Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 24

2 May 2018

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CASE I: AP17-0483 (JPC 4101485).

Signalment: 1-year-old, female, Zebrafish (*Danio rerio*), pisces.

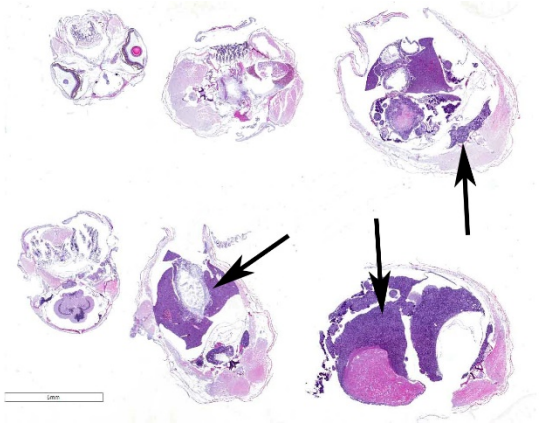
History: A subset of TP53 mutant zebrafish from the colony were found with significantly distended coelomic cavities, which necessitated their removals from the study.

Gross Pathology: The coelomic cavities of the affected fish were grossly distended and, upon opening, contained variably sized, firm, tan masses that compressed and effaced organs and infiltrated the body wall.

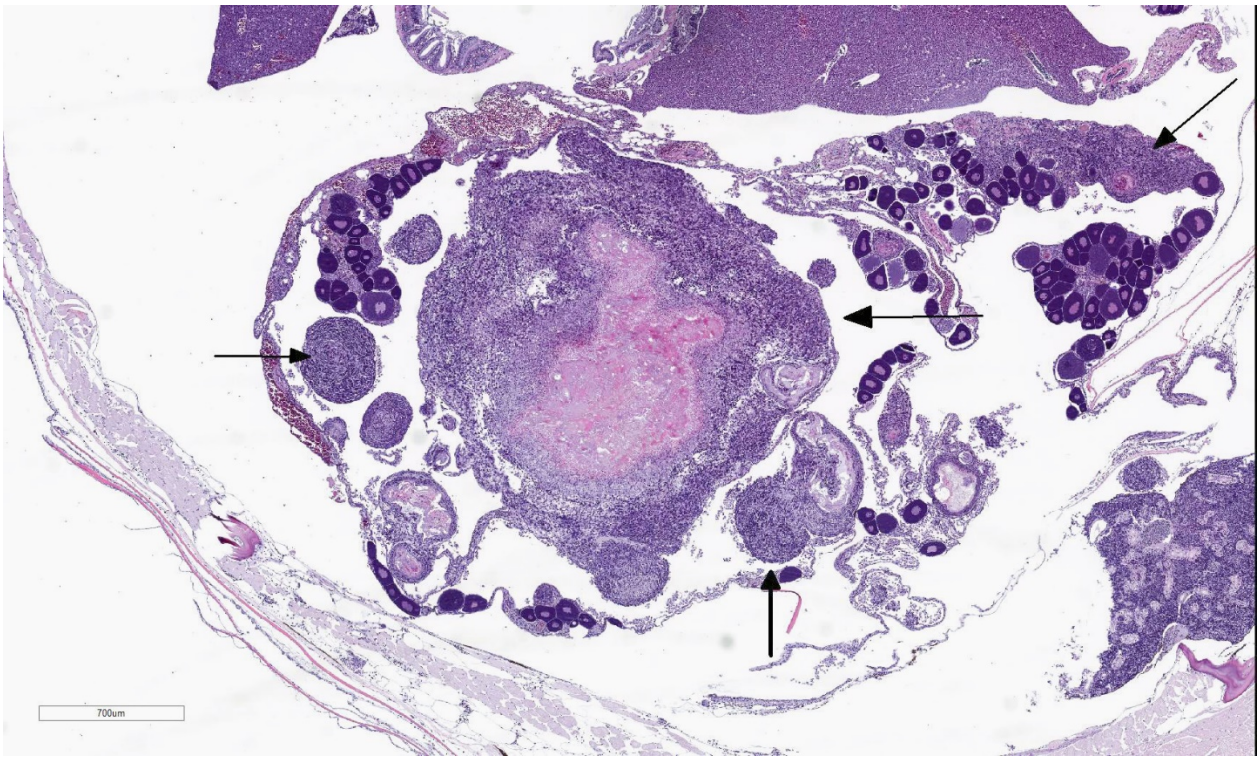
Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): None provided.

Microscopic Description:

The coelomic cavity is expanded by a large, unencapsulated neoplasm that is arranged in variably sized and tightly arranged bundles with regions of indistinct nuclear palisading and situated within an eosinophilic, collagenous matrix. Neoplastic cells have poorly defined cellular borders, are spindle-shaped and have moderate amounts of cytoplasm. Nuclei are oval to fusiform in shape and have either reticulated or hyperchromatic patterning with 1 to 2 nucleoli that are variable in prominence based on the orientation of the tumor cells. There is marked anisocytosis and anisokaryosis with atypical giant cells



Transverse sections, zebrafish. Several cross-sections of the fish are submitted for examination. 3 0f 6 s4ections contain a large infiltrative partially necrotic (lower left) neoplasm which partially fills the coelomic cavity. (HE, 5X)

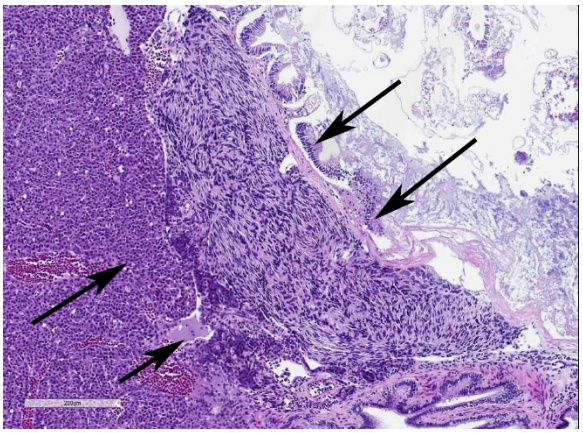


Coelomic cavity, zebrafish. Higher magnification of the neoplasm (arrows) present within the coelomic cavity. Tumor nodules often have large central pink areas of necrosis, and the tumor infiltrates the ovary and separates follicles. (HE, 30X)

scattered throughout the tumor. Mitoses range from 0-4 per 400x/hpf. There are varying combinations and concentrations of small lymphocytes, heterophils and histiocytes that are distributed throughout the neoplasm as well as randomly scattered foci of necrosis that often coalesce together. There are also rare, small sized granulomas that are centered on clear spaces as well as entrapped remnants of ovarian tissues in different states of degeneration. Neoplastic cells contact and extend into portions of the body wall. Metastasis is not observed.

Contributor's Morphologic Diagnosis:
Coelomic cavity: Malignant peripheral nerve sheath tumor, Zebrafish

Contributor's Comment: The tumors that were observed in the *Tp53*-mutant zebrafish are classified as malignant peripheral nerve sheath tumors (MPNSTs) based on cellular pleomorphism, invasiveness, the presence of necrosis, and, most importantly, the clinical course of the disease that was documented for this colony. In humans and animals peripheral nerve sheath tumors (PNSTs) may arise throughout the body and are largely defined by their morphologic patterning and include neurofibromas, schwannomas and perineurinomas. Further subdivision into benign versus malignant tumors is currently based on the degree of cellular differentiation, mitotic count, presence of necrosis and clinical staging. Schwann cells are the putative cells of origin for most PNSTs. Benign PNSTs most consistently express S100, which is frequently used to distinguish them from other neuroepithelial and mesenchymal neoplasms with similar morphologies.[3,5,9] PNSTs are not currently documented in zebrafish outside of experimental manipulations affecting *Tp53* status or ribosomal protein genes, although



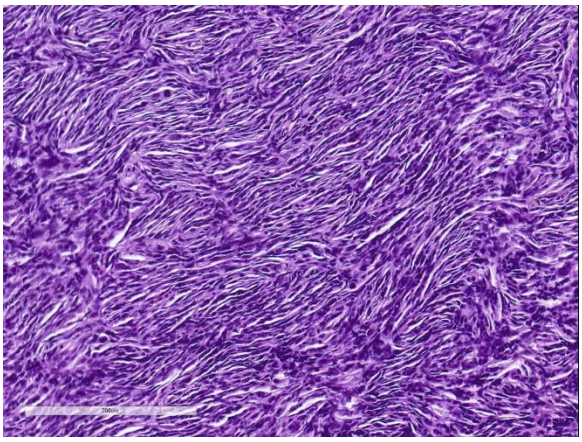
Coelomic cavity, zebrafish. The tumor infiltrates between the liver and intestine. (HE, 40X)

viral infection resulting in neurofibromatosis has been described in damselfish.[1,7,10] One study has confirmed that sarcomas arising in laboratory zebrafish, which had mutations of both T*p53* and B*rca2*, are derived from Schwann cell populations as assessed by the positive labeling of the tumor cells with anti-CD57 and anti-S100 antibodies.[13]

The zebrafish has been an important animal model for studying human MPNSTs, especially in the context of hereditary cancer syndromes, because *t* mutated zebrafish MPNSTs often have comparable genetic alterations as human tumors.[12,14] Since many of the functions of P53 are conserved the spectrum of tumors that may arise from perturbations to P53-mediated cellular processes frequently result in lymphomas, leukemias, sarcomas and carcinomas in both humans and animals. However, there are often species specific differences in the frequencies of certain tumors, such as the predisposition for zebrafish to form malignant rather than benign PNSTs.[6,8,12] These differences suggest that a combination of time and environmental dependent factors are important for the types of P53 dependent tumors that develop and their biological behaviors.[2] While these differences in biology between humans and animal species exist, these dissimilarities are being used to further understand the essential genetic and environmental events that are important for the biology of PNSTs.

