Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

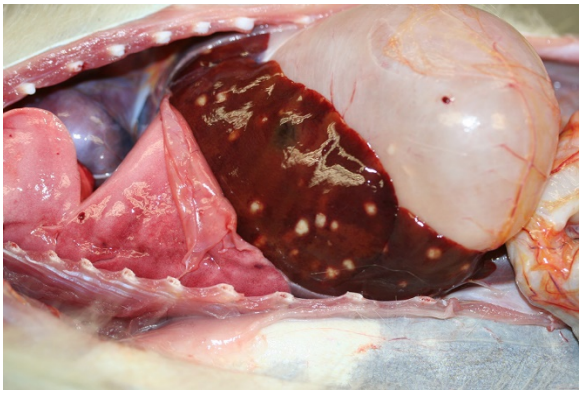
C o n f e r e n c e 1

22 August 2018

CASE I: RUSVM-1 (JPC 4020132).

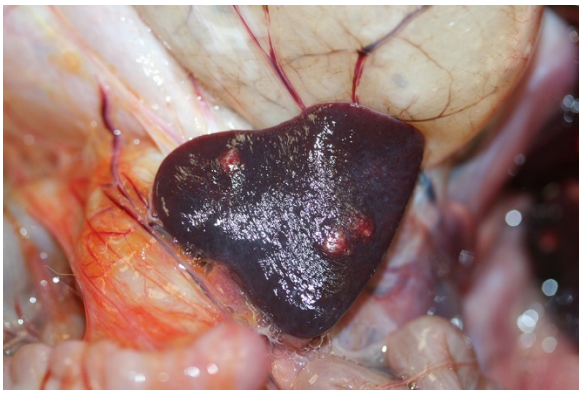
Signalment: 4-year-old, male, African green monkey (*Chlorocebus aethiops sabaeus*)

History: This monkey was euthanized during a recent outbreak of acutely fatal enteric disease in a colony of captive African green monkeys (*Chlorocebus aethiops sabaeus*) in the island of St. Kitts, West Indies. On clinical examination, the monkey had bloody diarrhea, was pyrexic and severely dehydrated. Previous to this case submission, multiple monkeys in the same enclosure had died after a short period of illness characterized by depression, diarrhea and dehydration, or had been found dead in the enclosure. Necropsies performed by the referring veterinarian revealed multifocal, variably-sized, white foci throughout the splenic and hepatic parenchyma. All affected monkeys were part of a large, breeding population maintained by the Behavioral Sciences Foundation, Estridge Estate, St. Kitts, West Indies. Maintenance, testing and all procedures carried out in this facility are approved by the Animal Care Committee of the Behavioral Sciences Foundation, acting under the auspices of the Canadian Council on Animal Care.



Liver, African green monkey. There are multifocal to coalescing variably sized 2-8mm white foci scattered throughout the liver. (Photo courtesy of: Department of Pathobiology, Ross University School of Veterinary Medicine, St. Kitts, West Indies, www.rossu.edu)

Gross Pathology: At necropsy, the monkey was in poor body condition (BCS 2/5), with scant fat reserves and muscle mass. The carcass was moderately dehydrated and the perineum was stained with blood-tinged feces. Multifocal areas of petechiation were present throughout the subcutaneous tissue, and mucous membranes were diffusely pale. The liver and the spleen had multifocal, variably-sized (2 mm-8mm) white foci. The spleen was slightly enlarged. On cut surface, the foci were moderately firm and had a caseated appearance (abscessation /necrosis). The stomach was markedly distended with gas. The mucosa of the small intestine was

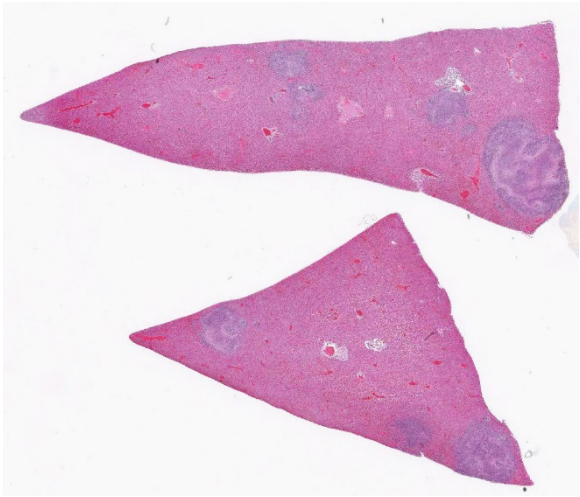


Spleen, African green monkey. There are similar foci scattered throughout the spleen. (Photo courtesy of: Department of Pathobiology, Ross University School of Veterinary Medicine, St. Kitts, West Indies, www.rossu.edu)

diffusely reddened. The cecum and the colon contained blood-tinged mucus and there were numerous 1-2 cm white, slender nematodes present (*Trichuris* sp.). The mesenteric lymph nodes were moderately enlarged and slightly edematous. No other gross lesions were present elsewhere.

Laboratory results: Bacteria recovered from hepatic and splenic swabs collected during necropsy examination were identified by routine culture and biochemical methods as gram-negative, cytochrome oxidase-negative rods. MicroID kits identified the isolates as *Yersinia* spp. Further molecular diagnosis provided by amplification and sequencing of the 16S SSU rRNA gene confirmed the isolates as *Y. enterocolitica*. Leukograms of affected monkeys indicated a leukocytosis most commonly composed of monocytosis, basophilia, lymphocytosis (combined increase in lymphocytes and large granular lymphocytes), an occasional mature neutrophilia, and rarely a left shift. Monocytes were commonly vacuolated. Lymphocytes commonly had increased amounts of pale blue glassy cytoplasm, an occasional reniform nucleus, and more open chromatin. Large granular lymphocytes were commonly larger than a neutrophil with variable numbers of granules and reniform to amoeboid nuclei. Neutrophils commonly had open chromatin and were pale staining. Döhle bodies, cytoplasmic basophilia, and vacuolation (toxic changes) were not common. Many ruptured cells ("basket cells") were observed. Increased PCV was uncommon, and red cell abnormalities included schistocytes and microcytes; plasma was often pink-tinged. Platelet clumping was common. The biochemical profile occasionally indicated cholestasis, less commonly, hepatocellular damage, and infrequent increases in UN and phosphorus.

Microscopic Description: Sections of the liver and the spleen reveal random, multifocal to coalescing areas of liquefactive necrosis characterized by extensive loss of normal tissue architecture and cellular detail. Areas of necrosis consisted of a central core of pale eosinophilic and karyorrhectic cellular debris, degenerate neutrophils and a pale, eosinophilic, fibrillar material (fibrin) associated with variably-sized gram-negative colonies (up to 200µm in diameter) of coccobacilli. Numerous macrophages, and



Liver, African green monkey. There are multifocal to coalescing areas of lytic necrosis scattered throughout the two sections of liver. (HE, 5X).

