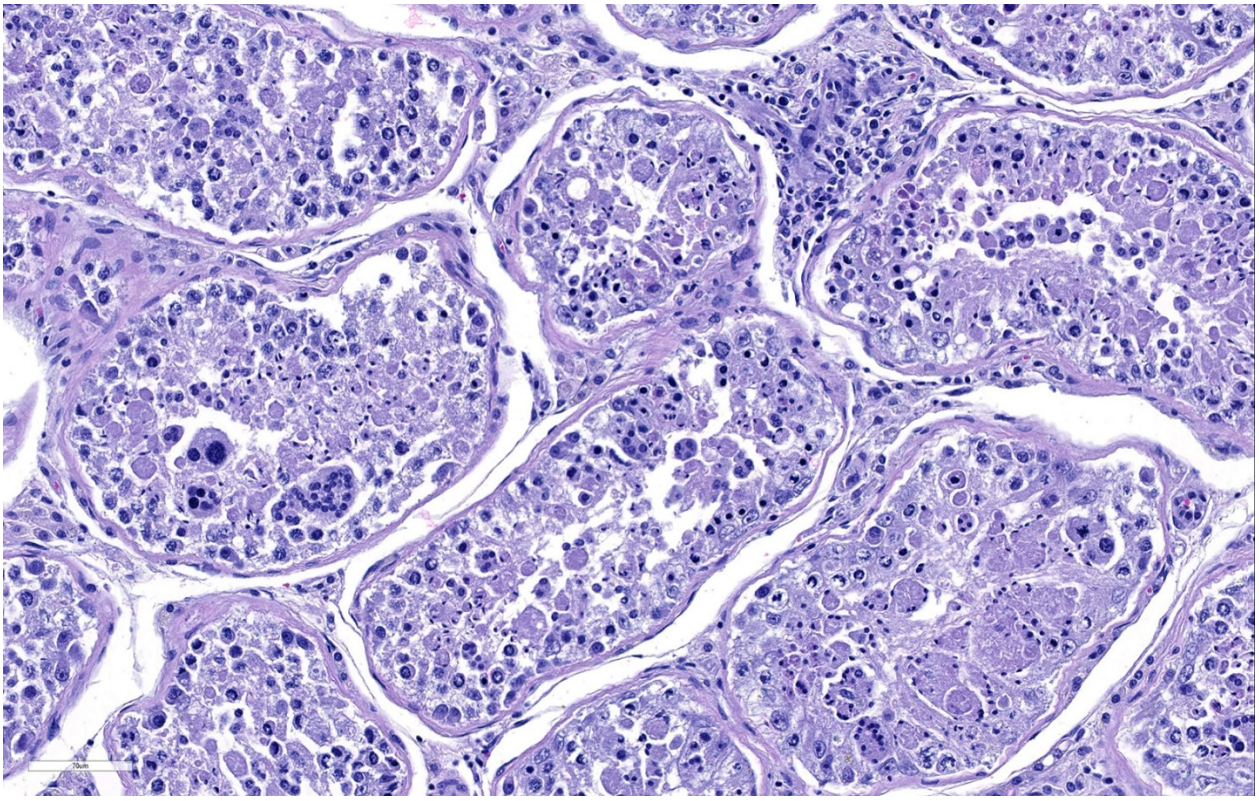
necrosis of the myocardium, adrenal gland and liver can be present.[7]

Among the differential diagnosis for vasculitis in dogs, other causes of secondary vasculitis should be considered, such as autoimmune diseases (such as lupus erythematosus), infections with canine circovirus, *Leishmania* spp, *Angiostrongylus vasorum* and *Dirofilaria immitis*, and exposure to therapeutic products, such as carprofen and meloxicam.[4,9]

Contributing Institution:
California Animal Health and Food Safety Laboratory
San Bernardino Branch
105 W Central Ave
San Bernardino, CA 92408
www.cahfs.ucdavis.edu

JPC Diagnosis: 1. Testis, arterioles: Vasculitis, necrotizing, random, multifocal, severe, with seminiferous tubule necrosis. 2. Testis, seminiferous tubules: Degeneration and atrophy, diffuse, moderate, with spermatid giant cell formation.

JPC Comment: The contributor has provided an excellent discussion of Rocky Mountain spotted fever in the dog. Humans and dogs are the only two species in which clinical disease (and life threatening infections) may result from *Rickettsia rickettsiae* infection. While a number of small mammals, including rodents, rabbits, and opossums may provide a reservoir for the bacterium, clinical disease has not been documented in these species. Recent inoculation studies in horses failed to elicit clinical disease.



Testis dog. Three is necrosis of spermatogonia and Sertoli cells within seminiferous tubules. The presence of spermatid giant cells is likely a pre-existent degenerative change. (HE, 295X)

Clinical symptoms of RMSF in humans arise abruptly and generally include high fever, headache, nausea, vomiting, and generalized myalgia. A generalized macular rash appears two to four days later which may or may not be accompanied by eschar at the site of the tick bite, and often starts on the wrists, ankles, and forearms. In 50-60% of patients, the lesions evolved into petechiae or purpura. More severe lesions include gangrene, pulmonary and cerebral edema, myocarditis, renal failure and disseminated intravascular coagulation. 5-10% of patients will die despite treatment.[6]

The contributor refers to "secondary vasculitis". This particular nomenclature comes from human medicine. The term "primary" vasculitis refers to blood vessel wall injury arising without an apparent cause, and "secondary" vasculitis in which an apparent cause (infectious, immune-mediated, or neoplastic) is identified. Few well-defined syndromes of primary vasculitis exist in veterinary medicine, to include the so-called "beagle pain syndrome", as well as a number cutaneous vasculitides in the dog. The vasculitis seen in this case, secondary to *R. rickettsii* infection, would be classified as "secondary" vasculitis. A retrospective study of 42 cases by Swann et al in 2015, compared cases of vasculitis which fell into both categories. Significant differences between the two groups were not many, with female dogs more likely to develop primary vasculitis, and dogs with primary vasculitis were more likely to have elevated serum globulin concentrations. The authors concluded that there does not appear to be well-defined syndromes of primary and secondary vasculitis in the dog as there are in humans.[9]

In veterinary medicine, the determination of "small" vs. "medium" arterioles is not well defined, due to the range of species we often examine (i.e., in a gerbil, all arteries are small). In human medicine, the definition is much more well defined, with luminal measurements defining "large", "medium", and "small". The moderator suggested that in any animal species, the aorta and the pulmonary artery are should be considered large, direct branches off of them would be "medium" and a third or more distal branches represent "small" arteries.

References:

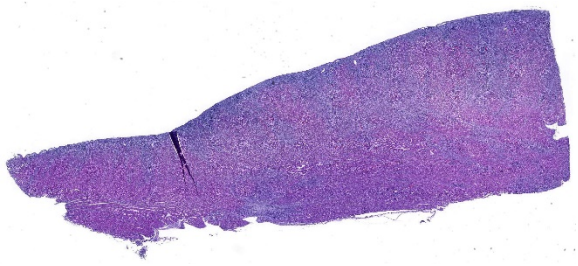
1. Chen LF, Sexton DK. What's new in Rocky Mountain spotted fever? *Infect Dis Clin N Am* 2008; 22 (3): 415-432.
2. Greene CE, Kidd L, Breitschwerdt EB. Rocky Mountain and Mediterranean spotted fevers, and typhus. In: Greene CE, ed. Infectious diseases of the dog and cat. 4th ed. St Louis, MO: Elsevier; 2012: 259-276.
3. Grindem CB, Breitschwerdt EB, Perkins PC, Culins LD, Thomas TJ, Hegarty BC. Platelet-associated immunoglobulin (antiplatelet antibody) in canine Rocky Mountain spotted fever and Ehrlichiosis. *J Am Anim Hosp Assoc* 1999; 35 (1): 56-61.
4. Li L, McGraw S, Zhu K, Leuttenegger CM, Marks SL et al. Circovirus in tissues of dogs with vasculitis and hemorrhage. *Emerg Infect Dis* 2013; 19 (4): 534-541.
5. Nicholson WL, Allen KE, McQuiston JH, Breitschwerdt EB, Little SE. The increasing recognition of rickettsial pathogens in dogs and people. *Trends Parasitol* 2010; 26 (4): 205-2012.
6. Parola P, Paddock DC, Socolovschi C, Labruna MG, Mediannikov O, Kernif T, Abdad MY, Stenos J, Bitam I, Fournier P, Raoult. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev* 2013; 26(4):657-702

7. Robinson WF, Robinson NA. Cardiovascular system. In: Maxie MG, ed. Jubb, Kennedy and Palmer's Pathology of domestic animals. 6th ed Vol 3. St. Louis, MO: Elsevier; 2015: 1-101.
8. Shaw SE, Day MJ, Birtles RJ, Breitschwerdt EB. Tick borne infectious diseases of dogs. *Trends Parasitol* 2001; 17 (1): 74-80.
9. Swann JW, Priestnall SL, Dawson C, Chang YM, Garden OA. Histologic and clinical features of primary and secondary vasculitis: A retrospective study of 42 dogs (2004-2011). *J Vet Diagn Invest* 2015; 27 (4): 489-496.
10. Ueno TE, Costa FB, Moraes-Filho J, Agostinho WC, Fernandes WR, Labruna MB. Experimental infection of horses with *Rickettsia rickettsii*. *Parasit Vectors* 2016; 9(1):499.