JPC Diagnosis: 1. Omentum, ovary, pancreas: Malignant peripheral nerve sheath tumor, Zebrafish (*Danio rerio*), pisces.
2. Kidney, ovaries, omentum: Granulomas, multiple.

Conference Comment: In humans, malignant peripheral nerve sheath tumors (MPNSTs) are rare but devastating tumors, asthey are aggressive and have high rates of relapse following chemotherapy. In approximately half of cases, MPNSTs occur in association with neurofibromatosis type I which results from loss of function mutations to the tumor suppressor neurofibromin. Neurofibromatosis type I, an autosomal dominant condition, is the most common human syndrome which predisposes to cancer. Clinical signs include: cutaneous hyperpigmentation (known as “café-au-lait spots”) and multiple cutaneous neurofibromas which can transform into MPNSTs.



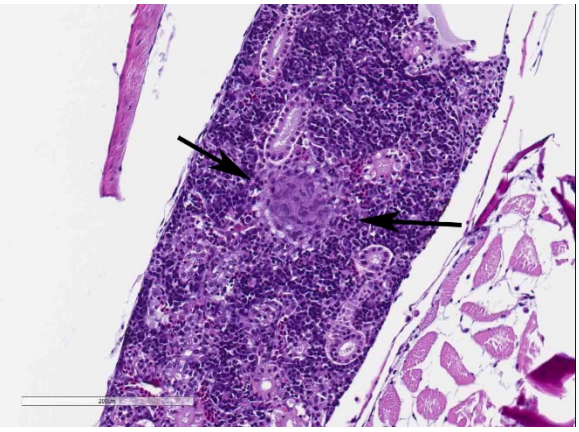
Coelomic cavity, zebrafish. Neoplastic spindle cells are arranged in short streams and bundles. Nuclei are ellipsoid to spindled with moderate anisocytosis and anisokaryosis. (HE, 200X)

In addition to NF-1, TP53 mutations have also been associated with development of MPNSTs – this is where zebrafish are particularly useful.[4] Historically, human cancer studies utilized rodent models. However, disadvantages such as increased time for tumor development and cost encouraged identification of a new model. Zebrafish are more economic and undergo neoplastic transformation and growth quicker. Most importantly, many aspects of carcinogenesis in humans are similarly expressed in zebrafish.

Several methods are used to generate effective models for research including: chemical carcinogenesis, forward or reverse genetics screens, transgenic models, and xenotransplantation in embryos.[11] Currently, zebrafish are used as models for the following types of cancer:

Table 1: Zebrafish models of cancer[11]

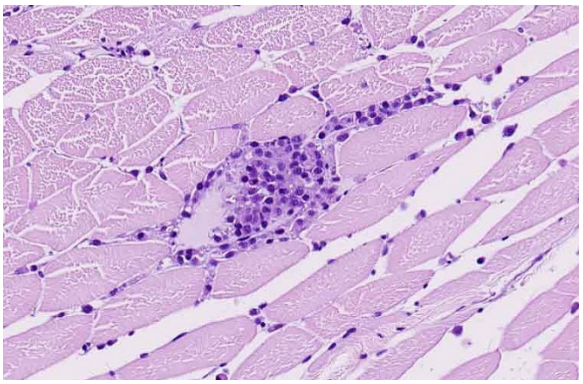
Tissue of origin	**Cancer model**
Cutaneous neoplasia	Benign nevus; melanoma; papilloma; epidermal tumors
Muscular, adipocytic, and vascular neoplasia	Embryonal rhabdomyosarcoma; rhabdomyosarcoma; liposarcoma; hemangiosarcoma
Intestinal, pancreatic, and hepatic neoplasia	Intestinal adenoma; malignant intestinal tumors; pancreatic acinar cell adenoma; pancreatic carcinoma; hepatoma; hepatocellular carcinoma
Hematopoietic, lymphoid, and small round blue cell neoplasia	T-cell leukemia; T-cell or B-cell acute lymphocytic leukemia; T-cell lymphoblastic lymphoma; acute myeloid leukemia; myeloproliferative neoplasm; small round blue cell tumors
Neural and neuroendocrine neoplasia	MPNST; neuroblastoma; neuroepithelioma; pituitary corticotroph adenoma; pancreatic neuroendocrine carcinoma



Head kidney: Small granulomas are present within several organs including the kidney, ovary, and omentum. (arrows). (HE, 40X)

Peripheral nerve sheath tumors can be further differentiated as benign and malignant Schwannoma, neurofibroma, or neuro-fibrosarcoma. In most cases, the Schwann cell likely the progenitor cell. A common rule out in fish is chromatophoroma (pigment cell tumors). The bi-color damselfish was once thought to be a naturally-occurring model of neurofibromas before it was discovered they were virally induced.[10]

During the conference, the moderator pointed out that there is increased hematopoietic tissue and vacuolated tubular epithelial cells



Abdominal wall, zebrafish. Rare muscle fibers are necrotic with infiltration of numerous macrophages. (HE, 100X)

within the anterior kidney, multiple granulomas, and histiocytes focally within skeletal muscle. Conference participants discussed what constitutes a "malignant" PNST and concluded that invasiveness is a key indicator but that spread to other organs via blood or lymphatics is not required.

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CASE II: IP17-300 (JPC 4101145).

Signalment: Juvenile, undetermined sex, Nile tilapia (*Oreochromis niloticus*), pisces.

History: In a group of 50,000 fish, after 21 days of culture, with an approximate weight between 0.8 and 1 gram, a considerable amount of mortality was reported. Some of these fish had difficulty swimming and were found on the pond's surface. There were whitish spots distributed throughout the body. At least 300 fish were found dead on the surface and countless more were found on the pond's bottom. Wet mounts of gills and skin (including fins), were performed to calculate the mean intensity for mono-geneans, trichodinids and *Ichthyo-phthirius multifiliis*; the values obtained were 5, 15 and 47 parasites per infected fish respectively. The prevalence for these same parasites was 46%, 53.3% and 100% respectively.

Gross Pathology: Multifocal to coalescing raised pinpoint 2mm white spots covered most of the skin, gills and oral cavity; in some fish these pinpoint lesions affected the cornea as well.

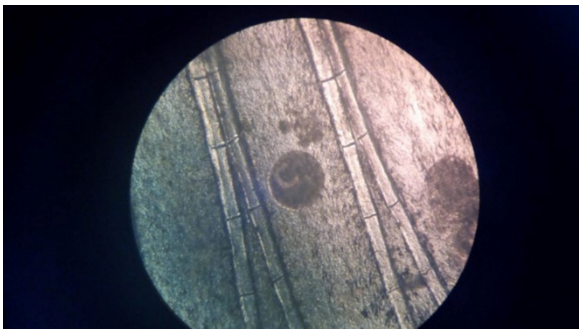
Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): Wet mounts of gill clippings obtained at necropsy demonstrated large numbers of round, 50-300 µm diameter theronts and trophonts consistent with *Ichthyophthirius multifiliis.*

Presentation, tilapia. Numerous white spots are scattered across the scales, eyes, and fins of affected fish. (Departamento de Patología (Pathology Department). Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. Mexico City, Mexico.
Web site:
http://fmvz.unam.mx/fmvz/departamentos/patologia/acerca.html)

Microscopic Description:

Gills: Multifocally, there is moderate hyperplasia of the gill epithelium with blunting and fusion of secondary lamellae and numerous irregularly round, single-cell, up to 200 um diameter, intraepithelial protozoal cysts with a 1-2 um thick hyaline wall, abundant, finely granular to vacuolated basophilic cytoplasm containing numerous host erythrocytes, and a 30 x100 um, crescent-shaped, deeply basophilic macronucleus (trophont). Goblet cell hyperplasia was also observed. Variable amounts of mucus and necrotic debris admixed with filamentous bacteria was present in some sections. Attached to the lamella or freely between them, numerous saucer shaped, hemispheric, dumbbell shaped, or sac like or flattened cylindrical protozoa, consistent with trichonids, were also observed.

