**Joint Pathology Center**
**Veterinary Pathology Services**

# WEDNESDAY SLIDE CONFERENCE 2021-2022

## C o n f e r e n c e 18

**9 February 2022**

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**CASE I:** NO21-0000475 (JPC 4170014)

**Signalment:**
10-year-old male African Elephant *(Loxodonta africana*)

**History:**
This 10-year-old male African elephant had been diagnosed and treated over the last week for elephant endotheliotropic herpesvirus (EEHV) infection. It had chronic anemia for several years. After several days of rapidly progressing debilitation, it went down in the elephant restraint device and died, despite antiviral treatment.

**Gross Pathology:**
The epicardium, myocardium, and endocardium were diffusely dark red to black and there was a 5 x 5 cm region of hemorrhage at the base of the pulmonary artery. Petechial hemorrhages were noted on the gastrointestinal serosa, mesentery, lymph nodes, and there was extensive mural edema multifocally in the intestine. The bone marrow was pale.

**Laboratory Results:**
Perimortem, elevated EEHV-2 levels were noted with plasma levels at 435,000 vge/ml.

**Microscopic Description:**
Within the heart, capillary vessels are frequently disrupted and infiltrated by neutrophils. Edema and hemorrhage are extensive in these areas, disrupting the adjacent cardiomyocytes which are shrunken, fragmented, and hypereosinophilic with moderate to marked myofiber disarray. Endothelial cells rarely contain intranuclear inclusions.

**Contributor's Morphologic Diagnoses:**
Heart: Severe myocardial necrosis, degeneration, and hemorrhage with intranuclear endothelial inclusion bodies**.**

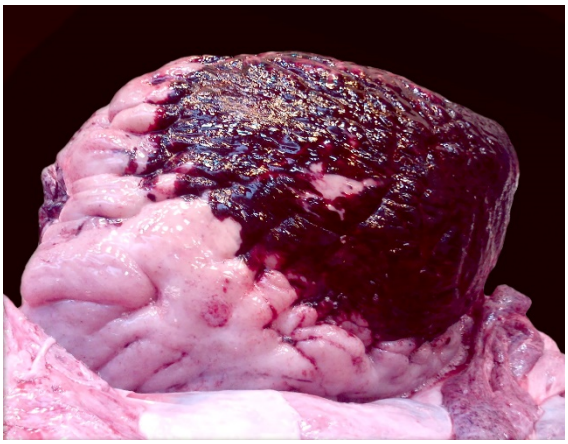

**Figure 1-1. Heart, elephant: There is suffusive hemorrhage over the epicardium. (*Photo courtesy of:* National Institutes of Health, Bethesda, MD 20892, https://www.ors.od.nih.gov/sr/dvr)**

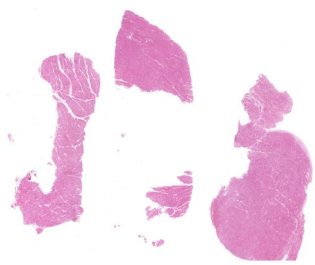

**Figure 1-2. Heart, elephant. Multiple sections of cardiac muscle are submitted for examination. There are multifocal areas of hemorrhage scattered randomly throughout each section. (HE, 7X)**

**Contributor's Comment:**

The histologic findings are consistent with elephant endotheliotropic herpesvirus (EEHV) infection. In this case, EEHV-2 was isolated. Elephant endotheliotropic herpesvirus (EEHV) is a double stranded DNA, beta herpesvirus that causes an acute hemorrhagic syndrome (EEHV-HD) that mostly affects young Asian elephants. Studies of Asian and African elephants have shown that most Asian elephants carry strains EEHV1b, EEHV4 and EEHV5, while African elephants carry EEHV2, EEHV6 and EEHV7. Despite its widespread presence in African elephants, there have been only 13 confirmed cases of EEHV in African elephants with an approximately 50% mortality rate EEHV is known to infect endothelial cells, causing edema, hemorrhage, and coagulopathies, with capillary endothelial cells being the most affected. The heart is also often severely affected. Nonclinical African elephants may have cutaneous papillomas along the trunk and lymphoid hyperplasia in the urogenital mucosa and lungs. Currently, it is not known why some EEHV-infected elephants develop EEHV-HD, while others do not. The cause of chronic anemia in this elephant was not determined, but there was a marked decrease in the bone marrow in the samples examined.

The described virus: dsDNA, betaherpesvirus
Target → primarily young elephants
Cause: acute hemorrhagic syndrome (EEHV-HD)
Asian Elephants EEHV1b, EEHV4, and EEHV5

- only 13 cases; 50% mortality; targets endothelial cells; coagulopathy
- non-clinical → papillomas along trunk; lymphoid hyperplasia in urogenital mucosa and lungs
- Unknown why some develop disease and not
- Unknown why anemia in this particular elephant, though marked bone marrow decrease

African Elephants: EEHV2, EEHV6, and EEHV7

**Contributing Institution:**

National Institutes of Health
Bethesda, MD 20892
https://www.ors.od.nih.gov/sr/dvr

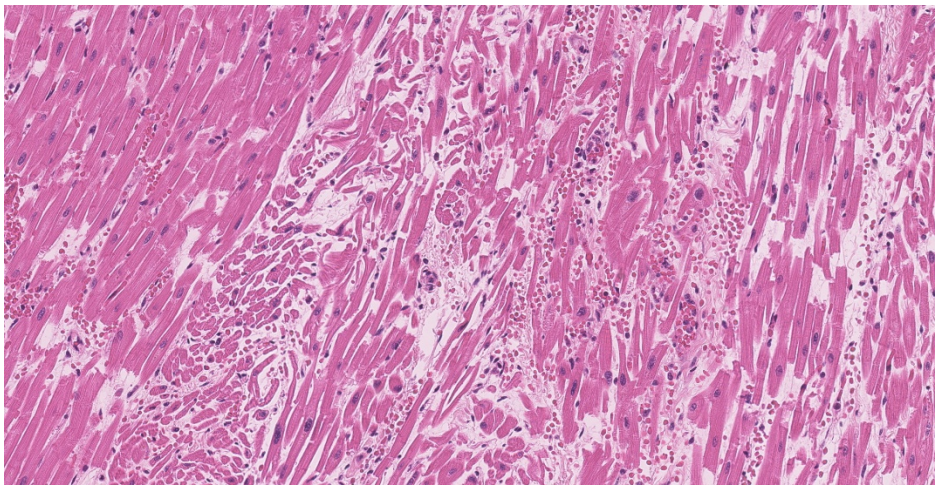

**Figure 1-3. Heart, elephant. In some areas of hemorrhage, there is marked edema and separation of myofibers. (HE, 105X**

**JPC Diagnosis:**
Heart: Vasculitis, necrotizing, multifocal, moderate, with myocardial hemorrhage and edema, and rare endothelial intranuclear viral inclusions.

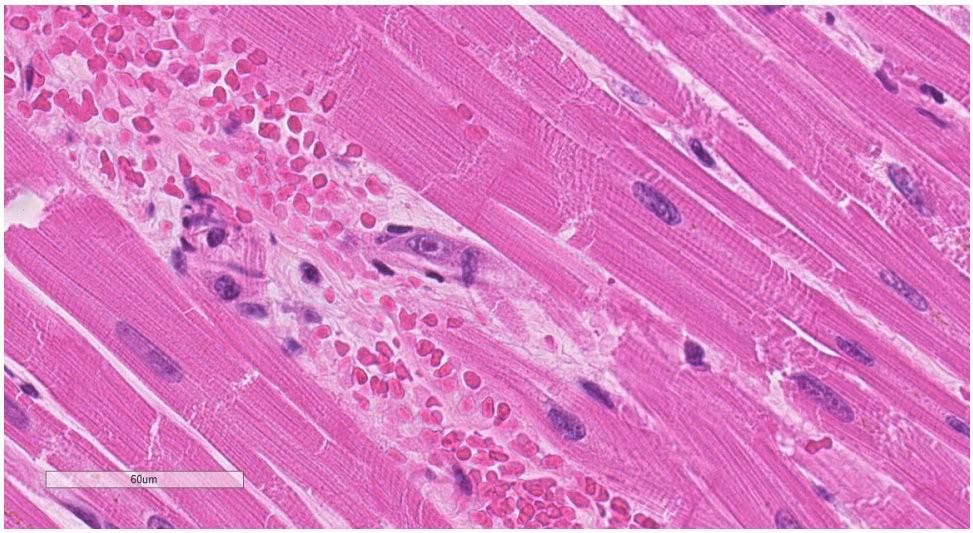


**Figure 1-4. Heart, elephant. Rarely, endothelial cells within damaged vessels contain a single intranuclear viral inclusion. (HE, 600X)**

**JPC Comment:**
The contributor provides a concise review of elephant endotheliotropic herpesvirus (EEHV), a significant cause of mortality in predominately juvenile Asian and African elephants.

