



WEDNESDAY SLIDE CONFERENCE 2015-2016

Conference 14

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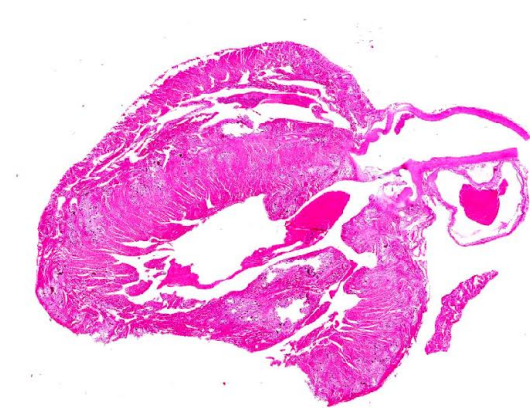
CASE I: AFIP case#1 (JPC 4032259).

Signalment: Seven male Hartley guinea pigs, 14 weeks-1.5 years of age (*Cavia porcellus*).

History: Tissues from several of 7 Hartley guinea pig boars, 14 weeks-1.5 years of age, found dead (4) or euthanized due to lethargy and hindlimb weakness (3) over a 3 week period. Animals were housed at an outside research institution, fed a commercial laboratory guinea pig diet, and used for several different research or training IACUC protocols by multiple investigators. Fixed tissues were received for histologic evaluation.

Gross Pathology: Hearts reported to have “severe multifocal to diffuse white infiltrates at several locations.” Heart weight-to-body weight ratios were reported for two animals, 0.779% and 0.737% (normal 0.422%).⁴

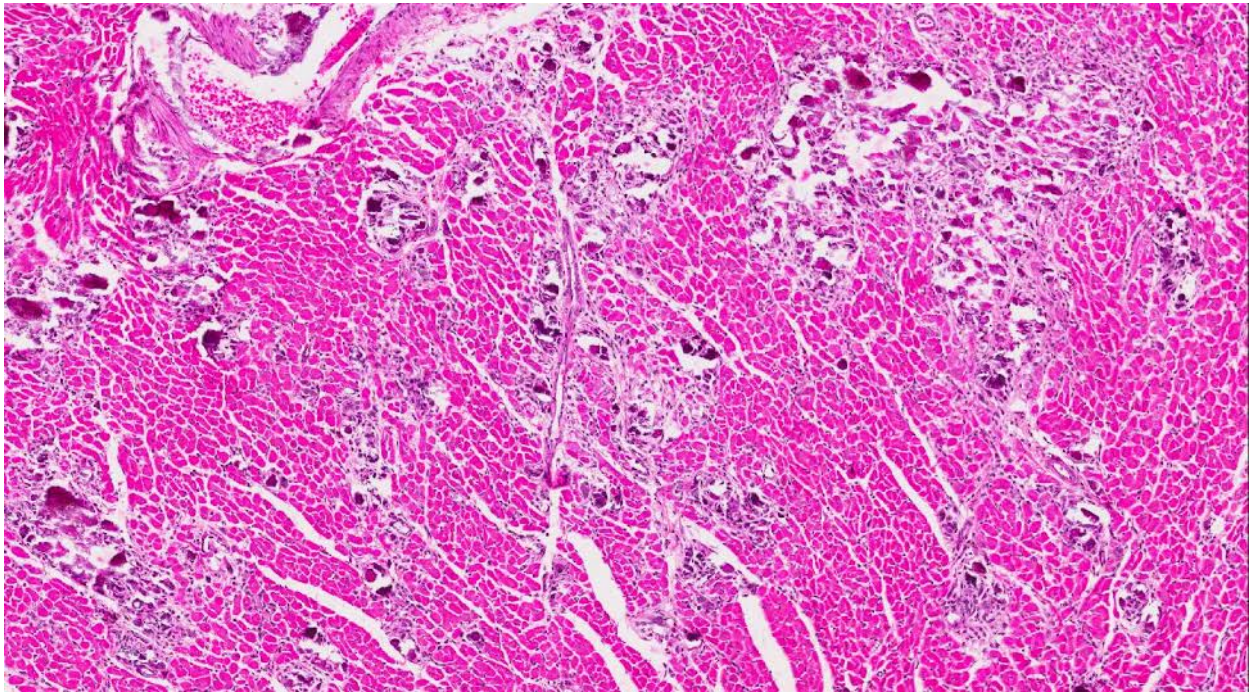
Laboratory Results: An independent analysis of the feed showed actual vitamin D3 levels to be 160 fold higher than the



Subgross of a sagittal section of the heart and great vessels. At low magnification, there are visible areas of myofibers loss, predominantly in the interventricular septum and left ventricular free wall. (HE, 5X)

label analysis reported. The specific lot of this diet was one of several later recalled for elevated vitamin D levels.

Histopathologic Description: Heart (longitudinal sections): Affecting from 30 to 70% of the ventricular myocardium (LVFW and IVS>RVFW) are multiple coalescing foci of clusters or less commonly individual cardiomyocytes notable for their intensely hyaline refractile amphophilic



Heart, guinea pig. In this higher magnification of the interventricular septum, there are multifocal to coalescing areas of myofiber loss, fibrosis, and mineralization. (HE, 63X)

cytoplasm that is often fractured (mineralization). In some less affected cardiomyocytes, mineralization is finely punctate within the cytoplasm (mitochondrial mineralization). There is moderate to robust bland histiocytic to granulomatous inflammation, the latter including Langhans and foreign body type giant cells. There is significant organizing immature fibroplasia circumscribing inflammation and replacing cardiomyocytes. In some sections mineralization is also evident in the atrial myocardium as well as the intima and media of the aorta and pulmonary and coronary arteries. A von Kossa stain confirmed mineralization.

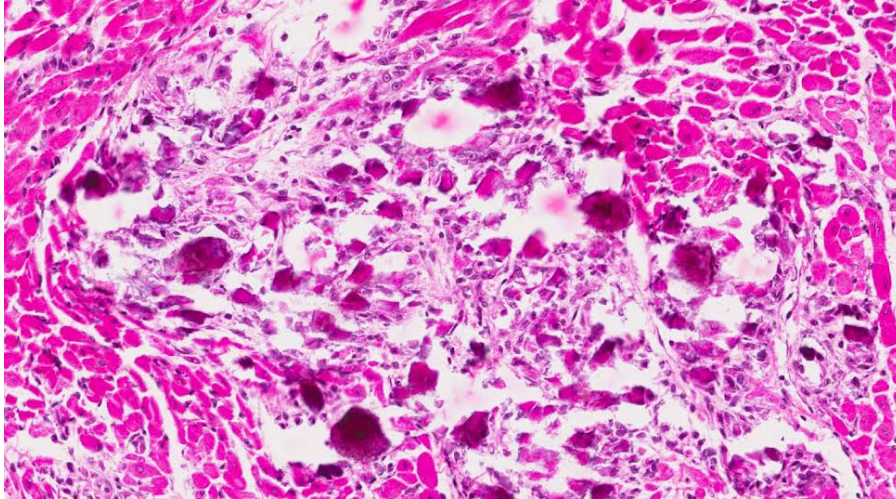
Mineralization of hepatocytes and renal tubular epithelium was also observed, and rarely the lung and spleen were mineralized (not submitted). In some animals there was centrilobular atrophy of the liver and

hemosiderin laden alveolar macrophages (heart failure cells) in the lungs.