Skin and oral cavity: Multifocally, there are nodular foci within the epidermis that are composed of hyperplastic epithelium that



Gill filaments, tilapia. A wet mount of the gill clippings contains a 50-300um theront with a prominent horseshoe nucleus characteristic of Ichthyophthiris multifiliis. (Departamento de Patología (Pathology Department). Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. Mexico City, Mexico. Web site: http://fmvz.unam.mx/fmvz/departamentos/patologia/acerca.html)

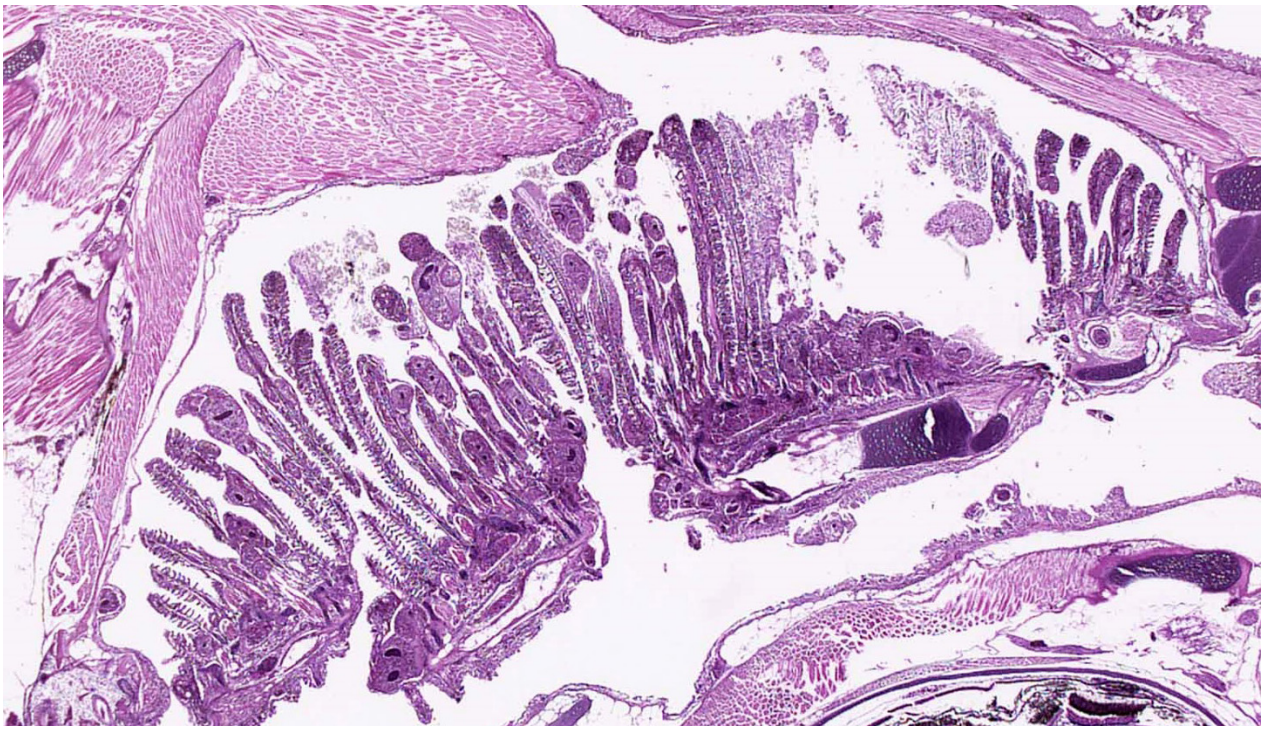
piles up to 6 to 9 cell layers. Hyperplastic epithelium often surrounds previously described intraepithelial protozoa.

Contributor's Morphologic Diagnosis:
Gill: Epithelial hyperplasia, nodular, moderate, diffuse, with numerous protozoa (trophonts and theronts) consistent with *Ichthyophthirius multifiliis* and trichodinids.

Skin and oral cavity: Epithelial hyperplasia, nodular, multifocal, moderate with protozoa (trophonts and theronts) consistent with *Ichthyophthirius multifiliis.*

Contributor's Comment: Histologic findings were similar in all fish examined. In addition to proliferative branchitis and dermatitis, affected fish exhibited a similar lesion in the oral cavity associated with organisms identical to those observed in the gills. *Ichthyophthirius multifiliis* is a ciliated protozoan parasite that infects the skin and gills of freshwater fish and causes "Ich" or white spot disease. The life cycle begins with a small migratory and infective stage known as theront attaching to the epidermis and gills, where it feeds and continue its development to a trophont, which elicits a reactive response (epidermal hyperplasia). At this stage, trophonts increase dramatically in size due to enlargement of their macro-nucleus, production of food vacuoles and liposomes, and formation of new mucocysts. The trophont breaks through the epithelium, drops off the host, and forms a capsule (tomont) that adheres to the bottom of the tank. Tomonts undergo binary fission within the cyst to produce tomites which break through the cyst and eventually become infective motile theronts. *Ichthyophthirius multifilis* are 75 μm to 1 mm in diameter and uniformly ciliated with a crescent-shaped nucleus. *I. multifiliis* causes localized lymphocytic infiltration, focal necrosis, and varying degrees of epithelial proliferation in the skin and gills. In severe cases, sloughing of the epidermis has been observed.[1,2,3,10] In experimentally infected channel catfish (*Ictalurus punctatus*), infection of the peritoneal cavity has been reported; three possible enterance routes were speculated: penetration through esophageal wall, penetration of the pneumatic duct (a structure connecting the esophagus and swim bladder), or retrograde migration from the anus.[5] In saltwater fish *Cryptocaryon irritans* has the same life cycle as *I. multifilis*, where it is referred as "marine ich" and produces similar lesions.

Conversely, trichodinas/trichodinids are mobile peritrich ciliates that attach temporarily to the substrate while feeding and have been found in both freshwater and marine fish. They are found in the skin and gills. Although they are typically considered commensal organisms, they may become numerous in stressed or debilitated fish. Depending on the orientation of the parasites in tissue sections, they may appear as saucer shaped, hemispheric, dumbbell shaped, and sac like or flattened cylindrical organisms. Heavy infection with this parasite, has been



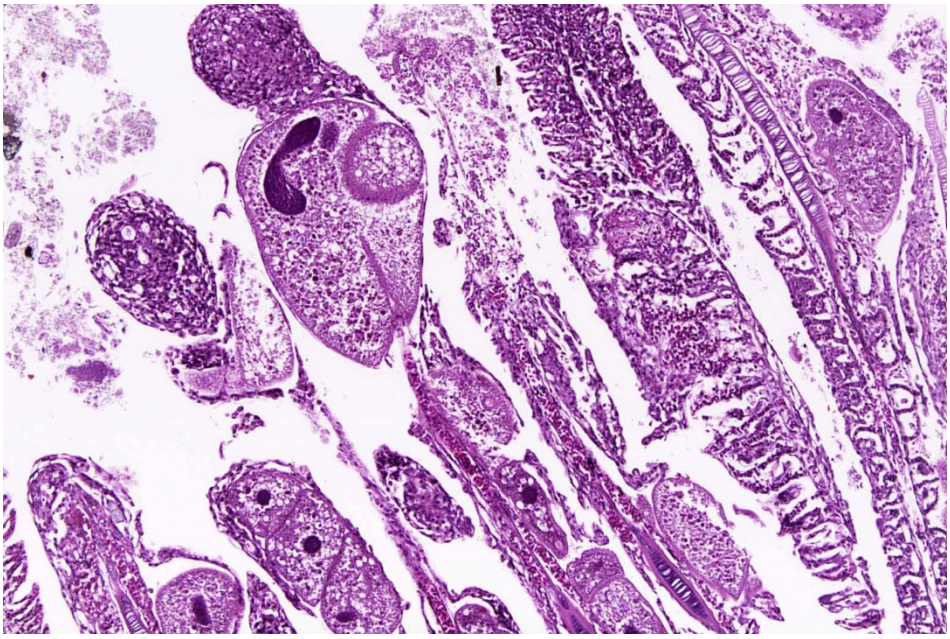
Gill, filaments: Gills are diffusely thickened and variably hypercellular. Many filaments are expanded by the presence of large trophonts with a horseshoe-shaped macronucleus (arrows). (HE, 40X)

associated with excessive secretion of mucous, and can result in hypertrophy and hyperplasia of gill epithelium with subsequent fusion of secondary gill lamellae.[1,2]

In addition to *I. multifilis* and trichodinids, several other potential etiologic agents were observed microscopically that could have contributed to some extent to the lesions in gills, skin and oral cavity. Depending on the slide, there were also few monongeneans, filamentous bacteria and epitheliocystis. In this group of fish, *I. multifilis* is believed to be the primary pathogen when one takes into account the previously stated prevalence and mean intensity.