In 1988, a young Indian elephant in a Swiss circus died from an unknown acute hemorrhagic disease, which was followed by nearly 20 similar cases in North America and Europe during the 1990s. In 1995, a veterinary pathology resident at the Smithsonian National Zoo, Dr. Laura Richman, identified herpesvirus-like particles using electron microscopy while investigating endothelial cell nuclear inclusion bodies in heart and liver samples from an Asian elephant from their collection. Four years later, Richman and her colleagues published a report describing the detection of DNA from a novel herpesvirus called "elephant endotheliotropic herpesvirus". Since its discovery, EEHV has been identified in both captive and wild elephants and is the most common cause of mortality in juvenile Asian Elephants (*Elephas maximus*) in both North America and Europe.[5] As a testament to the virus' significance, EEHV was responsible for the deaths of 29.6% of Asian elephants born in captivity in the United Kingdom and Ireland between 1995 and 2013.[3]

One retrospective review[6] of 27 cases of EEHV in Indian elephants found the most common gross lesion to be myocardial hemorrhage, followed by multisystemic hemorrhage, blue or purple tongue discoloration, pericardial effusion, edema, and ascites. Common areas of non-cardiac hemorrhage included the gastrointestinal mucosa and serosa, liver, pancreas, and lungs. Histologically, all cases demonstrated hemorrhage within the heart, spleen, and thymus, with the most severe hemorrhage noted in the heart and spleen. Cardiac hemorrhage was most severe in the sub-endocardial and sub-epicardial regions. Microthrombi were present in 63% of cases, most commonly in the lungs in addition to other organs such as the kidneys and liver. 100% of lung sections demonstrated endothelial cell damage characterized by separation, sloughing, denudation, or loss, followed by edematous expansion of the tunica intima (95%) and migrating leukocytes (67%). Similar lesions were present within the hepatic blood vessels, predominantly within the sinusoids and portal capillaries. The most consistent organs

demonstrating endothelial viral nuclear inclusions were the heart and liver.[6]

The same study concluded disseminated intravascular coagulation (DIC) is likely associated with end stage EEHV, with key histologic features including the presence of microthrombi and widespread hemorrhage in multiple tissues. DIC is an acquired coagulopathy that commonly precedes multiorgan failure, cardiovascular failure, and death. The most likely pathogenesis associated with EEHV stems from endothelial cell damage with secondary exposure of subendothelial tissue factor. Exposure of tissue factor results in both platelet activation and initiation of the extrinsic coagulation pathway. Extensive endothelial damage, as is likely in cases of EEHV, can overwhelm localized homeostasis control mechanisms, resulting in generalized activation of the coagulation cascade. If the underlying cause is unable to be resolved, both platelets and coagulation factors become depleted and hemostasis can no longer be maintained, resulting in multifocal areas of hemorrhage.[6]

Asian elephants with EEHV have successfully been treated with famciclovir, an antiviral medication, in addition to supportive therapy such as rectal and intravenous fluids, conspecific plasma, diuretics for edema, antibiotics, and oxygen. However, administration of antivirals and supportive care is most likely to be successful when initiated prior to onset of clinical signs. Subclinical viremia has been detected in Asian elephants up to 28 days prior to onset of clinical signs. Therefore, the optimal method suggested for identification of subclinical cases is regular PCR analysis of blood samples of young Asian elephants.[3] Additional methods of sample collection for surveillance may include trunk washes, conjunctival swabs, and saliva swabs as viral shedding typically commences 10 days after onset of viremia.[1] A recently published study demonstrated the ability to detect EEHV in elephant feces using qPCR, although sensitivity was significantly lower compared to saliva swabs. However, a similar method may one day provide a suitable non-invasive method of early detection of EEHV in captive elephants while also facilitating surveillance of shedding in wild herds.[1]

A notable feature of EEHV is severe and lethal disease is practically non-existent in animals less than one year of age, suggesting maternal antibodies likely provide protection against disease, if not infection.[5]

During the conference, the moderator discussed encephalomyocarditis virus (ECMV) as a differential diagnosis in this case. In comparison to EEHV, ECMV is typically associated with more severe myocardial degeneration and necrosis, less hemorrhage, and endothelial intranuclear viral inclusions are not associated with this entity. However, based on the moderator's experience, viral inclusions are infrequently present cases of EEHV and additional diagnostics (e.g. PCR) are frequently required for definitive diagnosis.

**References:**


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**CASE II**: LE-2 (JPC 4120289)

**Signalment:**
3-year-old neutered male rabbit (*Oryctolagus cuniculus* forma domestica

**History:**
Two days after its partner-animal died without premonitory signs, the presented rabbit was vaccinated against myxomatosis and rabbit hemorrhagic disease virus (RHDV). The used vaccines were Nobivax Myxo-RHD® (owner of license: Intervet international) and Filavac VHD K C+V® (owner of license: Filavie). As the animal showed a good state of health, it was placed in a new group of rabbits 3 days after vaccination. On the next day it was found dead without any premonitory signs.

**Gross Pathology:**
The animal was in a good body condition. The liver showed a diffuse orange to reddish brown coloration and a moderate friable consistence. Multifocal mild to moderate acute hemorrhages were detectable within the lung. Bilateral in the axillary subcutis a lipoma (each 2 cm in diameter) was visible. No further pathomorphological alterations could be observed.

**Laboratory Results:**
The molecular testing of a pooled sample of liver and lung by real-time PCR to detect rabbit hemorrhagic disease virus (RHDV) was finished with a positive result. The sequence alignment of the PCR-product showed a 97% homology to RHDV type 2.
The parasitological investigation of the intestine was finished with a negative result.

**Microscopic Description:**
Liver: Multifocal to coalescing a moderate to severe hepatocellular necrosis is detectable, which can predominantly be found in periportal and midzonal areas of the hepatic lobules. The affected hepatocytes are characterized by a hyper-eosinophilic, frequently shrunken and fragmented hepatocellular cytoplasma as well as karyopyknosis and karyolysis. Some necrotic areas show slight hemorrhages and are scattered infiltrated by mostly degenerated neutrophils. Due to dissociation of the damaged hepatocytes, the liver cords are multifocally discontinuous and irregular. Numerous of the remaining hepatocytes are enlarged and show a fine foamy cytoplasm, frequently revealing tiny lipid droplets or sometimes vacuoles. Infrequent, fatty cysts are visible. Binucleated hepatocytes are a common finding. Multifocally, the periportal areas show a proliferation of the connective tissue, characterized by a mild to moderate fibroplasia and scattered early proliferation of bile ducts. In these areas a mild to moderate mononuclear portal/periportal infiltration, predominantly characterized by lymphocytes as well as several plasma cells

and macrophages can be detected. In several of the remaining hepatocytes, Kupffer cells and periportal located macrophages, a mild storage of a dark brown coarse-grained pigment can be observed, consistent with hemosiderin (staining by Berlin-Blue: positive).