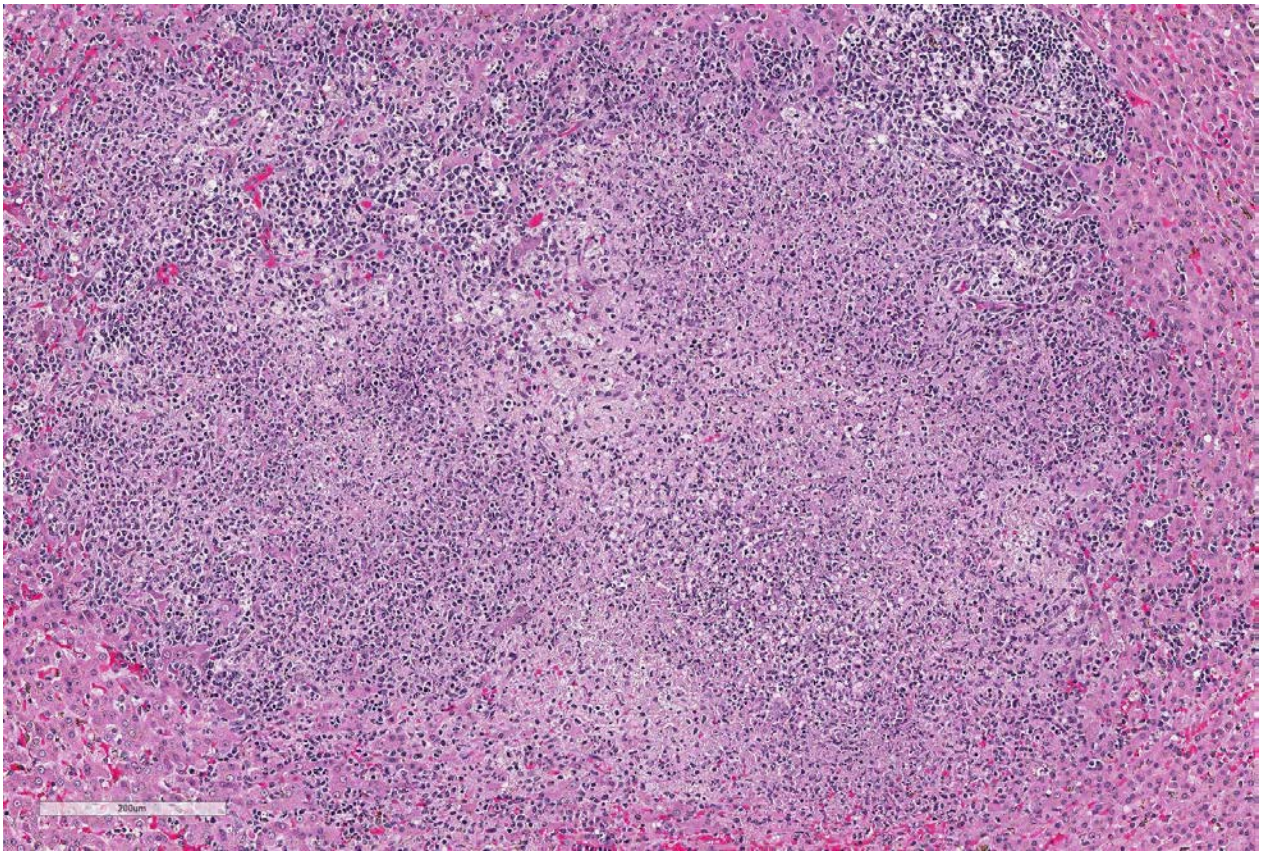
lesser numbers of lymphocytes and plasma cells surrounded the areas of necrosis. Additional microscopic lesions present in the liver include disorganization of hepatic cords, centrilobular and sinusoidal congestion and a mild periportal lymphocytic and plasmacytic hepatitis. Numerous hepatocytes and Kupffer cells contained a yellow-brown granular pigment in the cytoplasm and small bile casts were occasionally observed in canaliculi. Additional microscopic lesions present in the spleen included follicular lymphoid hyperplasia and splenic histiocytosis (please note that not all slides contain a section of the spleen). Similar microscopic lesions were present in the mesenteric lymph nodes and the small intestine (not submitted).

Extramedullary hematopoiesis is present.

Contributor's Morphologic Diagnoses: Liver and spleen: Necrotizing and suppurative hepatosplenitis, multifocal, subacute, with gram-negative bacterial colonies, African green monkey.

Contributor's Comment: *Yersinia enterocolitica* is a member of the genus *Yersinia*, a facultative anaerobic bacterium from the Enterobacteriaceae family. *Yersinia enterocolitica* is associated with diverse clinical disease manifestations in humans and a variety of animal species, including non-human primates.[6,7]

Yersiniosis is an important condition in non-human primates. Non-human primates are very sensitive to pathogenic *Yersinia*, and yersiniosis has been described in these species as an acutely fatal enteric disease, or

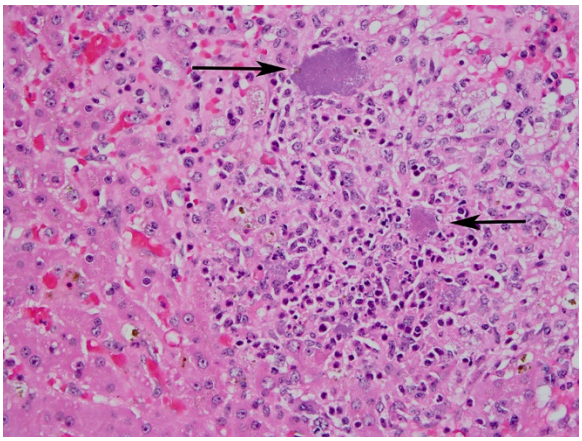


Liver, African green monkey. Higher magnification of an area of lytic necrosis. (HE, 130X)

as a chronic, debilitating, primarily enteric disease characterized by anorexia, diarrhea and weight loss.[1,3-5] Enteric yersiniosis in non-human primates is caused by *Y. pseudotuberculosis* and *Y. enterocolitica. Yersinia pestis*, the causative organism of plague, is thought to have evolved from *Y. pseudotuberculosis* and initially was considered an enteric pathogen.[6] Numerous reports have been published describing outbreaks of acute fatal yersiniosis with high morbidity and mortality in captive colonies of non-human primates.[1,3,5] Host susceptibility to *Yersinia* may differ depending on monkey species.[6] Outbreaks are characterized by variable degrees of weight loss, anorexia, diarrhea and dehydration. Clinical disease manifestation is variable, and highly dependent on bacterial strains.[5,6]

Yersinia spp. are widespread in the environment. Fecal contamination of water and the environment, as well as food contamination, are commonly recognized sources of infection.[1,3] Pigs, wild rodents, and wild birds are major reservoirs of pathogenic *Yersinia* spp. From the point of view of public and animal health, additional sanitary precautions and extreme caution are necessary in order to avoid intra and interspecies transmission of pathogenic *Yersinia* spp.

The source of infection in this outbreak has yet to be established, however, wild birds and rodents were commonly observed around the enclosures. It has also been postulated that stress and behavioral factors may precipitate the presentation of severe clinical disease in captive populations of non-human primates since prevalence of infection appears to be high.[1,4] Age may also be an important factor in clinical disease manifestation, with asymptomatic infections being common in adults.[5] In the outbreak described, the majority of mortalities occurred in juveniles; however as the outbreak progressed, adult monkeys were also clinically affected.



Liver, African green monkey. Large colonies of bacilli (arrows) are scattered throughout areas of necrosis. (HE, 200X) (Photo courtesy of: Department of Pathobiology, Ross University School of Veterinary Medicine, St. Kitts, West Indies, www.rossu.edu)

Characteristic postmortem findings reported in non-human primates include severe enterocolitis, necrotic foci in the liver and the spleen and mesenteric lymphadenitis.[3-5] Microscopic findings are characterized by the presence of multiple necrotic foci of variable size, containing cellular debris and large gram-negative colonies of coccobacilli. Differential diagnoses for bacteria that appear microscopically as large colonies include *Yersinia* spp., *Actinomyces* spp., *Actinobacillus* spp., *Corynebacterium* spp., *Staphylococcus* spp. and *Streptococcus* spp. (resulting in the mnemonic YAACS).

In this case, the clinical signs, the gross findings and the characteristic microscopic lesions were consistent with *Yersinia* spp. infection. Amplification and sequencing of the 16S SSU rRNA gene confirmed the isolates as *Y. enterocolitica.* Antimicrobial susceptibility testing of bacterial isolates obtained indicated tetracycline, gentamycin, chloramphenicol, amikacin, imipenem, ceftiofur, kanamycin, trimethoprim/sulfamethoxazole, ceftriaxone, ciprofloxacin, ceftazidime, pip/tazo con 4, aztreonam, levofloxacin, and cefepime susceptibility. Isolates were resistant to

sulfisoxazole, amoxicillin/clavulanic acid 2:1 ratio, ampicillin, amoxicillin, erythromycin, vancomycin, and clindamycin. Outbreak mortalities were significantly reduced after tetracycline administration.

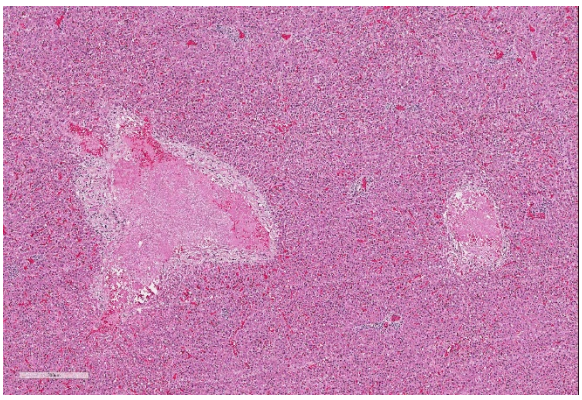
JPC Diagnosis: 1. Liver: Hepatitis, necrotizing, multifocal to coalescing, marked, with large colonies of bacilli.
2. Spleen: Splenitis, necrotizing, multifocal, marked with large colonies of bacilli. (spleen not present on all slides.

