CASE I – S55/00 (AFIP 2888672)

Signalment: 14-week-old, female, ZIKA-hybrid rabbit, Oryctolagus cuniculus.

History: This animal was inoculated experimentally with $10^2$ hemagglutination units/ml of an infectious agent. 4 days p.i. the rabbit developed progressive jaundice, became anorectic and was euthanized on day 10 p.i. At this time a large, continuously bleeding hematoma had appeared on the ear after blood sampling the previous day.

Gross Pathology: At necropsy, the moderately emaciated rabbit was severely icteric. All lobes of the diffusely brownish-yellow liver showed multifocal to confluent, sharply demarcated, irregularly shaped, reticulated, greyish to red, gritty areas (Fig. 1). The kidneys were enlarged and bright yellow. The thymus was atrophic, the spleen was moderately enlarged, and the lungs showed diffuse, severe, alveolar emphysema.

Laboratory Results: RT-PCR and in situ hybridization revealed RHDV-positive-strand RNA in the liver, located mainly in macrophages of periportal areas and, rarely, in periportal hepatocytes. Macrophages and reticulocytes in the sinuses and red pulp of the spleen were also positive for RHDV-RNA (Fig. 2).

Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrotizing and histiocytic, subacute, centrilobular to bridging, severe, with calcification and biliary hyperplasia. Etiology consistent with Rabbit hemorrhagic disease virus (RHDV).

Contributor’s Comment: Periportal and midzonal hepatocytes are variably increased in size, some of them binucleate with basophilic cytoplasm, large, chromatin-poor nuclei and two, or sometimes more, prominent nucleoli (regeneration). Most midzonal hepatocytes contain large cytoplasmic lipid vacuoles, some of them bile droplets. Centrilobular hepatocytes are hypereosinophilic, pyknotic, or karyorrhectic (degeneration). Many centrilobular areas show dystrophic, granular calcification and are surrounded by large numbers of histiocytes and lymphocytes. Bridging of necrotic...
areas obliterates the sinusoidal and lobular architecture of the liver. Portal tracts show marked hyperplasia of bile ducts accompanied by moderate to severe lymphocytic and histiocytic infiltration and mild fibrosis. 

This animal was inoculated with liver homogenate, obtained from a rabbit that had died from “Rabbit hemorrhagic disease” caused by RHDV strain “Eisenhüttenstadt”. However, the clinically subacute course as well as the gross and histopathologic presentation of this case is rather uncommon for RHD. RHDV, a positive-stranded RNA virus, has been recently classified as type species within the genus *Lagovirus*, family *Caliciviridae*. Experimentally infected and susceptible domestic and wild rabbits (*Oryctolagus cuniculus*) usually exhibit hepatic necrosis and pulmonary hemorrhages 30 h p.i. with subsequent involvement of lymphatic tissues and kidneys. In adult rabbits the disease is peracute (usually not more than 3 days) with a high morbidity (100%) and mortality (80-90%), whereas rabbits 45 days and younger are susceptible to RHDV infection but do not develop clinical signs. Submassive necrosis of the liver is thought to be central to the pathogenesis leading to a primary or secondary depletion of coagulation factors and endothelial lesions causing the hemorrhagic syndrome after which the disease was named. The initial necrosis of the liver is caused by viral replication within hepatocytes. The current hypothesis is that alteration of these cells is responsible for the activation of clotting factors. Hepatic diseases leading to severe tissue destruction stimulate fibrinogen synthesis and release massive amounts of tissue thromboplastins. The defective clearance of activated clotting factors by the liver, combined with decreased levels of coagulation inhibitors in the plasma trigger disseminated intravascular coagulation (DIC), which in turn further promotes hepatic necrosis. Fatty degeneration of hepatocytes and centrilobular bridging necrosis are interpreted as final stages of the temporary severe hypoxia to the liver, coinciding with the DIC during the acute phase of the disease. Monocytes and macrophages are considered to represent further cellular targets and their infection may also be relevant to the development of DIC. Using *in situ* hybridization to detect RHDV-RNA in formalin-fixed, paraffin-embedded tissues of infected rabbits, it was shown that 10 days p.i. hybridization signals in hepatocytes become sparse, and predominantly macrophages show strong signals in the liver (Fig. 2).