Contributor's Morphologic Diagnosis:

Heart, ventricular myocardium: Mineralization, multifocal to coalescing, subacute to chronic, severe, with granulomatous inflammation and organizing fibroplasia.

Contributor's Comment: In the summer of 2012, a laboratory animal feed manufacturer recalled several batches of feed, including the specific lot fed to these animals, because of excessively high vitamin D levels. Similar mortality nearly wiped out a small colony of a guinea pig model of human genetic disease at another institution.⁵ Interestingly, only one of 9 guinea pigs in that report had significant cardiac mineralization, and renal disease was the predominant manifestation.



Heart, guinea pig. Within affected areas, there is marked myofiber loss and replacement with fibrous connective tissue and crystalline mineral (which has fractured on sectioning.) Remaining myofibers are shrunken and atrophic, as well as at the periphery. (HE, 168X)

one of the guinea pigs suggested the probability of vitamin D toxicosis. Plants high in vitamin D glycosides include day blooming jessamine (*Cestrum diurnum*), *Trisetum flavescens*, and members of the *Solanum* (nightshade) family including *S. malacoxylon*.¹ The latter agent produces the disease *enteque seco* or *espichamento* in Argentina and Brazil. In addition to diet, accidental or deliberate rodenticide

The major action of vitamin D (1,25(OH)₂D₃) is to increase calcium and phosphorus absorption from the intestine via increased synthesis of calbindin by enterocytes.¹ Vitamin D also acts on the bone, stimulating osteoclastic resorption and making osteocytes responsive to parathyroid hormone. Vitamin D can stimulate calcium resorption in the kidney as well.

The major differential etiology for these lesions is metastatic mineralization, a poorly understood and likely multifactorial disease predominantly affecting male guinea pigs >1 year of age.^{3,7} Diet, particularly when high in phosphate and low in magnesium, is reported to be a significant factor. Only 4% of 140 animals examined in the Sparschu and Christie study had demonstrable cardiac mineralization, but lesion appearance and ventricular localization was similar (but less severe) than that reported herein. It is interesting to note that the guinea pigs in that study were given kale, a vitamin D rich plant.

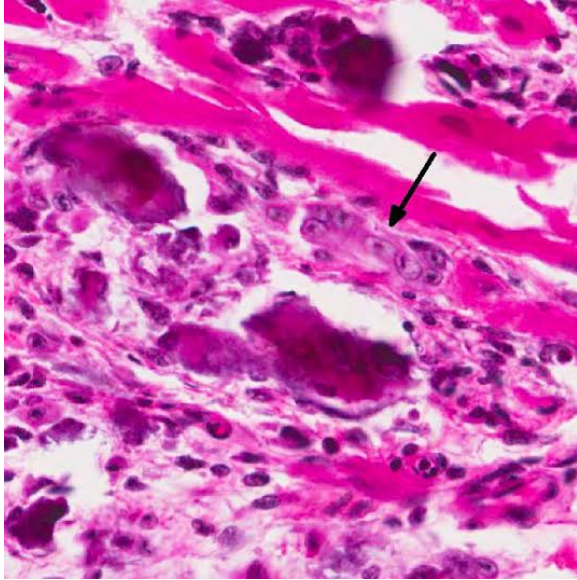
The epizootic nature of the cases here combined with the young age (14 weeks) of

poisoning should be ruled out.

JPC Diagnosis: 1. Heart, myocardium: Myocyte, degeneration, necrosis, atrophy and loss, multifocal to coalescing, severe with marked fibrosis, granulomatous myocarditis and mineralization.

2. Aorta: Mineralization, intimal and mural, multifocal, moderate.

Conference Comment: The initial steps in the synthesis of vitamin D involve the production of vitamin D₃ (cholecalciferol) in the skin via exposure to ultraviolet radiation but can also be obtained through dietary intake of vitamins D₂ or D₃. In the liver, vitamins D₂ and D₃ are converted to 25-hydroxyvitamin D_{2/3} which are found in circulation and can be measured. In the kidney, 25-hydroxyvitamin D is converted to the active form of vitamin D, which is known as calcitriol and abbreviated 1, 25(OH)₂D. Excess vitamin D can be ingested due to feed mixing errors as in this case, or via the ingestion of toxic plants, which is more commonly seen in ruminants. This condition results in elevated levels of



Heart, guinea pig. There are scattered attempts at myofiber regeneration throughout the section (arrow). (HE, 324X)

25-hydroxyvitamin D, while the active form, calcitriol is not elevated. It may be possible that when present at toxic levels, the inactive forms are involved in stimulation of the vitamin D receptor.⁴ The result of elevated vitamin D levels is an increase in calcium absorption in the intestine, increased resorption from bone and reduction in renal excretion followed by hypercalcemia, and hyperphosphatemia. In addition to the endocardium, other targets for mineralization include the kidneys, gastric mucosa, lungs and atrial walls.²

Gross lesions described by Holcombe et al. in a colony of guinea pigs similarly affected included white discoloration of multiple tissues including the gastrointestinal tract, skeletal muscle, and kidneys in addition to the cardiac muscle. Histologic lesions included varying degrees of mineralization in the tissues described grossly, as well as the lungs. Fibrosis and granulomatous inflammation were associated with mineralization, as seen in this case. Bone lesions were also demonstrated in affected guinea pigs, including osteosclerosis (most

prominently in the tibia), and changes in the articular cartilage. Additional lesions described in those cases included fatty change in the liver and chronic interstitial nephritis. Clinicopathologic changes included hyperphosphatemia and elevated serum alkaline phosphatase. Serum calcium levels (ionized and total) were not significantly elevated in affected guinea pigs, and are not a reliable indicator of vitamin D status in the guinea pig. However, the values of calcium multiplied by that of phosphorus was greater than 70 for the majority of affected animals in that study.⁴

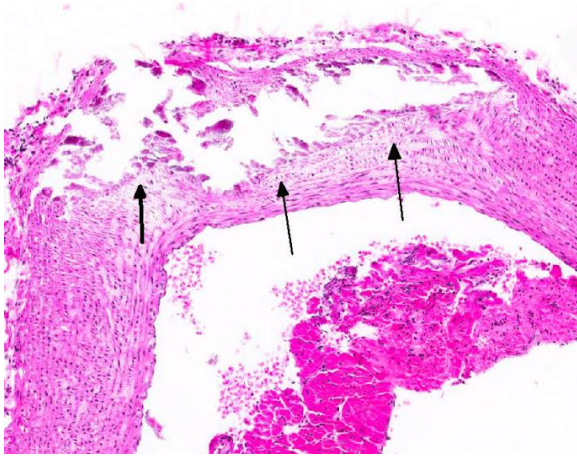
Conference participants described degeneration, necrosis and loss of cardiac myocytes secondary to mineralization. Participants discussed the differential diagnoses for metastatic calcification including renal disease, neoplasia such as lymphoma, multiple myeloma and tumors affecting the parathyroid gland resulting in primary hyperparathyroidism, as well as vitamin D toxicosis. Diagnostic techniques for investigating an outbreak of nutritional or toxic disease case involving multiple animals were also a point of discussion.