Conference Comment:

The genus *Yersinia* is a member of the family Enterobacteriaceae and consists of 14 species and gram-negative bacilli, three of which, *Y. enterocolitica*, *Y. pseudotuberculosis*, and the causative agent of plague, *Yersinia pestis* are pathogenic for humans and nonhuman primates. An additional species, *Y. ruckeri* is a pathogen of fish. Nonhuman primates and man are considered extremely susceptible to infection by these pathogens, although *Y. enterocolitica* was not considered as a human or veterinary pathogen until the late 1960s.[6] In contrast, a number of domestic and wildlife species including pigs, rodents, and wild birds, are and important reservoirs of these pathogens and considered significantly less susceptible to their pathogenic effects. (Interestingly, pork chitterlings (intestine) have been often identified as a source of food-borne illness to young children, during the cleaning and preparation phase.) *Y. enterocolitica* has over 60 serotypes, but less than ten are pathogenic in management and nonhuman primates. Stress and behavioral factors may also precipitate the conversion of asymptomatic carriage of pathogenic *Yersinia* serotypes to active infection; one case report describing an outbreak of fatal yersiniosis was attributed to the administration of metronidazole to treat colony trichomonad infections.[1]

It is difficult, if not impossible, to distinguish the lesions caused by *Y. enterocolitica* from those caused by *Y. pseudotuberculosis* either grossly or histologically, and advanced diagnostics (as done in this case) are recommended in all cases where definitive speciation is required. One study[5] suggested that the pathogenicity of *Y. enterocolitica* is lower than that of *Y. pseudotuberculosis*, as sudden death may occur in the absence of clinical signs with the latter. This study postulates that the *ypm* gene carried by *Y. pseudotuberculosis* (but not *Y. enterocolitica*) may encode a superantigenic toxin resulting in enhanced pathogenicity.

An interesting historical fact about *Y. enterocolitica* serogroup 0:8 (a particularly pathogenic serogroup in both man and non-human primates) is the story of some of its earliest outbreaks. In September 1976, two hundred school children in Holland Patent, NY, developed nausea, vomiting, diarrhea, and abdominal cramping. Thirty-six children were hospitalized, sixteen of whom subsequently had an appendectomy. Contaminated chocolate milk was identified as the common source in this outbreak.[8] A second major outbreak occurred in a coed summer camp in Liberty, NY, five years later. At least 35% of 455 campers and staff members had similar GI signs as the previous



Liver, African green monkey. Arterioles and venules are multifocally occluded by fibrin thrombi. (HE, 100X)

outbreak, with 53% complaining of abdominal pain. Appendectomies were performed on five of the seven campers who were hospitalized.[8] This likely reflects the affinity of "hot" gram-negative bacilli for lymphoid tissue, and the ability of this particular bacterium to mimic a surgical emergency.

Conference participants discussed various aspects of the pathogenesis of yersinial invasion into the GI tract and its affinity for M cells (similar to other "hot" gram-negatives), as well as similarities and differences of *Y. pseudotuberculosis and enterocolitica* as compared to *Y. pestis* (which has an enteric form with lesions quite similar to those seen in this case.) Additionallly, another ruleout for abdominal infection in African green monkeys, hypermucoviscous *Klebsiella pnemoniae* (WSC 2015, Conference 1, Case 2) was briefly discussed.

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CASE II: BC/EP/NTP (JPC 4023574).

Signalment: 2-year-old female, Wistar-Han, *Rattus norvegicus*, rat

History: Tissue from a rat used in used in a National Toxicology Program (NTP) chronic toxicity/carcinogenesis study submitted for peer review. Clinically, the rat was reported as thin, lethargic, breathing abnormally and having a ruffled hair coat.

Mesentery, rat. Mesenteric arteries are tortuous and markedly increased in size. (Photo courtesy of: Experimental Pathology Laboratories, Inc., www.epl-inc.com)

Gross Pathology: The individual animal necropsy record stated that multiple mesenteric blood vessels were enlarged (up to 10 x 5 x 5 mm). Other findings included bilateral granular appearing renal cortices and a 2 x 15 x 15 mm mass in the left inguinal mammary gland. The right ovary contained a 10mm diameter clear, fluid-filled cyst.

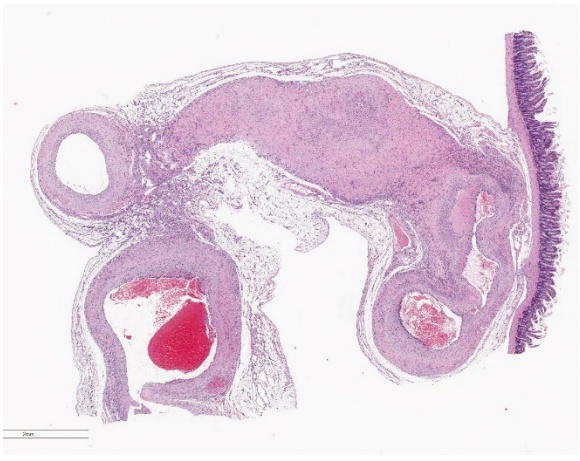
Laboratory results: None.

Microscopic Description: The section is of small intestine (ileum) and attached mesentery. The mesenteric artery is dilated and has a thickened wall surrounded by a varying amount of neovascularized connective tissue (granulation tissue). There is margination of neutrophils with focal necrosis of endothelium, inflammatory cell infiltrates, and variable amounts of fibrinoid material in some segments/sections. The tunica media is thickened by fibromuscular tissue and focal accumulations of lymphocytes and plasma cells are present at the periphery. Marked medial hypertrophy and thrombosis are present in some sections.

Contributor's Morphologic Diagnoses: Artery, mesenteric – arteritis and periarteritis, chronic-active and proliferative, severe with multifocal necrosis and thrombosis.

Contributor's Comment: The slide presents a typical case of spontaneous periarteritis (polyarteritis) nodosa of rats. Polyarteritis nodosa (PN) is a multisystemic, transmural necrotizing vasculitis of small or medium-sized muscular arteries.[11] PN is the most conspicuous inflammatory vascular lesion of rats; the incidence varies among different strains. Incidences have been reported in the August (45.4% in males and 43.0% in females), Fischer 344 (1.8% in male and 0.9% in females), Long-Evans (4.5% in males and 2.6% in females) and in Wistar (9.1% in males and 2.6% in females)[7] and spontaneously hypertensive rats (SHR) (76.1% in males and 10.3% in females).[11]

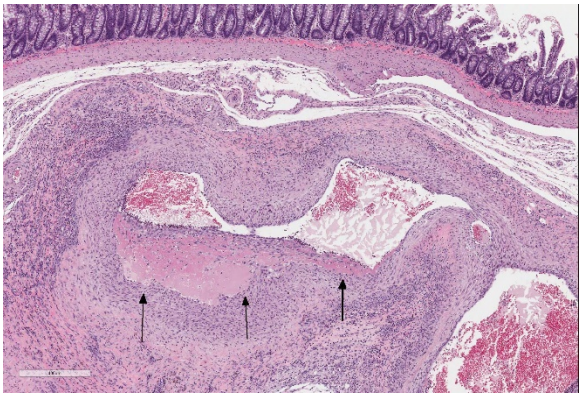
In the well-studied SHR rat, PN mainly affects the spermatic artery, mesenteric arteries, kidney, adrenal, and pancreas and is seen less frequently in the tongue, paratracheal tissue, salivary gland, liver, esophagus, thyroid, spleen, urinary bladder and intestine.[12] Suzuki, et al. reported that PN was never seen in the lung, brain and aorta.[12] Though PN lesions develop later in the mesenteric arteries than in testicular arterioles, the lesions of PN progress much faster in mesenteric arteries.[10] Lesions of PN develop earlier and have a higher incidence



Mesentery, rat. At subgross magnifications, mesenteric vessels are tortuous and walls are thickened up to 5 times normal. (HE, 5X).

in stroke-prone spontaneously hypertensive rats (SHRSP) than in stroke resistant (SHRSR) SHRs.[12] PN lesions are often found at bifurcations of medium-sized arteries, small arteries and arterioles.[12]

A number of chemicals have been reported to produce vascular lesions.[5,6,9,10,12] Fenoldopam mesylate (FM), a selective post-junctional dopaminergic (DA1) vasodilator, causes lesions of large caliber splanchnic arteries (100-180 micrometers) in the rat characterized by necrosis of medial smooth muscle cells and hemorrhage.[5] FM does not induce lesions in other vascular beds of the rat, or in dogs or monkeys.[5] Dopamine is an alpha- and beta-adrenoreceptor and dopaminergic receptor agonist.[5] Dopamine, like FM, causes hemorrhagic lesions of large caliber splanchnic arteries in the rat, as well as fibrinoid necrosis of small caliber arteries (less than 100 micrometer) of the splanchnic, cerebral, coronary and renal vascular beds.[5] Co-exposure to dopamine and an alpha adrenoreceptor antagonist (phenoxybenzamine) blocked fibrinoid necrosis of distal branches of the mesenteric arcade but increased severity of hemorrhagic lesions of larger arteries.[5] Dopamine alone did not induce medial necrosis and hemorrhage of the rat pancreatic artery but phenoxybenzamine pretreatment resulted in characteristic lesions of the type induced by FM at this site.[5] No rats exposed to dopamine and a combination of phenoxybenzamine and a DA_1 receptor antagonist developed arterial lesions of any kind.[6] The arterial lesion produced by FM and dopamine was not the result of alpha-adrenoreceptor-mediated vasoconstriction because phenoxybenzamine pretreatment provided no protection.[5]