**Contributor's Morphologic Diagnoses:**
Liver:

1. Hepatocyte necrosis, acute, multifocal to coalescing, moderate to severe, consistent with the diagnosis of viral hemorrhagic disease of rabbits
2. Proliferation of connective tissue, periportal, multifocal, mild to moderate with mononuclear infiltration, multifocal, mild to moderate

**Contributor's Comment:**
The rabbit hemorrhagic disease (RHD), also referred as viral hemorrhagic disease (VHD) of rabbits, is an acute and highly contagious viral hepatitis with a mortality rate ranging between 70% and 100%. RHD affects both European domestic and wild rabbits (*Oryctolagus cuniculus).*[1,6] First described in China (1984), the disease rapidly spread worldwide and occurs on almost all continents, being endemic in most parts of Europe, Asia, parts of Africa, Australia and New Zealand.[1] The causative agent is the rabbit hemorrhagic disease virus (RHDV), a

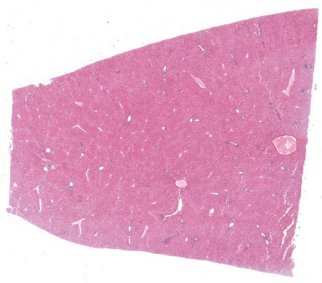

**Figure 2-1. Liver, rabbit: There is a diffuse retiform pattern of pallor in the submitted section of liver and mild hypercellularity in portal areas. (HE, 5X)**

calicivirus belonging to the genus *Lagovirus.*[12,22,25,28,29,33] Like other caliciviruses, it is a small sized (about 35-40 nm in diameter), non-enveloped, icosahedral single-stranded RNA-virus.[25,29,36,39]

RHDV is mainly transmitted directly by contact to infected animals which shed viral particles in their secretions and excretions (oral, nasal, conjunctival, parenteral), but indirect transmission (fomite-contaminated food, water, bedding, cages, clothing, equipment) as well as a vector-borne transmission (scavenging mammals, birds, blood-feeding insects insects) is described.[4,7,9,24,39] In natural infections the fecal-oral route is considered to be the preferential way of transmission.[4,24] Virus injected into bovine liver, which was used to mimic RHDV in rabbit carcasses, was still viable after 3 months.[14] Due to this high resistance and stability, carcasses of RHDV-infected animals are considered as an important source for viral spreading in the field.[14,21,23]

The incubation period of RHD ranges between 24 and 72 hours, whereas the symptoms occur suddenly, often without conspicuous signs, and usually with an elevated temperature of up to 40-41.5°C.[23] Three clinical courses of the disease can be differentiated: peracute, acute and subacute/mild.[23,39] No clinical signs are visible in the peracute form. In contrast, animals with acute infections show unspecific signs as anorexia and apathy, but also neurological symptoms (e.g. excitement, paralysis ophistotonus, ataxia) and occasionally respiratory signs (tracheitis, dyspnea and cyanosis), foamy or bloody nasal discharge, lacrimation, ocular hemorrhage, epistaxis, blood in feces, icteric coloration of the subcutis and skin of the ears, coagulation disorders, anemia and leuco-cytosis are described.[11,16,20,23,39] Subacute/

mild forms present similar but milder clinical symptoms and most rabbits survive and develop antibodies against RHDV, which confer protection upon re-infection.[23,29]

Naturally occurring RHD due to infection with the "classical" RHDV is rarely seen in rabbits less than two months of age and the most studies on experimentally-infected animals were only able to detect viral antigens in rabbits older than 4-weeks.[20,30,34,39] The viral dissemination in these young (so-called "resistant") animals is so far unclear, but is possibly due to differences in the leucocyte response to hepatocyte infection between adult and juvenile rabbits. In this context the lymphocytic rather than the heterophilic response is observable in younger animals, which possibly reflects a protective host response to viral antigens on the hepatocyte surface.[1,8]

In adult rabbits the primary target tissues of RHDV are liver, lung and spleen.[2,10,16,25,32] A viral replication in the cytoplasm of hepatocytes is detectable within the first hours post infection, reaching a maximum after 36-48 hours.[2,11,15,30] Moreover, RHDV can be observed in macrophages within the red pulp of the spleen and lymph node sinuses as well as within alveolar macrophages of the lung and Kupffer cells of the liver but also in circulating monocytes of unaffected organs such as enteric submucosae, myocardium and thymus.[2,16,30,32] Therefore it is supposed, that macrophages are important for spreading the infection.[16,32]

The most conspicuous lesions at necropsy are mostly found in liver, trachea and lungs.[20] The pale yellow or grayish and friable liver can be enlarged and is often characterized by a fine lobular appearance and sometimes interspersed with haemorrhages.[5,11,15,16,20,28] Histomorphologically, the characteristic finding is a severe hepatocellular necrosis especially in the peripheral zones of lobules, which is infiltrated by neutrophils (acute necrotic hepatitis).[10,11,16,20,30] As also shown

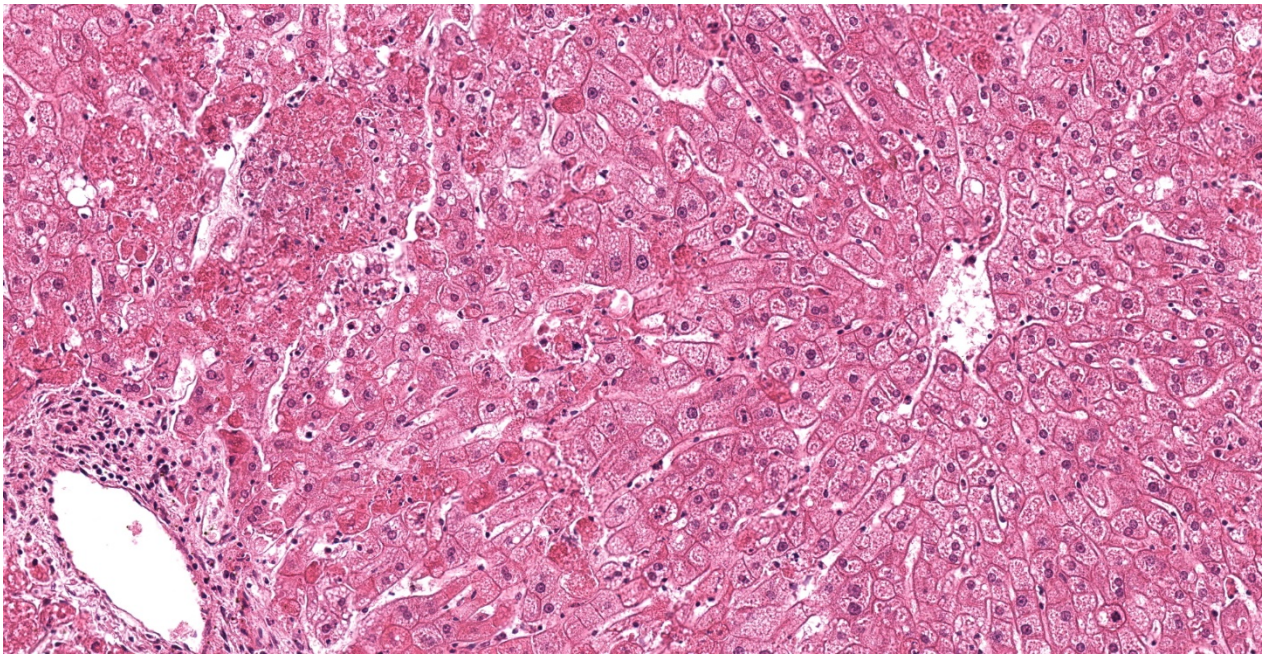

**Figure 2-2. Liver, rabbit: There is diffuse swelling of hepatocytes by abundant intracytoplasmic lipid microvesicles. Randomly, individual and small aggregates of hepatocytes are rounded up with brightly eosinophilic granular cytoplasm and nuclear pyknosis, fragmentation and loss (apoptosis). (HE, 213X)**