Fish parasites are an integral part of water ecosystems and they are common in wild and cultured populations of fish. It is well known that fish live in balance with parasites, however, this balance can be broken by stressors such as sudden changes in water quality (i.e. temperature, oxygen, etc) and poor husbandry (i.e. high stocking densities, excessive handling, etc.). Therefore, diseases caused by parasites are much more frequently manifested in cultured fish, which suffer from numerous stress factors that influence their ability to effectively protect themselves against parasitic infections. Infections caused by protozoan and metazoan parasites occur very frequently in cultured fish and can cause significant economic losses to fish farms due to mortality, but parasites may also exert considerable impact on growth and behavior of fish, on their resistance to other stress factors, susceptibility to predation, etc. Many of these parasites can cause severe injury to different organs and tissues and they can provide portals of entry for bacteria in fish, generating co-infections.[4,7,9]

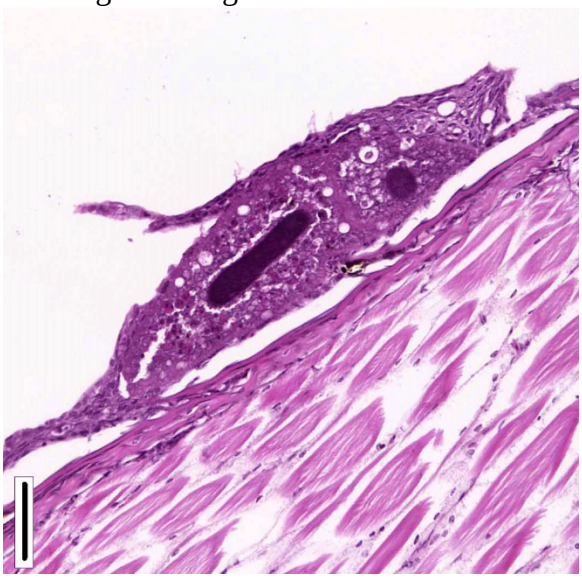
JPC Diagnosis: 1. Gills, pharynx, skin: Epithelial hyperplasia, blunting and fusion of



Gill, filaments: Gills are diffusely thickened and variably hypercellular. Many filaments are expanded by the presence of large trophonts with a horseshoe-shaped macronucleus (arrows). (HE, 40X)

secondary lamellae and numerous embedded protozoal ciliates (theronts) consistent with *Ichthyophthirius multifiliis* and free trichodinids, Nile tilapia (*Oreochromis niloticus*), pisces.
2. Gills: Epitheliocystis, rare.
3. Gills: Filamentous bacilli, multifocal.
4. Gills: Monogenean, single.

Conference Comment: The gills of teleosts are the most vulnerable structures they possess; their external location ensures close association with the external environment and any contaminates or parasites that inhabit it. External protozoan and monogenean trematode parasites have a particular affinity for the gills because they have a rich blood supply and thus provide a nutrient-rich environment. Additionally, the gills are often the route of entry for various bacterial and viral agents (lymphocystis or *Herpesvirus salmonis* for example) which spread from the branchial vessels hematogenously and systemically either via leukocyte trafficking or cell-free dissemination. The gills are composed of two main parts: the primary lamellae (which extend out from the branchial arch) and the secondary lamellae (which protrude as smaller projections out from the primary lamellae). Within the primary lamellae are: epithelium, endothelium, pillar cells and supporting stroma (made up of fibrous and cartilaginous connective tissues). Admixed are specialized cells such as: mucous cells, salt cells, eosinophilic granule cells and fixed macrophages. When injured, the inflammatory response is limited, and the earliest microscopic lesions are swelling and degeneration of the lamellar



Skin, tilapia: Non -encysted theronts (invasive form) are present on the skin, as well as on the mucosa of the oral and branchial cavity. (HE, 400X)

epithelial cells, or edema of the subepithelial connective tissue. In general, lamellar edema and epithelial necrosis are the result of acute exposure to direct acting toxins or chemical pollutants such as heavy metals, red tides, phytoplankton or jellyfish and ultimately lead to hemorrhage. On the other hand, lamellar hyperplasia often results from chronic exposure to lower levels of a toxicant and can assume several morphologic forms: clubbing of secondary lamellae, mucous cell hyperplasia or metaplasia, and ultimately lamellar fusion. With good water quality and a modicum of time, most gill lesions heal.[6,8]

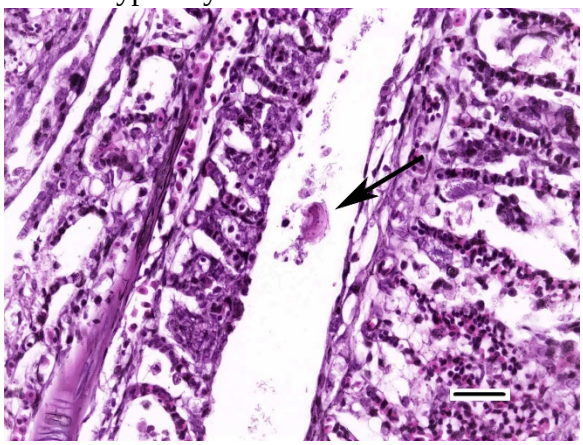
Table 1: Gross and microscopic differentials for *Ichthyophthirius multifilis*[6,8]

Protozoan		
Cryptocaryon irritans (marine ich)	Saltwater fish	Penetrates the epithelium; saltwater equivalent of *Ichthyophthirius multifilis*
Trichodina spp.	Marine or freshwater fish	Grossly similar to *Ichthyophthirius multifilis*; identified on wet mount as disk shaped organism scooting on the surface of tissues
Chilodonella spp.		Same life cycle and gross pathology as *Ichthyophthirius multifilis*; more severe tissue damage
Amyloodinium sp. (marine velvet disease)	Warm water marine fish (elasmobranches (sharks, rays) and teleost (ray fin fish)	Dinoflagellate; affects gills, skin, and eyes; larger than *Ichthyophthirius multifilis*
Piscinoodinium spp. (freshwater velvet disease, rust disease)	Freshwater fish	Freshwater analogue of Amyloodiniosis
Ichthyobodo spp. (*Ischthyobodo necator* complex)	Immunosuppressed and young fish; freshwater primarily	Smallest ectoparasite of fish (size of red blood cell); epithelial hyperplasia with increased mucus production (makes fish bluer and extra slimey)
Fungal/algae		
Saprolegniales sp. (water mold)	Freshwater (especially estuarine tropical fish)	Cottony, proliferative growth on skin or gills
Bacterial		
Epitheliocystis sp.	Freshwater and marine fish	Intracellular, Gram-negative; causes epithelial and dermal cell enlargement
Viral		

Lymphocystis (pscine iridovirus)		Hypertrophied fibroblasts with basophilic intracytoplasmic inclusion bodies

Icthyophthirius multifilis (also known as "ich" or white spot disease) is the largest protozoan parasite in fish; trophozoites can reach 100 µm in diameter and have a prominent oval or horseshoe-shaped nucleus. Ich is common in aquarium and hatchery-reared freshwater fish and can result in respiratory impairment in severely infected fish. Microscopically, the trophozoites are found in the skin or gill lamellae surrounded by epithelial hyperplasia. *I. multifilis* has a direct life cycle in which encysted trophozoites (trophonts) leave the fish and settle at the bottom of the tank where, in their tomont form, they divide into numerous motile tomites (theronts). It is the motile theront form that infects the skin of the fish. Their total life cycle only takes 4 days but can be quicker in warmer water temperatures.

Trichodina spp. is a saucer-shaped, 50 µm in diameter, peritrichal ciliated protozoan with a macro- and a micronucleus. Microscopically, it appears as a characteristic ring of interlocking denticles. Low numbers are not typically associated with disease and are frequently environmental contaminates. However, in increased numbers, with concurrent disease, or in an immunosuppressed host, they can cause increased skin and gill mucus and respiratory distress. *Trichodina* spp. have a simple life cycle and reproduce by binary fission.[6,8]



Gills, tilapia: Occasionally trichodinid ciliates are present between hyperplastic gill filaments. (HE, 400X)

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CASE III: 68197 (JPC 4084211).

Signalment: Nine-month-old, male, Meller's chameleon (*Trioceros melleri*), reptile.