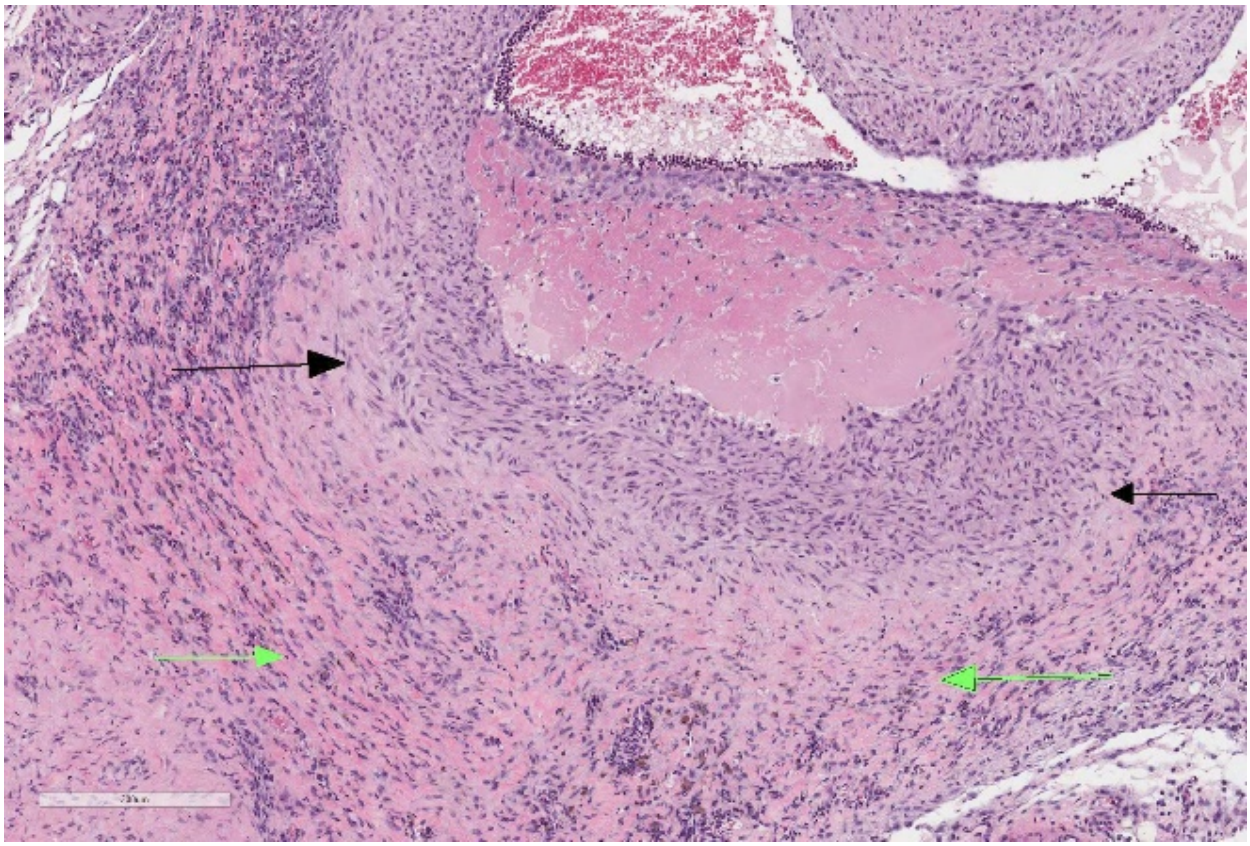


Mesenteric artery, rat. Segmentally, the tunica intima is effaced by bright pink extruded protein which extends in the tunica media (arrows). (HE, 52X).

Phosphodiesterases (PDE) are a family of enzymes responsible for the metabolism of the intracellular second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Phosphodiesterase-4 (PDE4) is the major cAMP metabolizing enzyme in inflammatory and immune cells and makes a significant contribution to cAMP metabolism in airway smooth muscle cells.[6] PDE4 inhibition produces bronchodilation, reduces vascular leakage in airways and modulates airway inflammation.[6] Some PDE4 inhibitors produce arterial lesions in the mesentery of the rat; however, the lesions appear to begin in the mesenteric fat rather than in the arterial wall.[6]

Theophylline, an alkyloid and non-specific phosphodiesterase inhibitor found in cocoa and tea, is used medicinally as a bronchodilator and has been reported to induce mesenteric arteritis in male F344/N rats exposed chronically for 2 years at 75mg/kg and in both sexes in acute (16 day) studies at 40mg/kg given once daily.[7] The mesenteric and pancreatic arteries of rats are particularly sensitive to excessive vasodilator activity.[7]

JPC Diagnosis: Mesenteric artery: Arteritis, necrotizing and proliferative, multifocal, chronic-active, marked, with fibrinoid



Mesenteric artery, rat. Subjacent to the lakes of extruded protein, the tunica media is hypercellular and smooth muscle is disordered (black arrows) and the tunica adventitia is markedly thickened with abundant fibrosis and aggregates of lymphocytes and macrophages (green arrows). (HE, 52X).

necrosis, mural smooth muscle hyperplasia and marked medial and adventitial fibrosis.

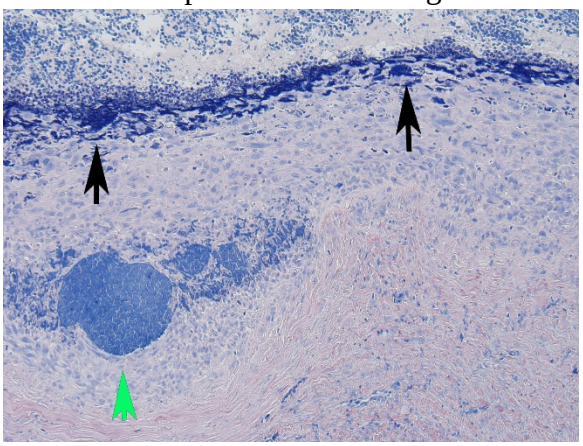
Conference Comment: Lesions consistent with polyarteritis nodosa are not exclusive to the rat but have been documented in a wide range of species including mice, sheep, raccoons, pigs, dogs, non-human primates, and man.

While PN in many instances and in many species is idiopathic, a number of plausible theories have been purported to explain its cause. In humans, PN is a disease primarily of young adults which affects the small-or medium-sized muscular arteries typically involving renal vessels but sparing the pulmonary circulation.[2] Lesions are very similar to those seen in this case, down to the often segmental, non-circumferential areas of fibrinoid necrosis, with a predilection for vascular branching points.[1] Approximately 30% of PN cases in humans or the result of infection with hepatitis B, as antigen-antibody complex is within affected vessels off and are immunopositive for hepatitis B antigen. [2] The lesions of PN blue foxes infected with *Encephalitozoon cuniculi* [7] have also been suggested as a potential infectious cause for the classic Type III hypersensitivity lesions in this condition.

A number of related theories abound regarding the development of PN in rats administered a variety of structurally unrelated vasodilators listed in the contributor's comments. The reduction in mural tension of splanchnic vessels in the

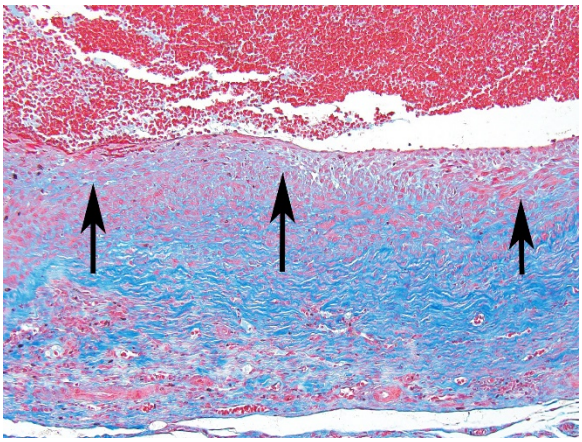
face of the positive inotropy caused by these drugs may result in increased shear forces to endothelium and damage to the underlying tunica media, as well as the possibility of interference with the diffusion of essential nutrient's into the wall affected arteries increasing there are susceptibility to injury.[9] In affected rats, mesenteric and pancreatic arteries are particularly sensitive to these types of drugs, which may be the result of the lack of supporting tissue for the splanchnic vasculature.[9] It has also been suggested that arterial necrosis develops due to alternating dilation and vasoconstriction of these muscles, which may help to explain the prevalence of this condition in spontaneously hypertensive rats.