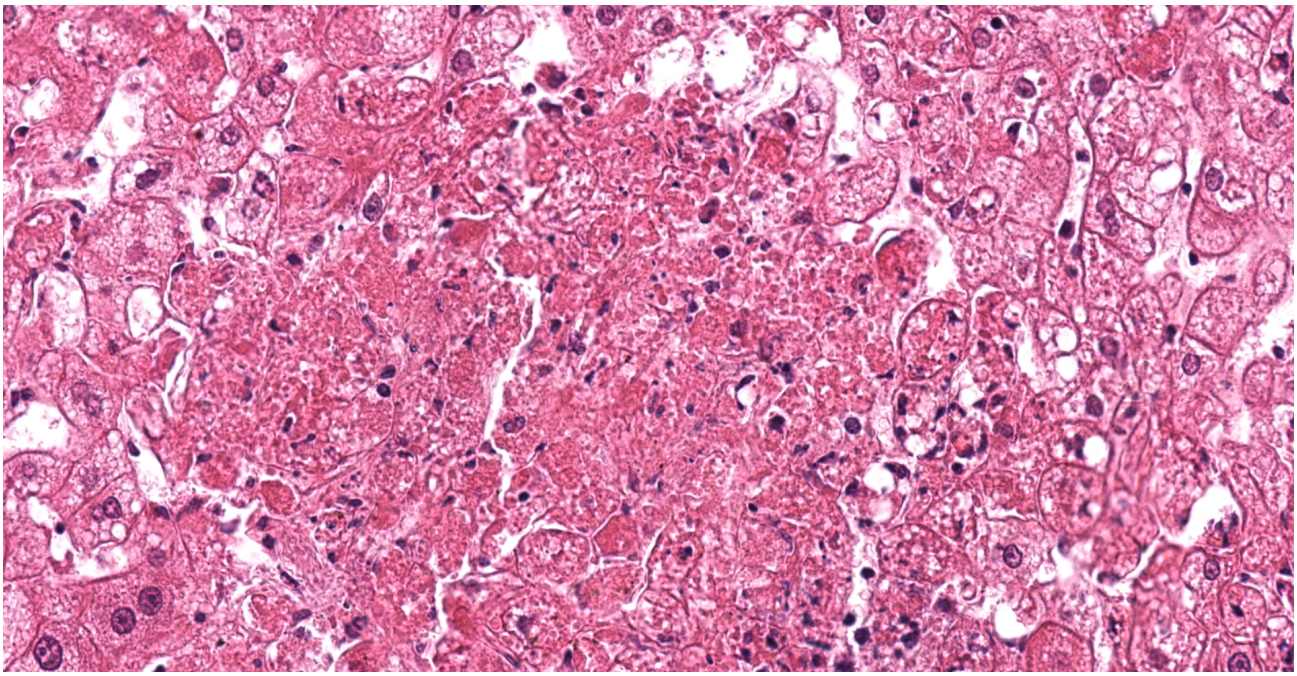

**Figure 2-3. Liver, rabbit: Aggregates of hepatocytes are apoptotic with loss of sinusoidal architecture. Within areas of apoptosis, Kupffer and sinusoidal lining cells are likewise necrotic. (HE, 481X)**

in the present case, affected hepatocytes are characterized by hyper-eosinophilia, karyopyknosis and karyorrhexis. The liver cords become discontinuous and irregular as a consequence of hepatocyte dissociation and small, scattered intralobular foci of hemorrhage are present.[10,20,30] Further hepatocytic lesions, also observed in the remaining hepatocytes of the present case, are a fine foamy cytoplasma (tiny lipid droplets or sometimes vacuoles) as well as bile pigment and/or iron pigment deposition.[10,20,30] In the present case, hepatocellular necrosis can not only be detected in the peripheral but also in the midzonal areas of the lobules. Only few, mostly karyorrhectic neutrophils are visible, whereas several hepatocytes are enlarged and binucleated. Moreover, the (peri-)portal areas show a proliferation of connective tissue, characterized by a mild to moderate fibroplasia and scattered early proliferation of bile ducts, accompanied by mild to moderate mononuclear infiltration of the portal tracts. Similar findings could be made in experimentally RHDV-infected animals killed 7 days *post infectionem*.[10] Therefore the alterations can be interpreted as early signs of regeneration or rather reparation, which is in line with a subacute process and indicates a subacute/mild form of RHD in the present case.

As a characteristic feature of disseminated intravascular coagulation (DIC) in RHD a frequent histopathological finding are microthrombi in small blood vessels especially from liver, spleen and kidney but also in the brain and alveolar capillaries.[20,30] Consequential, almost all organs, particularly lungs, heart and kidney are characterized by petechial haemorrhages.[10,20,32] Moreover, congestions, especially of the lung, trachea, kidney and spleen, splenomegaly as well as an oedema of the lung and abundant frothy fluid in the trachea are frequent features.[5,10,11,15,16,20,28,32]

As infrequent findings in RHD profuse bloody effusion in the thoracic and abdominal cavities, poor blood coagulation, icterus, catarrhal gastritis with mucosal

erosions and hyperplastic enlargement of mesenteric lymph nodes, karyorrhexis and karyopyknosis in cells of the spleen and other lymphoid tissue (BALT, GALT, thymus, lymph nodes), leading to depletion and lymphopenia are described.[5,20,30,39] Few authors report membranous glomerulonephritis, non-suppurative encephalomyelitis, gastritis, adrenocortical necrosis, endometrial congestion and hemorrhages, degeneration of the pancreatic acinar epithelial and islet cells, focal necrosis in the mucosa of the gall bladder, and inflammatory cells and serous exudate filling pulmonary airways and alveoli.[39] In pregnant rabbits, fetuses with multi-organ focal hemorrhages were found.[5]

In summary, a severe necrotizing hepatitis, especially in the peripheal zones of lobules as well as a DIC, followed by hemorrhages and congestions, particularly in the lungs, heart and kidney and splenomegalia are the most consistent pathological findings in RHD.[2,10,11,16,23,28,32]

In 2010, a new lagovirus was identified in France, spreading throughout Europe within a couple of years now reaching Australia.[17,18,26,27,31] This new virus showed a capsid protein sequence identity of about 80 per cent with RHDV and was able to cause RHD, also in vaccinated animals.[17] Therefore it was termed RHDV type 2 (RHDV2). As also shown in the presented case pathomorpholgical findings in rabbits infected by RHDV2 are consistent with those of RHD caused by the "classical variant" of RHDV.[5,17]

In contrast to the "classical variant" of RHDV, RHDV2 causes not only RHD also in young rabbits (4 weeks old), but also a RHD-like disease in different hare species, including European brown hares (*Lepus europaeus)*.[5,13,18,19,31,37] These special

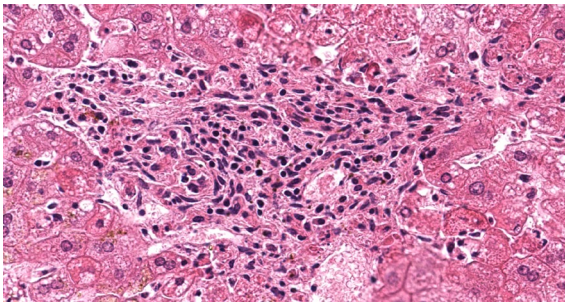

**Figure 2-4. Liver, rabbit. Portal areas are infiltrated by moderate numbers of lymphocytes and plasma cells. Remaining hepatocytes often contain intracytoplasmic lipofuscin granules. (HE, 600X)**

features of RHDV2 strongly suggest, that the virus did not originate from RHDV but rather that it had recently emerged from an unknown source.[6]

While in experimental infections with the early detected strains of RHDV2 a mortality rate of only 20-30 per cent could be detected, experimental infections of rabbits with two Italian RHDV2-strains isolated in 2014 and 2015 induced at least 80 % mortality rate.[6,18] These findings approaches the usual mortality rate of RHDV and is four times higher than that found in the early RHDV2-isolates.[6,18]

Nowadays, RHDV2 is the dominant virus causing RHD in most European countries, but "classical variants" of RHD still coexists in some areas, also in Germany.[6,35] Therefore, it is advisable to vaccinate not only against RHDV2 but also against the "original" RHDV strains.[6,35] In the present case this advice was fulfilled by using two different vaccines: Nobivax Myxo-RHD® (owner of license: Intervet international) and Filavac VHD K C+V® (owner of license: Filavie). While the first one (live vaccine, antigens RHD/myxomatosis) is used to protect against myxomatosis and the "original" RHDV-strains, the second contains an inactive strain of RHDV and RHDV2, and is therefore used for the immunization of rabbits against "original"

RHDV-strains as well as the new RHDV2-variant.[35] Nevertheless the presented animal died following RHDV2-infection. There are no datas given, nor regarding the state of health within the new animal group the rabbit was placed in, nor regarding the cause of death of the partner-animal. A complete immunization by Filavac VHD K C+V® is accomplished not until seven days after vaccination.[35] Therefore, there are two possible sources of RHDV2 thinkable in the present case: First, the dead partner-animal and second an infected animal within the new group. In this context it has to be considered, that the histomorphological findings (proliferation of the connective tissue, fibroplasia, early proliferation of bile ducts, mononuclear infiltration of the portal tracts) are in line with a subacute process. Due to these findings, it can be assumed that the RHDV2-infection already occurred before vaccination. Therefore, the peracute death of the partner-animal two days before vaccination can, in all probability, be assigned to a peracute form of RHD and can be considered as the source of RHDV2 in the present case. Most rabbits survive subacute forms of RHD and develop antibodies.[23,29] In the present case it is thinkable, that vaccination of an already infected animal worsened the course of disease.

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**JPC Diagnosis:**
Liver: Hepatitis, necrotizing, acute, multi-focal, random, moderate, with hepatocellular vacuolar change.