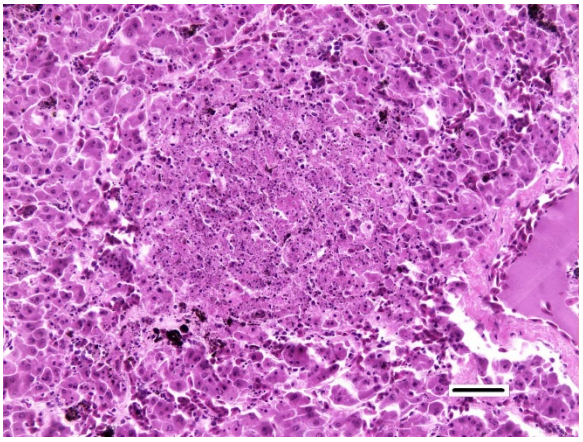
History: A cohort of five Meller's chameleons (two male, three female) were group-housed in an outdoor zoological exhibit for approximately five months during the summer. One month prior to presentation, they were moved to an indoor enclosure for the winter. Routine complete blood cell count and serum chemistry panels performed two months prior to presentation were unremarkable.

Liver, chameleon. There is a diffuse loss of normal hepatic architecture and focal areas of pallor. (HE, 6X)

All five animals died within a span of one month. The first mortality occurred without premonitory signs. One week later, three other individuals in the group presented with acute onset dehydration, lethargy and anorexia. Despite supportive care and environmental changes, these three individuals became moribund and were euthanized ten days after presentation. Two days later, the final group member exhibited intermittent mouth gaping, decreased appetite, and a cutaneous vesicle near the tail base. Blood work performed at that time showed hyperglycemia, hyperphosphatemia, and a leukocytosis with reactive heterophils and monocytes. Despite gavage feedings and treatment with famcylcovir, ceftazadime, subcutaneous fluids, and meloxicam the animal developed serous oculonasal discharge and multifocal oral and dermal petechiae, and was found dead 10 days after onset of clinical signs.

Gross Pathology: All animals presented in thin body condition. The first four animals did not have any other significant gross findings, while the fifth animal exhibited mild transudative coelomic effusion and petechial hemorrhages affecting the tongue and kidneys.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): Fixed liver tissue from the first chameleon and a pooled sample of fresh frozen liver from the following three chameleons were sent to the San Diego Zoo Institute for Conservation Research for Ranavirus qPCR testing. All samples were positive for Ranavirus. PCR



Liver, chameleon. There is diffuse loss of normal plate architecture. Foci of necrosis (center) lytic necrosis contain abundant cellular debris as well as melanin pigment liberated from necrotic melanomacrophages. (HE, 200X)

and sequencing of the neurofilament-like and major capsid protein genes further identified the virus as a member of the Frog Virus 3 (FV3) group.

Microscopic Description:

Liver: Randomly distributed throughout the hepatic parenchyma are numerous, variably-sized areas of necrosis characterized by disruption of chordal architecture and replacement by eosinophilic and karyorrhectic debris admixed with fibrin, mild hemorrhage, and dark brown to black granular material (presumptive melano-macrophage granules). Adjacent hepatocytes are often shrunken and hypereosinophilic with pyknotic to faded nuclei. Throughout the section, hepatocytes and biliary epithelial cells frequently contain one to multiple, variably-sized, basophilic intracytoplasmic viral inclusion bodies. Biliary epithelial cells are also multifocally necrotic, and the periductular connective tissue is often mildly expanded by clear space (edema). Rarely, and with some section variation, the walls of small blood vessels are segmentally disrupted by necrotic cellular debris and brightly eosinophilic fibrillar material (fibrinoid necrosis). Aggregates of small (1-2μm) intravascular rod-shaped bacteria are also present in some sections.

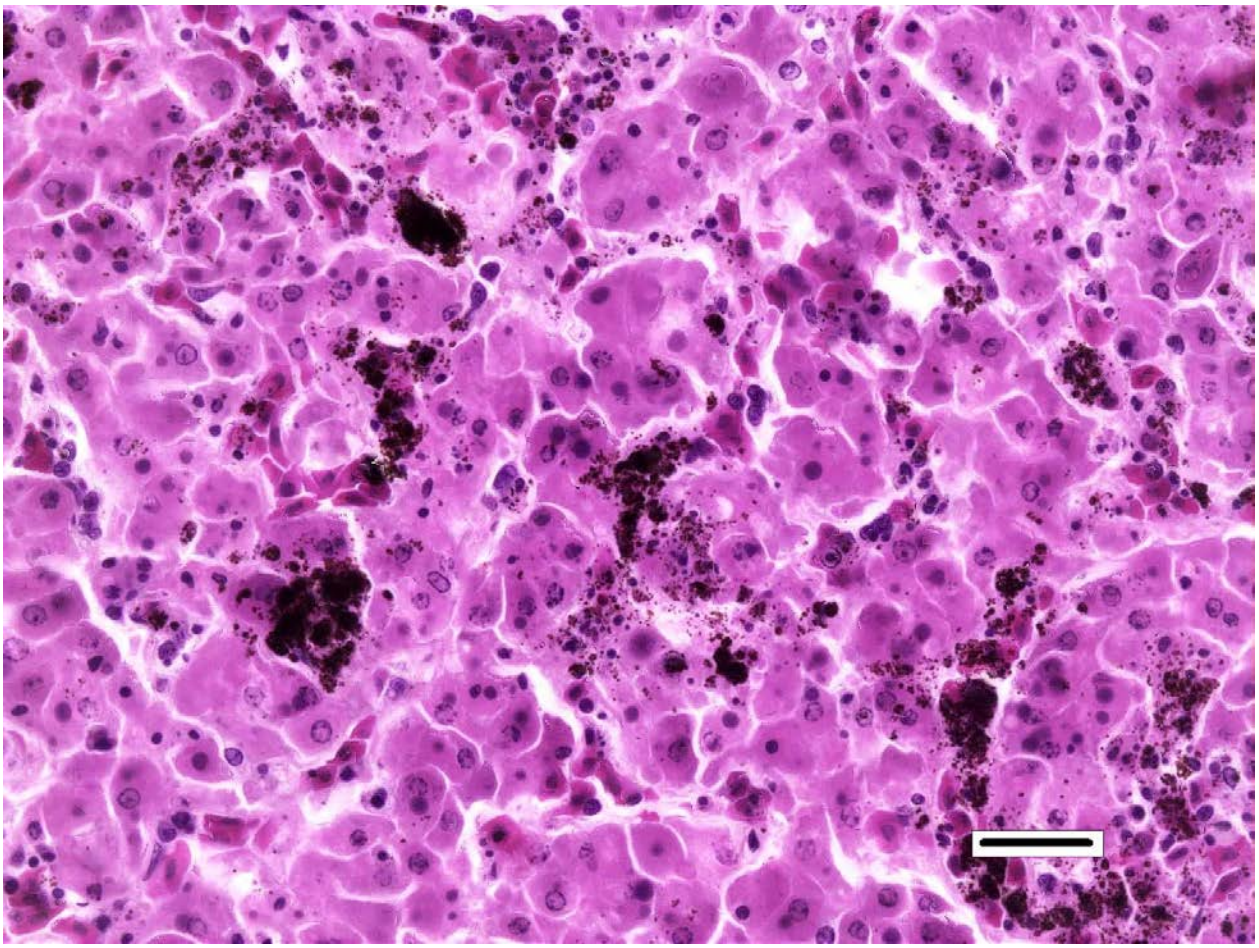
Contributor's Morphologic Diagnosis:

Liver, necrosis, multifocal to coalescing, acute, moderate, with intracytoplasmic viral inclusion bodies and intravascular bacteria

Contributor's Comment: Death in these chameleons is attributed to systemic ranaviral infection. Ranaviruses are increasingly recognized pathogens of fish and amphibians, which have contributed to infection, disease, and die-offs worldwide.[3] As such, ranaviral infection in amphibians is reportable to the World Organization for Animal Health.[12] In reptiles, ranavirus infections are well documented in turtles and tortoises, with sporadic reports in snakes and lizard species.[13] While other iridoviruses have been previously isolated from chameleons[7], to the author's knowledge this is the first documented case of ranavirus-related disease in chameleons. To date, ranavirus infection of mammals and birds has not been reported.