CASE II: L14 10949 (JPC 4066258).

Signalment: Two 2.5 week old male intact, Spots breed, *Suidae*, domestic pigs (litter mates).

History: Healthy litter of pigs - 2 1/2 weeks old. Owner noticed rapid breathing in one piglet, and it was given penicillin G and iron dextran injections. The pig died immediately. A second pig in the litter developed similar breathing problems and died after being given Florfenicol. A third piglet with similar signs received Florfenicol, Flunixin and Dexamethasone with some improvement noted. All three piglets developed signs in the span of a few hours. The two dead piglets were submitted for necropsy.



Heart, piglet. There is a dense cellular infiltrate most prominently along the epicardial surface which replaces up to 40% of the parenchyma. (HE, 7X)

Gross Pathology: Gross alterations were similar in both pigs. The pericardial sac contained approximately 5 ml of serosanguinous fluid. The epicardium had multifocal to coalescing white areas that on cut surface extended into the myocardium. The lungs were diffusely wet, non-collapsing and mottled light red to light purple, with prominent interlobular septa. Approximately 30 ml of serosanguinous fluid was present in the peritoneal cavity, and scattered delicate fibrin strands covered the serosal surface of the intestines. Diffuse accentuated lobular pattern was evident throughout the liver, which appeared to be more friable than usual.

Laboratory results: Bacteriology: Lung - No bacteria isolated
Parasitology: Colon contents - No parasite eggs seen
Virology:

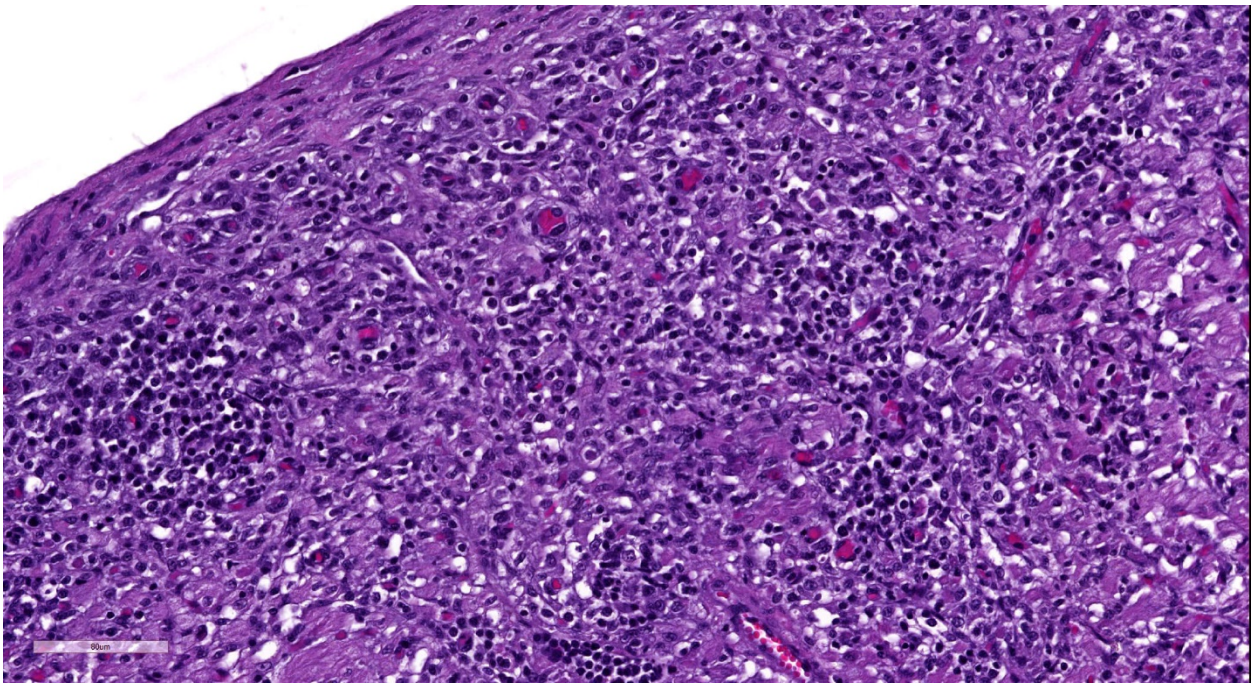
Lung and spleen - Negative for Porcine Reproductive and Respiratory Syndrome virus (PRRSV) by fluorescent antibody

Lung, intestine, and spleen - Negative for Porcine Circovirus-2 (PCV2) by fluorescent antibody

Intestine - Negative for Transmissible Gastroenteritis (TGE) virus and Porcine Rotavirus by fluorescent antibody assay, and for enteric viruses by electron microscopy

Heart - Negative for Encephalomyocarditis virus (EMCV) by PCR

Immunohistochemistry (performed at the University of Pennsylvania):



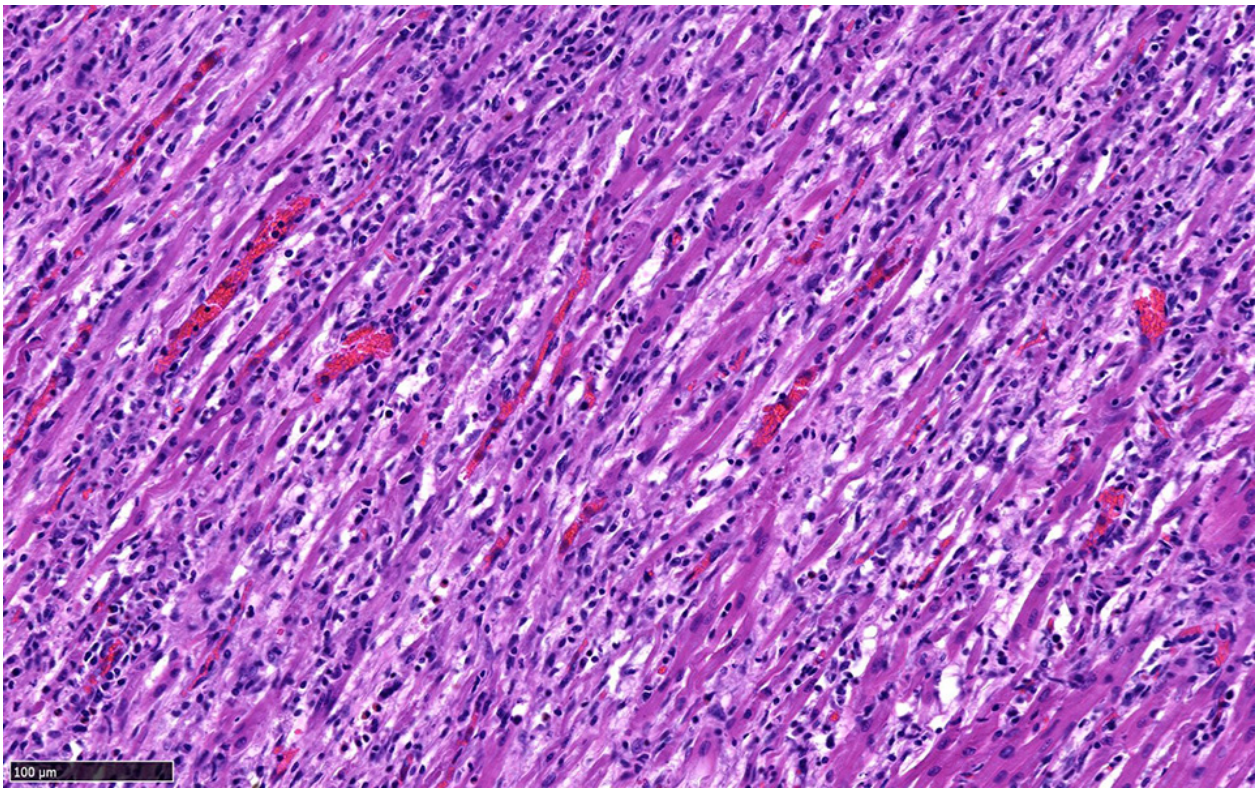
Heart, piglet. The cellular infiltrate is composed primarily of lymphocytes, with fewer macrophages and plasma cells. (HE, 278X)