**JPC Comment:**
The contributor provides an outstanding and thorough review of the emergence, clinical signs, pathogenesis, and host factors associated with both classical RHDV and RHDV2, two significant causes of mortality in lagomorphs. Since the submission of this case to the WSC in 2019, RHDV2 has been reported in North America, with outbreaks occurring in both Canada and the western United States.[3,38]

An outbreak of RHDV2 in the southwestern United States was first detected in New Mexico during March 2020. Positive cases were later identified in Texas, Colorado, Arizona, Utah, Nevada, and southwestern California by August 2020. Affected species predominantly included wild lagomorphs such as the black-tailed jackrabbit (*Lepus californicus*) and desert cottontail rabbit (*Sylvilagus audubonii*). In addition, RHDV2 was isolated from a domestic rabbit on July 10$^{th}$, 2020, indicating spillover into the domestic rabbit population. In response, the California Department of Food and Agriculture banned the entrance of rabbits, hares, and any associated products and equipment from any affected state within the previous 12 months. In addition having a negative economic impact, this disease also poses a significant threat to the endangered riparian brush rabbit (*Sylvilagus bachmani riparius*).[1]

An additional RHDV2 outbreak in the United States was identified along the northwestern coast of Washington State in July 2019, with affected regions including Orcas Island, San Juan Island, Whidbey Island, and Clallam County located on the Washington State mainland. This outbreak likely extended from a 2018 RHDV2 outbreak along the coastal region of British Columbia, Canada. Affected rabbits included a 2-year-old, 4-H pet, Norwegian dwarf buck, feral domestic rabbits, and 108 of 145 rabbits at rescue organization. Samples of liver and spleen (14 total) were submitted to the USDA-APHIS Foreign Animal Disease Laboratory

(FADDL; Plum Island, NY, USA) from the Washington Animal Disease Diagnostic Laboratory (WADDL) for antigen ELISA and reverse transcriptase PCR (RT-PCR), confirming RHDV2 in all submitted samples. Although histologic lesions of rabbits affected in this outbreak were similar to those described in European outbreaks of RHDV2, there were notable differences regarding gross and histologic findings. Icterus has been historically described to be a prominent feature of this entity, which was absent in all cases submitted to the WADDL. Histologically, hepatic lesions in the WADDL cases were randomly distributed, with all lobular zones affected, which contrasts with the clear periportal hepatocellular necrosis described in European cases. The underlying cause of this alternative pattern of distribution is unknown but may be due to differences in cellular tropism, pathogenesis, host factors, or other variables. Therefore, RHDV2 should be considered as a differential in cases of unexpected lagomorph death, including those cases lacking periportal hepatocellular necrosis.[38]

There was spirited discussion amongst participants in regard to the distribution of hepatic lesions in this case. A significant minority favored a periportal to midzonal distribution, as typically associated with this entity. However, random hepatocellular necrosis has also been reported with this entity, as previously discussed with reference to the 2019 outbreak of RHDV2 in Washington State. In addition, conference participants noted periportal lymphocytic and heterophilic inflammation, which is not a typical finding associated with rabbit hemorrhagic disease. The cause of this inflammation is unclear, though participants suggested *Eimeria stiedae,* a common apicomplexan parasite of rabbit biliary epithelium, as a differential. However, other features of this entity, such as biliary hyperplasia and the presence of apicomplexan gametocytes, schizonts, and oocysts were not observed.

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**CASE III**: EX57_19 (JPC 4152810)

**Signalment:**
2-years-old, male, inland bearded dragon (*Pogona vitticeps*)

**History:**
The bearded dragon was referred with a history of anorexia and jaundice. The reptile died spontaneously before therapy and a complete necropsy was performed by the referring veterinarian. The entire liver was submitted for histopathology.

**Gross Pathology:**
The liver was severely enlarged (9x5.5x2 cm) and characterized by diffuse yellowish discoloration and by multifocal to coalescing, white-yellowish nodules bulging from the surface and ranging from 0.5 up to 2 cm in diameter.

**Laboratory Results:**
None provided.

**Microscopic Description:**
Approximately 70% of the hepatic parenchyma is substituted by multiple nodules ranging from 350 µm to 10 mm in diameter and composed of a multifocal to coalescing, variably demarcated, unencapsulated, densely cellular neoplasms with expansile to infiltrative growth patterns. Neoplastic nodules consist of cells arranged in palisades, nests, rosettes, and pseudorosettes, supported by a fine fibrovascular stroma. Neoplastic cells are polygonal, 15-20 microns in diameter, with variably distinct margins and high nuclear-cytoplasmic ratio. Cytoplasm is scant, finely granular, and weakly eosinophilic. The nucleus is paracentral, oval to round, 10-15 microns, with granular to marginated chromatin, and 1-2 basophilic, paracentral, 2-3 microns prominent nucleoli. Anisocytosis and anisokaryosis are moderate. Mitotic figures range from 0 to 3/HPF and are often atypical. Numerous apoptotic cells and occasional bi- and tri-nucleated cells are also present. The center of some of the nodules is diffusely necrotic with variable amount of fibrin. Some neoplastic cells impinge and infiltrate vascular walls producing endoluminal projections. Scattered in the lumen of liver sinusoids, neoplastic emboli are also detectable. Viable hepatocytes bordering neoplastic nodules are atrophic, in association with rows of hepatocytes characterized by lipidosis. Mild to moderate, diffuse hyperplasia and hypertrophy of the melanomacrophage centers is evident in normal hepatic parenchyma.

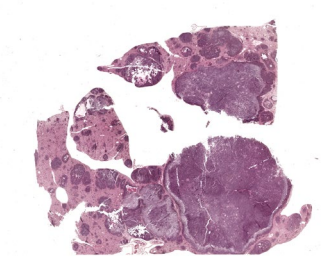

**Figure 3-1. Liver, bearded dragon. Approximately 70% of multiple sections of liver is replaced by a densely cellular, multinodular neoplasm. (HE, 5X)**

**Contributor's Morphologic Diagnoses:**
Liver. Neuroendocrine carcinoma.

**Contributor's Comment:**
Neuroendocrine carcinomas ("carcinoids") are rare neoplasms arising from neuroendocrine cells residing in several organs but most commonly develop in the gastrointestinal and respiratory tracts.[6] Neuroendocrine carcinomas have been reported in dogs and cats,[5] as well as occasionally in cattle[4,10] and horses.[9,13]

Captive reptiles have an incidence of neoplasia comparable to that of mammals and birds.[3] Snakes are the most commonly reported class, followed by lizards, chelonians, and crocodilians.[3] In lizards, hematopoietic, cutaneous, and hepatic systems are the most frequently affected by neoplasia, with bearded dragons (*Pogona vitticeps*) and green iguanas (*Iguana iguana*) being over-represented in recent years, likely based on their increasing popularity as pets.[3] Lymphoma, squamous cell carcinoma, and peripheral nerve sheath tumors are among the most common neoplasms described in bearded dragons.[3,16] Few cases of gastric neuroendocrine carcinomas have been described in young animals of this species.[1,7,8,14,16]

In this case the liver was the only tissue submitted. Neuroendocrine carcinomas primary developing in the liver are rare.[17] The angiocentric organization of neoplastic nodules observed in the current case suggests a metastatic nature of the neoplasm. Indeed, liver is a common site of metastasis of gastric neuroendocrine carcinomas in bearded dragons.[1,7,8,14,16] Other common sites for metastasis are the kidneys, followed by other organs like pancreas, intestine, heart, oviduct, ovary, lungs, adrenal gland, and spleen.[1,16]

Clinical sings commonly associated with neuroendocrine carcinomas in bearded dragons include anorexia, weight loss, weakness, and vomiting, occasionally combining with clinicopathological abnormalities like anemia and hyperglycemia.[1,7,14,16]

Neuroendocrine carcinomas are easily recognized by the typical histological pattern of palisading nests, solid masses, or anastomosing ribbons of cells separated by a thin fibrovascular stroma, with occasional formation of rosettes or acinar-like structures containing eosinophilic material.[11] In humans, neuroendocrine neoplasms have been historically classified using a range of site-specific terminologies and criteria, with consequent confusion among pathologists.[15]

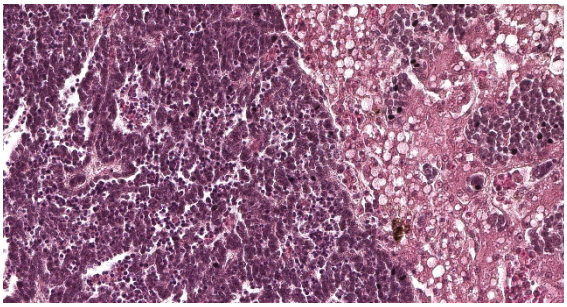

**Figure 3-2. Liver, bearded dragon. Neoplastic cells expanded sinusoids (right) and form large nodules (left), where they are arranged in cords and trabeculae and often palisade along the stroma. Remaining hepatocytes are often expanded by large discrete lipid vacuoles. (HE, 328X)**