The Ranavirus genus belongs to the family Iridoviridae, which are large viruses (120-300 nm) with double stranded DNA and an icosahedral capsid containing a lipid component.[9] There are six species within the Ranavirus genus, the most well-characterized being Frog Virus 3 (FV3).[5]

In chelonians, ranavirus infection has been associated with sudden death, cervical / palpebral edema, and necroulcerative stomatitis / esophagitis. Histologic lesions generally include fibrinoid vasculitis, hepatic and splenic necrosis, enteritis, and pneumonia.[2,6,9] Reports in snakes are rare; one report in a group of green pythons described nasal mucosal ulceration, hepatic necrosis, and necrotizing pharyngitis.[4] Ranavirus has been detected in a total of 8



Liver, chameleon. In less affected areas, hepatocytes demonstrate degenerative and necrotic changes. Hepatocytes often contain one of more 2-4um round intracytoplasmic viral inclusions (arrows). (HE, 400X)

lizard species, with signs ranging from no overt disease to granulomatous dermatitis, necroulcerative glossitis, and hepatic necrosis.[1,10,13] A recent report on ranaviral disease in lizards indicates that ranaviral infection may be an important differential diagnosis for skin lesions in lizards.[13] In all hosts, subtle lesions of ranaviral infection may be obscured by secondary bacterial or fungal infection, especially when cytoplasmic inclusions are rare or inapprarent.[11]

The chameleons in this report presented predominately with non-specific clinical signs or sudden death. One animal presented with petechial hemorrhage and multifocal papular epidermitis progressing to vesicle formation and ulceration. The most significant microscopic findings in these cases were multifocal necrosis, most notably affecting the spleen, liver, kidney, adrenal tissues, and nasal cavity. Moderate to abundant numbers of basophilic intracytoplasmic intrahepatocytic and intrahistiocytic viral inclusions were present in the liver and nasal cavity, respectively. While stomatitis was not appreciated in this cohort, all animals exhibited varying degrees of necrotizing rhinitis with secondary bacterial and, in one animal, fungal infection. Occasional intravascular bacterial colonies were also observed in the liver of the

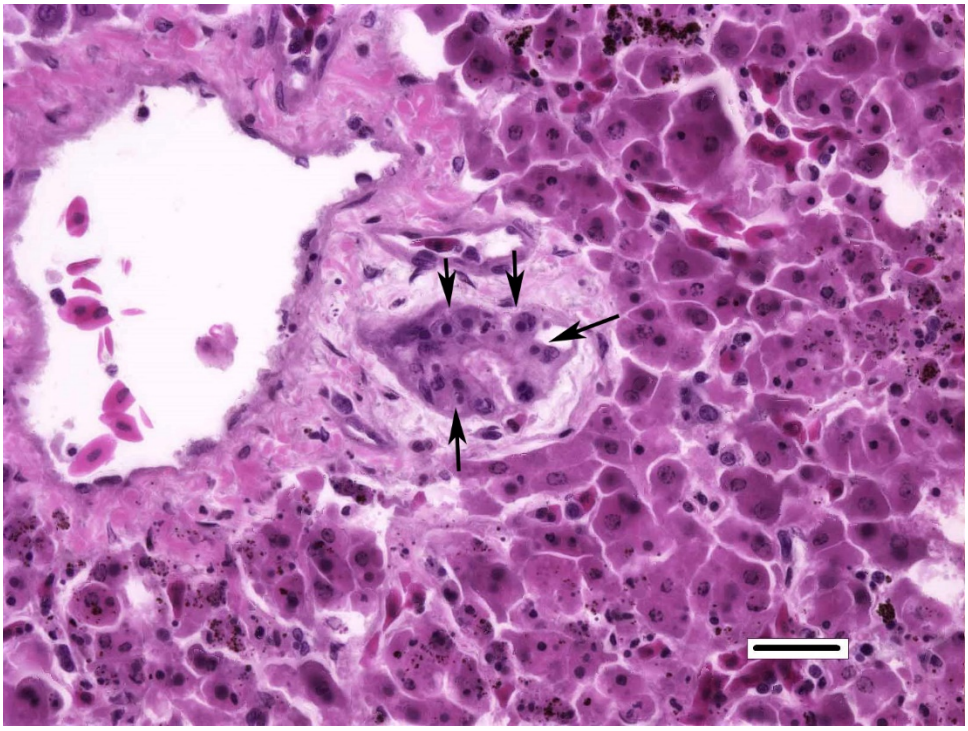
submitted chameleon, suggestive of intercurrent bacteremia/septicemia.

Antemortem diagnostic tests for ranavirus include identification of basophilic intracytoplasmic inclusions in leukocytes, PCR of blood or oral / cloacal swabs, or ELISA of plasma. Postmortem diagnostic tests include necropsy and histologic identification of basophilic intracytoplasmic inclusions, virus isolation, PCR of affected tissues, and electron microscopy. Immuno-histochemistry has been demonstrated in research settings, but is not available commercially[10]. Molecular testing for ranavirus is becoming more readily available, however many tests rely on reactivity with the highly conserved major capsid protein (MCP), which identifies the Ranavirus genus, but is not reliable for speciation. For these chameleons, quantitative polymerase chain reaction (qPCR) targeting the ranavirus MCP gene was followed by sequencing of the MCP and neurofilament-like genes, which further identified the virus as a member of the Frog Virus 3 (FV3) group.

In summary, ranavirus is an emerging disease of fish, amphibians, and reptiles that exhibits high morbidity and mortality. It is becoming increasingly recognized as a pathogen of lizards and thus, should be considered a differential in lizards that present with sudden death, rhinitis, skin lesions, and splenic / hepatic necrosis.

JPC Diagnosis: Liver: Hepatitis, necrotizing, diffuse, severe with numerous intracytoplasmic viral inclusions, Meller's chameleon (*Trioceros melleri*), reptile.

Conference Comment: The family *Iridoviridae* contain viruses which affect a very broad host range (arthropods, fish, amphibians, and reptiles) and produce numerous disorders to include systemic necrosis (genera *Ranavirus* and *Megalocyticirus*), and non-neoplastic skin lesions (genera *Lymphocystivirus*). On electron microscopy, iridoviruses form paracrystalline arrays within the cytoplasm which can be seen microscopically as prominent basophilic intracytoplasmic viral inclusions. Iridovirus virions are structurally similar to those of *Asfarviridae* (the causative agent of



Liver, chameleon. Intracytoplasmic viral inclusions are also present within biliary epithelium as well. (HE, 400X)

African swine fever) and functionally, as there is a limited amount of initial replication which occurs in the nucleus, followed by more extensive replication in the cytoplasm later in the disease.[8]

There are two known strains of ranaviruses in amphibians, ranavirus type I (Frog virus-3) and ranavirus type III (tadpole edema virus).[14]

Frog virus-3 (FV-3, the etiologic agent in this case), the first ranavirus identified, was originally isolated from leopard frogs infected with ranid herpesvirus-1 (Lucke's renal adenocarcinoma). Despite the initial presumption of ranaviruses being a "benign" infectious agent, it became evident that their pathogenicity could cause a wide spectrum of diseases, ranging from cutaneous hemorrhage and necrosis to diffuse necrosis of numerous visceral organs; the aforementioned etiological agent is responsible for mass die-offs in North American frogs. Tadpoles are the most susceptible, but most wild amphibian populations are at risk of widespread epizootics. Affected tadpoles present initially with gross lesions resembling redleg, a common presentation of Gram-negative septicemia.

With ranavirus infections, the most severe lesions are in the kidneys, characterized by glomerular endothelial necrosis and hemorrhage with multifocal tubular necrosis, mild hemoglobin nephrosis, and free melanosomes within glomeruli. Additionally, there are extensive areas of hemorrhage and necrosis in the stomach and periportal to lobar necrosis in the liver. Basophilic intracytoplasmic inclusion bodies are present within glandular epithelial cells in the stomach and hepatocytes.[14]

Tadpole edema virus (TEV), an acute fatal infection of wild tadpoles of bullfrogs (*Rana catesbeiana*), bufonids (*Bufo americanus, Bufo woodhousei fowleri*), and pelobatids (*Scaphiopus intermontana*) has similar gross and microscopic findings to FV-3. [14]

Table 1: Viruses in the family *Iridoviridae*[8]

Genus	**Virus**
Iridovirus	Invertebrate iridescent virus-6, 1, 2, 9, 16, 21, 22, 23, 24, 29, 30, 31
Chloriridovirus	Invertebrate iridescent virus-3
Ranavirus	Frog virus-3 (tadpole edema virus, tiger frog virus) Ambystoma tigrinum virus (regina ranavirus) Bohle iridovirus Epizootic hematopoietic necrosis virus European catfish virus (European sheatfish virus) Santee-Cooper ranavirus (largemouth bass virus, doctor fish virus, guppy virus-6) Singapore grouper iridovirus, Grouper iridovirus
Megalocytivirus	Infectious spleen and kidney necrosis virus
Lymphocystivirus	Lymphocystis disease virus-1
Unclassified	White sturgeon iridovirus; Erythrocytic necrosis virus

Conference participants debated at length regarding the presence of bacteria in vessels and sinusoids and concluded that, although ranavirus commonly occurs in conjunction with other agents, the bacteria was most likely not part of the pathogenesis and excluded it from the morphologic diagnosis.

