The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator:
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WEDNESDAY SLIDE CONFERENCE 2009-2010

Conference 9

2 December 2009

Conference Moderator:

Dr. Tim Walsh, DVM, Diplomate ACVP

CASE I: BA42/09 (AFIP 3134367).

Signalment: 5-year-old male nyala (*Tragelaphus angasii*).

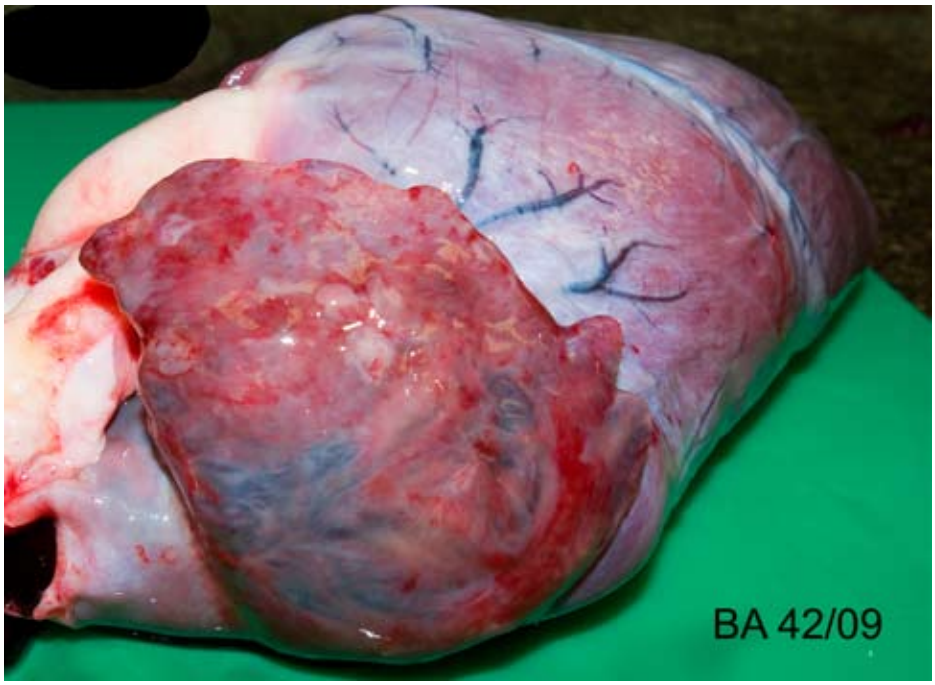
History: This animal was reported to be quiet and lethargic for several hours before being found dead by zoo staff. A clear brown, watery fluid was aspirated from the cardiac region by the referring veterinarian while attempting to take a post mortem blood sample.

Gross Pathology: The carcass was in good nutritional condition. The pericardium contained approximately 40ml of red tinged watery fluid, and a cream to red, soft, smooth 8 x 0.5 x 0.2 cm clot of coagulated protein. The epicardium was multifocally mildly roughened to granular. The right atrium and atrial appendage were dilated and thin walled, and the right atrial appendage contained multifocal intramural nodules measuring 2-5mm in diameter which were firm and often had a mottled red to brown appearance, with a pale yellow / brown, firm to gritty central area (**fig. 1-1**). The right atrial wall at the atrioventricular junction was mildly thickened and spongy. The heart weighed 560g, 0.66% of body weight (maximum normal for cow and goat is 0.66%).[6] The trachea and bronchi contained a moderate amount of white frothy fluid. The dorsal aspects of both lungs contained multifocal to coalescing regions of dark red discoloration (hemorrhage), which did not extend into the parenchyma. The liver had rounded borders, and oozed moderate amounts of blood from the cut surface. Skeletal muscles were macroscopically unremarkable.

Laboratory Results: Liver: vitamin E 34.22 µmol/kg FT (ref. range bovine = 3-18 µmol/kg); selenium 1.37 mg/kg DM (ref. range bovine: >23 mg/kg).

Histopathologic Description: Right atrium, one section is examined. Regionally extensive and coalescing areas of the myocardium are replaced by loosely organized knots and whorls of plump spindloid cells (fibroblasts) (**fig. 1-2**). There are also numerous clusters of small blood vessels in these areas (neovascularization). They are lined by plump endothelial cells, and the spindle cells tend to form circumferential rings around them. Occasional eosinophilic hyaline droplets are closely associated with the small vessels, interpreted to be plasma. The surrounding stroma and myocardium is infiltrated by large numbers of foamy macrophages, moderate numbers of pyknotic eosinophils, and smaller numbers of lymphocytes and neutrophils. There are also moderate to large numbers of extravasated red blood cells (hemorrhage). These regions are often surrounded by mature dense collagen bundles and moderate to large numbers of macrophages, which contain coarsely granular, brown pigment (hemosiderophages).

*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists, and the C. L. Davis Foundation.



1-1. Heart, Nyala. The right atrium and atrial appendage are dilated and thin walled, with multifocal intramural, firm, mottled red to brown nodules measuring 2-5 mm in diameter which occasionally have gritty centers. Photograph courtesy of Veterinary Pathology Unit, Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, Scotland, sionagh.smith@ed.ac.uk.

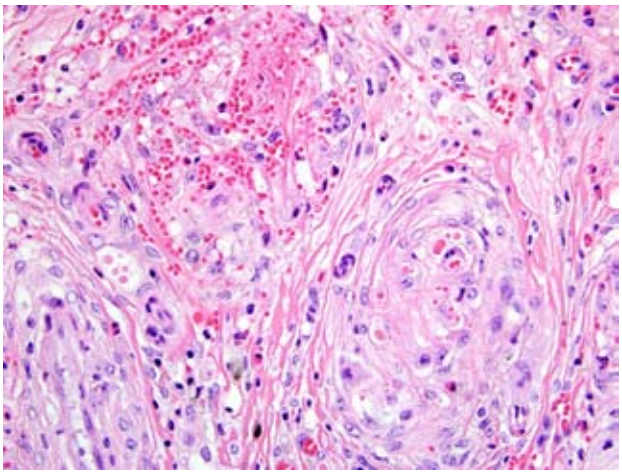
Some blood vessels contain smudged eosinophilic material (fibrin thrombi). Occasional larger blood vessels contain moderate amounts of intramural smudged eosinophilic material (fibrin), creating a "starburst" appearance. These vessels are surrounded by a layer of foamy macrophages and fibroblasts, hemorrhage, pyknotic granulocytes, and lymphocytes (vascular fibrinoid necrosis) (**fig. 1-3**). Myofibers in the remaining myocardium are occasionally shrunken, hypereosinophilic, and fragmented (myodegeneration). Moderate numbers of plasma cells and lymphocytes are scattered throughout the myocardium, together with moderate numbers of fibroblasts. Multifocal to coalescing regions of the myocardium are replaced or separated by finely granular, palely eosinophilic material (fibrosis). Multifocal areas contain irregular fragments of trabecular bone, and one fragment of bone has a paler central core which contains numerous large, pale blue grey cells within lacunae (chondrocytes, cartilage) (**fig. 1-4**).