Heart - Immunopositive for PCV2 antigen in cardiomyocytes, endothelial cells, and infiltrating macrophages

Microscopic Description:
Heart: Approximately fifty percent of the myocardium of the right and left free ventricular walls as well as of the interventricular septum is multifocally disrupted and replaced by inflammatory infiltrates of lymphocytes and plasma cells, fewer histiocytes and eosinophils, and sporadic multinucleated giant cells. Inflammatory cells also multifocally infiltrate the epicardium and endocardium. Cardiomyocytes are frequently characterized by loss of cross-striations, sarcoplasmic vacuolation and fragmentation, and nuclear swelling or pyknosis (myocardial degeneration and necrosis). Occasional cardiomyocyte abortive regeneration is suspected based on the presence of some giant cells with mildly basophilic cytoplasm and clusters of large euchromatic nuclei. Lost myocardium is often replaced by fibrous connective tissue.
Additional not shown histologic findings in both pigs consisted of centrilobular hepatocellular degeneration and necrosis, erythroid hyperplasia in the bone marrow, and extramedullary hematopoiesis in multiple tissues suggestive of anemia, as well as subacute pulmonary edema and congestion consistent with subclinical cardiac insufficiency.

Contributor's Morphologic Diagnoses:
Heart: Myocarditis, lymphoplasmacytic and histiocytic, multifocal to coalescing, chronic, severe, with fibrosis

Contributor's Comment: Porcine circovirus type 2 (PCV2) is a small, non-enveloped DNA virus. It was first described as the causative agent of postweaning multisystemic wasting syndrome (PMWS), a disease with a wide variety of clinical signs and lesions in multiple organ systems. Classically, PMWS is characterized by

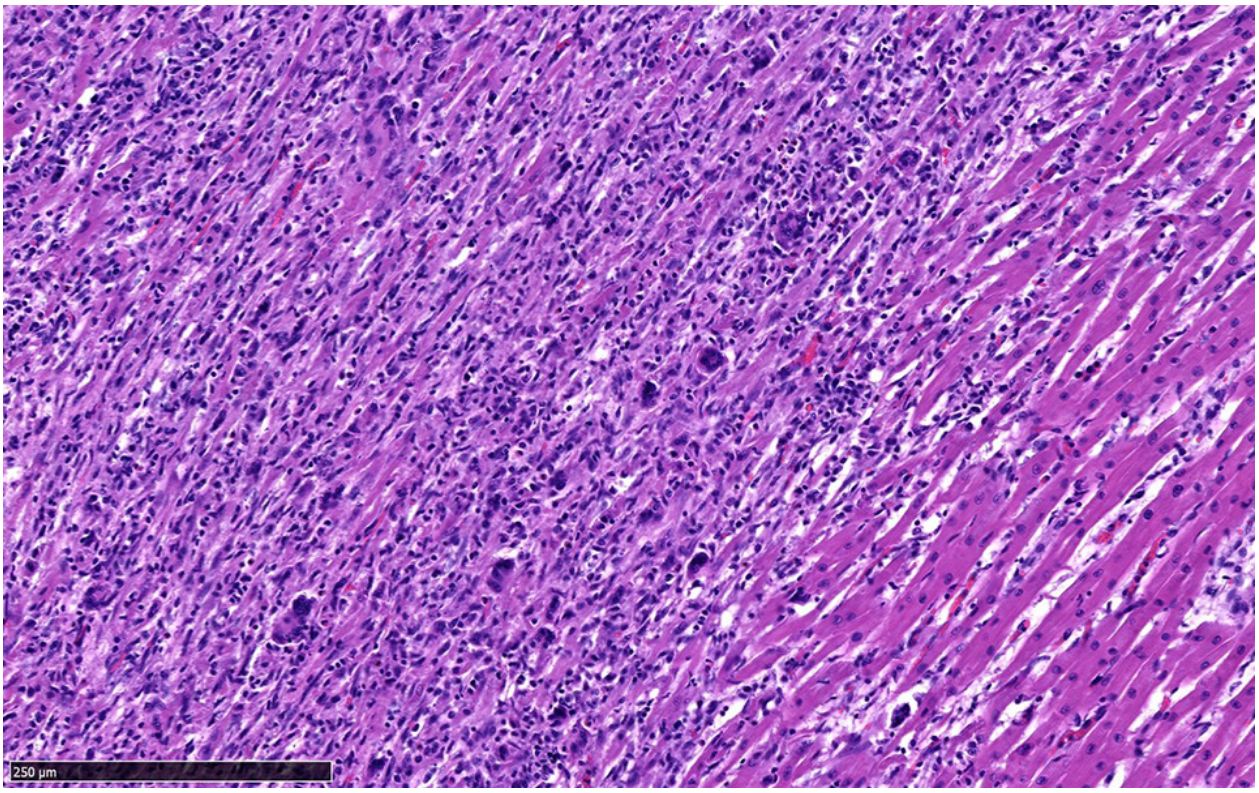


Heart, piglet. Cardiomyocytes exhibit marked variation in fiber size, fragmentation, and are separated by loosely arranged collagen fibers. (HE, 200X)

dyspnea, diarrhea, pallor, and jaundice with severe lymphoid depletion in pigs between 5 and 8 weeks of age. In addition to causing PMWS, PCV2-infection has been associated with an array of conditions, including respiratory and enteric diseases, porcine dermatitis and nephropathy syndrome (PDNS), and reproductive failure.[7]

The submitted case is an example of PCV2-associated myocarditis. PCV-associated myocarditis is most commonly seen in aborted, mummified, and weak-born fetuses that were infected during gestation.[6,7,11] However, chronic myocarditis has also rarely been reported in piglets infected at 1-3 days of age,[2,3,4,8] and we believe that this is what occurred in the current case. In previous reports, cardiac lesions tended to be more severe in animals co-infected with porcine parvovirus.[2,8] We did not test for parvovirus; we can therefore not rule it out as a contributing factor to disease in our case. Additional causes of myocarditis in swine including bacteria (e.g. *Streptococcus suis* and other bacteria associated with septicemia) and other viruses (e.g. PRRSV and EMCV) were ruled out by negative test results.[5]

In the current case, tissues routinely tested for the presence of PCV2 antigen (multiple lymph nodes, spleen, and lung) yielded negative results by immunofluorescence. These tissues also had no inflammatory lesions on histology. The heart, however, was positive by immunohistochemistry for PCV2 antigen, and this correlated well with the presence of the cardiac lesions. These findings illustrate the importance of being able to recognize potential PCV2-associated lesions in order to then submitting the affected tissues to PCV2 antigen testing. Similarly, only affected tissues should be used for detection of PCV2 by PCR, since the mere presence of PCV2 DNA in the absence



Heart, piglet. Numerous multinucleated giant cells are scattered throughout the areas of inflammation. (HE, 200X)

of lesions is not sufficient for a definitive disease diagnosis.[7]

Contributing Institution:
Louisiana Animal Disease Diagnostic Laboratory
http://www1.vetmed.lsu.edu/laddl/index.html

JPC Diagnosis: Heart: Pancarditis, lymphoplasmacytic, histiocytic, and necrotizing, multifocal to coalescing, severe with multinucleated giant cells.