The protracted nature of the infection in this case possibly may have been caused by factors such as immaturity at time of inoculation, innate immunity, or breed, alone or in combination. Bile duct proliferation, hepatocellular necrosis and regeneration together with a moderate inflammatory reaction can be summarized as early stages of cirrhosis of the liver. These chronic changes are uncommon findings with RHD, and even in endemic areas, end-stage livers are not a frequent condition in rabbits.

**AFIP Diagnoses:**
1. Liver: Hepatitis, necrotizing, acute, centrilobular, moderate, with hepatocellular regeneration, ZIKA-hybrid rabbit (*Oryctolagus cuniculus*), lagomorph.
2. Liver: Fibrosis, portal and bridging, multifocal, with lymphoplasmacytic and histiocytic cholangiohepatitis, and biliary hyperplasia.

**Conference Comment:** The contributor provides an excellent overview of RHDV and its mechanisms of hepatic necrosis. Cytokine release by activated macrophages and monocytes is thought to be a key component in the pathogenesis of many of the hemorrhagic diseases, including RHDV. The release of tumor necrosis factor (TNF) and interleukin-1 (IL-1) by activated macrophages induces the expression of procoagulant proteins on the endothelial surface, leading to DIC.  

Conference attendees considered toxic hepatopathy as the primary differential diagnosis due to the centrilobular pattern, paucity of inflammation, and lack of hemorrhage. Portal fibrosis and inflammation are not typical features of RHDV. It is unclear whether they are associated with the prolonged course in this case or are unrelated to the virus. Calcification, as described by the contributor, was not present on all slides.

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**References:**

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**CASE II - 40752 (AFIP 2890228)**

**Signalment:** 2 month-old, female, North American wood duck, *Aix sponsa*, avian.

**History:** This North American wood duck was hatched at the San Diego Zoo on 5 May 1999 and was housed in an outdoor exhibit with multiple species of birds. It was seen alive early on the morning of 28 July 1999, but was found dead later that morning.
Gross Pathology: The animal was in good body condition. There were numerous pinpoint tan foci throughout the liver. The spleen was speckled with dark red foci. At the esophageal-proventricular junction there was a 1mm wide, circumferential band of green discoloration of the mucosa. A similar wider circumferential band (3 to 4 mm) was present in a section of the distal small intestine. At this site the mucosal surface was covered with green-tan material and was rimmed by red margins.

Laboratory Results: None reported.

Contributor’s Morphologic Diagnoses:
1. Esophagus: Moderate diffuse epithelial hyperplasia and hypertrophy with multifocal acute erosive and ulcerative esophagitis and intranuclear and intracytoplasmic eosinophilic inclusion bodies.
2. Esophagus: Intraepithelial nematodes (not present in all sections).

Contributor’s Comment: Histologic examination of the esophageal-proventricular junction revealed diffuse hyperplasia of the esophageal stratified squamous mucosal epithelium. The epithelial cells showed a mild degree of disorderly maturation toward the luminal surface. Nearly all cells were markedly swollen with partial clearing of the cytoplasm and prominent intercellular desmosomal junctions. Nuclei had peripheralized chromatin and contained a 5 to 7 \( \mu \)m diameter round to oval eosinophilic inclusion body within the center. Similar smaller (2 to 3 \( \mu \)m diameter) eosinophilic inclusions were also present within the cytoplasm of the squamous epithelial cells.