Contributor's Morphologic Diagnosis: Myocardial degeneration and loss, severe, multifocal to coalescing, with fibrinoid vasculitis, neovascularization, thrombosis, hemorrhage and osseous metaplasia - right atrial appendage, Nyala (*Tragelaphus angasi*).

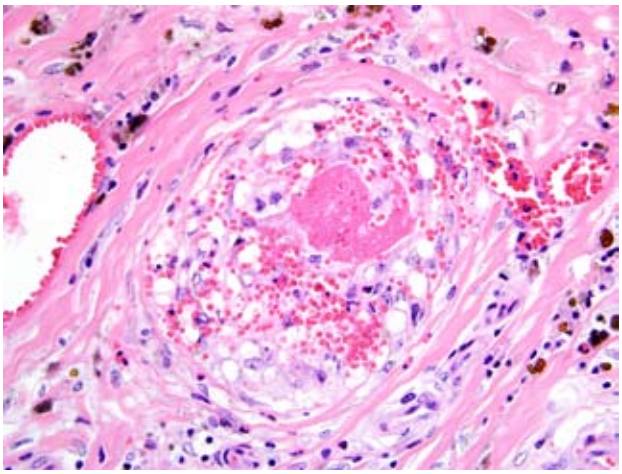
Contributor's Comment: Given the signalment, history, gross and histological findings, a diagnosis of nutritional myopathy due to vitamin E and/or selenium deficiency was considered to be most likely. Liu et al (1985) described histopathological features of nutritional myopathy in captive nyala, in particular noting coronary arteriolar fibrinoid necrosis in affected young animals.[4] An additional feature of this case is the striking neovascularization with small blood vessels surrounded by whorls of spindle cells. This is considered most likely to be a form of granulation tissue produced as part of the reparative process.

Vitamin E (as tocopherol) and enzymes containing selenium (glutathione peroxidase / glutathione reductase system) act as antagonists to free radicals and highly reactive unstable compounds produced either during normal cell function or as a result of disease or tissue injury.[8] Damage by free radicals is primarily caused by peroxidation of cellular and subcellular lipid membranes, resulting in loss of the ability to maintain essential differential ion gradients across the membrane. Free radicals may also cause damage to proteins of various cellular components, including mitochondria and endoplasmic reticulum.[1,2,4,8] Vitamin E and the selenium-containing glutathione enzymes act synergistically. However, they may remove different free radicals; therefore, a deficiency in either can lead to disease, with deficiencies in both leading to severe disease.[1,8] In this case hepatic vitamin E levels appeared to be adequate, whilst selenium levels were considered to be low.

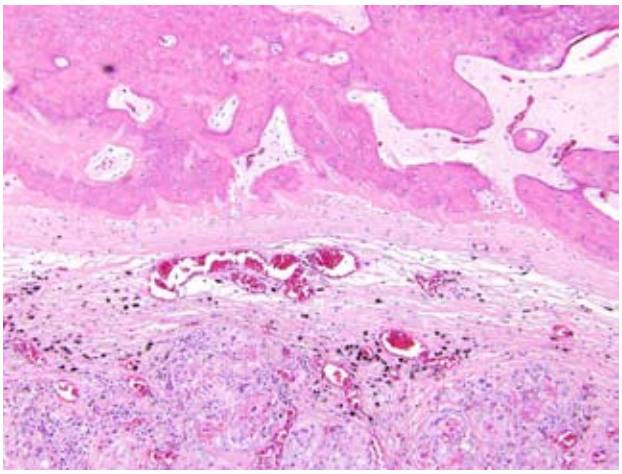
Oxidative damage is commonly seen in actively contracting muscle fibers where damage to the cellular membrane allows influx of calcium ions, which are actively moved away from the calcium sensitive myofibers and stored within mitochondria. This results in a



1-2. Heart, Nyala. Areas of the myocardium are replaced by streams and whorls of plump reactive fibroblasts which often center on small caliber blood vessels. Reactive fibroblasts are admixed with low numbers of lymphocytes, eosinophils, and neutrophils. (HE 400X)



1-3. Heart, Nyala. Vessel walls are replaced by brightly eosinophilic proteinaceous material, infiltrating inflammatory cells, and hemorrhage; the endothelium of affected vessels is markedly reactive. (HE 400X)



1-4. Heart, Nyala. Multifocally within the myocardium are areas of reactive fibrous connective tissue and bone. (HE 100X)

reduction in mitochondrial function and decreased energy production.[8] Calcium overload eventually causes a state of myofiber hypercontraction, causing degeneration of the myofilaments and coagulation of the contractile proteins.[8]

Nutritional myopathy (syn. white muscle disease, mulberry heart disease, nutritional myodegeneration, nutritional muscular dystrophy, stiff lamb disease) is recognized in many domestic and exotic species, most frequently associated with hypovitaminosis E and selenium deficiency. Reports in the nyala are common and it is considered to be a particularly susceptible species.[3-5,8] In domestic animals, nutritional myopathy is usually a disease of young animals, calves, lambs, swine and foals, with sporadic disease in adults. Affected young animals are usually well grown and thrifty, and affected adults are often those in good nutritional condition.[8] Factors related to vitamin E and selenium deficiencies may be rapid postnatal growth, poor quality feed, lush forage in heavily fertilized and watered pasture, selenium antagonists (e.g. copper, silver, zinc, sulphur), and grazing on dry pastures.[8]

AFIP Diagnosis: Heart, atrial appendage: Fibrinoid vascular necrosis and nodular proliferation, chronic-active, multifocal to coalescing, marked, with cardiomyocyte degeneration and loss, hemorrhage, fibrin thrombi, and fibro-osseous metaplasia.

Conference Comment: Conference participants interpreted the striking vascular changes in this case as the primary lesion, with the myocardial changes occurring secondarily as reflected in the preferred morphologic diagnosis. However, an alternative view is that the myocardial degeneration and necrosis is unrelated to the vascular changes, as is supposed in pigs with mulberry heart disease. In the latter, arterioles of the heart, kidneys, liver, stomach, intestine, mesentery, skeletal muscle, and skin exhibit changes ranging from endothelial swelling, with increased permeability, to fibrinoid change with thrombosis and smooth muscle cell necrosis. A deficiency in vitamin E, rather than selenium, is now thought pivotal in the development of mulberry heart disease.[6] While tissues from other organs were not submitted for histopathologic evaluation in this case, finding a similar distribution of

vascular lesions would be noteworthy. A list of selected diseases associated with vitamin E/selenium deficiency or imbalance in various animal species is available in WSC 2008-2009, Conference 14, case III.