Contributing Institution:

Department of Comparative Medicine
Penn State Hershey Medical Center
<http://www.hmc.psu.edu/comparativemedicine>

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Aorta, guinea pig: There are large areas of mineralization within the arterial wall (arrows). (HE, 80X)

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CASE II: CRL2 (JPC 4019884).

Signalment: Approximately 6 week old, male, New Zealand White rabbit (*Oryctolagus cuniculus*).

History: The rabbit was clinically observed with diarrhea.

Gross Pathology: Cecal contents were thin and watery. There were few formed fecal pellets.

Laboratory Results:

Heavy growth of *Escherichia coli* from cecum

Parasitology: Positive for *Eimeria media* and *Eimeria perforans* by fecal analysis.



Enteron, rabbit. There are three sections on the slide – colon (upper right), cecum (center), and ileum (lower left) (HE 6X).

Histopathologic Description: Cecum: There is moderate, multifocal degeneration, necrosis and loss of epithelial cells, predominantly lining the luminal surface but occasionally extending deeper into the crypts. Along the apical surface of the intestinal epithelia, clusters of small

coccobacilli bacteria are tightly adhered. Bacteria are numerous and affect a large percentage of the epithelial surface. The mucosa and submucosa is mildly edematous. The lamina propria is infiltrated by mild to moderate numbers of heterophils and lesser numbers of lymphocytes. There are increased mitoses in the crypts, and epithelial cells are crowded and piled up at the tops of the crypts, indicative of a proliferative response to superficial cells loss.

Additional sections of small and large intestine may be present on the slide. These tissues are similarly affected. The ileum is additionally characterized by numerous intraepithelial protozoal forms consistent with coccidiosis.

Contributor's Morphologic Diagnosis:

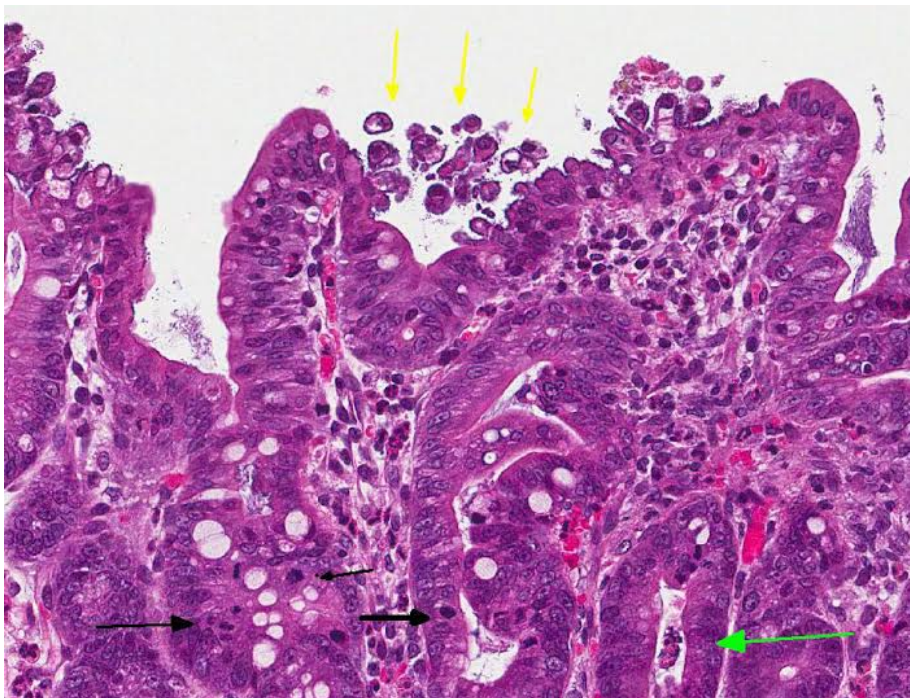
Cecum: Typhlitis, heterophilic and lymphocytic, moderate, diffuse with

moderate, multifocal adherent coccobacilli, consistent with *E. coli* infection.

Ileum: Enteritis, heterophilic, marked, diffuse with moderate adherent coccobacilli and coccidiosis.

Colon: Colitis, heterophilic and lymphocytic, mild, diffuse with mild multifocal adherent coccobacilli.

Contributor's Comment: Diarrhea in suckling and weanling rabbits can be a significant issue in rabbitries. Although diarrhea can be caused by a variety of factors, *Escherichia coli* is considered to be a common pathogen in young rabbits. *E. coli* can be classified into five categories: enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroadherent (EAEC), and enterohemorrhagic (EHEC).² Rabbits are usually affected with enteropathogenic *E. coli*, which does not produce toxins and does not invade the mucosa.⁴ Under normal circumstances, *E. coli* is not considered to be a part of the normal rabbit flora; or at least thought to be found in low numbers.^{5,6} While the mechanism of disease is not entirely clear, attachment and effacement of epithelial villi by the bacteria is thought to play a role with EPEC.³



Cecum, rabbit. There is necrosis and sloughing of luminal epithelium (yellow arrows), which are covered by a layer of short i. There are numerous mitotic figures (black arrows) in the underlying regenerative crypts. A single crypt abscess (green arrow) is present attesting to the necrotizing nature of this lesion. (HE, 204X)

In this case, the ileal, cecal and colonic epithelia have numerous adherent coccobacilli.

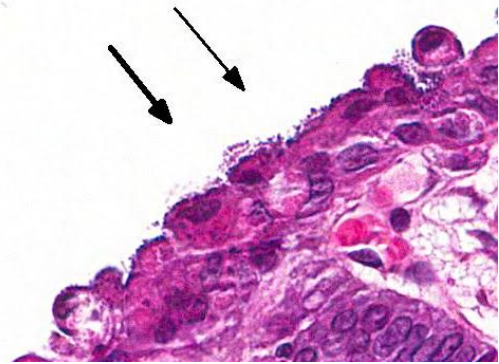
Gram staining revealed the bacteria to be gram-negative, and cultures of the intestines had heavy growth of *E. coli*. Concurrent coccidiosis may additionally contribute to *E. coli* proliferation in the intestines.

JPC Diagnosis: 1. Colon and cecum: Typhlocolitis, superficial and necrotizing, multifocal, mild with edema, crypt hyperplasia and moderate numbers of adherent mucosal bacilli.

2. Ileum: Enteritis, superficial and necrotizing, multifocal, mild, with edema, crypt hyperplasia, moderate numbers of adherent mucosal bacilli and moderate numbers of apicomplexan schizonts and gamonts.