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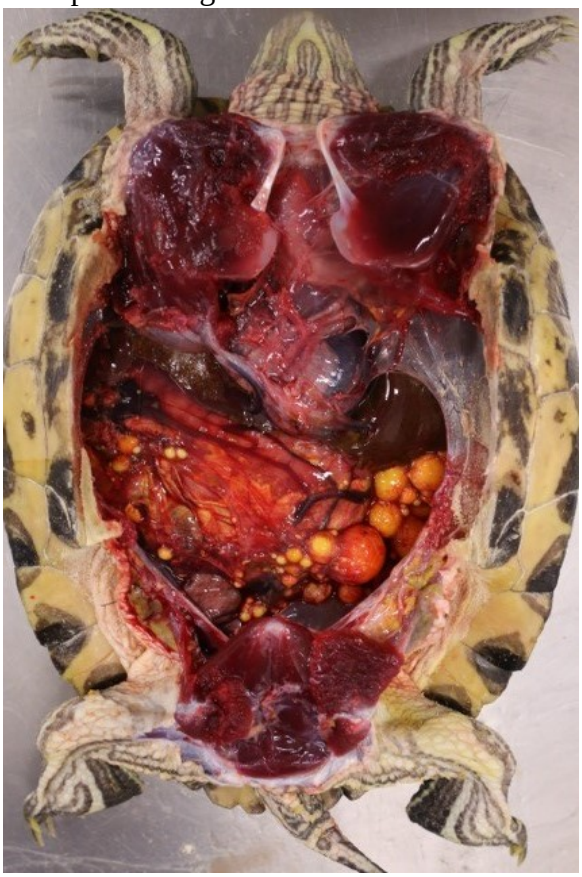
CASE IV: N17-104-1 (JPC 4101578).

Signalment: 22-year-old, female, intact, red-eared slider (*Trachemys scripta elegans*), reptile.

History: This turtle presented for persistent abnormal egg laying behavior, and had been treated for dystocia with 8 retained eggs several months prior at the referring veterinarian. No remaining eggs were observed on radiographs at presentation, and bloodwork revealed elevated liver enzymes (AST), azotemia, hyperkalemia, and hypoproteinemia. Coeloscopic ovario-hysterectomy was performed and revealed multiple enlarged ovarian follicles with numerous adhesions to the body wall and numerous yolk droplets on serosal surfaces. Due to the inability to completely surgically resect all ovarian tissue and poor prognosis for recurrent coelomitis, euthanasia was elected.



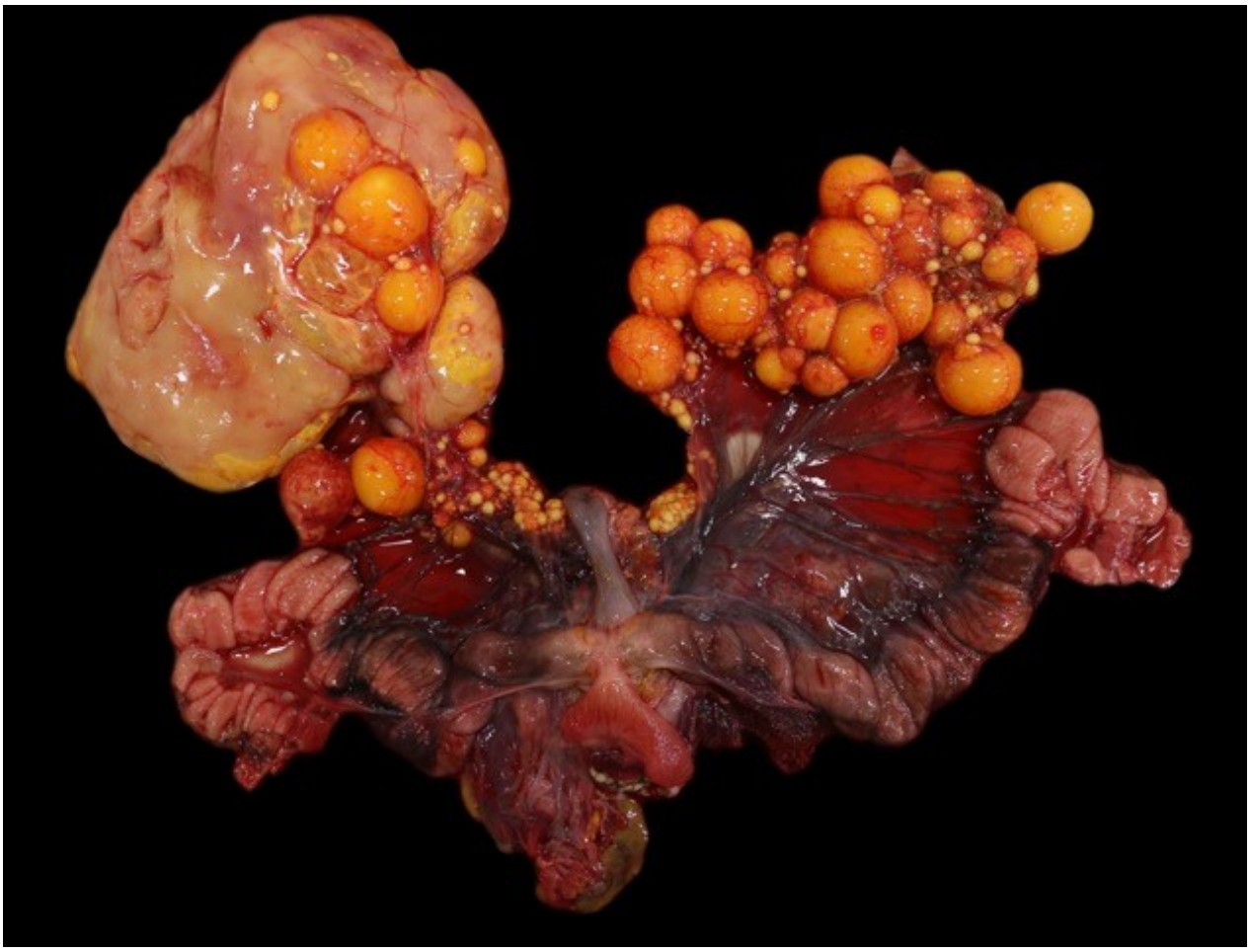
Presentation, turtle: A large reddish-tan mass is present within and expanding ovarian follicles. (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, http://vet.tufts.edu/foster-hospital-small-animals/departments-and-services/pathology-service/)

Gross Pathology: Within the caudal coelom, there is a mixture of healthy mature and immature follicles, bilaterally, with an 11 x 6 cm, yellow to tan, soft, friable, fatty, ovoid mass associated with the right-sided ovarian tissue and 2-3 mature follicles embedded within the capsule. On the surface of the opposing coelomic wall are patchy, thin, yellow, fibrinous adhesions. On cut section, the center of the mass is markedly friable and greasy, and homogenously pale tan. Sections of the mass did not float when placed into formalin.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): Cytologic impressions of the mass revealed small clusters of markedly pleomorphic, large, round to polygonal cells arranged singly or in loose aggregates on a moderately proteinacous background with scattered red blood cells. Cells range from 20 to 50 um in diameter, with distinct cell borders and contain moderate, pale basophilic, occasionally vacuolated cytoplasm. Nuclei are large and round to oval with reticular chromatin and contain multiple prominent nucleoli. Binucleation and multinucleation is frequent. Anisocytosis and anisokaryosis are marked, and mitotic activity is moderate.

Microscopic Description:

Ovary: Displacing previtellogenic and vitellogenic ovarian follicles, is an un-encapsulated, poorly demarcated, expansile neoplasm composed of sheets of round to polygonal cells, occasionally forming indistinct lobules, separated by very thin collagenous stroma. Neoplastic cells have distinct cell borders and moderate amounts of homogenous, pale eosinophilic cytoplasm.