Conference participants considered a number of potential etiologies, including plant toxins (i.e. cardiac glycosides), an endotheliotropic virus, encephalomyocarditis virus, and malignant catarrhal fever. This case illustrates the importance of ancillary testing in a diagnostic setting, and conference participants discussed practical approaches to making the correct etiologic diagnosis in the field. While reserving fresh frozen tissues is ideal for analysis in cases of suspected toxicity or dietary deficiency, the conference moderator reminded participants it is feasible to quantify many minerals from formalin-fixed tissues. For cases of suspected selenium deficiency, when tissue samples are unavailable from the affected animal, viable alternatives include testing samples from herdmates (i.e. animal tissues, blood, and milk), soil, or forages and grains for selenium levels. In cases where animals have been administered parenteral or enteral supplemental selenium in response to a clinical suspicion of deficiency, blood and milk levels of selenium rise rapidly, obscuring the deficiency and precluding a diagnosis. Alternatively, measuring whole blood glutathione peroxidase (GSH-PX) is still useful because its enzymatic activity depends on incorporation of selenium into erythrocytes during erythropoiesis, which requires four to six weeks for enzyme levels to rise following selenium supplementation. By contrast, plasma GSH-PX activity, which does not rely on incorporation into erythrocytes, rises more quickly following supplementation.[7]

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http://www.vet.ed.ac.uk/cliniclaserv/VPU/VPU.htm

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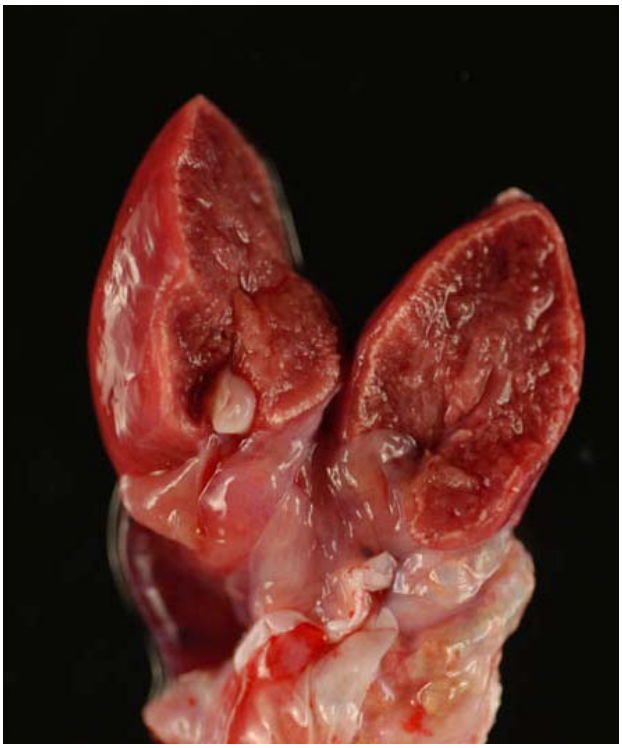
CASE II: N08-420 (AFIP 3134650).

Signalment: 6-year-old male green iguana (*Iguana iguana*).

History: Presented to the UF VMC as an emergency. It had been profoundly and progressively lethargic for approximately 10 days. It was reported that the animal was anorexic and not drinking for at least 7 days. The animal was housed in an outdoor glass screened enclosure with access to sunlight. Diet consisted of a commercial iguana pelleted diet and lettuce. On physical exam, this iguana was cachectic, critically dehydrated (estimated 12% dehydrated) and unresponsive. The animal exhibited muscle stiffness and fasciculations. Therapy (fluids, calcium gluconate) was initiated, but this animal spontaneously died approximately 10 hours post-admission.

Gross Pathology: The iguana is in poor nutritional condition, with depleted fat bodies. The kidneys are pale tan with numerous pin-point white foci visible beneath the capsule and on cut section within the parenchyma (**fig. 2-1**). There is a thin, tan-white streak immediately subtending the epicardium of the ventricle (**fig 2-2**). The aorta is expanded by white-yellow, hard, gritty plaques which elevate the intima. There are small fragments of wood in the stomach.



2-1. Heart, Iguana. There is a thin, tan-white streak immediately subtending the epicardium of the ventricle. Photograph courtesy of Department of Infectious Diseases and Pathology, University of Florida, 2015 SW 16th Ave, Room V3-156, Gainesville, FL 32610, farina@vetmed.ufl.edu.

Laboratory Results: Calcium 9.8 mg/dL; Ionized calcium 0.68 mmol/L (normal 1.22-1.62 mmol/L); Blood pH 6.8; Phosphorus 30.4 mg/dL; CK 133,460 U/L; Uric acid 258 mg/dL; AST 788 U/L; GGT 125 U/L; Potassium 7.6 mEq/L; WBC 21,200/µL; Heterophils 17,384/µL with mild degranulation and +2 toxicity.

Histopathologic Description: Multifocally, there is extensive mineralization of the basement membranes, mesangium and visceral and parietal epithelium of glomeruli. Diffusely, there is marked mineralization of the tubular basement membranes. Multifocally, the mineralized basement membranes are markedly expanded, and the tubular epithelium is also multifocally mineralized. Multifocally, there is mild to moderate degeneration and necrosis of tubular epithelial cells, and occasional tubular regeneration. Tubules sometimes contain small amounts of amorphous eosinophilic material and sloughed necrotic cellular debris, and occasionally, tubules are expanded by basophilic, radiating, acicular material (urate tophi) (**fig. 2-3**). The epithelial cells surrounding the urate tophi are often partially effaced and/or appear necrotic. Tubules also occasionally contain amorphous, basophilic, mineralized debris. There is multifocal mineralization of arteries and veins which expands the intima, elevating the endothelium, and extensively extends into the media. There are often aggregates of mineralized material luminally within vessels; this material sometimes has a radiating appearance.

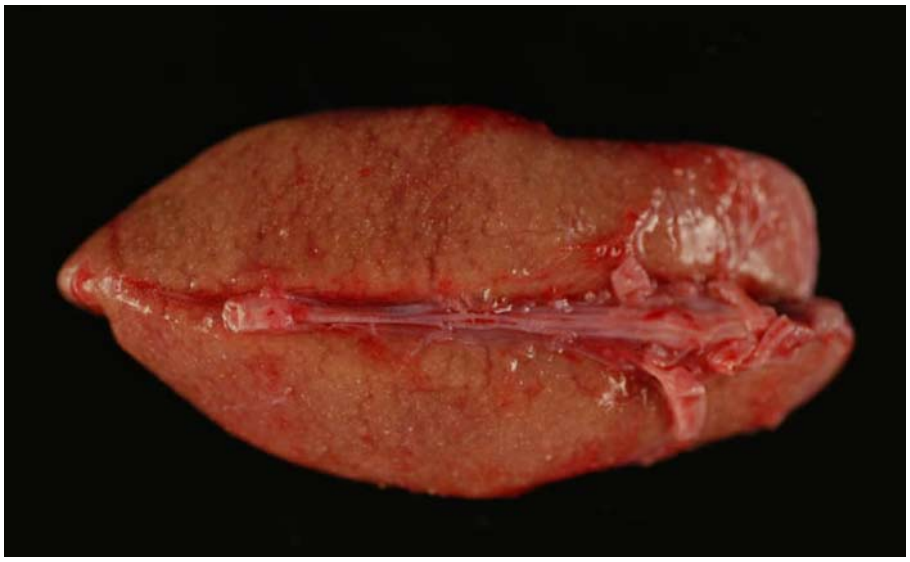
Multifocally, there is often extensive mineralization of the gastric mucosa (**fig. 2-4**). The mineralization affects all levels of the mucosa including the luminal and crypt epithelium and the connective tissue of the lamina propria. In more severely mineralized foci, there is often fibrosis in the lamina propria and loss of glands. There is multifocal moderate mineralization of vessels in the submucosa and lamina propria. There is mild mineralization of the outer aspects of the tunica mucosa and the serosa.