JPC Comment: The family Circoviridae is a rapidly expanding family of small, non-enveloped, circular, single-stranded, DNA viruses. Although chicken anemia agent, an early addition, has now been booted to the Anelloviridae, circoviruses have been identified in swine, a wide variety of bird species (including psittacine beak and feather disease), dogs, bats, mink, pandas, hermit crabs, and insects.[12]

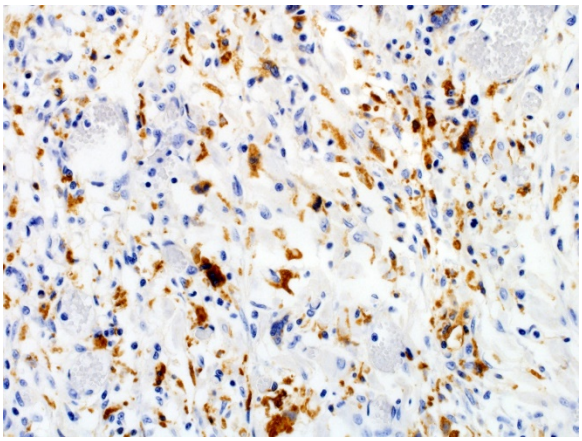
A number of interesting syndromes have been ascribed to PCV-associated diseases (PCVAD). Previous WSC submissions falling into this category include a previous case of PCV-associated myocarditis (which also has an excellent comment on PCVAD and post-weaning multisystemic wasting syndrome (PMWS) – WSC 2016, Conf 8, Case 2), cerebellar vasculitis and necrosis (WSC 2014, Conf 21, Case 4), granulomatous lymphadenitis and hepatitis (WSC 2013 Conf 25 Case1) and tubulointerstitial nephritis (WSC 2011, Conference 7, Case 3.

Lymphohistiocytic myocarditis is considered a hallmark lesion in the prenatal infection of pigs with porcine circovirus-2 (PCV-2). Experimental infection of swine fetuses at

day 52 is associated with myocardial viral tropism versus later infection (after day 92) which is generally associated with lymphoid tropism. [1] In affected fetuses, myofiber degeneration, necrosis, and loss and replacement by collagen and mineral, as well as inflammatory changes similar to those seen in this case and considered hallmark signs. Classic PCV-2-associated botryoid intranuclear inclusions (not seen in this section) may also be present. Cardiomegaly (predominantly right sided and associated with pericardial effusion) has been reported in piglets aged 4-7 weeks in heart failure.[7]

Vasculitis is also a well-described lesion in association with PCV-2 infection. Porcine dermatitis and nephritis syndrome is characterized by a necrotizing and neutrophilic vasculitis of arterioles and capillaries of the skin and kidney (to include glomerular capillaries). Fibrinoid necrosis of septal capillaries within the lung and resultant focal alveolar hemorrhage and edema has been widely reported in the US and Europe[7]. Finally, cases of myocarditis in piglets are often associated with lymphohistiocytic coronary arteritis and periarteritis.[7,8]

Within the last several years, a novel swine circovirus (PCV-3) has been identified by independent groups that has been identified with cardiac and multisystemic infection.[10,11] In one study, both individual swine and a 2% of a thousand-animal herd displayed common lesions of lymphohistiocytic myocarditis and cardiac arteriolitis. Metagenomic testing identified a new circovirus showing 55% and 33% with bat circovirus and porcine circoviruses.[11] A separate group in Kansas identified a similar virus, also identified as PCV-3 in sows that died acutely with lesions consistent with porcine dermatitis and nephritis syndrome.[10]



Heart, piglet. A variety of cells including cardiomyocytes, macrophages, and multinucleated giant cells stain positively for PCV-2 antigen. (anti-PCV2, 400X)

The moderator reviewed circoviruses in general, in pigs, dogs, and bears among other species, and a differential diagnosis for the lesion in this suckling piglet, including porcine circovirus-3, parvovirus, and aphthovirus was discussed.

References:

1. Cushing T, Steffen D, Duhamel GE. Pathology in Practice. *JAVMA* 2013; 242(3):317-319.
2. Ellis J, Krakowka S, Lairmore M, et al. Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. *Journal of Veterinary Diagnostic Investigation* 1999;11:3-14.
3. Hirai T, Nunoya T, Ihara T, Kusanagi K, Kato T, Shibuya K. Acute hepatitis in a piglet experimentally inoculated with tissue homogenates from pigs with postweaning multisystemic wasting syndrome. *Journal of Veterinary Medical Science* 2003;65:1041-1045
4. Kennedy S, Moffet D, McNeilly F, et al. Reproduction of lesions

of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus 2 alone or in combination with porcine parvovirus. *Journal of Comparative Pathology* 2000;122:9-24.

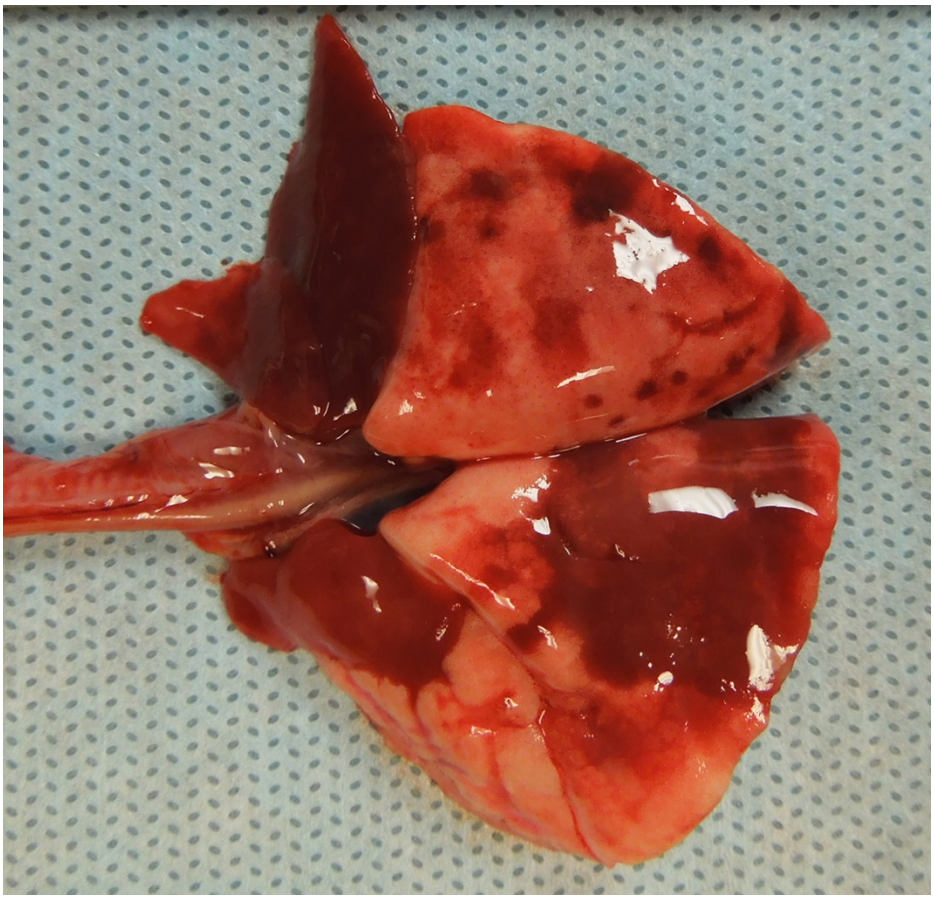
5. Loynachan AT. 2012. Cardiovascular and Hematopoietic Systems – Diseases of the Myocardium. In Zimmerman JJ, Karriker LA, Ramirez A, et al. (Eds.), Diseases of Swine. Ames, IA: Wiley-Blackwell. p. 192-193.
6. Mikami O, Nakajima H, Kawashima K, et al. Nonsuppurative myocarditis caused by porcine circovirus type 2 in a weak born piglet. *Journal of Veterinary Medical Science* 2005;67:735-738.
7. Opriessnig T, Langohr I. Current state of knowledge on porcine circovirus type 2-associated lesions. *Veterinary Pathology* 2013;50:23-38.
8. Opriessnig T, Janke B, Halbur P. Cardiovascular lesions in pigs naturally or experimentally infected with porcine circovirus type 2. *Journal of Comparative Pathology* 2006;134:105-110.
9. Palinski R, Pineyro P, Shang P, Yuan F, Guo R, Fang Y, Byers E, Hause BM. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. *J Virol* 2017 91(10:e01879-16.
10. Phan TG, Giannitti F, Rossow S, Marhaler D, Knutson T, Li L, Deng X, Resende T, Vannuci F, Delwart. Detection of a novel circovirus PCV-3 in pigs with cardiac and multi-systemic inflammation. *Virol J* 2016; 13:184.
11. Sanchez R, Nauwynck H, McNeilly F, et al. Porcine circovirus 2 infection in swine foetuses inoculated at different stages of gestation. *Veterinary Microbiology* 2001;**83**:169-176.
12. Sheykhi A, Sheiki N, Charkhkar S, Brujeni GN. Detection and characterization of circovirus in canary flocks. *Avian Dis* 2018; 62:137-142.