Phosphodiesterase-inhibiting drugs, which have positive inotropic effects on the heart, have also been shown to produce distinctive arterial changes of the extramural coronary arteries with subsequent myocardial necrosis and atrial and endocardial hemorrhage in the dogs as well.[3] (See WSC 2009-2010, Conference 2, case III). At 7 days of treatment or less histologic changes include medial necrosis and hemorrhage, and more chronic treatment protocols are required to achieve the proliferative changes of the intima and adventitia which characterize PN. Interestingly, associated clinical signs of fever, weight loss, cervical neck pain, and neutrophilic leukocytosis are seen in idiopathic polyarteritis of beagles ("beagle pain syndrome"), but not with administration of vasoactive compounds.[2]



Mesenteric artery, rat. In areas of endothelial loss, there is polymerized fibrin within the inner tunica media which stains dark blue (black arrows). A lake of extruded protein within the tunica media stains less intensely with this stain (green arrow) (PTAH, 200X)

An interesting view on the development of



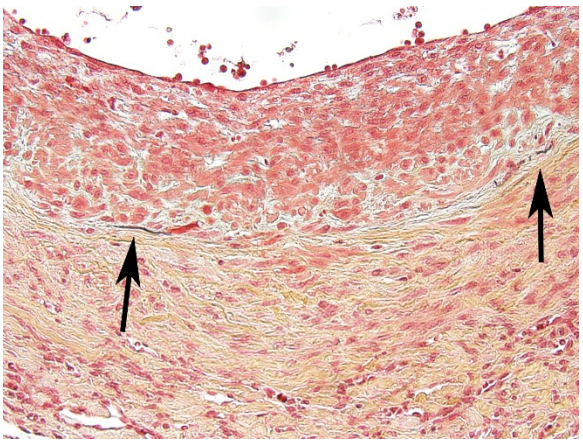
Mesenteric artery, rat. There is moderate amount of fibrosis separating medial smooth muscle cells and extensive collagen deposition and aggregates of lymphocytes, macrophages, and plasma cells within the tunica adventitia (Masson's trichrome, 200X)

PN in rats following administration of a phosphodiesterase type for inhibitor was put forth an article by Mecklenburg et al.[6] In a timed dosage study, rats administered a phosphodiesterase inhibitor developed a mesenteritis as early as 3 days after the initiation of drug treatment, which was characterized by macrophage infiltration, fibroblast proliferation, neovascularization, and atrophy and loss of adipocytes (similar to that seen in this case). A segmental necrotizing panarteritis was subsequently seen but only after 3 or 4 weeks of treatment, and only in a limited subset of individuals. The authors of this study[5] postulate that phosphodiesterase inhibitors do not cause a primary vasculitis in rats but the predominant toxic effects are seen in the mesentery, and that cytokines, growth factors, and other mediated results of inflammation in the

adjacent mesentery may result in vascular lesions.

A recent article has been published on spontaneous arteritis and its comparison with drug-induced arteritis in Gottingen minipigs.[3] Arteritis is a common background finding in Gottingen minipigs used in preclinical safety studies, and is most often seen in cardiac vessels, vagina, oviduct, rectum, epididymis, spinal cord, pancreas, urinary bladder, kidney, and stomach. It is of unknown etiology, and may be seen in association with thrombocytopenic purpura syndrome, an often fatal disease in this breed. The article discusses the relative resistance of minipigs to drug-induced arteritis as compared to the rats, dogs, and monkeys, and the absence of lesions associated with admonition of endothelin receptor antagonists and phosphodiesterase inhibitors.[3]

The moderator started the discussion by reviewing a recent article by Bacares[1], detailing an excellent method for the histologic examination of mesenteric artery of the rat. A review of special stains for this case (Masson's trichrome and Movat pentachrome) demonstrated a number of important features including the loss of the internal elastic lamina, discontinuity of the external elastic lamina, and the marked fibrosis of the tunica media and adventitia.



Mesentery artery rat. The external elastic lamina is largely effaced with small remnants (arrows). (Movat Pentachrome, 400X).

Contributing Institution:
Experimental Pathology Laboratories, Inc., www.epl-inc.com

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CASE III: PV2017 (JPC 4102985).

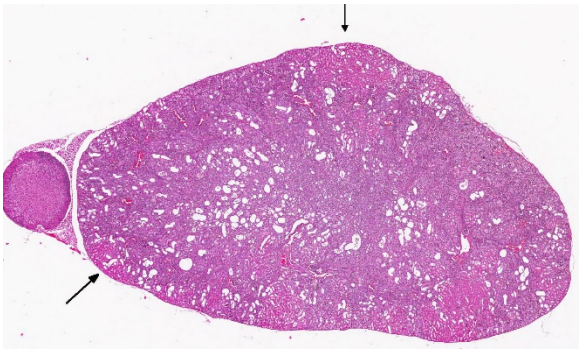
Signalment: Female NOD-SCID mouse

History: N/A.

Gross Pathology: The kidneys were described as pale by the technician.

Laboratory results: None.

Microscopic Description: Kidney: The kidney appears reduced in size. The capsular surface is irregular and there is a marked reduction in the number of normal proximal convoluted tubules. Most of the cortex is occupied by small tubules lined by cells that stain more lightly than normal. Many of these cells have karyomegalic nuclei with chromatin margination and eosinophilic intranuclear inclusion bodies. Their cytoplasm is pale and contains granular eosinophilic material. Other degenerative changes seen in the pale tubules include lining by a reduced number of cells, flattening, loss and anisokaryosis and anisocytosis of lining cells and accumulation of intraluminal debris. There is multifocal mild to moderate tubular dilation and the dilated tubules show similar degenerative changes. There is mild multifocal interstitial



Kidney, mouse: Subgross examination reveals an irregular outline to the kidney, with the majority of tubules in all levels of the cortex are hypercellular and basophilic. (Areas of normal tubules are marked by arrows.) There are numerous ectatic tubules, primarily in the deeper areas of the cortex. (HE, 5X)

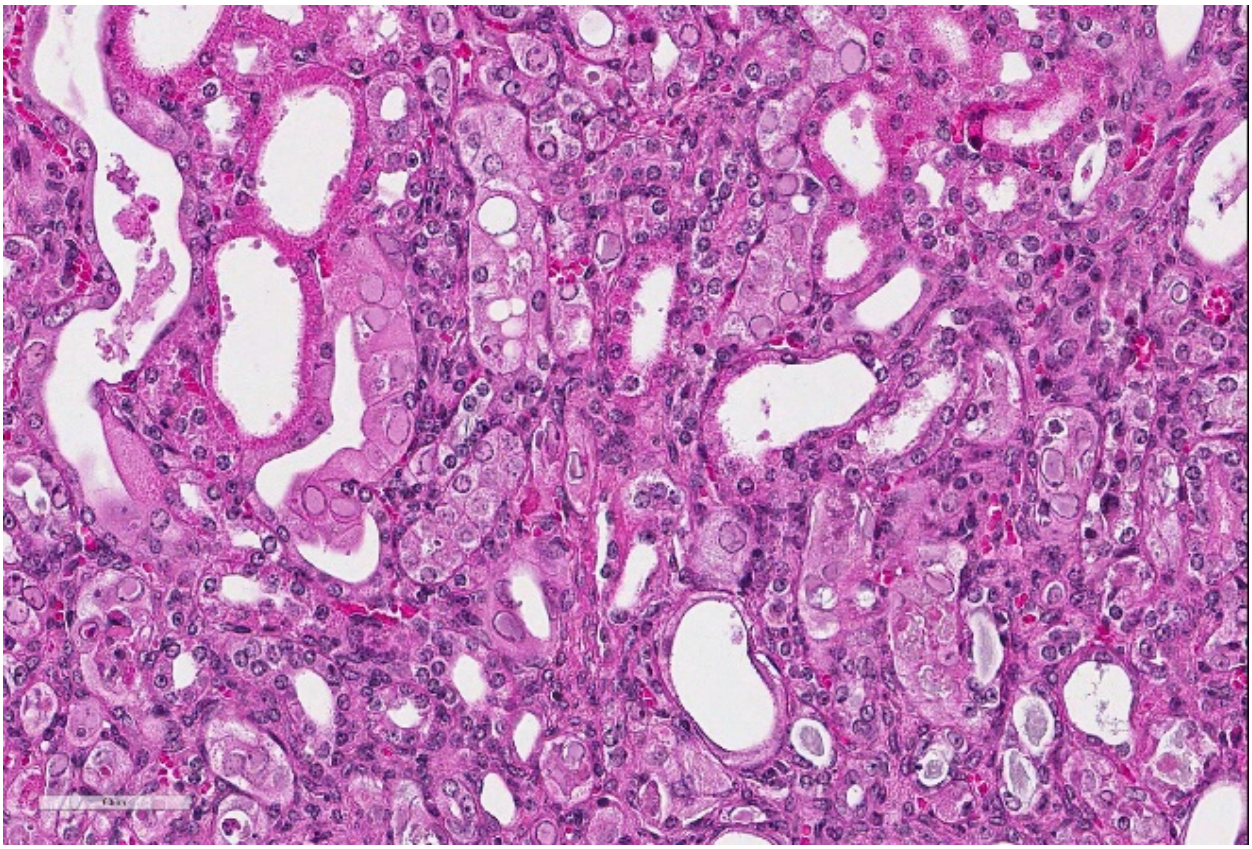
fibrosis and scattered accumulation of granular brown material in tubular lining cells, and possibly in macrophages.