Multifocally and extensively, there is mineralization of cardiac myofibers. The mineralization is evident as a linear band in the outer myocardium and also as scattered myofibers throughout the myocardium. There is extensive mineralization of the vessels in the myocardium and epicardium. There are moderate amounts of fibrous connective tissue associated with the mineralized myofibers in the linear band region (**fig. 2-5**). Surrounding myofibers are frequently markedly vacuolated and occasionally necrotic and infiltrated with moderate numbers of macrophages.

Contributor's Morphologic Diagnosis:

1. Metastatic mineralization, multifocal, chronic, moderate-severe, with:
   a. Glomerular, tubular, and vascular mineralization, chronic, multifocal, severe, kidneys.
   b. Myocardial and vascular mineralization, chronic, multifocal, marked, with mild myofiber degeneration and necrosis and mild myocardial fibrosis, heart.
   c. Gastric mucosal and vascular mineralization, chronic, multifocal, moderate, with fibrosis and loss of glands.
   d. Mineralization and fibrosis, chronic, multifocal, severe, aorta (not included in slide set).
2. Renal tubular urate tophus deposition, acute, multifocal, minimal to mild, with tubular necrosis.

Contributor's Comment: On microscopic examination, there was extensive mineralization of the tunica intima and tunica media of vessels in nearly all of the tissues examined as well as extensive mineralization of the renal tubules (predominantly basement membranes), glomeruli, myocardium, pulmonary interstitium, and other tissues. The mineralized tissue largely appeared relatively normal, consistent with metastatic mineralization.



2-2. Kidney, Iguana. The kidneys are pale tan with numerous pin-point white foci visible beneath the capsule and on cut section within the parenchyma. Photograph courtesy of Department of Infectious Diseases and Pathology, University of Florida, 2015 SW 16th Ave, Room V3-156, Gainesville, FL 32610, farina@vetmed.ufl.edu.

Causes of metastatic soft tissue mineralization include hypervitaminosis D (due to excess supplementation or ingestion of vitamin D-containing rodenticides or plants [*Cestrum* spp. and *Solanum* spp.]) and elevated calcium and/or phosphorus (nutritional or renal secondary hyperparathyroidism, primary hyperparathyroidism, hypercalcemia of malignancy).[4] Specifically, animals are considered at risk for soft tissue mineralization when the calcium-phosphorus product is greater than 60-70.[4]

Green iguanas require UV-B light to convert provitamin D3 to active vitamin D3, and oral supplementation of vitamin D3 (instead of a UV-B source) has been determined to be ineffectual.[7] A study on iguanas at the National Zoological Park presenting for necropsy revealed widespread mineralization of soft tissues, especially of vessels and basement membranes, cardiac and skeletal muscle degeneration and necrosis with mineralization, and occasional mild fibrous osteodystrophy. In these animals, circulating levels of vitamin D3 were 7-36 ng/ml (normal is >400ng/ml).[7,8]

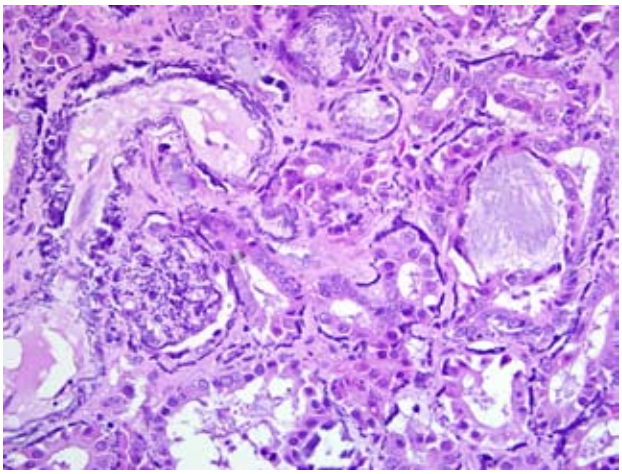
Possible causes for paradoxical soft tissue mineralization in these animals which were proposed were chronically low vitamin D levels leading to hypocalcemia, resulting in an exaggerated PTH response and excessive calcium mobilization from bone with resultant mineral deposition in soft tissues; or metabolic derangements altering the calcium-phosphorus ratio, which could result in an elevated calcium-phosphorus product which could predispose to mineralization.[7,8]

In this case, [Ca] = 9.8 mg/dL (normal 10.9-14.4), [P] = 30.4 mg/dL (normal 2.8-7.8), and [Ca] x [P] = 297.92. Therefore, in this case, we may have an explanation for mineralization based on elevated [Ca] x [P] product. Vitamin D3 levels were not determined. Given the sub-optimal husbandry reported by the clinician in this case, calcium and phosphorus abnormalities could have been nutritional and/or related to lack of adequate vitamin D3. Renal dysfunction was likely secondary to mineralization. While a few tophi were identified in renal tubules, these were acute lesions without associated inflammatory response, and likely developed terminally, possibly secondary to dehydration.

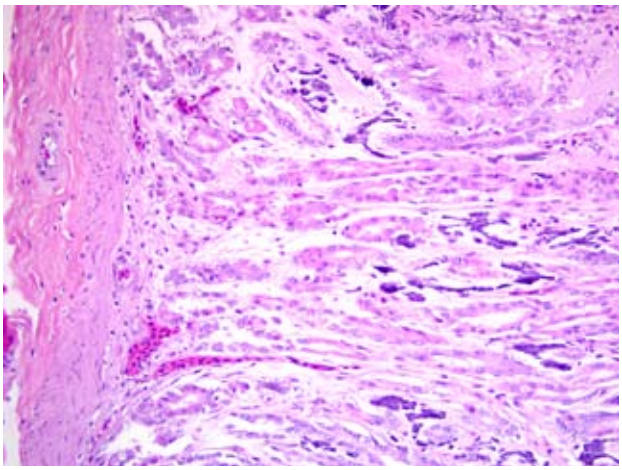
This animal did not have any evidence of fibrous osteodystrophy.

AFIP Diagnosis: 1. Kidney: Glomerular, vascular, and tubular basement membrane mineralization, diffuse, with tubular epithelial necrosis, interstitial fibrosis and few gout tophi.
2. Stomach: Mucosal and vascular mineralization, multifocal, with epithelial degeneration and necrosis and interstitial fibrosis.
3. Heart, ventricle: Myocardial and vascular degeneration, necrosis, and mineralization, multifocally extensive, moderate, with fibrosis.