CASE III: D12-44205-1A or B UMN VDL (JPC 4032592).

Signalment: One-month-old, intact male, white Hartley guinea pig (*Cavia porcellus*).

History: The guinea pig, purchased and shipped from a source colony, was placed in quarantine for an acclimation period of 7 days. The guinea pig was released from quarantine on day 7, placed on study day 9 involving an induction dose (6 intradermal injections of 0.1 ml of test article extract in normal saline, normal saline and/or Freund's Complete Adjuvant). The guinea pig lost body condition and exhibited progressive respiratory distress over days 10 and 11. The guinea pig was found dead on day 12.

Gross Pathology: The guinea pig was in good nutritional condition with mild postmortem autolysis. The thoracic cavity contained approximately 0.5 ml of watery, red, clear fluid. The left and right cranial lung lobes were diffusely dark red, heavy and wet. Sections of the left and right cranial lung lobes sank in 10% neutral buffered formalin.

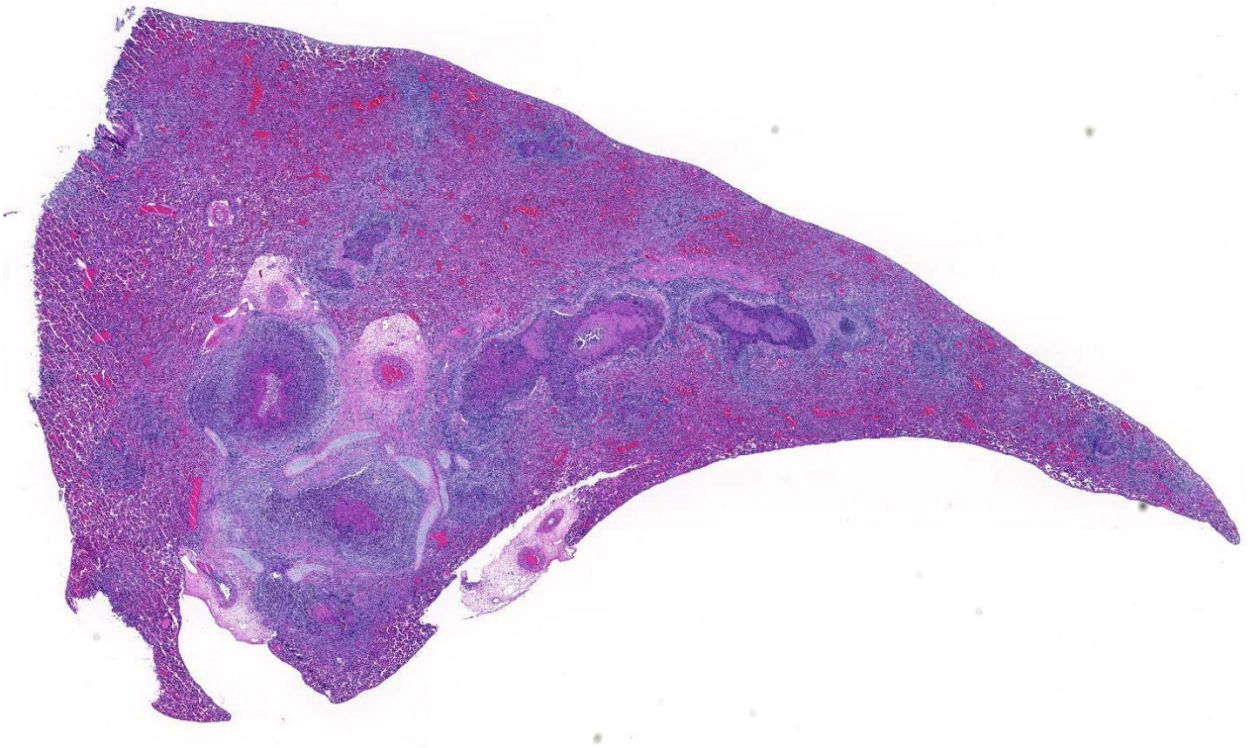


Lung, guinea pig: The right and left cranial lung lobes, right middle lung lobe, right accessory lung lobe were diffusely dark red and firm. The left and right caudal lung lobes contained multifocal to coalescing, dark red, firm, irregular, smooth, flat foci. (Photo courtesy of Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu)

The right middle and right accessory lung lobes were diffusely dark red and firm. The left and right caudal lung lobes contained multifocal to coalescing, dark red, firm, irregular, smooth, flat foci.

Laboratory results: Bacteriology: Lung - No bacteria isolated
Guinea pig adenovirus quantitative real-time polymerase chain reaction (qPCR) testing and aerobic culture of lung were performed at Charles River Research Animal Diagnostic Services. The lung sample was positive for guinea pig adenovirus via qPCR. Aerobic culture of the left cranial lung lobe yielded rare colonies of *Escherichia coli*.

Microscopic Description: Multifocally, comprising 60 – 70% of the tissue section, within the bronchi, bronchioles and alveoli, there are infiltrates of numerous degenerate and nondegenerate neutrophils, macrophages, lymphocytes, few plasma cells and extravasated erythrocytes. The lumina of the bronchi and bronchioles are frequently mildly to moderately ectatic, containing large amounts of cellular and karyorrhectic debris, extravasated erythrocytes, fibrin and sloughed degenerate to necrotic respiratory epithelial cells that occasionally contain a single, large, 5 – 15 um diameter basophilic intranuclear inclusion body. Multifocally bronchi and bronchioles are lined by flattened, attenuated, degenerate to necrotic epithelium that frequently form epithelial syncytia or are multinucleated. Multifocally alveolar septa are mildly to moderately expanded by lymphocytes, mononuclear cells, type II pneumocytes and congested blood vessels. Multifocally interstitium is mildly to moderately expanded by neutrophils, lymphocytes and plasma cells as well as edema. The tunica media of multiple small to medium caliber vessels and muscularis of



Lung; guinea pig. Multifocally bronchi, bronchioles and alveoli are filled with necrotic material and a cellular infiltrate which extends into the surrounding parenchyma. (HE, 14X)

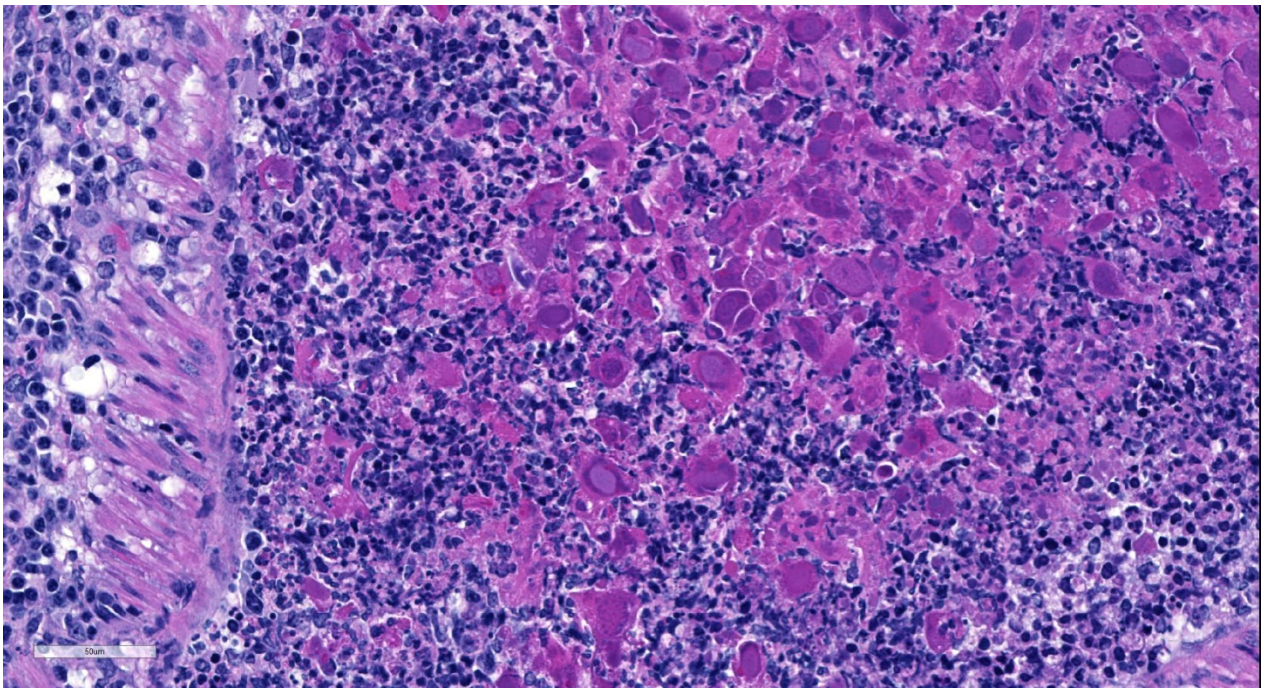
the bronchi and bronchioles exhibits vacuolar degeneration of the smooth muscle.