Contributor's Morphologic Diagnoses: Multifocal severe tubular degeneration and loss with karyomegalic and intranuclear inclusion bodies

Contributor's Comment: Karyomegalic with intranuclear inclusion bodies (INIB) and chromatin margination in renal epithelial cells is reported in different types of immunodeficient mice.[1,2] The condition appears to affect female more than male mice and was termed murine inclusion body nephropathy in one reference.[2] The distribution of the INIB is reportedly random but they are more common in the cortex than in the medulla and concentrate near the corticomedullary junction.[1,2]

Ultrastructurally, the INIB consist of a collection of flocculent electron-lucent material. No viral particles or other pathogens haven been identified.[1,2]. Histochemical analysis showed that they are not composed of nucleic acid or carbohydrate, leaving protein as the most likely major chemical constituent.[3]

Investigations using PCR (murine polyomavirus, papillomaviruses, circoviruses, anelloviruses), immunohistochemistry (adenovirus) and serologic tests for a broad range of murine pathogens were negative.[1,2] Toxic etiology was suspected, and indeed, cannot be ruled out.

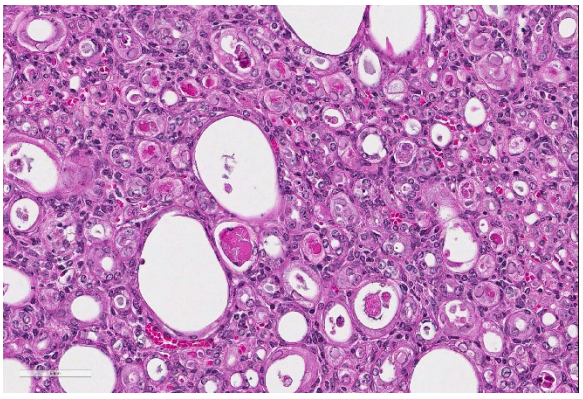


Kidney, mouse. Tubular epithelial cells are multifocally and markedly enlarged,with nuclear expansion by a homogenous karyomegalic intranuclear inclusion which peripheralizes the chromatin. Inclusions may or may not fill the expanded nucleus. (HE, 232X)

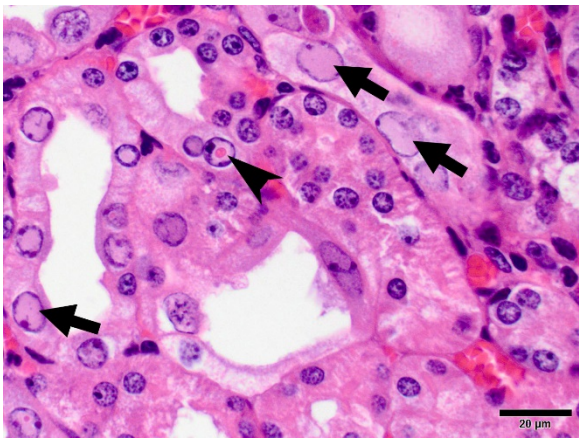
Lead poisoning is a well-known cause of inclusion bodies in multiple species, but the INIB in immunodeficient mice are not acid fast and their ultrastructural features differ from lead inclusion bodies which have an electron-dense core surrounded by a zone of fibrillar structures.[1,2]

Interestingly, the INIB were positive in immunohistochemistry for heat shock cognate 70 (Hsc70). This protein is a molecular chaperone that protects cells from physical and chemical damage. It is normally located in the cytoplasm but it can be recruited to the nucleus, where in concert with other cellular proteins, it assists repair. Once nuclear damage has been corrected, Hsc70 exits the nucleus. Thus, detection of HSc70 in nuclei with INIB indicates nuclear damage in these cells, but does not shed light on the underlying cause.[1,2] The same study also found that the INIB react specifically with anti-bovine papillomavirus type 1 L1 antibody, but the weight of the evidence suggests that the binding is due to a shared epitope rather than an indication of infection with murine papilloma virus.[2]

Both reports describe inclusion body nephropathy in sentinel mice.[1,2] and one report states that the nephropathy did not appear to affect the health of the mice.[1] In our samples, the nephropathic changes are pronounced and it seems likely that they would cause compromised renal function. Unfortunately, biochemical and urinalysis of this mouse were not carried out. The occurrence of other lesions in kidneys with inclusion body nephropathy appears to be inconsistent.[1,2]



Kidney, mouse. Tubules within deeper levels of the cortex are often ectatic, and contain various amounts of eosinophilic protein and sloughed granular cellular debris. (HE, 232X)

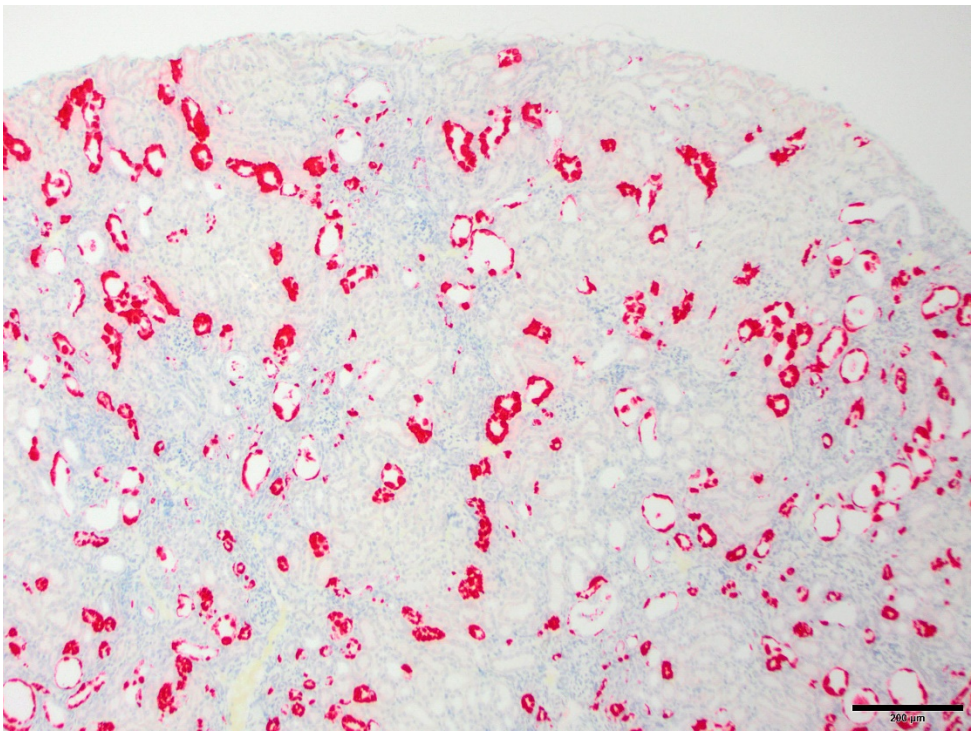


Kidney, mouse. Two types of inclusions are present in this section: many large pale amphophilic inclusions filling the nucleus (arrows) but also occasional (much fewer) small densely eosinophilic inclusions (arrowhead). (HE, 600X) (Image courtesy of: Dr. S. Monette, Memorial Sloan Kettering Cancer Center).

In summary, the cause of murine inclusion body nephropathy is currently not understood. From a practical point of view the authors of the papers describing this condition suggested to view them as a "degenerative change" [1] or as part of the "normal background pathology of immunodeficient mice".[2]

JPC Diagnosis: Kidney, tubular epithelium: Karyomegaly, with numerous eosinophilic intranuclear inclusions.

Conference Comment: Shortly after the publication of the first version of the results for this conference, the JPC was contacted by



Kidney, mouse. There is intense staining for viral RNA of numerous tubules in the cortex (Mouse kidney parvovirus RNA ISH, 100x). (Image courtesy of: Dr. S. Monette, Memorial Sloan Kettering Cancer Center).

Dr. Sébastien Monette, who is part of a multi-institutional research group that has been investigating this condition over a number of years. (Much of the discussion below is the result of personal communications from Dr. Monette and Dr. Ben Roediger).