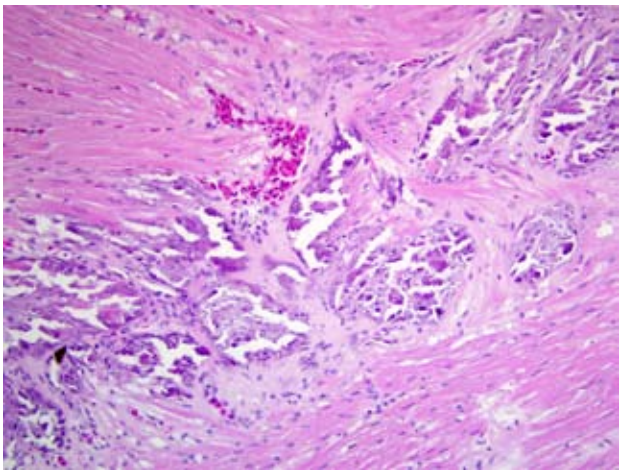
Conference Comment: Conference participants reviewed several unique reptilian anatomical structures that were demonstrated in this case. Because lizards and snakes have a sexually dimorphic kidney, participants could surmise that this was a male iguana. Males possess a sexual segment of the nephron located between the distal segment and the collecting tubules, characterized by prominent hypereosinophilic intracytoplasmic granules.



2-3. Kidney, Iguana. Multifocally displacing cortical parenchyma are large, up to 100 um diameter radiating acicular crystals (gouty tophi). Diffusely, the basement membranes of tubules, glomeruli, and blood vessels are expanded by mineral. (HE 200X)



2-4. Stomach, Iguana. Multifocally the mucosa is necrotic and the epithelial basement membranes and the lamina propria are expanded by accumulations of mineral. (HE 200X)



2-5. Heart, Iguana. Diffusely at the junction of the stratum compactum and the stratum spongiosum is an approximately 500 um thick band of mineralized myocardial tissue admixed with fibrosis. (HE 400X)

The secretory product that the sexual segment produces is incorporated into the seminal fluid.[2] With regard to the histologic anatomy of the heart, the ventricular myocardium consists of an outer stratum compactum and an inner stratum spongiosum. In most slides myocardial mineralization centered at the junction between the two muscular strata, and participants theorized the finding might be due to the abrupt change in myofiber orientation at this location, resulting in greater susceptibility to injury during contraction.

This case is an excellent example of metastatic mineralization of soft tissues, and the contributor provides a succinct review of the pathophysiology of the condition, including hypovitaminosis D in iguanas. Conference participants briefly reviewed the following causes of metastatic mineralization in animals:[1,3,5]

1. Kidney failure → phosphate retention → renal secondary hyperparathyroidism
2. Hypervitaminosis D (e.g. ingestion of calcinogenic plants [*Solanum malacoxylon, Cestrum diurnum*, *Trisetum flavescens*] or cholecalciferol-containing rodenticides) → secondary hyperparathyroidism
3. Hyperparathyroidism (primary or secondary) or pseudohyperparathyroidism (due to PTH-related protein production associated with canine lymphoma or adenocarcinoma of the anal sac)
4. Granulomatous disease (e.g. canine blastomycosis, bovine paratuberculosis)
5. Osteolytic bone lesions (e.g. primary or metastatic neoplasia)

In general, tissues predisposed to metastatic mineralization include the gastric mucosa, renal tubules, alveolar septa, and subpleural intercostal connective tissue.[5,6] In cattle with Johne's disease, intimal mineralization may occur in the thoracic aorta.[3] In dogs with blastomycosis, mineralization is thought to be caused by 1,25-dihydroxycholecalciferal production by activated macrophages.[1]

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CASE III: S16/08A, S17/08B (AFIP 3133958).

Signalment: 8-month-old, male (S 16/08) and female (S 17/08), Bearded Dragons (*Pogona vitticeps*), reptile.

History: The animals derived from a collection of several animals (the exact number is unknown), from which four animals did not gain weight, even though they were force-fed with crickets from the beginning. The rest of the collection developed normally. A scat sample tested for parasites was without diagnostic findings. The animals were treated with vitamin B and calcium, and fed with a "critical care" diet and crickets. They did not respond to treatment and were humanely killed.

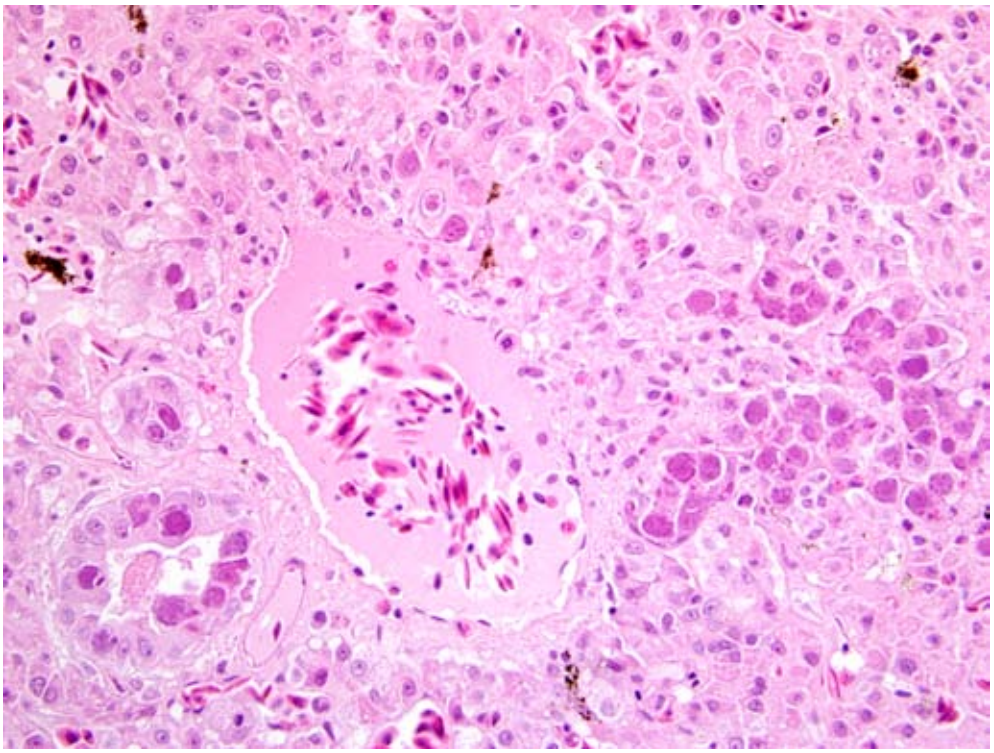
Gross Pathology: At necropsy, the lizards were in a poor body condition and up to 200-300 µl of a translucent transudate was found inside the coelomic cavities. There were no other gross lesions detected.