Immunohistochemistry for general adenovirus on tissue sections of lung were prepared by the Veterinary Medical Diagnostic Laboratory of the University of Missouri. Multifocally lumina of bronchioles contain numerous red to dark red, strongly immunopositive adenovirus particles, both free within the lumen admixed with cellular debris and within nuclei and cytoplasm of sloughed necrotic epithelial cells.

Electron microscopy was performed at the University of Minnesota Veterinary Diagnostic Laboratory. Bronchiolar lumina contain necrotic cells with markedly enlarged nuclei exhibiting discontinuous nuclear membranes, clumping of chromatin and lacking a clear distinction between the nucleus and the cytoplasm. The necrotic cells contain numerous loosely arranged, nonenveloped, round to icosahedral, 70 – 90 nm diameter, variably electron dense, icosahedral viral particles consistent with adenovirus.

Contributor's Morphologic Diagnoses: Lung, bronchopneumonia, necrosuppurative, with intraepithelial intranuclear inclusion bodies (adenovirus), multifocal, marked, subacute.

Contributor's Comment: Adenoviruses are non-enveloped, linear double stranded DNA viruses with icosahedral symmetry. Adenoviruses infect a wide variety of animals with clinical signs ranging from subclinical to enteric or respiratory manifestations. Guinea pig adenovirus (GpAV) has been classified as a distinct serotype within the genus Mastadenovirus.



Lung; guinea pig. Numerous necrotic bronchiolar epithelial cells contain a single large basophilic intranuclear inclusion. (HE 400X)

GpAV has the highest level of homology with other animal mastadenoviruses (mammalian adenoviruses) and human subgroups A, C and F.[5,14] Adenoviruses derived from birds (Aviadenovirus, Atadenovirus), frogs (Siadenovirus), fish (Ichtadenovirus) and some reptiles (Atadenovirus) are serologically distinct from Mastadenoviruses.[9]

Guinea pig adenovirus was first reported in 1981 in guinea pigs from 2 commercial breeders and a closed colony, as the cause of necrotizing bronchitis and bronchiolitis with low morbidity and high mortality.[11] The adenovirus observed in this outbreak was described as viral particles of 58 – 72 nm diameter, in a hexagonal crystalline array, observed as large, basophilic, intranuclear inclusion bodies by light microscopy. Additional outbreaks of GpAV in guinea pigs were characterized by necrotizing bronchitis and bronchiolitis with similar prominent basophilic intranuclear inclusions in sloughed, necrotic epithelial cells.[1] Inclusions contained typical adenoviral particles of 68 – 72 nm diameter, arranged individually or in crystalline arrays. GpAV infection was experimentally reproduced via intranasal inoculation of newborn guinea pigs and was found to be noninfectious in additional mammalian species of hamsters and rats.[7,10] Subclinical infection has been reported, but the asymptomatic animals were found to have microscopic lesions of minimal bronchial epithelial necrosis with typical large basophilic intranuclear inclusions.[4]

Bronchopneumonia due to GpAV is typically of low morbidity with up to 100% mortality. Clinically affected animals are often young, but infections have also been reported in adult guinea pigs in breeding colonies.[5,6,13] Infection of guinea pigs often presents as sudden death or death after a short period of malaise. On gross necropsy, the cranial lung lobes are usually consolidated or diffusely red, while the caudal lung lobes show

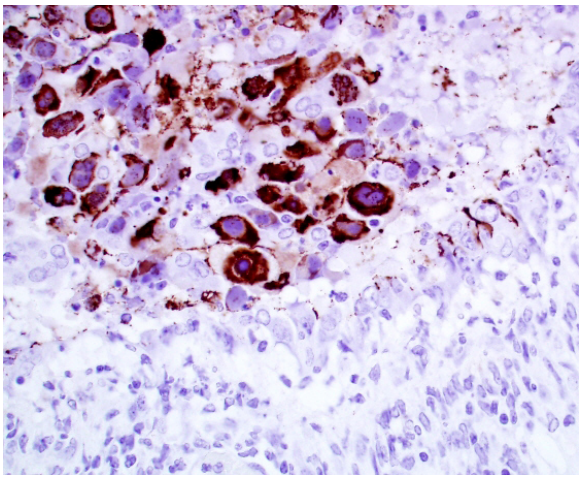
multifocal consolidation. Histopathology findings are characterized by a necrotizing bronchitis and bronchiolitis with sloughing of necrotic epithelial cells into the airways, often resulting in occlusion of the affected airways with necrotic cells admixed with cell debris, leukocytes and fibrin. The necrotic epithelial cells often exhibit karyomegaly with extremely large or bizarre basophilic, round to oval, 7 – 15 um diameter, intranuclear inclusion bodies.[1,7,11]
Diagnosis of GpAV infection can be confirmed via immunohistochemistry, electron microscopy, serology or polymerase chain reaction. Other differentials for pneumonia in guinea pigs include parainfluenza virus, cytomegalovirus and bacteria such as *Bordetella bronchiseptica*, *Streptococcus* species, *Staphylococcus* species and *Pseudomonas aeruginosa*.[13]

Contributing Institution:
Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu

JPC Diagnosis: Lung: Bronchitis and bronchiolitis, necrotizing, diffuse, severe with diffuse lymphohistiocytic peribronchiolitis and atelectasis, and numerous karyomegalic viral inclusions

JPC Comment: Adenoviruses are common viruses in a wide range of animal species, and display tropism for a wide range of tissues – endothelium (dog, deer cattle), liver (chicken, primates), and in immunosuppressed animals (and humans) , a vast array of cell types may be targeted.

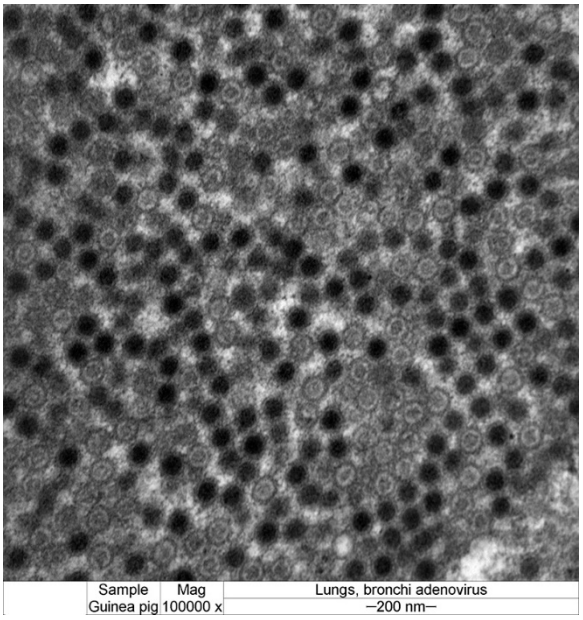
A number of pneumotropic adenoviruses may result in a similar clinical and histologic picture, although immunosuppression may be required for lesion development. Canine adenovirus-2 may result in pulmonary damage in (usually immunosuppressed, often



Lung: guinea pig. Cellular debris and necrotic epithelial cells within bronchiolar lumen exhibit strong immunopositivity to adenovirus. (Photo courtesy of Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu)

by canine distemper virus) individuals. In experimental infections in young beagles, bronchiolitis obliterans was seen 3-4 weeks following infection[2] (although long-term followup was not conducted to see if the lesions resolved.) Bovine adenovirus-3 will also result in a severe necrotizing bronchiolitis with viral intranuclear inclusions in calves administered dexamethasone.[12]

Pneumotropic adenoviral infections are well-known in primate species. In 1953 adenoviruses were first isolated by Rowe who was studying the growth of poliovirus in adenoidal tissue. Today, over 60 human adenovirus serotypes have been identified. Disease in humans varies with age, immune status and population characteristics. Adenoviral pneumonia associated with serotypes 3,7,14, 21, and 55 have been associated with potentially fatal outcomes, and infants, immunosuppressed individuals, and the elderly are especially hard hit. Extrapulmonary complications include meningoencephalitis, hepatitis myocarditis, nephritis, neutropenia, and DIC.[8]