Conference Comment: Classification of *E. coli* strains generally references the virulence factors and mechanisms which cause disease. Mechanisms of injury include alterations in cell membrane fluid / ion transport (enterotoxigenic forms) or necrosis of enterocytes caused by bacterial toxins and inflammatory mediators / enzymes (enteropathogenic and enterohemorrhagic forms). Enterohemorrhagic strains invade enterocytes but enteropathogenic and enterohemorrhagic forms do not. Enterohemorrhagic strains result in death of enterocytes, followed by inflammation and hemorrhage. Gross lesions in enterotoxigenic forms are often indistinct, in contrast to enteropathogenic and enterohemorrhagic forms where gross lesions can include a thickened granular appearing mucosa with hemorrhage and fibrin exudation, which varies in severity depending on strain. Enteroinvasive forms, which result in septicemic colibacillosis, begin as an intestinal infection and bacteria and enterotoxins gain access to systemic

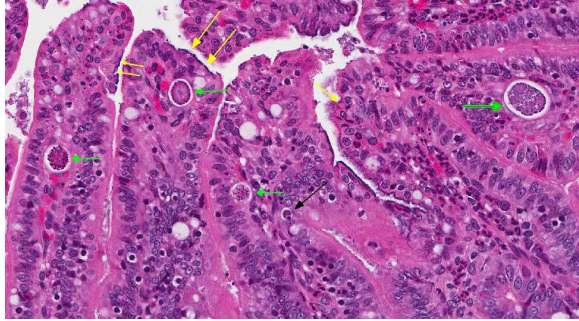
circulation through vessels in the lamina



Cecum, rabbit. Overlying the edematous lamina propria, degenerate luminal epithelium is attenuated with lined by a layer of attached bacilli consistent with enteropathogenic E. coli (HE, 400X)

propria underlying damaged mucosa.⁸

Enteropathogenic *E. coli* (EPEC), also referred to as attaching and effacing *E. coli* (AAEC), is often reported in rabbits but also affect other species such as calves, dogs and pigs. Bacteria attach to the microvillus border of intestinal epithelium by cups, which results in the formation of pedestals that can be seen ultrastructurally. Disruption of the microvillar border results in alterations in ion/fluid transport and a malabsorptive and maldigestive diarrhea; enterocyte death, fluid secretion and an inflammatory response also play a role in the pathogenesis.¹ Bacterial attachment is mediated by the protein intimin; EPEC also express fimbriae and EPEC adherence factor. EPEC produces bacterial proteins EspA, EspB, EspD, which enter cells and disrupt signal transduction pathways and microvilli. Some strains of EPEC produce a verotoxin which plays a role in the death of enterocytes and cells of the lamina propria.⁸ Infection of juvenile animals with EPEC / AAEC is often accompanied by co-infection with other intestinal pathogens such as rota or coronavirus, particularly in calves.¹



Ileum, rabbit. Within the epithelium, there are low numbers of developing coccidial gametocytes (green arrows) and a single developing schizonts (black arrow). There are numerous patches of aical epithelium which are lined by a dense band of enteropathogenic *E.coli* (yellow arrows). (HE, 400X)

Laboratory rabbit intestinal flora may include a wide variety of *E. coli* strains, including EPEC, which can be present in the absence of clinical signs; non-clinical animals may serve as reservoirs for infection. It has been suggested that inflamed intestine provides a favorable environment for pathogenic *E. coli* strains, which remain subclinical until other factors such as co-infections, stress or diet changes trigger an outbreak of clinical disease. Subclinical infection with various *E. coli* strains is important not only because of the reservoir potential, but also because many strains of *E. coli*, including EPEC, can be zoonotic.⁷

This case generated considerable discussion on different potential histologic diagnoses. Like the contributor, some conference participants preferred a mild to moderate typhlocolitis and enteritis; whereas others favored a more pathogenesis-oriented approach focused primarily on the EPEC-induced changes in the superficial mucosal epithelium (i.e. superficial mucosal epithelial necrosis) leading to the secondary lesions in lamina propria (i.e. inflammation, edema, and crypt hyperplasia). In sections of the small intestine intraepithelial protozoal schizonts and gamonts were described as

well as rare oocysts in the intestine lumen. Conference participants also noted the absence of adipose tissue in the small sections of mesentery that are present. Such mesenteric changes are suggestive of fat atrophy and could be related to this animal's nutritional status. However, the absence of adipose tissue could also be an incidental, age-related finding in a young rabbit.

Contributing Institution:

Charles River Labs Research Animal Diagnostic Services
CRIVER.COM

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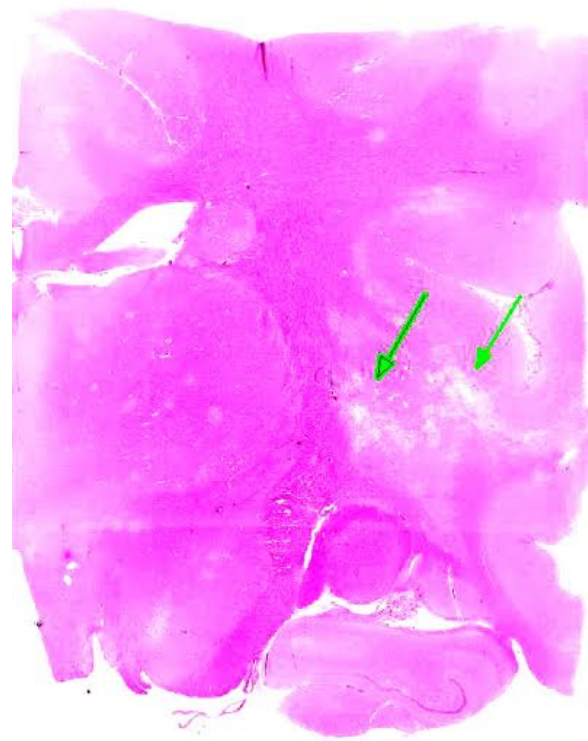
CASE III: 12A-892 (JPC 4033747).

Signalment: 11-year-old male Indian rhesus macaque (*Macaca mulatta*).

History: This animal was born at ONPRC and housed outdoors until 3 years of age, then kept indoors for the remainder of his life. He received two mucosal doses of SIV Mac239 approximately 5 years before his death. He was found deceased in the cage and was presented for necropsy the following day. He had been sedated multiple times for bronchoalveolar lavage over the course of the protocol. The most recent was performed 9 months prior to death. He had a history of intermittent epistaxis with the first report 10 months prior to death. On 12/29/2012 he was reported for epistaxis, not looking well, and hesitancy to stand. A cage side evaluation by a technician revealed a mild right head tilt and epistaxis. He took and ate treats readily when offered and was bright and interactive with the observer. He appeared slow-moving and hunched when ambulating. He was found dead in his cage the following day (12/30).

Gross Pathology: At necropsy, the animal was overweight (BCS 4/5). He had cyanotic oral mucous membranes, and epistaxis of

the right naris was evident. There was marked edema and congestion of all lung



Cerebrum, rhesus. At subgross magnification, there are areas of malacia within the white matter. (HE, 5X).

lobes with greater severity on the left side (found in left lateral recumbency). Sectioned surfaces of lung oozed blood-tinged fluid on palpation. Femoral bone marrow was diffusely dark red. Liver was rubbery, congested, enlarged, mottled dark red-brown with an enhanced reticular pattern. Gallbladder, cystic duct, and common bile duct were thickened and contained scant pale yellow bile. Renal cortices exhibited swelling and streaked pallor and renal medullae were streaked and congested. There was mild meningeal hemorrhage and congestion. Post-fixation, sections of the cerebellar peduncles have multiple, irregular foci of pale tan discoloration and are softer than the surrounding tissue.