Laboratory Results: DNA was extracted from liver tissue and cell cultured material. According to two published protocols,[1,13] PCR amplification resulted in products of about 430 bp and 520 to 550 bp, respectively. The obtained sequences of the adenoviral polymerase gene of the eight-month-old bearded dragons were compared to published sequences of reptilian adenoviruses in the GenBank by use of the Basic Local Alignment Search Tool, (BLAST) which showed a close relationship between the sequence of these animals and another agamid atadenovirus.

Histopathologic Description: The architecture of the liver is disordered and contains multiple foci of necrosis accompanied by moderate multifocal infiltration of lymphocytes and histiocytes. Numerous hepatocytes as well as epithelial cells of the bile ducts contain large amphophilic intranuclear inclusion bodies, which distend the nuclei 2 to 5-fold, partly with margination of chromatin (**fig. 3-1**). A mild proliferation of bile ducts is present.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, lympho-histiocytic, multifocal, moderate, with hepatocellular degeneration and amphophilic intranuclear inclusion bodies, bearded dragon (*Pogona vitticeps*), reptile.

Contributor's Comment: The Adenoviridae family comprises four genera: the Mastadenoviruses, which infect mammalian species; the Aviadenoviruses, which infect birds; and the two recently established genera



3-1. Liver, Bearded Dragon. There is hepatocellular necrosis, and degenerate hepatocytes and biliary epithelium contain large amphophilic intranuclear viral inclusion bodies that measure up to 20 um diameter. (HE 400X)

Siadenovirus (frog, poultry) and *Atadenovirus* (ruminants, birds, snakes, lizards, and a marsupial).[3,6] A fifth genus is proposed for a sturgeon adenovirus.[1,6]

Generally, adenoviruses are host specific and are transmitted by the fecal-oral route or by direct contact via oro-nasal secretions. Adenoviral infection in reptiles is reported with and without concurrent disease and in the majority of communications only individual animals are afflicted. The most common changes observed in association with an adenoviral infection include nonspecific clinical signs like wasting and anorexia, accompanied by necrosis and inflammatory changes in the gastrointestinal tract and the liver.[5,7-9,11,12] Others observed nephritis [7] or neurological signs like head tilt and circling,[8] but there are also cases of sudden death without prior signs of illness described.[9]

Additionally, the two animals investigated showed intranuclear inclusions in the tubular epithelial cells of the kidney identical to those seen in the liver associated with a moderate, multifocal, lympho-histiocytic interstitial nephritis. Furthermore, a moderate, multifocal, lympho-histiocytic, interstitial pancreatitis with numerous intranuclear inclusion bodies in epithelial cells of the pancreatic duct was detected. The microscopically visible intranuclear inclusion bodies are formed during the replication phase inside the nuclei of affected host cells. Using routine H&E stain, they are typically basophilic to amphophilic.[5,8,9,11,12] The virions form paracrystalline arrays, often causing severe condensation and margination of the host cell chromatin. Finally, they are released by cell lysis.[6] In order to substantiate the diagnosis, the products of the PCR were sequenced and compared to published sequences of reptilian adenoviruses in the GenBank by use of BLAST.[10]

AFIP Diagnosis: Liver: Hepatitis, portal and random, necrotizing and lymphohistiocytic, multifocal to coalescing, moderate, with mild biliary hyperplasia and many hepatocellular and biliary epithelial intranuclear amphophilic inclusion bodies.

Conference Comment: In addition to the degenerative hepatocellular changes reflected in the contributor's morphologic diagnosis, conference participants considered the necrosis a sufficiently prominent feature to merit incorporation in the morphologic diagnosis. Histochemical staining with reticulin highlighted the severity of disorganization in hepatic cords.

The contributor provides a useful synopsis of the pathogenesis of adenoviral infections in animals. In general, adenoviruses interact with host cells in one of three characteristic ways:[2]

1. Lytic infection: involves complete viral replication and host cell lysis, as described by the contributor

2. Latent or chronic infection: lymphoid tissue is often the site of latency
3. Oncogenic transformation (e.g., production of sarcomas in hamsters by simian and human adenoviruses): viral DNA is integrated into host cell DNA and T antigens are produced, but no infectious virions are produced

Recently, a fifth genus has been added to the Adenoviridae family: the *Ichtadenovirus* genus, containing *Sturgeon adenovirus A* as its sole species.[4] An example of canine adenovirus is available in WSC 2008-2009, Conference 3, case I, and an example of falcon adenovirus is available in WSC 2007-2008, Conference 23, case III.

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CASE IV: XN2892 (AFIP 2940472).

Signalment: Adult female goldfish (*Carassius auratus*).

History: An indoor, room temperature, 45 liter aquarium was established in July 2003 with a sparsely-planted gravel floor, fluorescent lighting and a canister filter powered by an electric pump. It was allowed to equilibrate for two weeks, then stocked with two goldfish (Carassius auratus), one orange and white and the other orange, purchased from an aquarium shop. The two goldfish remained together for six weeks and were fed on commercial goldfish pellets. In August 2003, two new goldfish, one a black moor and the other a panda moor, were purchased from a different aquarium shop and introduced to the aquarium. One week later, the original orange and white goldfish was found dead and submitted for postmortem examination.

Gross Pathology: A postmortem examination was conducted on the adult female goldfish within 15 minutes of death. The fish weighed 7 g and had a length of 90 mm from the mouth to the tail tip. Petechial hemorrhages were evident on the gills.

Laboratory Results: No parasites were detected in wet preparations of gills, skin and fins examined by light

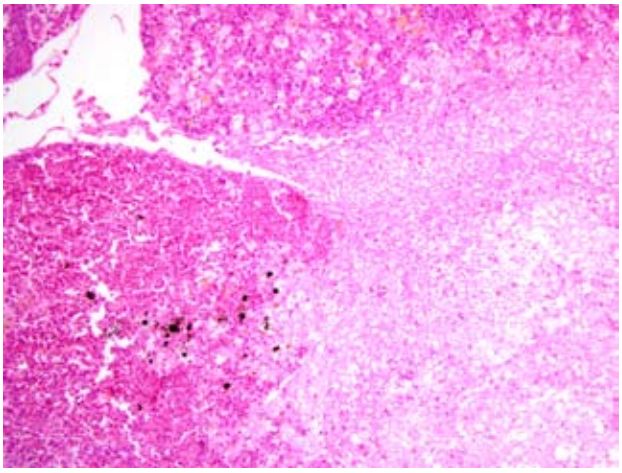
microscopy.

Histopathologic Description: Multifocal necrosis is evident in the renal hematopoietic tissue, spleen, pancreas, and intestine of this goldfish, along with multiple eosinophilic to amphophilic intranuclear inclusion bodies (**figs. 4-1, 4-2, and 4-3**). Lymphohematopoietic hyperplasia is evident in some less severely affected areas. Mild degenerative changes are also present in renal tubules and glomeruli. There are lymphoplasmacytic inflammatory infiltrates in segments of intestinal mucosa and submucosa. Some histological sections do not include all of these affected tissues. The gills from this goldfish had lamellar fusion and chronic bronchitis, with infiltrates of eosinophilic granule cells, epithelial hyperplasia and individual epithelial necrosis. A few monogenean flukes were visible histologically in the interlamellar spaces of the gills.