While milder lesions associated with renal tubular inclusions were previously reported in mice[1,2], as discussed by the contributor, description of the severe form of murine inclusion body nephropathy (IBN), as seen in the present case, was first reported in 2017[4] as part of an article on aging changes in NOD *scid* gamma (NSG) female mice. The article described moderate to marked renal tubular degeneration and necrosis with two types of intranuclear inclusions within renal cortical and medullary tubular epithelial cells (numerous large pale inclusions filling the nucleus, and occasional small densely staining inclusions surrounded by a clear halo; as observed in this case – the presence of these two distinct types of inclusions may be of diagnostic value to pathologists encountering these lesion for the first time.) These lesions were associated with marked elevation of serum BUN and creatinine concentrations, and were determined to be a significant cause of morbidity and mortality in the colony being studied. Renal samples were negative on PCR for mouse adenovirus 1 and 2, murine cytomegalovirus, and murine polyomavirus. TEM performed on 2 cases failed to identify viral particles (similar to the results obtained on this case at the JPC following the conference).

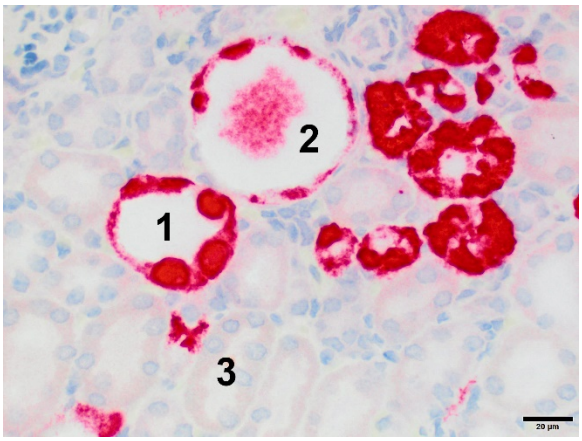
Unbiased viral metagenomic analysis of affected kidney samples lead to the discovery of a novel viral sequence, and this virus was shown to be the cause of murine IBN in a series of observational and experimental transmission studies, performed by scientists in multiple organizations, and described in the paper by Roediger et al[3], published a month after the conference. These findings have finally shed light on the elusive etiology of a lesion that has puzzled pathologists for decades. The etiology of the lesion, an atypical parvovirus, was shown to cause

naturally occurring morbidity, mortality, and marked lesions in highly immunodeficient mice such as NSG and Rag1 knockout mice, and subclinical infection and mild lesions in immuno-competent mice and athymic nude mice.

The novel parvovirus, termed "mouse kidney parvovirus" (MKPV), is highly divergent from the known mouse parvoviruses, mouse parvovirus (MPV) and the minute virus of mice (MVM) and is not detected by serology and PCR for these viruses. Based on analysis of its sequence, this virus apparently belongs to a new genus with the proposed name chapparvovirus, recently found in bats, wild rats, and pigs, but without disease association. In mice, it causes unusual pathology compared to typical parvoviruses. Unlike most parvoviruses which tend to require actively dividing cells to replicate (intestinal crypts, hematopoietic cells, external germinal cells of the developing cerebellum) this virus replicates predominantly in renal tubular cells, an unusual target for a virus of this family. The infection is chronic, and in immunodeficient strains leads to progressive tubular injury and loss, with interstitial fibrosis in advanced stages, over a period of months.

Attempts at isolating the virus in several cell lines have failed; therefore a pure inoculum could not be obtained and Koch's postulate could not be fulfilled. The causal relationship between MKPV and IBN was demonstrate in this article by a sum of evidence[3], including:

- MKPV virus was detected by PCR from kidney tissues in cases of IBN and never detected in the kidneys of normal mice.
- By in situ hybridization (ISH) staining for viral RNA, the virus was detected in kidneys with IBN but not in normal kidneys. Importantly, within affected kidneys the staining was localized to abnormal tubules



Kidney, mouse. There is intense staining for viral RNA in abnormal tubules displaying karyomegaly (1) or ectasia, epithelial attenuation, and luminal debris (2) while morphologically normal tubules (3) are not stained (Mouse kidney parvovirus RNA ISH, 600x). (Image courtesy of: Dr. S. Monette, Memorial Sloan Kettering Cancer Center).

with karyomegaly and degenerative changes but not present in normal tubules, suggesting that the viruses causes the lesions and is not simply a bystander.
- Viral load in urine and kidney, as measured by quantitative PCR, showed concordance with clinical progression, histopathologic severity of lesions, and serum BUN concentration.

The investigators have found evidence of this virus in laboratory mice in multiple research facilities in the US and Australia, in samples collected over a period of eleven years. Material from this particular case was sent to Dr. Monette and evaluated via RNA ISH and was strongly positive for this virus, with a staining pattern as previously described, adding evidence that this pathogen is likely widespread throughout the world.

While the virus has been identified in laboratory mice in several facilities, the prevalence of infection by MKPV within these facilities was not clear, as detection of cases was primarily based on histo-pathological observation of the lesions

followed by confirmation by PCR and ISH. Because immunocompetent mice develop much milder lesions that may be missed, and no clinical signs, determination of the true prevalence will require surveys by PCR or serology. Nevertheless, these findings suggest that MKPV may have significant negative impacts on research performed with mouse models, as it can cause unexpected morbidity and mortality in highly immunodeficient strains, but also because it may have subtle, but potentially significant effect on the outcome of research performed in all mice, including immunocompetent strains. Further research is ongoing to determine the impact on this virus on biomedical research that relies on mouse models.

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http://www.weizmann.ac.il/vet/

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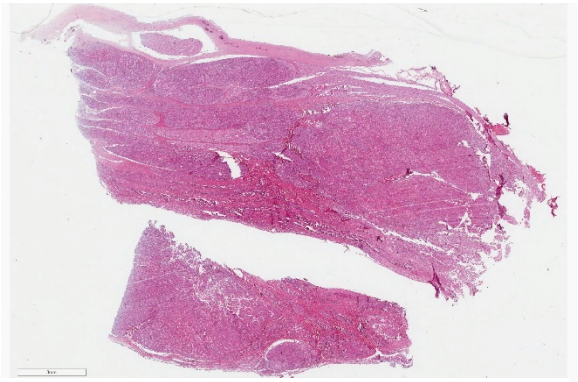
CASE IV: 15/191 (JPC 4068156).

Signalment: 19 years old sportpony mare (*Equus caballus*)

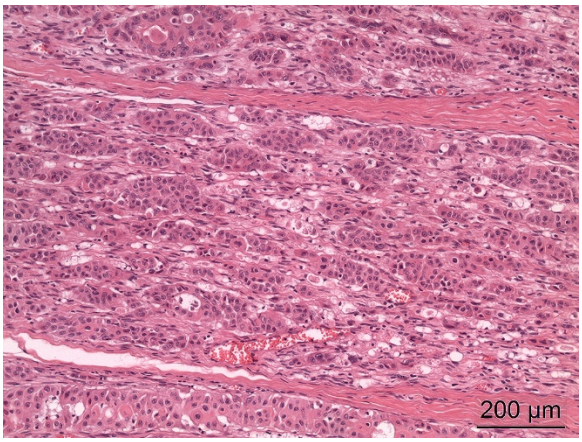
History: The pony had a history of eye problems of 2-3 weeks (exact duration not known). It had a corneal ulcer of the left eye that increased in severity, with left sided progressive neurological clinical signs, external ophthalmoplegia, ceased tear production, absent corneal sensitivity, dry mucosal membranes of the left nasal cavity. Keratitis and uveitis were also noted clinically. The neurological clinical examination indication a lesion affecting multiple cranial nerves associated with the ipsilateral fissure orbitalis.



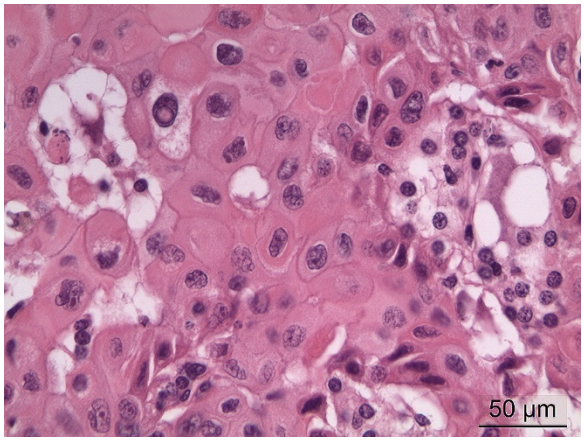
Cranial nerve and ganglion, horse: Sections of a cranial nerve (top) and the associated ganglion (bottom) are largely effaced by a moderately cellular infiltrative neoplasm. (HE, 5X)

Gross Pathology: In the left eye, there was a focal extensive ulceration of the cornea with yellow discoloration of the central and ventral areas and a narrow rim of intact cornea laterally and dorsally. At the lower limbus, there was severe hyperemia. After removal of the eye, the mandible and the arcus zygomaticus of the maxilla, the orbital fissure was inspected. A few small nodular proliferations, 2-3 mm in diameter, of a light grey firm tissue was detected associated with nerves in the area. Multiple sections was made in the cranium parallel to the optical canal, cutting the canal from the fissura orbitalis to the cranial cavity obliquely in several sections. Nerves within this canal was thickened with a light grey to white firm tissue and, in continuation with this, similar tumor-like tissue was also detected laterally to the infundibulum of the pituitary gland and optical chiasm. The left trigeminal ganglion also appeared grossly to be involved. The same structures on the right side was unaffected.