Laboratory Results: Culture of the right naris grew *Pseudomonas* and *Staphylococcus aureus*. Culture of the lung grew Gram positive *Bacillus* spp. and α -hemolytic *Streptococcus* spp. His most recent complete blood count (1 month prior to necropsy) had only a minimal basophilia and the most recent serum chemistry (4 months prior to necropsy) was within normal limits.

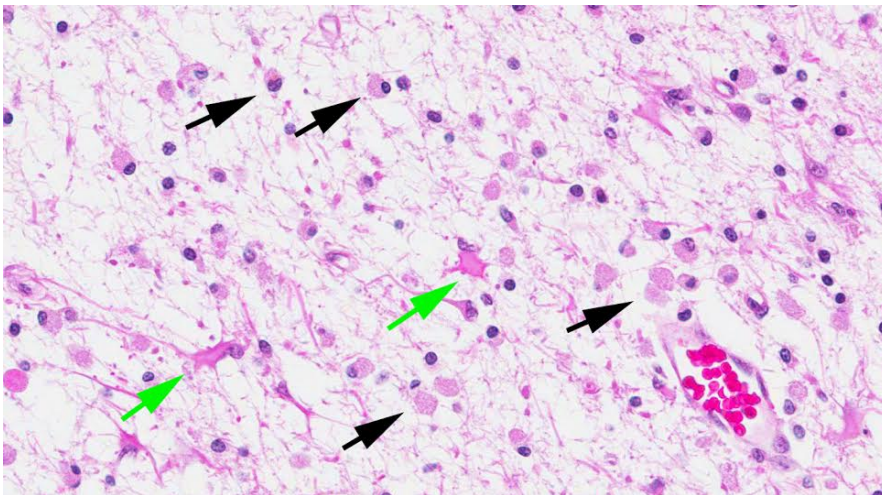
Immunohistochemistry of brain sections for SV40 antigen revealed moderate to strong staining of astrocyte nuclei and nuclei of (presumably) oligodendroglia within the white matter of the cerebrum and the cerebellum. Luxol fast blue staining of cerebrum and cerebellum highlighted loss of myelin with minimal myelin particulates in gitter cells.

Histopathologic Description: Within the cerebral white matter and extending into the white-gray matter junction are multifocal, irregular regions characterized by loss of neuropil and gliosis with prominent gemistocytic astrocytes that rarely contain glassy, intranuclear, amphophilic or granular, basophilic viral inclusions that

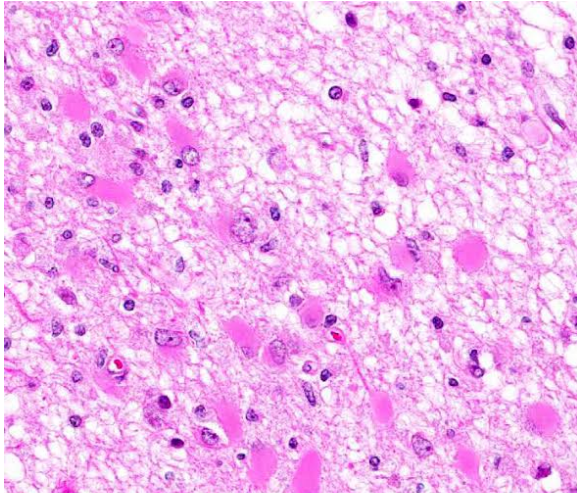
marginate the chromatin. Astrocytes are cyto- and karyomegalic, many with misshapen or bizarre nuclei and large or bizarre nucleoli. Affected areas are infiltrated by foamy gitter cells and fewer lymphocytes. In and around affected regions, Virchow-Robbins space of vessels is markedly expanded by clear space (edema) with variable numbers of lymphocytes, macrophages, and rare eosinophils. There is multifocal, minimal to mild extravasation of red blood cells into Virchow-Robbins space and rarely into surrounding parenchyma.

Contributor's Morphologic Diagnosis: Cerebrum: Leukoencephalitis, multifocal, chronic, severe with demyelination, gliosis, gitter cells, gemistocytic astrocytosis, and intranuclear viral inclusions within oligodendroglia and bizarre astrocytes.

Contributor's Comment: This case represents one manifestation of SV40 infection in immunosuppressed macaques. In this case, immunosuppression was due to experimental inoculation with SIV. The head tilt, posture, ambulation abnormalities, and death are attributable to brain lesions of SV40, while the epistaxis was unrelated and due to bacterial infection. Initial examination of the brain did not have grossly evident malacia, but multiple recuts of fixed tissue revealed discolored foci in the white matter. In addition to the characteristic histopathology, immunohistochemistry confirmed the presence of viral antigen within brain lesions.



Cerebrum, rhesus. At higher magnification, the areas of pallor are cavitations developing into glial scars. There is total loss of axons and myelin sheaths, with few remaining Gitter cells (black arrows). The remainder of the cells are astrocytes (green), whose proliferating processes are forming the glial scar. (HE, 400X). arrows



Cerebrum, rhesus. At the edge of the scar, there are numerous large astrocytes with abundant cytoplasm and large nuclei (gemistocytes). (HE, 256X).

Polyomaviruses are small, double-stranded DNA viruses and have been identified in birds, rodents, nonhuman primates, and humans. They were named for their propensity to cause tumors in either their natural host or atypical host(s), typically in differentiated cells. Much like herpesviruses, infection by polyomaviruses in their natural hosts typically causes an asymptomatic, life-long infection. The polyoma virion is divided into early and late-coding regions. The early region codes T antigens, the late codes capsid proteins. Small (t) and large (T) transforming/tumor antigens are conserved across all polyomavirus species and these proteins are fairly well-characterized. The small t antigen is not necessary for productive infection in cell culture, but the large T antigen is essential for production of progeny virions. T antigen plays a role in DNA replication as well as tumorigenesis. Some polyomaviruses, including SV40, have additional T antigens.¹