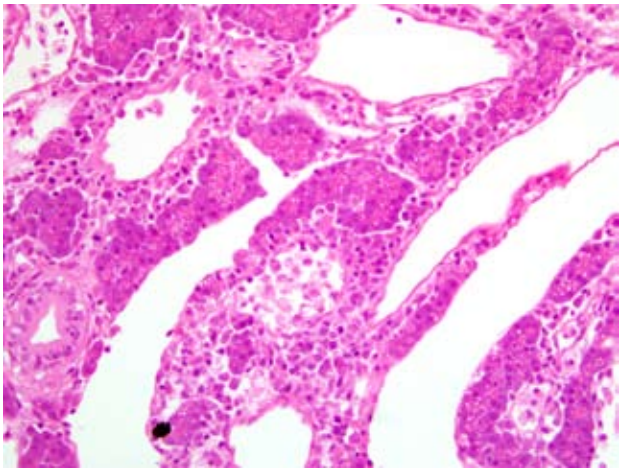
Contributor's Morphologic Diagnosis: 1. Renal hematophoietic tissue: Necrosis, multifocal, locally extensive, moderate to severe, with eosinophilic intranuclear inclusion bodies, etiology consistent with herpesvirus hematopoietic necrosis, Goldfish, ornamental, *Carassius auratus*.
2. Spleen: Necrosis, multifocal, moderate, with eosinophilic intranuclear inclusion bodies.
3. Pancreas: Necrosis, multifocal, mild to moderate, with eosinophilic intranuclear inclusion bodies.
4. Intestine: Necrosis, submucosa and mucosa, segmental, multifocal, mild to severe, with eosinophilic intranuclear inclusion bodies in epithelial cells, also inflammation, lymphoplasmacytic, segmental, moderate.

Contributor's Comment: The multifocal renal hematophoietic, splenic, pancreatic, and intestinal necrosis observed histologically in this case, along with the eosinophilic intranuclear inclusion bodies, are consistent with herpesvirus hematophoietic necrosis (HVHN) of goldfish.[1,5,9,10] This disease was first observed in Japan in 1992 [9] and has subsequently been diagnosed in North America [5], Taiwan [1], and Australia [10]. It has been associated with severe outbreaks of disease and high mortality in cultured goldfish.[1,5,9] Affected fish are often lethargic and inappetent or anorexic, may exhibit increased respiratory effort and may remain at the bottom of their ponds.[1,5,9,10] Gross lesions include pallor of the gills, swelling and pallor of the spleen and kidneys, occasionally with multiple white foci, pallor of the liver and an empty intestinal tract.[5,9,10] Histological lesions are characterized by multifocal to diffuse necrosis of renal hematopoietic, splenic, pancreatic, intestinal and branchial tissue, with eosinophilic intranuclear inclusion bodies.[1,5,9,10] Gill lamellar fusion and epithelial hyperplasia have been described in affected goldfish [5,10] In addition to multifocal necrosis of the splenic parenchyma, necrosis of sheathed arterioles in the spleen has also been reported.[9] Degeneration and necrosis may occur in the oropharynx and the epidermis and dermis of the skin, but these changes are not consistent.[5] Multifocal necrosis has been described in the heart of affected goldfish [1], and diffuse necrosis has been reported in the thymus.[10]

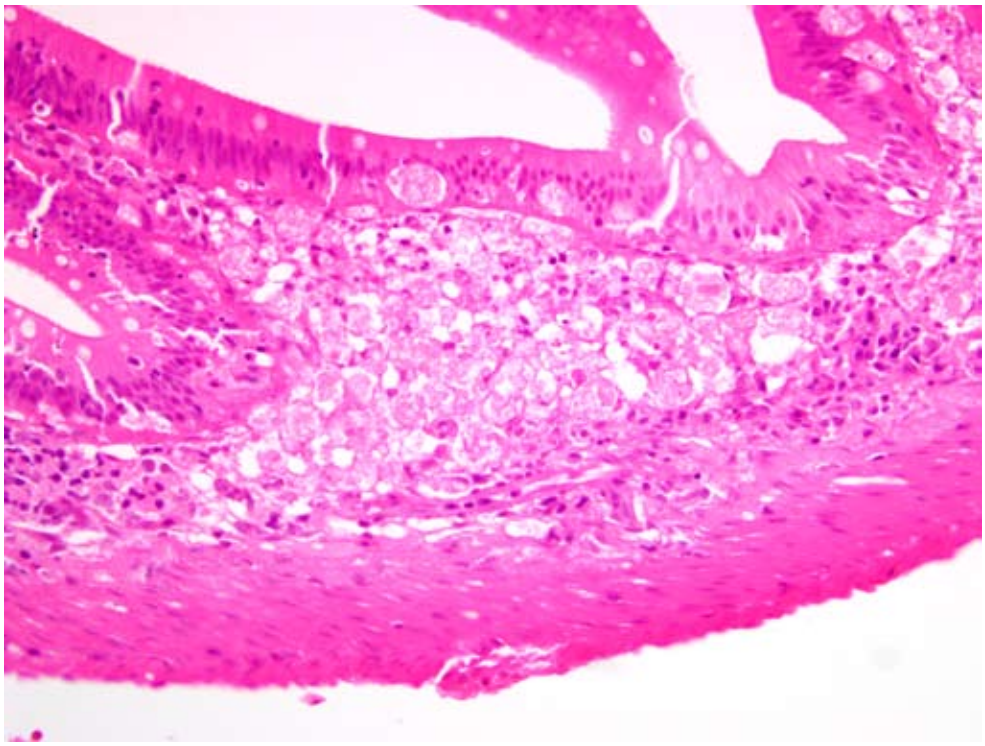
A herpesvirus, designated goldfish hematopoietic necrosis virus (GFHNV), has been isolated from affected goldfish and shown to reproduce HVHN experimentally.[9] Herpesvirus-like particles can be detected by electron microscopy in