Laboratory results: None.



Cranial nerve and ganglion, horse. Nests of polygonal cells, rarely making tubules, infiltrate the nerve. Between nests of neoplastic cells, remaining axons are swollen (spheroids) with dilated axon sheaths. (HE, 100X) (Photo courtesy of: Institute of Basic Sciences and Aquatic Medicine, Norwegian University of Life Science, School of Veterinary Medicine, www.nmbu.no)



Cranial nerve and ganglion, horse. Neoplastic cells have distinct cell borders and a moderate amount of brightly eosinophilic keratinizing cytoplasm. Similar to keratinizing squamous epithelium, some neoplastic cells have numerous vacuoles in proximity(Photo courtesy of: Institute of Basic Sciences and Aquatic Medicine, Norwegian University of Life Science, School of Veterinary Medicine, www.nmbu.no)

Microscopic Description: In the trigeminal ganglion, the tumor tissue to the left of the pituitary gland, and the above-mentioned cranial nerves, there was a non-encapsulated, infiltrative, cell-rich tumor consisting of squamous epithelial tumor cells growing in islands and trabeculae with moderate amount of stroma. The tumor cells were large with abundant amphophilic to eosinophilic cytoplasm with keratinization of single cells or groups of cells, and large round, oval to irregular nucleus with coarsely stippled chromatin and 1-2 nucleoli with variable size. Anisocytosis and anisokaryosis were prominent, and there was one mitotic figure per 10 40X HPFs. In multiple areas, the tumor cells formed narrow slit-like or larger irregular cyst-like lumens, often with papillary epithelial formations. There was a multifocal mild mononuclear inflammatory cell infiltrate dominated by lymphocytes and multifocal mild mineralization in the stroma. In nerves and in the ganglion remnants surrounding the tumor tissue, there were multifocal degenerative changes.

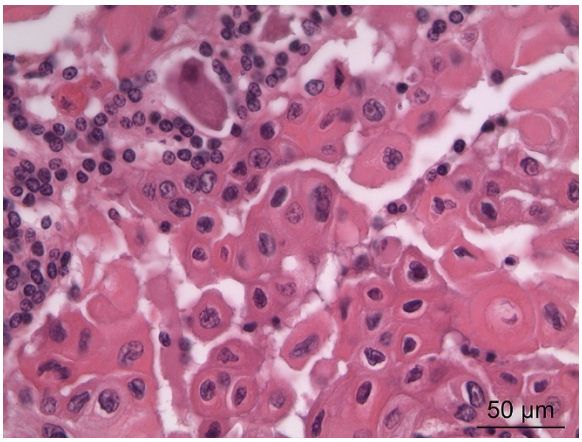
Contributor's Morphologic Diagnoses: Cranial nerves and trigeminal ganglion: malignant craniopharyngioma, papillary

Contributor's Comment: Craniopharyngiomas are rare epithelial tumors of humans and animals arising in the sellar or suprasellar region. They are thought to arise in remnants of craniopharyngeal duct ectoderm that normally forms the Rathke's pouch,[1,4,8] and they may occur in two forms, adamantinomatous and papillary, the former shows morphologic similarities with odontogenic lesions.[6] In animals, they usually consist of solid and cystic areas composed of cuboidal, columnar to squamous epithelial cells.[1] Craniopharyngiomas are usually described as benign tumors, but some malignant examples of the tumor have been reported in humans and anmials.[1,5,6]

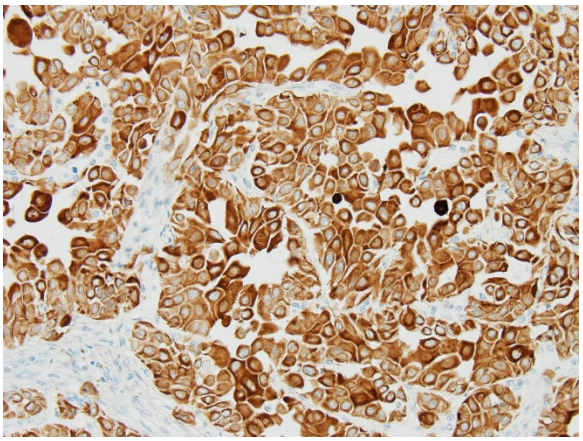
The tumor in this horse was diagnosed as malignant based on severe pleomorphism and extensive infiltrative growth. Tumors arising in the sellar or suprasellar region may cause different clinical signs depending on which structures they involve; pituitary hormone secretion may be affected either due to compression or involved of the pituitary gland itself, cranial nerve function deficits and CNS dysfunction due to extension into the overlying brain.[1] In this horse, the cranial nerve dysfunction was most prominent, and the clinicians suspected that nerves associated with fissura orbitalis was involved. Several cranial nerves enter the lower medial orbita associated with the fissura orbitalis and the foramen rotundum, including the ophthalmic nerve (V1 of trigeminal nerve, cranial nerve no. 5), maxillary nerve (V2 of trigeminal nerve), trochlear nerve (cranial nerve no. 4), oculomotor (cranial nerve no. 3) and abducent nerves (cranial nerve no. 6), however in the horse there is a separate foramen for the trochlear nerve, the foramen trochleare.[2,3] Most, if not all, of these nerves were affected by the tumor tissue. The tumor did not exert any pressure on the optic chiasm and the optic nerve was unaffected. The pituitary infundibulum and overlying brain tissue was also unaffected, and in the pituitary gland there was diffuse hypertrophy and hyperplasia of the pars intermedia in addition to a microadenoma of 2 mm in diameter.

JPC Diagnosis: Cranial nerve and ganglion: Craniopharyngioma.

Conference Comment:
Craniopharyngiomas are benign tumors that have primarily been identified in small animals and laboratory species. Their sellar or suprasellar location may make them grossly indistinguishable from other tumors of the hypophysis such as pituitary adenomas, and their growth may result in destruction of the pituitary gland or hypophysis resulting in clinical signs of



Cranial nerve and ganglion, horse. Some areas of the neoplasm are populated by smaller, less differentiated neoplastic cells (upper left). (HE, 100X) (Photo courtesy of: Institute of Basic Sciences and Aquatic Medicine, Norwegian University of Life Science, School of Veterinary Medicine, www.nmbu.no)



Cranial nerve and ganglion, horse: Neoplastic cells are strongly immunopositive for cytokeratin. (anti - AE1-AE3, 400X)

hypopituitarism or diabetes insipidus. These neoplasms, however, tend to arise in individuals much younger than expected to develop tumors of the pituitary pars intermedia or glandularis.[7]

Grossly, these neoplasms may advance along the ventral aspect of the brain incorporating and effacing cranial nerves as seen in this case. Extensive growth should not be interpreted as a sign of malignancy.[7]

The histological characteristics of this tumor is unique and does not replicate any other neoplasm that may be found in this location of the cranial vault. The distinct features of keratinization results from the ectodermal origin of this tumor as previously discussed by the contributor; and cytokeratin (as performed in this case at the JPC) should be strongly positive. Neoplastic cells are also immunopositive for alpha-fetoprotein suggesting their embryonic level of differentiation; however the AFP stain performed by the JPC was noncontributory, as it is likely not optimized for equine tissues.

Both solid and cystic areas are often seen in these neoplasms.[7] Neoplastic cells will occasionally form colloid-like structures containing eosinophilic secretory material (but this was not a significant feature in this case).

Valentine et al.[9] has suggested that these tumors be classified as germ cell tumors rather than craniopharyngiomas. In a study of five craniopharyngiomas in young dogs, diagnosis of germ cell tumor is based on 3 criteria: A) a midline suprasellar location, B) the presence of several distinct cell types within the tumor, a germ cell phenotype being one, as well as other cells suggesting differentiation into a secretory or squamous phenotype, and C) positive staining for alpha-fetoprotein.[9] The AFP run at the JPC was non-contributory, and neoplastic cells were negative for NSE, GFAP, and S-100. (Interestingly, satellite glial cells were strongly positive for GFAP; enhanced expression of which has been reported by satellite glia in response to nerve injury).

Conference participants discussed potential differentials based on location in the hypothalamus: pituitary adenoma, suprasellar germ cell tumor, and craniopharyngioma. While we strongly considered the contributor's diagnosis of malignant craniopharyngioma, conference attendees favored a benign neoplasm due to the lack of appreciable pleomorphism or vascular invasion in the distributed sections or any description of metastasis in the submitted history.

Contributing Institution:
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www.nmbu.no

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