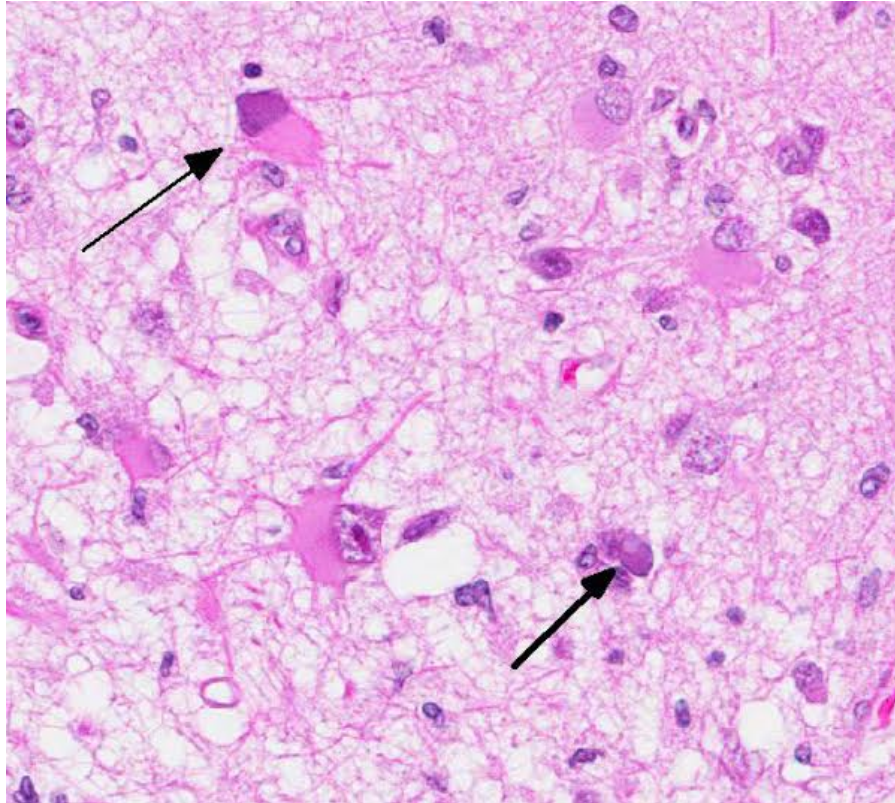
Simian virus 40 (SV40) is an endemic polyomavirus in captive and free-ranging macaques. Seroprevalence increases with age in socially-housed animals, and the

majority of animals are seropositive by 1 year of age.⁹ Infection does not cause lesions in immunocompetent animals, but immunosuppression, such as SIV infection or immunosuppressive drug regimens, allows reactivation of latent infections or primary infections to cause disease. Disease in immunosuppressed macaques can manifest as progressive multifocal leukoencephalopathy (PML), nephritis, or pneumonia.⁶

SV40 is of particular utility in research due to its comparative ease to grow in culture as well as it causing PML in SIV or SHIV-infected macaques similar to disease in human AIDS patients infected with JC virus. SV40 is potentially zoonotic, identified as a contaminant in early polio vaccines and is seroprevalent in people working with macaques.^{3, 4} There is public concern over SV40 vaccine contamination, and there has been extensive research into oncogenicity of SV40 in people as it is known to cause a host of neoplasms in hamsters.⁷

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Neuropathology of SV40 can be PML or meningoencephalitis, though some overlap does occur.² PML is hypothesized to occur in animals with underlying SV40 infection that subsequently are immunosuppressed, while macaques with more inflammatory



Cerebrum, rhesus. Nuclei of both astrocytes and oligodendroglia are expanded by a glassy, basophilic viral polyomaviral inclusion (HE, 324X).

than demyelinating lesions were SV40-negative at the time of SIV infection with subsequent experimental SV40 infection.^{8, 2} PML occurs due to infection and subsequent loss or dysfunction of myelin-producing oligodendroglia. In addition to the demyelination, a unique histopathologic feature of PML in macaques are the gemistocytic astrocytes with abundant eosinophilic cytoplasm and bizarre nuclei.⁶ The gitter cell infiltration and gliosis are a secondary reaction to the breakdown of myelin. The more inflammatory manifestation of neurologic SV40 is characterized by leptomeningeal edema and perivascular and multifocal plaque-like infiltrates of lymphocytes and macrophages with extension of inflammatory cells into cortical gray matter.²

JPC Diagnosis: Cerebrum, subcortical white matter: Leukoencephalomalacia, multifocal, marked with demyelination, numerous pleomorphic gemistocytic astrocytes, and glial intranuclear inclusion bodies.

Conference Comment: The contributor provides an excellent review of SV40 infection and corresponding lesions above. There are seven polyomaviruses that have been identified in non-human primates. In addition to SV40 in

macaques, these include SV12 in baboons, B-lymphocyte papovavirus (LPV) in African green monkeys, *Polyomavirus papionis 2* in baboons, cynomolgus polyomavirus (CPV), SV40-CAL in macaques, and chimpanzee polyomavirus. These viruses are common as latent infections as seen with SV40 and discussed above.⁵ Renal lesions are associated with initial SV40 infection and the characterization of renal lesions and their relationship to the sequence of infection and immunosuppression vary between different polyomaviruses in non-human primates and humans.² With SV40 infection lesions include nonsuppurative tubulointerstitial nephritis with basophilic intranuclear inclusion bodies in tubule epithelial cells. Sloughed epithelial cells are present in tubule lumina and evidence of regeneration may be present. Pulmonary lesions secondary to SV40 infection may include interstitial pneumonia; other pulmonary

lesions may be also present and associated with immunosuppression, not secondary to SV40 infection.⁶

The pathogenesis of infection and corresponding CNS lesions in this case was also discussed. Recrudescence of the virus secondary to immunosuppression results in infection of oligodendrocytes and astrocytes, with loss of oligodendrocytes resulting in the demyelinating lesion seen histologically.

Multifocal to coalescing areas of necrosis and loss of the white matter constitute approximately 10% of the section, and are located primarily at the junction of the grey and white matter which contain numerous gemistocytic astrocytes and gitter cells. Additional characteristics include mild spongiosis and multifocal dilation of axon sheaths. There was a discussion regarding the chronicity of these lesions with some participants interpreting areas of pallor as having abundant astrocytic fibers, less debris and fewer gitter cells, indicating a developing astrocytic scar. Immunohistochemistry for glial fibrillary acidic protein proved that many of the filaments traversing these areas are indeed astrocytic processes.

Contributing Institution:

Oregon National Primate Research Center
<http://www.ohsu.edu/xd/research/centers-institutes/onprc/>

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CASE IV: Rt13-623 (JPC 4070249).

Signalment: 9-month-old, female, Thicket Rat (*Grammomys surdaster*).

History: A thicket rat was inoculated with 200,000 *Plasmodium chabaudi* sporozoites intravenously and blood was collected for evaluation every other day. While blood was being collected, the rat died.

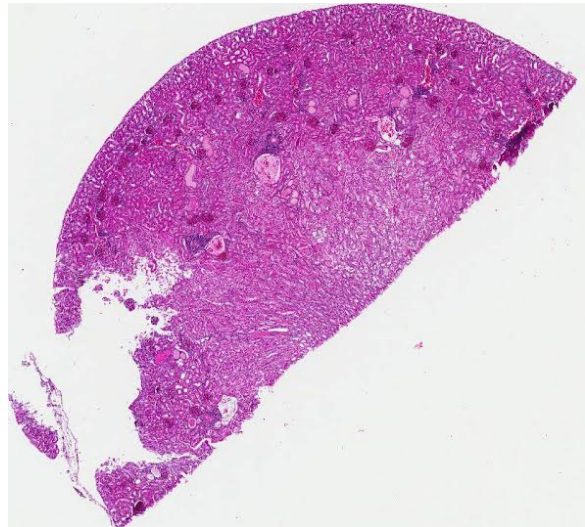
Gross Pathology: There was anasarca and approximately 1 ml of a clear pleural effusion. The spleen was very dark and was approximately four times normal size.