In most sections, at the esophageal-proventricular junction there was an abrupt transition between the hyperplastic epithelium and a large esophageal ulcer, which extended through the lamina propria and into the underlying muscular tissue. The ulcer bed was lined by necrotic cellular debris and fibrin with superficial colonies of bacteria. There were prominent aggregates of inflammatory cells along the margins of the ulcer, composed predominantly of heterophils and a few macrophages. The majority of the heterophils were necrotic. A mild infiltrate of heterophils with few macrophages and lymphocytes was present within the lamina propria of the proventriculus. Multifocal mild erosions were seen elsewhere in the esophageal epithelium in many sections. Varying degrees of necrosis and inflammation with intranuclear inclusions bodies were present in liver, intestines, cloaca, spleen, and thymus in this case.

As an incidental finding, there were cross-sections of adult nematodes within the esophageal mucosa. These nematodes were approximately 100 to 130 \( \mu \)m in diameter with a 1\( \mu \)m thick smooth cuticle, prominent platymyarian musculature, a digestive tract with uninucleate cells and a brush border, and a single oval reproductive tract. These most likely represent Capillaria sp. However, none of the examined sections contain hypodermal bands or esophageal gland cells (stichocytes). Nematodes were not present in all submitted slides.

The above findings of epithelial hyperplasia and ulcerative esophagitis with intranuclear and intracytoplasmic inclusion bodies are diagnostic for duck viral enteritis,
an acute contagious disease of Anseriforme birds (ducks, swans, geese) caused by the \( \alpha \)-herpesvirus Anatidae herpesvirus 1.\(^4\) It affects both wild and domestic waterfowl, however it most commonly causes large outbreaks in duck or domestic waterfowl raising facilities. Transmission is via the oral route through direct contact with infected birds or contaminated soil or water.\(^1\) At domestic facilities it is most often transferred by wild waterfowl flying over the farms.\(^4\) Vertical transmission from a carrier bird to its young has also been reported.\(^2\) There is a variable susceptibility between Anseriformes. Pintails (\textit{Anas acuta}) and the European teal (\textit{Nettion crecca}) are resistant to disease despite infection. Mallards (\textit{Anas platyrinchos}) are infected and in some cases can be inapparent shedders. Muscovy ducks (\textit{Cairina moschata domesticus}) seem to be the most highly susceptible.\(^3,4\) Recovered animals may shed virus orally and through excretions for up to 4-5 years.\(^1,4\) To date the natural reservoir has not been identified.\(^3\)

Clinical signs develop within 3 to 14 days (typically 3 to 7) and may include diarrhea, dehydration, extreme thirst, weakness, bloodstained vent, cyanotic bill, photophobia, drooping plumage and in males, phallic prolapse.\(^1,4\) When moved, animals may also demonstrate head, neck and whole body tremors.\(^2,4\) In most cases, however, there are typically no clinical signs and dead birds are found floating on the surface of the water.

Common gross findings include pinpoint areas of necrosis throughout the liver, petechiae in the liver, pancreas and along the intestinal serosa, and a hemorrhagic enteritis. The enteritis is characterized by a dark red mucous membrane and band shaped areas of ulceration covered by a thick tan to yellow diphtheritic cast. The esophagus and cloaca may also show similar ulceration.\(^1-3\) In our experience, intestinal lesions are uncommon; lesions are most often seen in the esophagus and cloaca.

The reported histologic findings are similar to those described above. The intranuclear inclusions are a consistent finding; intracytoplasmic inclusions have been described\(^5\) and are not unusual in our experience. The intranuclear inclusions may also be found in hepatocytes and pancreatic acinar cells surrounding areas of necrosis, as well as renal tubular epithelial, interstitial cells of the kidney and bursa of Fabricius, and in mononuclear cells of the spleen.\(^1,4\) Lymphoid organs may show reactive follicles, lymphoid depletion and necrosis.\(^4\) Secondary opportunistic fungal and bacterial infections are also a common finding.\(^1\)

On ultrastructural examination, viral particles are 105 to 115 nm in diameter, hexagonal and have an electron dense core. They are found both in cytoplasmic vesicles and budding through the nuclear envelope. When present, intracytoplasmic inclusion bodies may form clusters. Enveloped virions (200 to 250 nm in diameter) are occasionally seen free in the cytoplasm.