In order to reduce inconsistencies and contradictions in the classification scheme, the WHO recently introduced the distinction between differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs), supported by genetic evidence at specific anatomic sites as well as clinical, epidemiologic, histologic, and prognostic differences.[15] However, too few cases have been observed to date in domestic animals to determine the most appropriate prognostic and other features for these neoplasms.[11]

Further subclassification of neuroendocrine tumors can be based on hormone production by neoplastic cells and the presence or absence of a clinical syndrome related to hormone secretion.[16] Indeed, these neoplasms may be non-functional, secrete a single product, or exhibit multihormonal expression.[16] In domestic animals, functional neuroendocrine carcinomas have been reported in the intestinal tract of dogs.[11] Immunohistochemistry provides the gold standard for confirmation of neuroendocrine origin and for functional subclassification based on detectable secretory products.[16] Markers to determine neuroendocrine differentiation include antibodies against secretory granule proteins (i.e. chromogranins A and B), synaptic vesicle proteins (i.e. synaptophysin), and enzymes (i.e. neuron-specific enolase) specific for neuroendocrine cells.[16] However, staining for these generic markers has been rarely reported in bearded dragon carcinoids with the exclusion of somatostatin that is the most frequent marker utilized and observed in these tumors.[1,7,8,16] This has led to the final diagnosis of somatostatinoma for all carcinoids reported in bearded dragons, this observation being consistent with the hyperglycemia occasionally observed and resembling what reported in human somatosostatinomas.[14]

Furthermore, the primary gastrointestinal location of the somatostatinomas in bearded dragons is reminiscent of those associated with neurofibromatosis type 1 (NF-1, also called Von Recklinghausen's disease) in humans.[7,16] Indeed, neuroendocrine carcinomas associated with NF-1 show decreased expression of neurofibromin. This feature was also observed by Ritter et al. (2009)[16] in all the bearded dragon carcinoids examined by immunohistochemistry, possibly suggesting that a decrease in functional neurofibromin protein may be involved in the pathogenesis of neuroendocrine carcinomas, and specifically somatostatinomas, in this species.

**Contributing Institution:**
https://www.dimevet.unimi.it/

**JPC Diagnosis:**
1. Liver: Neuroendocrine carcinoma.
2. Liver, hepatocytes: Lipidosis, diffuse, marked.

**JPC Comment:**
The contributor provides an excellent review neuroendocrine tumors in domestic and exotic species, a broad subset of neoplasms that arise from cells within the diffuse endocrine system found throughout the body, predominantly in the gastrointestinal and respiratory tracts.[12]

Although the neuroendocrine tumor in this case likely occurred as the result metastatic disease, primary neuroendocrine tumors may also arise in the liver and biliary tree. Previously referred to as "carcinoids", these neoplasms are believed to originate from the diffuse neuroendocrine cell population within the biliary epithelium or from hepatic progenitor cells, also known as oval cells.[12]

One retrospective study[12] of 13 cases of primary canine gallbladder neuroendocrine carcinoma found Boston terriers to be overrepresented, suggesting a possible breed predilection. Common findings amongst the cohort were emesis, often in conjunction with hematemesis, and elevated liver enzymes with 100% demonstrating elevated alanine aminotransferase and alkaline phosphatase activity. Histologically, all 13 cases shared similar features as previous described by the contributor, in addition to positive immunoreactivity for neuron specific enolase, chromogranin A, synaptophysin, and gastrin. Greater than 50% of neoplastic cells in every case demonstrated immunoreactivity for synaptophysin, with similar results for gastrin (12/13 cases); 25-50% of neoplastic cells were immunopositive in the one outlying case. Chromogranin immunoreactivity was reported in each case, ranging from <25% immunoreactivity to >50%. As with primary neuroendocrine tumors arising in other locations, vascular invasion and metastasis were common, identified in 61.5% and 43% of the cases, respectively.[12]

The correlation between emesis and hematemesis in addition to 100% of canine gallbladder neuroendocrine carcinomas demonstrating strong immunopositivity for gastrin was strongly suggestive of a paraneoplastic syndrome, variants of which are further discussed below. Interestingly, gastrin secretion by gallbladder neuroendocrine carcinomas is associated with a better prognosis in humans (MST 3.7 years) compared to hepatic neuroendocrine carcinomas (MST 3 days). This disparity is likely multifactorial, however, early tumor identification as a result patients seeking treatment for paraneoplastic syndrome associated symptoms is likely a significant contributing factor.[12]

A historical review of neuroendocrine tumors begins in 1870 with German physiologist Rudolf Heidenhain being the first note a group of gastrointestinal cells with "yellow" chromate staining properties that were distinctly different from chief, parietal, and enteric cells. Nikolai K. Kultschitzky, a Russian anatomist and histologist who later become the education minister of the Russian empire, further increased awareness of these cells 1897, for which they became known as "Kultschitzky's cells". The term "enterochromaffin" was introduced by Carmelo Ciaccio in 1907 and Harry Kull noted these cells demonstrated a similar morphology to chromaffin cells in 1925. In 1914, Antonin Gosset and Pierre Masson, a French surgeon and French-Canadian pathologist, respectively, used silver impregnation techniques to demonstrate the argentaffinity (i.e. argyrophilia) of carcinoid tumors and suggested they may arise from enterochromaffin cells.[2]

The term "carcinoid" was introduced by Siegfried Oberndorfer, a German pathologist, in 1907 in a report describing four pea-sized, seemingly benign "Karzinoide Tumoren" in the ileum of a woman that died from tuberculosis. Notably, German pathologists Theodor Langhans and Otto Lubarsch likely described similar lesions but did not recognize them as distinct entities decades earlier in 1867 and 1888, respectively. Two decades after "discovering" carcinoids, Oberndorfer reversed his earlier opinion of these lesions being benign, reporting evidence of metastasis as an indication of malignancy. However, the significance of this finding faded over time, largely due to Oberndorfer's departure from Germany as a result of his ethnic background prior to World War II. Although his research continued at the University of Istanbul in Turkey, his publications were printed in Turkish and failed to gain significant attention in the scientific community. As a result, misconceptions of the malignant behavior of carcinoids remained for several decades. In 1995, Italian pathologist Carlo Capella and other neuroendocrine tumor experts revised the classification scheme of neuroendocrine tumors and suggested the term "carcinoid tumor" no longer be applied and instead use the term "neuroendocrine tumor".[2]

Functional neuroendocrine tumors are commonly associated with paraneoplastic syndromes and include entities such as gastrinoma, insulinoma, glucagonoma, cholecystokininoma, and somatostatinoma.[2]

In 1955, US Surgeons Robert Zollinger and Edwin Elson described two cases of severe peptic ulcers that were associated with pancreatic neuroendocrine tumors. Three years later, gastrin was extracted from a pancreatic neuroendocrine tumor. Zollinger-Ellison syndrome has since been linked to gastrinomas.[2]

Cholecystokinin (CCK) is normally produced by specialized I cells in the gastrointestinal tract. Cholecystokininoma syndrome is characterized by nonwatery diarrhea, severe weight loss gall bladder, and peptic ulcers. Interestingly, peptic ulcers associated with cholecystokinomas occur due to CCK being a full agonist for the gastrin/CCK-B receptor. Therefore plasma

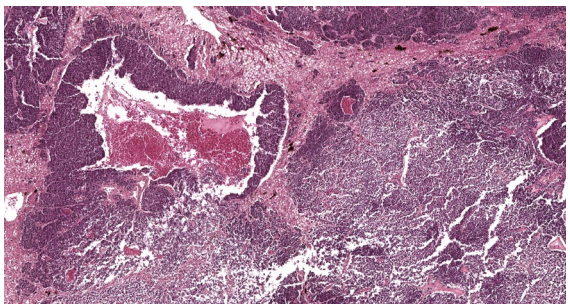

**Figure 3-3. Liver, bearded dragon. There are extensive areas of necrosis and hemorrhage within larger nodules of the neoplasm. (HE, 52XX)**

gastrin concentrations can be used to differentiate between gastrin and CCK secreting pancreatic tumors associated with peptic ulcers, which should not be elevated in cases of cholecystokininomas.[2]

US surgeon Allen Whipple and pathologist Kneeland Frantz identified key features of insulinomas (also known as 'Whipple's triad') to be 1) symptoms known or likely to be caused by hypoglycemia, 2) hypoglycemia measured at the time of symptoms, and 3) relief of symptoms upon return to normoglycemia.[2]

Glucagonomas typically arise in the pancreas and are associated with hyperglycemia.[2]

Somatostatinomas most commonly arise from the pancreas or the proximal duodenum in the periampullary region and are associated with diabetes mellitus, cholithiasis, weight loss, steatorrhea, and diarrhea.[2]

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**CASE IV:** 18042E (JPC 4136503)

**Signalment:**
4-year-old male, red-bellied short-necked turtle (*Emydura subglobosa*)

**History:**
The turtle was housed with 9 other turtles, in an appropriately sized tank. The animal presented to the rDVM with a history of progressive ascites and edema. A treatment of ceftazidime was initiated after thoracic radiographs were suspicious for pneumonia. The animal improved briefly, but then clinical signs recurred. At the submitting institution's exotic clinic, examination revealed ecchymoses, edema, ascites, and loss of keratin/epidermis on plastron. Due to poor prognosis, the owner elected euthanasia.