Laboratory Results: Parasitemia at time of death was 25.5%.

Histopathologic Description: Dark violet-red granules line capillary tufts in almost all glomeruli, with smaller granules in the mesangium and in the basement membrane of tubules. Some tubules are ectatic and contain protein casts. There are multifocal, perivascular lymphoid aggregates.

Other lesions included erythroid extramedullary hematopoiesis of the spleen, follicular hyperplasia of the splenic white pulp, multifocal nonsuppurative myocarditis, multifocal centrilobular hepatic lipidosis, and hemosiderophages in the liver, spleen, blood vessels, alveolar walls, and nasolacrimal ducts.

The granular material does not polarize and does not lose color when bleached.



Kidney, thicket rat. At subgross magnification, glomeruli are prominent due to their deep eosinophilia. (HE, 5X).

Special stains: Puchtler-Sweat and Perl's [Iron stains]: Blue granules were seen within tubular epithelium but not associated with the basement membrane. No staining was present in the glomeruli.

Masson's trichrome (-); PAS (-); and von Kossa and Alizarin Red (-).

Transmission Electron Microscopy:

Kidney, Glomerulus: Within the podocytes, there are 150-750 nm granular deposits adjacent to the basement membrane (white arrow). Similar but smaller deposits are seen within the basement membrane (black arrow). The foot processes are effaced with infrequent filter slits. The mesangium is moderately expanded without an increase in cellularity.

Kidney, Tubule: Small granules (<150 nm) are within the basement membrane (black arrow) and adjacent to the basement membrane (white arrow). There is a slight increase in lipid.

Elemental analysis of the granules was attempted but was inconclusive.

Contributor's Morphologic Diagnosis:

Kidney: glomerulonephropathy characterized by deposition of granular material along [capillary loops] glomerular basement membranes, diffuse, marked.

Kidney, tubules, basement membrane: granular pigmented material deposition.

Kidney: tubular proteinosis, multifocal, mild.

Kidney: chronic interstitial nephritis, multifocal, mild.

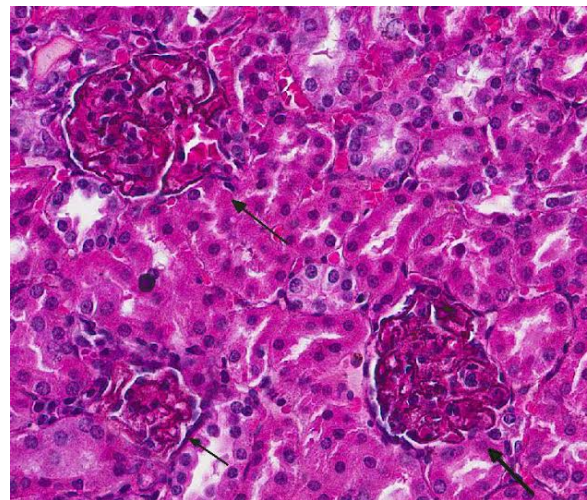
Contributor's Comment: According to the World Health Organization, there were approximately 198 million cases and 584,000 deaths due to malaria in 2014.¹² Although most of the cases are concentrated in (sub-Saharan) Africa, malaria can be found in Central and South America, India and Asia.¹¹ Mortality is highest in children under 5 years and in pregnant women.^{1,12} Humans are infected by *Plasmodium falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi*. Anopheles mosquitoes serve as the vector.¹ Severe disease due to Acute kidney injury is found in less than 1% of cases of *Plasmodium falciparum* infection but the mortality rate can be as high as 45%.³ Few cases of kidney injury are also seen with *Plasmodium malariae* and *P. vivax* infection.³ Acute kidney injury is primarily seen in adults, older children, and those who live in areas with a low incidence of infection. Renal insufficiency may be part of multi-organ failure or the only clinical presentation.^{3,8} cerebral malaria or anemia is most often associated with *Plasmodium falciparum* infection.^{1,7}

If there is a full recovery, there is no progress to chronic kidney disease.³ Microscopic changes are variable, but

tubular degeneration and necrosis predominate. Parasitized erythrocytes are sequestered in glomerular and interstitial vessels, and hemosiderin in tubular epithelium, hemoglobin casts, mild glomerular changes, and interstitial nephritis may also be present. Immune-mediated glomerulonephritis is not associated with acute kidney injury.^{3,7} Renal changes may be due to obstruction and sequestration of infected erythrocytes, glomerular and tubular inflammatory changes, fluid loss, and altered renal circulation.³

There have been reports of a “quartan malarial nephrotic syndrome” due to immune complex disease in children in Nigeria that was thought to be related to *Plasmodium malariae* infection.⁶ Subsequent papers have suggested that renal disease in children in Nigeria, as well as Uganda and Ivory Coast, was associated with, but not caused, by *Plasmodium malariae* infection.^{3,4}

Thicket rats found in sub-Saharan Africa are natural hosts for chronic *Plasmodium chabaudi*, *P. berghei*, *P. vinckei*, and *P. yoelii* infection and model chronic *Plasmodium falciparum* disease in man. Anopheles mosquitos serve as vectors for the both human and rodent plasmodial



Kidney, thicket rat. A deeply eosinophilic granular pigment is deposited within glomeruli. (HE 296).

infection. Although no one species mimics *Plasmodium falciparum* infection completely, there are enough similarities, especially with *Plasmodium chabaudi*, to make them a valuable tool for comparative study. In both *Plasmodium chabaudi* and *Plasmodium falciparum*, symptoms develop as parasitemia increases and then resolve when parasitemia decreases. Infected rodents develop anemia secondary to dyserythropoiesis, suppression of hematopoiesis, and thrombocytopenia as seen in man. Red blood cells sequester in the placenta, which serves as a model of malaria-related fetal and neonatal abnormalities. In several ways, *Plasmodium chabaudi* is not representative of infection by *Plasmodium falciparum*. Unlike *Plasmodium falciparum* in man, the organ of sequestration is the liver, and not the brain. Infected animals develop hypothermia instead of fever. Although there is splenomegaly, it is not correlated with level of parasitemia or sequestration.¹⁰

In a recent report, mice infected with *Plasmodia berghei* ANKA developed renal failure accompanied by increased inflammatory cytokines, expression of adhesion molecules, and products of oxidation. Resulting changes in the vascular endothelium led to interstitial edema and, over time, mice developed acute tubulointerstitial nephritis. Although there was no deposition of brown granular material apparent by examination of H&E slides, there was deposition of polarizable crystals identified as hemozoin in glomeruli and endothelium.⁵

The degree of pigment deposition in the glomeruli was unusual. Analysis by special stains, electron microscopy, and elemental analysis did not reveal the composition of the granular material. Given the high level

of parasitemia, we felt the material was directly related to infection with malaria.

JPC Diagnosis: 1. Kidney, cortex, glomerular and tubular basement membranes: Immune complex deposition, diffuse.