Prevention of disease in domestic ducks can be accomplished through vaccination.\(^1-4\)
AFIP Diagnoses:
1. Esophageal-proventricular junction: Esophagitis, ulcerative, acute, focal, moderate, with diffuse lymphoid necrosis, epithelial hyperplasia, and eosinophilic intranuclear and intracytoplasmic inclusion bodies, North American wood duck (Aix sponsa), avian.
2. Esophagus: Intraepithelial nematodes with diffuse epithelial hyperplasia.

Conference Comment: The contributor provides a concise review of duck viral enteritis. This case was reviewed in consultation with Dr. C. H. Gardiner, Parasitologist. The intraepithelial Capillaria sp. are not present in all slides. Cross-sections of the parasite demonstrate coelomyarian polymyarian musculature, hypodermal bands and, occasionally, stichosomes, which are features of aphasmid nematodes.

Herpesviruses are 150nm diameter, enveloped, double-stranded DNA viruses with icosahedral nucleocapsids. Intranuclear and occasionally, intracytoplasmic inclusions are present in cases of Anatidae herpesvirus 1. Intracytoplasmic inclusion bodies are not typically characteristic of herpesviruses, except in cytomegalovirus and gallid herpesvirus 2 (Marek’s disease)\(^5\). Herpesvirus replicates in the nucleus where virion DNA becomes encapsidated, and these nucleocapsids then become enveloped by budding through the inner layer of the nuclear envelope\(^6\). Intracytoplasmic inclusions in cases of duck viral enteritis have been shown to contain enveloped herpesviruses.\(^4,5\)

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References:
Signalment: Three year-old female spayed Border collie, *Canis familiaris*.

History: Animal has two-month history of dermal and subcutaneous nodules and draining tracts. Lesions initially observed on the left front foot, but now involve all four distal extremities.

Gross Pathology: Physical examination revealed pyrexia, generalized lymphadenopathy, and pitting edema of all four limbs. There were multiple ulcerated cutaneous lesions, nodules, and draining tracts involving all four distal extremities. The draining exudate was serohemorrhagic to purulent. Some ulcerative lesions were covered by an eschar. A single 5x5 cm nodule was present in the left caudal mammary gland. The prescapular and popliteal lymph nodes were enlarged approximately 5x normal.

Laboratory Results: Hematologic and serum biochemical analyses revealed an inflammatory leukogram, hyperglobulinemia, and mild hypercalcemia. Cytologic examination of a fine needle aspirate of the left popliteal lymph node revealed pyogranulomatous inflammation with numerous broad, infrequently septate hyphae. Culture of a fine needle aspirate of the left popliteal lymph node yielded *Lagenidium* sp. Identification of the cultured isolate was made on the basis of morphology as well as PCR amplification.

Contributor’s Morphologic Diagnoses:
1. Skin from rear leg: dermatitis, necrotizing and granulomatous, chronic, diffuse, severe with presence of broad, irregularly branching hyphae and multinucleated giant cells.
2. Popliteal lymph node: lymphadenitis, necrotizing and granulomatous, chronic, multifocal to diffuse, severe with presence of broad, irregularly branching hyphae and multinucleated giant cells.

Please note: Some sections do not show necrotic components in skin and lymph node. They show granulomatous inflammation with presence of hyphae and giant cells.

Contributor’s Comment: Oomycetes are soil- or water-dwelling organisms that belong to the Kingdom Stramenopila (Chromista). Although they are not true fungi, many grow like fungi on mycological media and produce vegetative hyphae that are morphologically similar to those of fungi in the class Zygomycetes. *Pythium insidiosum*, an aquatic organism best known as a cause of cutaneous lesions in horses and of gastrointestinal or cutaneous disease in dogs, has long been considered to be the only mammalian pathogen in the class Oomycetes. However in 1999, a second pathogenic oomycete was isolated from tissue taken from a dog with severe multifocal cutaneous lesions and regional lymphadenopathy. The dog died acutely following rupture of a caudal vena caval aneurysm, and necropsy revealed severe sublumbar lymphadenitis and pyogranulomatous vasculitis. Sequencing of a portion of the ribosomal RNA gene of the isolate recovered from this dog identified it as member of the genus *Lagenidium*.  