**Gross Pathology:**
The turtle was in extremely poor nutritional status (no fat stores appreciable within the carcass). The plastron was devoid of epithelium, and there was a focal 0.5 cm by 0.5 cm indentation in the left cranial portion of the plastron. The carapace had multifocal to coalescing ulcerations. Upon opening the carcass, bright yellow fluid spilled out, and when the plastron was removed, there was clear fluid within the coelomic cavity. All tissues were very pale and contained little blood (anemia). All muscles within the coelom had a thick layer of yellow firm pitted tissue (possible fibrin or myonecrosis). The neck muscles had a sharply demarcated line between the yellow outer material covering (or replacing the muscle) caudally underneath the carapace and more normal appearing muscle (albeit pale) cranially. The subcutaneous tissues and eyelids were edematous and there was approximately 75 mL of free fluid within the coelom and approximately 10 mL within the pericardial sac. There were multiple white nodules located in the joints of at least one digit from each limb. The nodules contained thick white material (possible gout). When the metacarpals of the front right limb were cut, tan material oozed out. The lungs looked unremarkable grossly. The kidneys were not readily apparent.

**Laboratory Results:**
Abnormal bloodwork included inclusion bodies of unknown origin in red blood cells, hypoglycemia, PCV 10% and Total Solid too low to read.

**Microscopic Description:**
Joint with surrounding muscle/tissue (foot). The joint space has marked accumulation of

amorphous fine, globular to dense deposits of mineralized material. Mineralization extends over joint surfaces and cartilage is eosinophilic with small numbers of lacunae containing cellular debris. Surrounding capsule has loss of epithelium with marked mineralization and occasional small numbers of granulocytes, lymphocytes and macrophages; latter are occasionally multinucleated. The adjacent skeletal muscle has widespread atrophy, degeneration with internalization of nuclei, loss of striations, increased eosinophilia, hypercontraction bands to fragmentation of cytoplasm and often blue stippled to dense deposits of mineral. Myocytes in affected areas are often ringed by a dense band of mineralized material with partial to complete loss of cytoplasm. In atrophic areas myocytes are separated by a very loose arrangement of fibrocytes and collagen.

In some slides, subcutaneous tissue has separation of fibrocytes and collagen; the latter has multifocal areas of light to dense mineralization. Vessels, when present, have occasional mineralized thin band within inner layer and multifocal deposits in arterial tunica muscularis.

Other tissues (not included) with similar, but less striking lesions: cornea (eye), kidney, mesentery and skin.

**Contributor's Morphologic Diagnoses:**
Severe, systemic mineralization with myonecrosis and pseudogout. (Hydroxyapatite deposition disease – HADD)

**Contributor's Comment:**
Mineralization is the term used to describe deposition of insoluble, inorganic minerals, and is often categorized as either dystrophic, metastatic, idiopathic or iatrogenic.[4] The nature of the mineral involved, in most species, is usually calcium paired with phosphate or carbonate.[4] However, when deposition of crystalline material is seen in a reptile's joint(s), one must be careful to differentiate the nature of the mineralization,

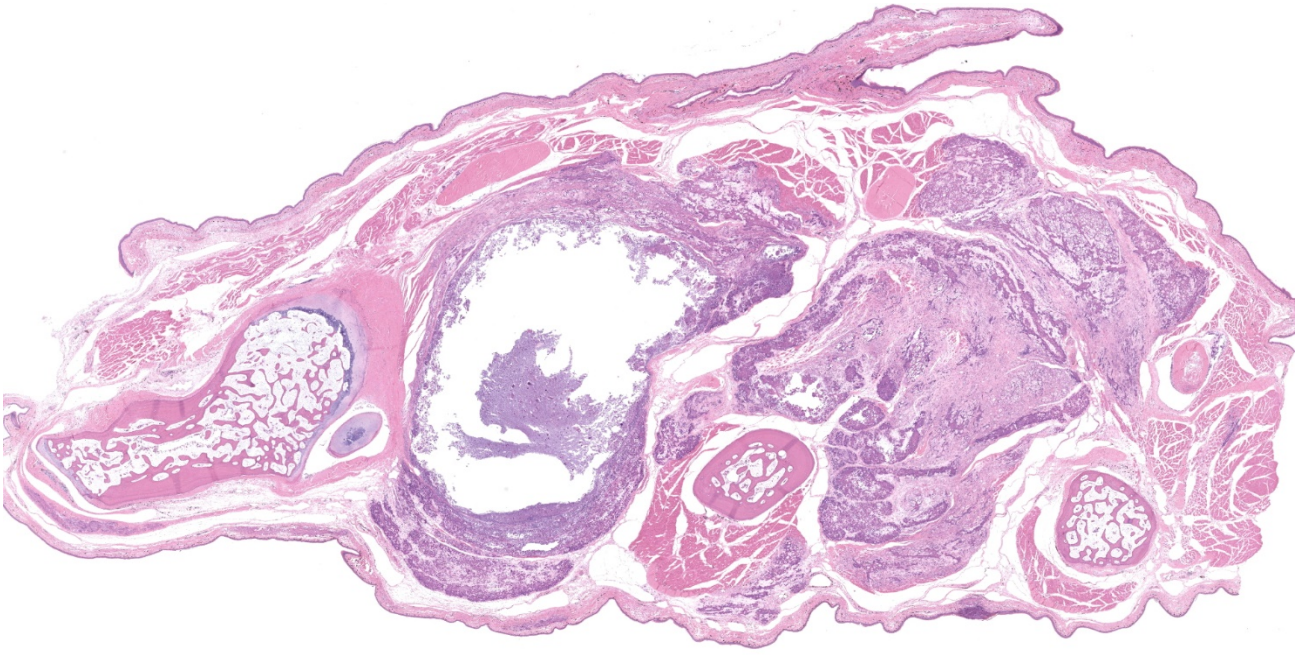

**Figure 4-1. Leg, turtle. A transverse section including the joints are submitted for examination. At subgross magnification, there are extensive lakes of crystalline mineral which effaces large areas of smooth muscle. (HE, 5X)**