2. Kidney, cortex: Tubular ectasia and proteinosis, multifocal, mild.

3. Kidney: Cortical perivascular lymphocytic infiltrates, multifocal, mild.

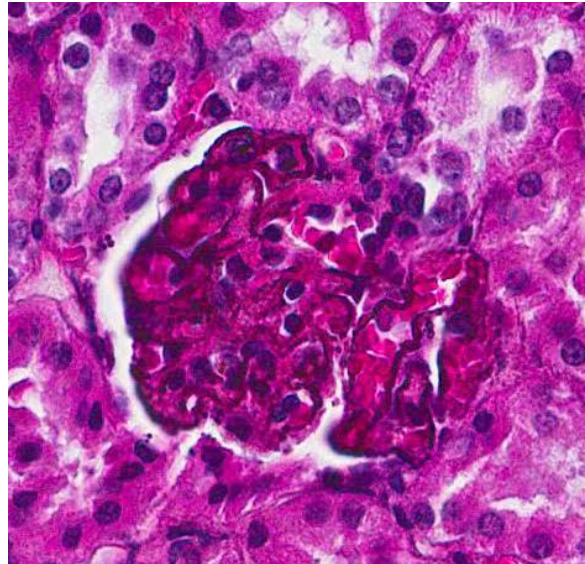
Conference Comment: This case was studied in consultation with Dr. Rachel Cianciolo co-director of the International Veterinary Renal Pathology Service; she interpreted the renal changes in the submitted microscopic slide as most consistent with immune complex-mediated membranous glomerulonephropathy and commented it is very unusual to observe the actual immune complex deposits with hematoxylin and eosin staining alone. This case was also studied in consultation with the Department of Nephropathology at the Joint Pathology Center, whose medical pathologists are familiar with glomerular disease as described in humans; they similarly postulated that the deposits may be immunoglobulin or complement, as would occur with membranous glomerulonephropathy, and further noted that, in humans, the index of suspicion would be elevated for lupus nephritis in this case.

A definitive diagnosis of immune-mediated glomerulonephropathy requires immunofluorescent or immunohistochemical confirmation of immunoglobulin and complement components in glomerular tufts. Ultrastructurally, electron-dense deposits may be seen in mesangial, subepithelial or subendothelial locations. Glomerulonephritis results following the deposition of immune complexes within the glomeruli or

following the formation of antibodies to antigens present in the basement membrane (anti-GBM disease). Immune complex glomerulonephritis is a sequela to persistent infections of various etiologies including viral, bacterial and parasitic infections, as well as autoimmune disease. Antigen-antibody complexes form in the presence of antigen excess or when antigen-antibody quantities are equal. The complexes deposit in glomerular capillaries, fix complement, and cause basement membrane damage, which is enhanced by the ensuing inflammatory response. Factors that can determine the extent of deposition include the size and charge of complexes, glomerular permeability and the antigen-antibody avidity. Small and intermediate complexes are the most damaging, as larger complexes are removed from circulation by the mononuclear phagocytic system.⁹

Glomerular lesions described in human malaria patients do include prominent mesangial proliferation with occasional basement membrane thickening and deposition of an eosinophilic granular material along capillary walls, within the mesangium and in Bowman's capsule.^{2,3} The origin/nature of this material is not well described. Other findings include immunofluorescence demonstration of IgM and C3 in mesangial capillary walls, although other studies have found immune complex deposition was not seen in basement membranes or the mesangium of glomeruli and clinical / clinical pathology findings apparently don't necessarily support the presence of immune complex deposits in all cases of acute renal failure related to malaria infection.³ However, in other reports in the literature glomerulonephritis is described in cases of acute renal failure in malaria infection and a distinction is made between chronic progressive glomerulopathy which occurs in

quartan malaria in Africa, versus acute renal failure associated with falciparum malaria in Southeast Asia, India and sub-Saharan Africa.² Ultrastructural findings described in human cases include subendothelial and



Kidney, thick-tailed rat. At higher magnification, the granular pigment outlines capillary basement membranes, and granular deposits are present within the mesangium as well. (HE, 400X).

mesangial electron dense deposits with the presence of granular, fibrillary and amorphous material.^{2,3} The absence of a prominent glomerular inflammatory response in this case leads to speculation regarding the role of the glomerular deposits in renal function.

In this case, diffusely and globally within glomeruli there are 1-2µm, prominent, eosinophilic globules lining capillaries and slight increase in cellularity within the mesangium. There are multifocal glomerular synechia, and the parietal epithelium is mildly hypertrophied. The eosinophilic material is also seen within the basement membrane of adjacent tubules, and there is tubular ectasis, proteinosis and attenuation of tubular epithelium with few areas of tubular epithelial degeneration and necrosis. During the conference, there was discussion

regarding the origin of the eosinophilic globules, including calcium, hemosiderin, lipofuscin, and melanin. A battery of histochemical stains performed by the contributor did not shed further light as to the nature the basement membrane deposits.

Nephrotic syndrome is well-documented in human malarial infection, and various histologic patterns of glomerular lesions are reported in the literature, including focal segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis, minimal change disease, and membranous glomerulonephritis, among others [Asinobi AO – 2015]. In a 1967 study of the histologic features of nephrotic syndrome in 77 people with quartan malaria (infection with *Plasmodium malariae*), approximately half had detectable malarial organisms, and all but one had glomerulonephritis, including nine cases histological classified as membranous glomerulonephritis [Kibukamusoke JW-1967], which corresponds with the preferred diagnosis offered by Dr. Rachel Cianciolo in this case. In the same study, 63% of the patients were children, thus presumably “non-immune”, and had a higher incidence of parasitemia as compared to adults. A more recent review article on the topic provided by the contributor summarizes the clinicopathologic findings of malaria-induced renal damage in people, including the various forms of malarial nephropathy and their association with a specific malarial etiology and the patient anamnesis⁴. General observations in the review included the finding that non-immune (naïve) patients have a higher risk of developing acute renal failure than semi-immune people (those living in endemic areas); in quartan malaria (*Plasmodium malariae*) cases originating from Nigeria and the Ivory Coast, the primary glomerular lesion with light microscopic examination was characterized as membranous nephron-

pathy; and fatal acute renal failure occurs in cases of falciparum malaria in up to 40% of non-immune patients with high parasitemia above 5% of parasitized erythrocytes.

During the conference, the moderator disclosed that this thickset rat was one of several in an experimental group that either died or was euthanized with similar clinical and histopathologic findings as the one presented in this case. The glomerular lesions, gross findings indicative of nephrotic syndrome (anasarca and pleural effusion), and the clinical history of an immunologically naïve animal with a high parasitemia lends to the possibility of an emerging animal model for malarial acute nephrotic syndrome/renal failure. Interestingly, glomerulonephritis is described in both rat and mouse models infected with variants of *Plasmodium berghei*, with primarily mesangial deposition of immunoglobulins (e.g. IgM, IgG, C3, among others) and malarial antigen. [George CR 1976; Ehrich JH – 1981). In the rat, the renal lesions were transitory and appeared to resolve within one to three months, unlike the lethal course of disease that occurred in this thickset rat.

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