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Presently, more than 30 dogs with serologic, histologic, and/or culture evidence of *Lagenidium* sp. infection have been identified.

The clinical and epidemiologic features of lagenidiosis that have thus far been identified are similar in many respects to those associated with cutaneous pythiosis. Affected animals are typically young to middle-aged dogs living in the southeastern U.S. Although most dogs have been from Florida or Louisiana, we have also identified cases in Texas, Tennessee, Virginia, and Indiana. A number of infected dogs have had frequent exposure to lakes or ponds. Infected dogs are typically presented for evaluation of progressive cutaneous or subcutaneous lesions (often multifocal) involving the extremities, mammary region, vulva, or trunk. Grossly, these lesions appear as firm dermal or subcutaneous nodules, or as ulcerated, thickened, edematous areas with regions of necrosis and numerous draining tracts. Regional lymphadenopathy is often noted, and may occur in the absence of cutaneous lesions. Animals with great vessel or sublumbar lymph node involvement often develop hindlimb edema. Similar to the clinical course associated with cutaneous pythiosis, skin lesions in dogs with lagenidiosis tend to be progressive, locally invasive, and poorly responsive to therapy. In contrast to pythiosis, however, the majority of dogs with lagenidiosis have been found to have lesions in distant sites, including great vessels, sublumbar and inguinal lymph nodes, lung, pulmonary hilus, and cranial mediastinum. *Lagenidium* sp. infection has not been identified in mammals other than dogs.

The histologic features of lagenidiosis are similar to those associated with pythiosis and zygomycosis, and are characterized by pyogranulomatous and eosinophilic inflammation associated with broad, irregularly branching, sparsely septate hyphae. In contrast to *P. insidiosum*, *Lagenidium* sp. hyphae are usually visible on H&E-stained sections. On GMS-stained sections, numerous broad, thick-walled, irregularly septate hyphae are easily recognized. *Lagenidium* hyphae typically demonstrate a great deal of variability in size (even within the same tissue section), but in general are much larger than *P. insidiosum* hyphae, ranging from 7 to 25 µ in diameter, with an average of 12 µ. Immunoblot serology for the detection of anti-*Lagenidium* antibodies in canine serum can provide a presumptive diagnosis of lagenidiosis, but must be interpreted in conjunction with results of serologic testing for *P. insidiosum* infection because of the potential for cross reactivity. A definitive diagnosis of *Lagenidium* sp. infection is best made by culture followed by identification of the pathogen via either ribosomal RNA gene sequencing or genus-specific PCR. This same PCR assay can also be used for the detection of *Lagenidium* DNA in infected tissue samples.

**AFIP Diagnoses:**
1. Haired skin: Dermatitis, pygranulomatous, multifocal and coalescing, severe, with ulceration and fungal hyphae, Border Collie, canine.
2. Lymph node, popliteal (per contributor): Lymphadenitis, pygranulomatous, diffuse, severe, with fungal hyphae.
The primary mechanism by which granulomatous disease causes hypercalcemia involves alterations in vitamin D metabolism. In vitro, macrophages can convert 25-hydroxycholecalciferol to its active form, 1,25-dihydroxycholecalciferol (calcitriol) by 1-alpha hydroxylase found in macrophage mitochondria. Normally, 1-alpha hydroxylation takes place in the proximal renal tubular epithelium. The extrarenal production of calcitriol by macrophages is unregulated and causes excess absorption of dietary calcium, reabsorption of renal calcium, and osteoclast resorption of bone calcium, leading to hypercalcemia. 7,8,9,10