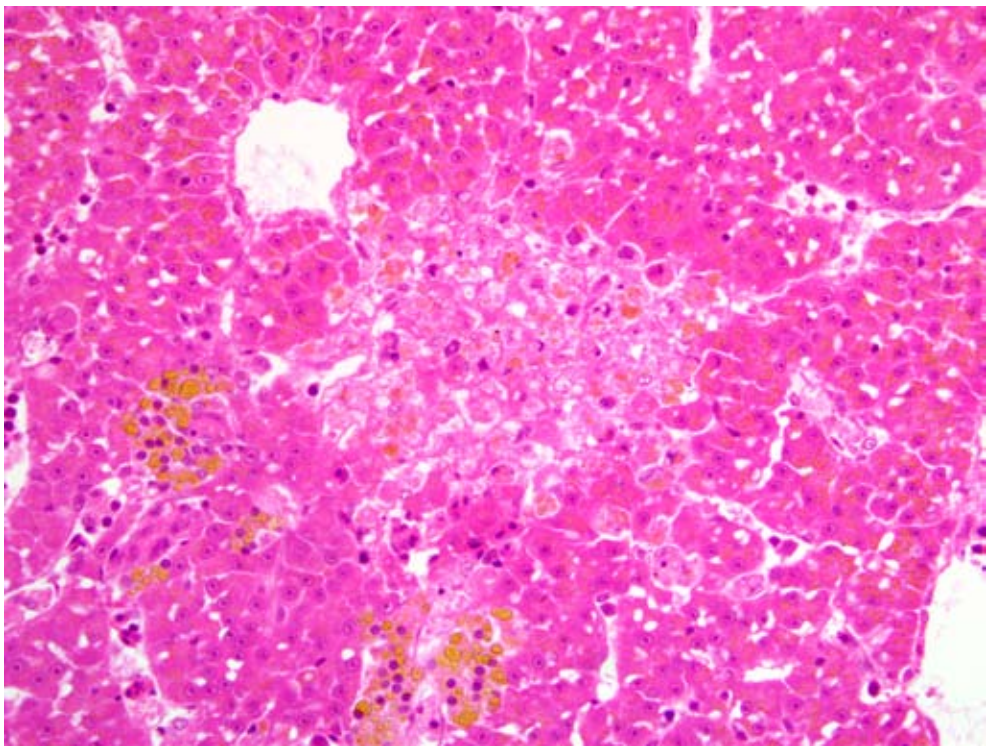
4-1. Spleen and kidney, Goldfish. Multifocally there is necrosis of lymphoid and hematopoietic tissue in the spleen and kidney. (HE 200X)



4-2. Pancreas, Goldfish. Multifocally, there is necrosis of pancreatic acini, and some inflammatory cells extend to the overlying serosal surface. (HE 400X)



4-3. Intestine, Goldfish. Within the submucosa there are focally extensive areas of necrosis. (HE 400X)



4-4. Liver, Goldfish. Multifocally within the liver there is multifocal hepatocellular necrosis. Intracellular and extracellular yellow to orange globular pigment is also present. (HE 400X)

affected tissues.[1,5,9,10] HVHN of goldfish has pathological similarities to carp nephritis and gill necrosis (CNGN), which affects koi carp (*Cyprinus carpio*) and is caused by koi herpesvirus (carp nephritis and gill necrosis virus).[6] GFHNV does not affect koi carp [1] and koi herpesvirus does not infect goldfish [6]. Outbreaks of CNGN were first observed in cultured koi carp in Israel and North America in 1998 [6] and have subsequently been reported in Japan, Indonesia and Europe, including the United Kingdom.[3,7] HVHN and CNGN are both characterized by multifocal necrosis of renal hematopoietic, splenic, pancreatic and intestinal tissue, with variable histological lesions in the gills, oropharynx and skin.[1,5,6,9,10] Necrosis, lamellar fusion and epithelial hyperplasia are observed in the gills of koi carp with CNGN, similar to gill lesions in goldfish with HVHN.[6] Koi herpesvirus can be detected in tissues from affected koi carp by electron microscopy and polymerase chain reaction.[2,4,6] Comparisons of the genomes of koi herpesvirus isolates from outbreaks of CNGN in different regions of the world by restriction fragment length polymorphism, protein gel electrophoresis and DNA hybridization have demonstrated that most isolates are nearly identical and are likely to have been derived from a common source.[2-4]

In outdoor ponds in Japan and North America, GFHNV usually causes disease in cultured goldfish in spring and autumn (fall), when the temperature of the water is in the range of 15 to 25 degrees Celsius.[5,9] Outbreaks of CNGN caused by koi herpesvirus have been reported on farms with water temperatures of 17 to 25 degrees Celsius.[6,7] Experimentally infected koi carp are susceptible to disease in water with a temperature range from 18 to 28 degrees Celsius.[3] Goldfish affected by major outbreaks of HVHN have usually been less than one year of age, with some epidemics occurring in juveniles less than two months of age, although goldfish of all ages may be affected.[1,5,9,10] Two-month-old goldfish affected by HVHN in North America were 25 to 40 mm long and weighed two to three grams.[5] A single goldfish diagnosed with HVHN in Australia weighed approximately five grams.[10] Koi carp of all ages may be affected by CNGN.[5] In the case of HVHN reported here, a single adult goldfish 90 mm long and weighing seven grams was affected. It could be speculated that this goldfish developed HVHN after first exposure to GFHNV following introduction of an infected fish. Alternatively, the goldfish may have harboured latent GFHNV that was reactivated following the stress caused by the introduction of new fish to the aquarium tank. Outbreaks of mortality due to HVHN in 20 to 25-day-old goldfish fry, as well as associated mortality in adult brood goldfish, were associated with the introduction of new fish to a hatchery in Taiwan.[1]

AFIP Diagnosis: 1. Pancreas: Pancreatitis, necrotizing, multifocal, moderate to marked, with intranuclear inclusion bodies and acute serosistis.
2. Intestine, lamina propria and submucosa: Necrosis, multifocal, with intranuclear inclusion bodies.
3. Spleen; and renal hematopoietic tissue: Necrosis, multifocal, with intranuclear inclusion bodies.
4. Liver: Hepatocellular degeneration and necrosis, multifocal, mild to moderate, with few intranuclear inclusion bodies.

Conference Comment: This case was submitted in 2004; since then, the cause of this lesion has been determined as Cyprinid herpesvirus-2. There are currently three members of the genus *Cyrinivirus* within the *Alphaherpesviridae* family:[8]

- Cyprinid herpesvirus-1: carp pox herpesvirus
- Cyprinid herpesvirus-2: hematopoietic necrosis herpesvirus of goldfish
- Cyprinid herpesvirus-3: koi herpesvirus

Despite substantial variation among the slides with respect to the organs and lesions represented, participants readily recognized a systemic herpesviral infection primarily targeting hematopoietic and lymphoid tissues. During the conference, the conference moderator cautioned participants that pancreatic necrosis in fish is often very difficult to distinguish from autolysis. However, the contributor in this case provided histologic sections from particularly well-preserved tissues as a good example of this important entity, and further complimented the slides with a thorough review. Additionally, conference participants commented on finding serositis in many slides, which is not surprising given the widespread distribution of pancreatic tissue in fish (**fig. 4-2**). Furthermore, most slides contain abundant intra- and extracellular accumulations of yellow to orange, granular to globular material throughout the liver (**fig. 4-4**). Conference participants discussed various potential pigments, including hemosiderin, hematoidin, bile, melanin, and lipofuscin. In this case, an abundance of iron-containing pigment (i.e. hemosiderin) was demonstrated by the presence of intense blue staining with the Prussian blue reaction. Staining using the Hall's bile method was negative.

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