Lung; guinea pig. Within necrotic bronchiolar epithelial cells are numerous 70 – 90 nm diameter, variably electron dense, round to icosahedral viral particles. (Photo courtesy of Veterinary Diagnostic Laboratory, University of Minnesota, www.vdl@umn.edu

An outbreak in a national primate center in 2009 in a closed colony of titi monkeys (*Callicebus* sp.). 23 of 65 developed upper respiratory symptoms which progressed to fulminant pneumonia and hepatitis; 19 of these animals died. A novel, highly divergent adenovirus (TMAdV) was isolated, sharing only <57% nucleotide identity with other known adenoviruses. The virus was also isolated from a researcher who demonstrated respiratory symptoms for 4 weeks, and a close family member who had never been to the primate colony.[15]

In 1997, an outbreak of adenoviral pneumonia resulted in respiratory illness in 9 infant olive baboons, with two fatalities. Six untypeable adenoviruses were isolated from these animals during the course of the illness; one novel adenovirus (BaAdV-3) was determined to the cause of the disease.[3]

Conference attendees noted the presence of abundant viral antigen within the cytoplasm of epithelial cells within the necrotic bronchioles, with relative sparing of nuclei containing karyomegalic inclusions in the image of the immunohistochemical stain provided by the contributor (Fig 2-4). The significance of this change was not apparent.

References:

1. Brennecke LH, Dreier TM, Stokes WS. Naturally occurring virus-associated respiratory disease in two guinea pigs. 1983;**20**:488-491.
2. Castleman WL. Bronchiolitis obliterans and pneumonia induced in young dogs by experimental adenovirus infection. *Am J Pathol* 1985; 119(3):495-504.
3. Chiu CY, Yagi S, Lu X, Yu G, Chen EC, Liu M, Dick EJ, Carey KD, Erd DD, Leland MM, Patterson JL. A novel adenovirus species associated with an acute respiratory outbreak in a baboon colony and evidence of coincident human infection. *mBio* 2013; 4(2):e00084-13.
4. Crippa L, Giusti AM, Sironi G, et al. Asymptomatic adenoviral respiratory tract infection in guinea pigs. 1997:**47**(2):197-199.
5. Feldman SH, Sikes R, Eckhoff G. Comparison of the deduced amino acid sequence of guinea pig adenovirus hexon protein with that of other Mastadenoviruses. Comp Med. 2001;**51**(2):120-126.
6. Harris IE, Portas BH, Goydich W. Adenoviral bronchopneumonia of guinea pigs. Aust Vet Journal. 1985;**62**(9):317-318.
7. Kaup, FJ, Naumann S, I Kunstyr, et al. Experimental viral pneumonia in guinea pigs: An ultrastructural study. 1984;**21**:521-527.
8. Khanal S, Ghimire P, Dhamoon AS. The repertoire of adenovirus in

human disease: the innocuous to the deadly. *Biomedicines* 2018; 6:30 doi: 10.3390/biomedicines6010030
9. King, AMQ, Adams MJ, Carstens EB, et al. Virus Taxonomy. London, UK: International Committee on Taxonomy of Viruses, Elsevier Inc; 2012.
10. Kunstyr I, Maess J, Naumann S, et al. Adenovirus pneumonia in guinea pigs: an experimental reproduction of the disease. 1984;**18**:55-60.
11. Naumann S, Kunstyr I, Langer I, et al. Lethal pneumonia in guinea pigs associated with a virus. Lab Animals. 1981;**15**:235-242.
12. Narita M, Yamada M, Tsuboi T, Kawashima. Bovine adenovirus type 3 pneumonia in dexamethoasone-treated calves. *Vet Pathol* 40:128-135.
13. Percy DH, Barthold SW. Pathology of Laboratory Rodents and Rabbits, Third Edition. Ames, Iowa: Blackwell Publishing Professional; 2007.
14. Pring-Akerblom P, Blazek K, Schramlova J, et al. Polymerase chain reaction for detection of guinea pig adenovirus. J Vet Diagn Invest. 1997;**9**:232-236.
15. Yu G, Yagi S, Carrion R, Chen EC, Liu M, Brasky KM, Lanford RD, Kelly KR, Bales KL, Schnurr DP, Canfield DR, Patterson JG, Chiu CY. Experimental cross-species infection of common marmosets by titi monkey adenovirus. PLoS One 2013; 8(7):e68558

CASE IV: 14A310 (JPC 4083744).

Signalment: 13.05 yr, female, Indian rhesus monkey, *Macaca mulatta*

History: Presented from the field cage in dystocia and an infant was successfully delivered by C-section but the dam died in recovery.

Gross Pathology: Hemorrhagic meninges primarily over the cerebellum, enterocolitis, hemorrhage in both adrenals.

Laboratory results: I-stat Results:

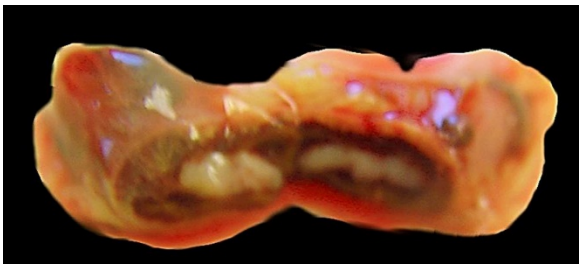
Na	144 meq/L	Ph	7.152
K	3.5 meq/L	PCO2	47.3 mmHg
Cl	111meq/L	HCO3	16.6 mmol/L
TCO2	18 mmol/L	BEECF	12 mmol/L
BUN	18 mg/dL	ANGAP	20 mmol/L

Bacterial culture of the meninges: *Streptococcus pneumoniae*

Microscopic Description:
In the cortex of the adrenal, primarily the zona fasiculata is severely congested and hemorrhagic with loss of cortical cell cords and pooling of RBC between surviving the fibrovascular supporting framework. Adjacent cortical cells are swollen and rare capillaries contain minimal aggregates of neutrophils and mononuclear leukocytes.

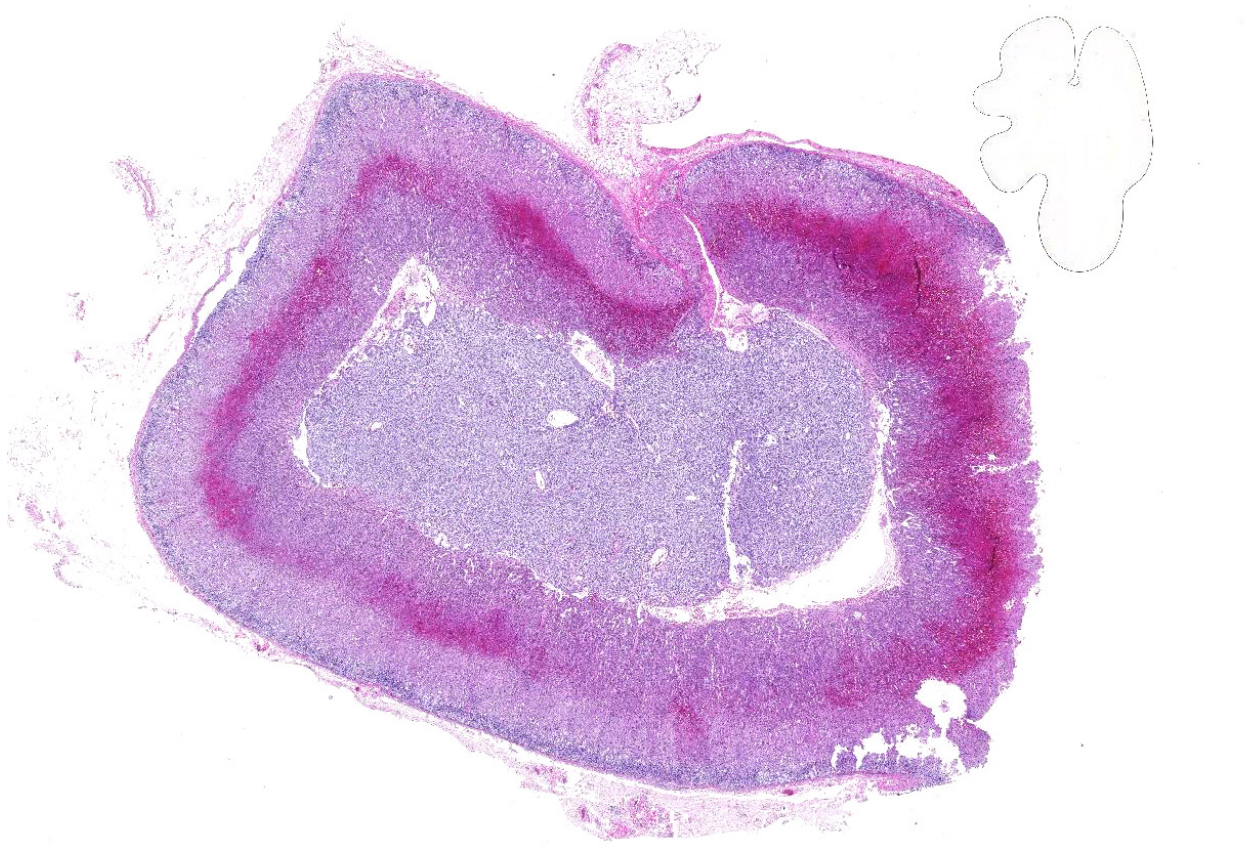
Contributor's Morphologic Diagnoses: Acute severe adrenal cortical hemorrhage with mild degeneration and minimal necrosis, rhesus macaque