Another proposed mechanism of macrophage-induced hypercalcemia is described in human sarcoidosis, a chronic granulomatous disorder. 12 Parathyroid hormone-related protein (PTHrP) is produced by many normal adult and fetal tissues, where it has autocrine and paracrine functions. PTHrP is a mediator of hypercalcemia of malignancy, and produced by neoplasms such as lymphoma and adenocarcinoma of the apocrine gland of the anal sac. PTHrP acts on PTH receptors in the bone and kidney to cause mobilization of calcium from bone by osteoclasts and calcium reabsorption in the kidney. Although PTHrP is normally secreted by macrophages, it was identified in the cytoplasm of macrophages and multinucleated giant cells in granulomas of human sarcoidosis, and was reported as the source of elevated PTHrP levels. 10,12

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References:

**CASE IV - NIAH-No.2 (AFIP 2888757)**

**Signalment:** 7.5-year-old, female, Bernese mountain dog, *Canis familiaris*, canine.

**History:** According to the owner, the dog exhibited anorexia, weight loss, and finally anemic jaundice. Markedly swollen spleen and liver were palpable at the veterinary clinic. The disease developed rapidly over a couple of weeks before termination.

**Gross Pathology:** Systemic anemia and jaundice, splenomegaly, and hepatomegaly with congestion and cloudy swelling were observed. The bone marrow was yellowish.

**Laboratory Results:** Blood examination showed elevation of ALT (189 IU/L), ALP (740 IU/L), total bilirubin (3.2 mg/dl) and anemia.

**Contributor’s Morphologic Diagnosis:** Spleen and lung: Malignant histiocytosis, hemophagocytic, Bernese mountain dog, canine.

**Contributor’s Comment:** In the spleen, the proliferating and infiltrating cells are atypical pleomorphic round or histiocytic cells with abundant cytoplasm and anisokaryosis. Large binucleated and multinucleated cells are also seen. These cells frequently engulf erythrocytes, pigment, cell debris, and have vacuolated cytoplasm. Mitotic figures are not prominent. Megakaryocytes are scattered without associated erythroblastic lineage. Immunohistochemically, the proliferating cells are positive for lysozyme, CD68, and MAC 387 in paraffin-embedded sections. In the lung, the tumor
cells were often larger than those in the spleen and other organs, and had a prominent nucleolus.

Well-defined proliferative histiocytic diseases in dogs include canine cutaneous histiocytoma, cutaneous histiocytosis, systemic histiocytosis, and histiocytic sarcoma (HS) / malignant histiocytosis (MH). The latter is known as a familial disease for Bernese mountain dogs. MH is indistinguishable from HS after dissemination.

In this case, the lung lesions had been confused with anaplastic lung carcinoma or giant cell variant of large cell anaplastic carcinoma. However, electron microscopy and immunohistochemistry revealed that the character of the tumor cells were identical.

AFIP Diagnoses:
1. Lung: Malignant histiocytosis, Bernese Mountain Dog, canine.
2. Spleen: Malignant histiocytosis.

Conference Comment: Malignant histiocytosis, also known as disseminated histiocytic sarcoma, is a rapidly progressive tumor of the mononuclear phagocyte system, and is an inherited disease in Bernese Mountain Dogs. It is also reported in Rottweilers, Golden Retrievers, Labrador Retrievers, and Flat-Coated Retrievers.5

Grossly, it causes solitary or multiple firm, white masses in the lung, liver, spleen, lymph nodes, bone marrow, or kidneys. Microscopically, multinucleated giant cells are a prominent feature and atypical histiocytes often show extensive erythrophagocytosis.5

The contributor mentions the differential diagnosis for proliferative histiocytic lesions in dogs. Systemic histiocytosis is a disease of non-neoplastic histiocytes that form dense perivascular cuffs, primarily in the skin and lymph nodes. Systemic histiocytosis, cutaneous histiocytosis, and cutaneous histiocytoma are considered reactive proliferative histiocytic diseases. Localized histiocytic sarcoma is a rapidly growing, solitary cutaneous or subcutaneous mass most frequently located on a distal limb adjacent to a joint. Occasionally it is found in the spleen, liver, gastric wall, or tongue. Malignant fibrous histiocyotma is characterized by neoplastic cells with features of fibroblasts and histiocytes.1,5,6

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References:

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