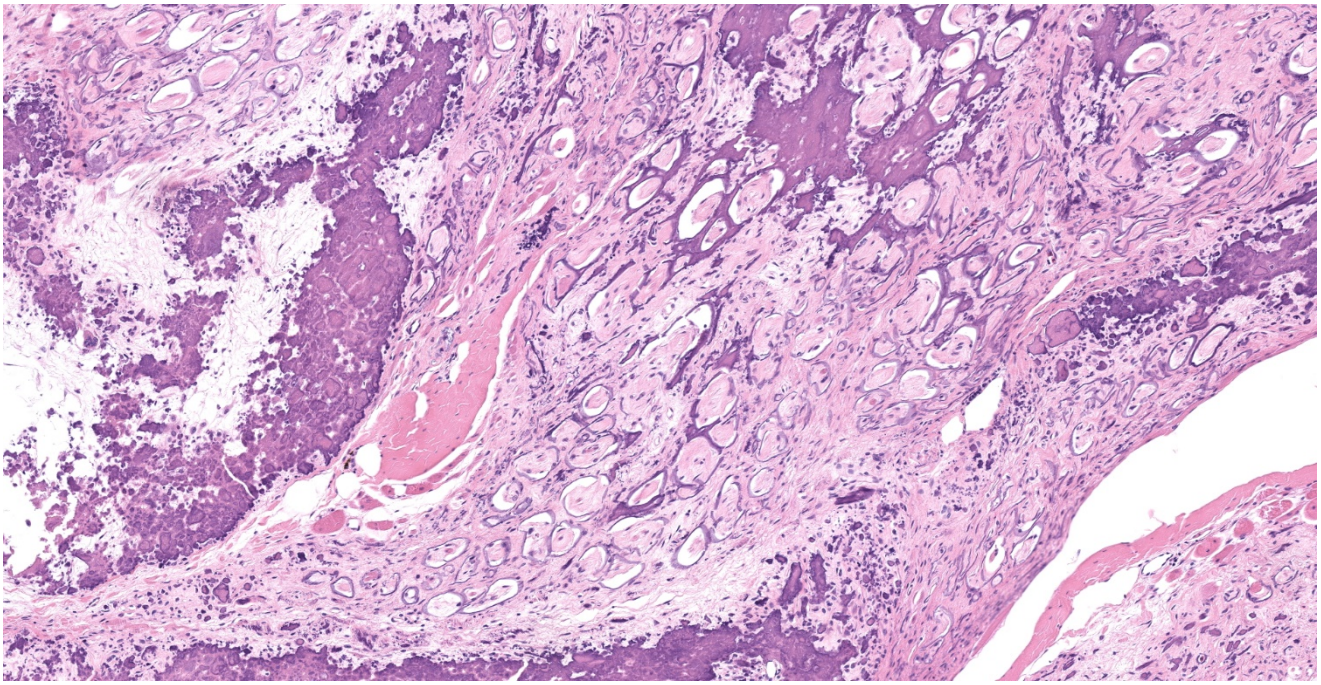

**Figure 4-2. Leg, turtle. There is progressive fibrosis and mineralization of skeletal muscle bundles. A honeycomb pattern of endomysial fibrosis and mineralization surrounds atrophic myocytes (right), and an older lesion at left is reduced to a large lake of crystalline mineral. (HE, 105X)**

to accurately diagnose as either gout (urate crystals) or 'pseudogout' (other, including calcium), considering that in these species; gout can manifest as solely articular, separate from visceral or renal involvement. Pre-mortem assessment can include cytologic preparation, where once stained and observed under polarized light, the crystals will appear as strongly birefringent and needle-like, or radiography, since uric acid deposits are radiolucent and could be differentiated from other mineral-based deposits.[3] However, caution must be exercised, since crystals can dissolve during staining and chronic gout lesions may mineralize[3], giving false negatives resulting in challenging interpretation.

The contributor believes this periarticular mineralization to be pseudogout, also known in the literature as false gout, tumoral calcinosis, calcinosis circumscripta, and hydroxyapatite deposition disease (HADD). In other species, similar lesions may be calcium pyrophosphate deposits. Terminology, when referring to these deposition conditions, have been inconsistent and makes literature review difficult.

Animals with HADD present with non-ulcerated, firm, multinodular swellings that may impede their mobility.[3] When radiographed, the nodules appear vibrantly radiopaque.[3] Recommendations are often directed at husbandry and diet to slow progression of lesions, as regression has not been reported. On transection, the nodules are sharply demarcated, filled with white to yellow, pasty to chalky substance within periarticular tissues.[3]

Histology shows affected tissues expanded by multiple irregular, nodular aggregates of granular to amorphous, basophilic or eosinophilic non-birefringent material.[3] Adjacent to mineral deposits, there is fibrous tissue and variable numbers of inflammatory

cells, predominantly epithelioid macrophages and multinucleated giant cells.[3]

The exact cause of HADD, or articular tumoral calcinosis in reptiles, has not been elucidated. In other species, similar lesions have been linked to trauma, renal failure, hyperparathyroidism, hypervitaminosis D, and other tissue or bone conditions (e.g., infection or neoplasia); however, evidence of any of the previously mentioned underlying conditions have not been linked to any reports published so far.[3]

Previous articles describing HADD were almost exclusively covering juveniles, and the majority of cases were in turtles. The literature reports HADD in 10 red-bellied short-necked turtles[1], painted turtles[2] and a red-eared slider[3]. There was also a report in a *Uromastyx sp.* lizard.[1] Between 2017 and 2019, the submitting institution has had 4 cases of periarticular mineralization: the current case and 3 red-eared s
liders, ages 25, 27 and another adult (exact age unknown).

The severity and extensive mineralization in multiple tissues seen in the present case was not reported in literature, and may indicate a systemic involvement, such as renal failure or calcium/phosphorus imbalance, and could not be ruled out due to constraints in testing pre-mortem.

**Contributing Institution:**
Veterinary Pathology – Western College of Veterinary Medicine (https://wcvm.usask.ca/departments/vet-pathology.php)

**JPC Diagnosis:**
1. Leg, skeletal muscle: Fibrosis and dystrophic calcification, multifocal to coalescing, severe, with myofiber atrophy and loss.
2. Bone marrow, adipose tissue: Atrophy, diffuse, moderate to severe.

**JPC Comment:**
The contributor provides an excellent review of hydroxyapatite deposition disease (HADD) in reptiles. Although HADD has predominantly been described in aquatic turtles, it has also rarely been reported in lizards and should be considered as differential diagnosis for reptiles presenting with gout-like lesions.

Various molecular forms of calcium salts may be deposited in tissues. Analysis of crystals forming periarticular deposits in cases of HADD are often composed of hydroxyapatite ($Ca_{10}(PO_4)2H_2O$), the primary mineral component of bone. Other species, such as humans, NHPs, and canines[1], and avians[6] are affected by similar periarticular calcium deposits. However, in these species, the calcium salt is often composed of structurally dissimilar calcium pyrophosphate dehydrate ($Ca_2P_2O_42H_2O$). Therefore, the term "hydroxyapatite deposition disease" has been suggested to specifically apply to disease associated with hydroxyapatite while "pseudogout" be applied to disease associated with calcium pyrophosphate dehydrate. Furthermore, human literature typically applies the term "tumoral calcinosis" for nodular periarticular deposits of hydroxyapatite whereas veterinary literature typically applies the term

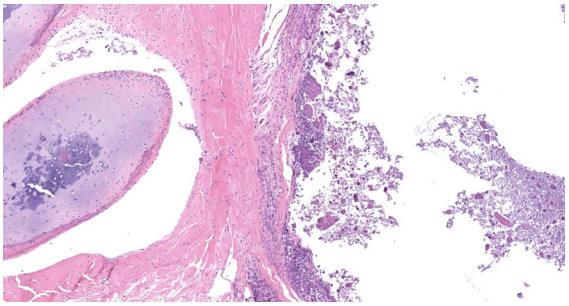

**Figure 4-3. Leg, turtle. Fibrosis and mineralization encroach upon the joint capsule. (HE, 220X)**

"calcinosis circumscripta" for similar lesions.[1]

Although unlikely in this case, the pathogenesis of gout is worthy of discussion given the commonality of the disease amongst reptiles. Gout occurs as the result of hyperuricemia, due to excess uric acid, and results in the deposition of monosodium urate crystals (known as tophi) in organs and soft tissues. In terrestrial reptiles, uric acid is the main break down product of protein, purines, and other non-protein sources of nitrogen. Excess uric acid may occur as the result of overproduction (i.e. primary gout), such as in cases of excessive dietary purines from insect rich diets, or may accumulate in the body due to decreased renal excretion (i.e. secondary gout) in cases such as starvation, dehydration, and damage to the renal tubules. Deposition of tophi is frequently associated with granulomatous inflammation and often results in nodular periarticular swellings observed in association with joints.[5]

A useful tool for differentiating gout from calcium-containing minerals such as calcium pyrophosphate when analyzing joint fluid is the combined use of a polarizing filter with a red compensator filter. Gout crystals composed of monosodium urate are negatively birefringent, demonstrated by the colors yellow and blue when aligned parallel and perpendicular to the direction of polarization, respectively.[5,6] Conversely, calcium pyrophosphate crystals in cases of pseudogout demonstrate positive birefringence, demonstrating a blue color when parallel to the slow axis of the compensator and yellow when perpendicular.[6] Histologically, von Kossa and Alizarin red S stains are useful for identification of calcium containing mineral in nondecalcified histologic sections, such as in cases of HADD and pseudogout.[6] However, confirmation of calcium deposition is not pathognomonic, since chronic gout lesions may also undergo mineralization, as previously noted by the contributor. In regard to this case, Von Kossa stain confirms the presence of calcium containing mineral within the section, further supporting the diagnosis of HADD.

An additional finding noted by conference participants is significant atrophy of adipose tissue within the bone marrow. Adipose atrophy is an indication metabolic demand chronically exceeding dietary intake, resulting in the mobilization and eventual depletion of fat stores. Although reptilian adipose tissue is typically concentrated in abdominal fat bodies or the tail, the bone marrow is a useful location to assess the nutritional status of an animal at the time of its death.

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