Contributor's Comment: The cesarean section for this animal was complicated by an



Adrenal gland, rhesus macaque. Hemorrhage was present within the adrenal cortex. (Photo courtesy of Tulane National Primate Research Center, Department of Comparative Pathology, 18703 Three Rivers Rd, Covington, LA 70433, http://tulane.edu/tnprc/)

abscess in the midbrain and meningitis from which *Streptococcus pneumoniae* was cultured. Meningococcus in humans is often a cause of acute adrenocortical insufficiency[2] although we see adrenal hemorrhage infrequently in our primate colony (62 cases in last 30 years). Worldwide, cerebral tuberculosis is the most often associated with pituitary and adrenal dysfunction[3]. The Waterhouse-Friderichsen syndrome in humans is described as a rare complication of bacteremia due to pneumococci, staphylococci, *Neisseria meningitides*, *Pseudomonas aeruginosa*, or *Haemophilus influenza* and also cytomegalovirus infection that results in systemic hemorrhages including purpura of the skin, with hemorrhage of the adrenal, serous membranes and other organs consistent with development of disseminated intravascular coagulation (DIC).[1,4,8] The basis for the hemorrhage, suggested to originate from the venous sinusoids of the medulla and suffuse into the cortex could be due to direct bacterial seeding of the endothelium, DIC, endotoxin-induced vasculitis or hypersensitivity vascultitis.[2]



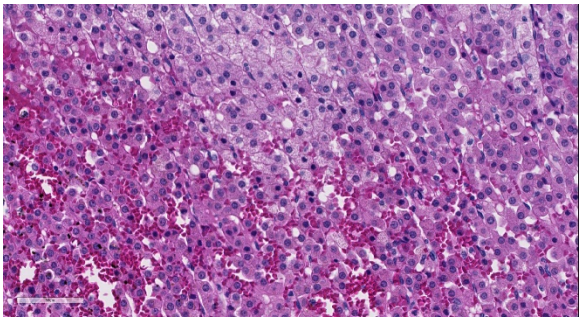
Adrenal gland, rhesus macaque. There is multifocal to coalescing hemorrhage within the deep zona fascicularis and superficial zona reticularis. (HE, 10X)

Contributing Institution:
Tulane National Primate Research Center
Department of Comparative Pathology
18703 Three Rivers Rd
Covington, LA 70433
http://tulane.edu/tnprc/

JPC Diagnosis: Adrenal gland, zona fasciculata and reticularis: Necrosis, multifocal, marked, with hemorrhage.

JPC Comment: The Waterhouse-Friderichsen syndrome (WFS) is a well-known entity (named for English physician Rupert Waterhouse (1873-1958) and Danish pediatrician Carl Friderichsen (1886-1979) in humans, non-human primates, and several other species. It was first described in 1911 as adrenal hemorrhage and subsequent adrenal crisis related to sepsis.[8]

In humans, this syndrome is usually associated with fulminant sepsis associated with pneumococcal or meningococcal bacteremia (with *Streptococcus pneumoniae* and *Neisseria meningitidis* the most commonly cultured bacilli, respectively) often in patients with splenic disorder or asplenia. Other reported bacterial causes include *N. gonorrhoeae*, *Pseudomonas aeruginosa*, *E. coli*, *Hemophilus influenzae*, and *S. aureus*.[6] It is often associated with hypotensive shock, disseminated intravascular coagulation, purpura fulminans, and adrenal gland failure. In affected individuals, adrenal hemorrhage may vary from microscopic hemorrhage at the corticomedullary junction to massive, grossly identifiable hemorrhage effacing the entire cortex. Several anatomic factors facilitate the hemorrhage: high rate of blood flow, contribution of several arteries to a single organ-wide capillary plexus, and a single large central vein draining this organ.[8] WFS in humans is an emergency situation, clinically characterized by rapidly falling levels of cortisol – high doses of corticosteroids are usually administered as well as antibiotics directed at the bacterial agent.[5] Mortality, even in treated cases, often exceeds 50%. While historically a postmortem diagnosis, the adrenal hemorrhage that characterizes WFS in humans may now be identified by ultrasonography and CT scans, increasing the odds of successful treatment.[5]



Adrenal gland, rhesus macaque. Within areas of hemorrhage in the zona reticularis, cortical cells are mildly shrunken, condensed, eosinophilic, and nuclei are hyperchromatic, but necrotic cells are rare. (HE, 400X)

In 2009, Hukkanen et al.[7] published a retrospective of five cases of systemic inflammatory response syndrome (SIRS) including the Waterhouse-Friedrichsen syndrome, acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC) and multiple organ dysfunction in five cases including both pigtailed macaques and olive baboons. In these animals, predisposing factors were similar to those in humans – major surgery, obstetric complications, and infection. The syndrome is thought to be seen in "high-responders" – subsets of nonhuman primates (and humans) which may produce 10 to 100 fold greater cytokines (IL-1b, IL-6, and IL-8 as well as TnF-alpha in response to infectious stimuli.[7]

References:

1. Adem PV, Montgomery CP, Husain AN, Koogler, TK, Arangelovich V, Humilier M, Boyle-Vavra S, Daum RS. Staphylococcus aureus sepsis and the Waterhouse-Friderichsen syndrome in children. N Engl J Med 353:1245-2451, 2005.

2. Cotran RS, Kumar V, Collins T. (1999). Robins Pathologic Basis of Disease (6^{th} ed) p 1160.

3. Dhanwal DK, Kumar S, Vyas A, Saxena A. Hypothalamic pituitary dysfunction in acute nonmycobacterial infections of central nervous system. Indian J Endocrinol Metab 15 (Suppl 3): S233-S237, 2011.

4. Emori K, Takeuchi N, Soneda J. A case of Waterhouse-Friderichsen syndrome resulting from an invasive pneumococcal infection in a patient with a hypoplastic spleen. *Case Reports in Crit Care* 2016; art. ID 4708086.

5. Fox B. Disseminated intravascular coagulation and the Waterhouse-Friderichsen syndrome. Arch Dis Childhood 46:680-685, 1971.

6. Hale AJ, LaSalvia M, Kirby JE, Kimball A, Baden R. Fatal purpura fulminancs and Waterhouse-Friderichsen syndrome from fulminant Strptococcus pneumoniae sepsis in an asplenic young adult. *IDCases* 2016: 6:1-4.

7. Hukkanen RR, Liggitt HD, Murnane RD, Frevert CW. Systemic inflammatory response syndrome in non-human primates culminating in multiple organ failure, acute lung injury, and disseminated intravascular coagulation. *Toxicol Pathol* 2009; 37(6):799-904.

8. Verzeletti A, Bonfanti C, Leide A, Assalini E, De Francesco MA, Piccineli G, De Ferrari. Streptococcus pneumoniae detection long time after death in a fatal case of Waterhouse-Friderichsen syndrome. *Am J Forens Med* 2017:38(1)18-19.

9. Vincentelli C, Molina EG, Robinson MJ. Fatal pneumococcal Waterhouse-Friderichsen syndrome in a vaccinated adult with congenital asplenia. Amer J Emerg Med 27:751.e3-751.e5, 2009.