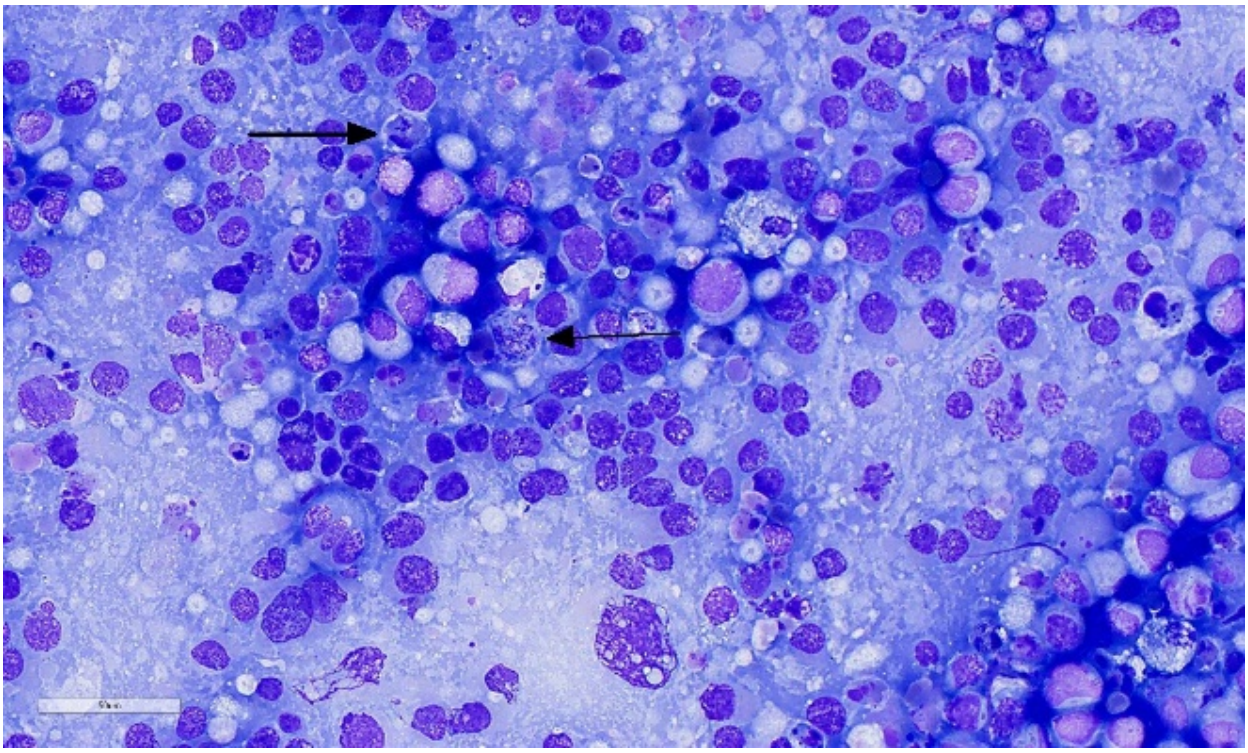


Ovary, turtle: A tan mass (at left) incorporates numerous follicles. (HE, 6X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, http://vet.tufts.edu/foster-hospital-small-animals/departments-and-services/pathology-service/)

Nuclei are round to oval, centrally located with vesiculate chromatin pattern and contain 1 to 3, prominent nucleoli. Anisocytosis and anisokaryosis are moderate. There are 19 mitotic figures in 10 hpf. There is frequent multifocal individual cell necrosis and low numbers of scattered lymphocytes.

Contributor's Morphologic Diagnosis:
Ovary, dysgerminoma

Contributor's Comment: Dysgerminomas are germ cell tumors that arise from undifferentiated, pluripotent germ cells in the ovary.[6,12,14,15] Other germ cell tumors, such as teratoma and embryonal carcinoma, are distinguished by somatic differentiation and maturation of neoplastic cells towards an embryonic tissue type(s). In chelonians, germ cell tumors are extremely rare.[3,5,6,9,12] Dysgerminomas have been reported in 2 red-eared sliders and a snapping turtle. Teratomas were reported in a red-eared slider and a snapping turtle. In other veterinary species, dysgerminomas are similarly rare, and have been reported in dogs, cats, horses, maned wolves, Eastern rosella, and mountain chicken frogs.[2,10,15] Maned wolves in captivity have been reported to have an increased prevalence of ovarian tumors, with suspected hereditary predisposition for dysgerminomas.[10] In humans, dysgerminomas can occasionally be hormonally functional and human chorionic

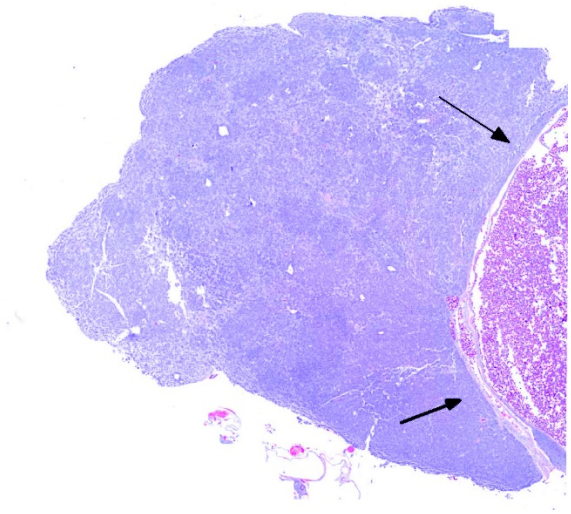


Ovary, turtle: An impression of the mass contain numerous pleomorphic and occasional multinucleated cells with numerous mitotic figures (arrows) on a blue proteinaceous background. (Wrights, 400X)

gonadotropin, produced by syncytio-trophoblastic giant cells, can contribute to aberrant follicular maturation and ovulation and pregnancy-like signs.[11,15] Similar hormonal influence by the tumor contributing to the clinical signs of chronic egg laying behavior in this turtle is speculative. Although dysgerminomas are considered malignant, metastasis is rarely observed.

Clinical signs across all species is largely non-specific, and related to the effects of a space-occupying mass within the abdomen. Turtles have shown signs of anorexia, lethargy, carapace dysecdysis, or inability to swim normally, and, in this case, abnormal egg laying behavior.[3,5,9] Grossly, in chelonians, dysgerminomas appear as unilateral, intracoelomic, white to yellow, friable, fat-like masses, ranging from 4 to 11 cm, and are associated with ovarian tissue. Microscopic examination reveals sheets of round to polygonal cells with large, round to oval vesicular nuclei with prominent nucleoli, and moderate pale eosinophilic cytoplasm. Mitoses are frequent. Scattered lymphocytes and multifocal regions of ischemic necrosis are occasionally noted.

Immunohistochemistry for germ cell tumors have not been established in chelonians, and was not performed in this case. Currently, a diagnosis of dysgerminoma in people is reliant on positive immunoreactivity with placental alkaline phosphatase (PLAP) and vimentin.[8,11] Recent development of germ cell-selective immunohistochemical markers, OCT3/4 and SALL4, are now also recommended for diagnosis. OCT3/4 is a nuclear transcription factor that plays a role in maintaining pluripotency in primordial germ and stem cells. SALL4 is a nuclear factor with which OCT3/4 interacts, and is involved in totipotency. Both OCT3/4 and SALL4 are strongly expressed in dysgerminomas, but, since they are both



Ovary, turtle. A densely cellular neoplasm opposes a vitellogenic follicle (arrows). (HE, 6X)

markers of pluripotency, they are also expressed in embryonal carcinomas and less differentiated teratomas. CD117 (c-kit) is a proto-oncogene expressed in dysgerminomas, and not in other germ cell tumors. However, only 30% of dysgerminomas demonstrate immuno-reactivity. Dysgerminomas are immuno-negative for α-fetoprotein, inhibin-α, and S-100.

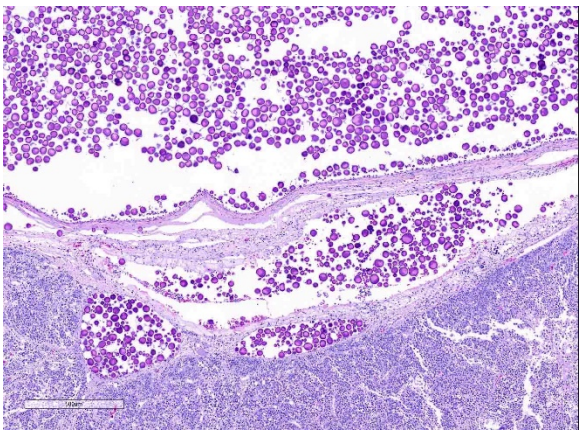
Immunohistochemical markers for dysgerminomas in veterinary literature are variable across species and, in some cases, show similarities to the immunophenotype observed in people.[2,4,15] OCT4 was expressed in dysgerminomas in mountain chicken frogs, but the tumors were immunonegative for vimentin, PLAP, and calretin.[2] In the dog, dysgerminomas do not express c-kit, but are immunopositive for SALL4 with variable expression of PLAP and vimentin. Immunohistochemistry with α-fetoprotein, inhibin-α, and S-100 are negative in dogs.[4]

Based on the gross and microscopic appearance in this case, diagnosis of dysgerminoma is strongly supported. Dysgerminomas should be considered as a differential diagnosis in turtles with a coelomic mass and antemortem identification with cytology may be helpful. As more cases are identified, immunophenotyping for germ cell tumors in turtles can be further elucidated. This is the first report of a red-eared slider with a dysgerminoma showing clinical signs of chronic reproductive behavior, suggesting the possibility of hormonal production by this tumor in this species.

JPC Diagnosis: 1. Ovary: Dysgerminoma, red-eared slider (*Trachemys scripta elegans*), reptile.
2. Cytologic impression: Round cell proliferation with intracytoplasmic, eosinophilic granules and foamy macrophages.

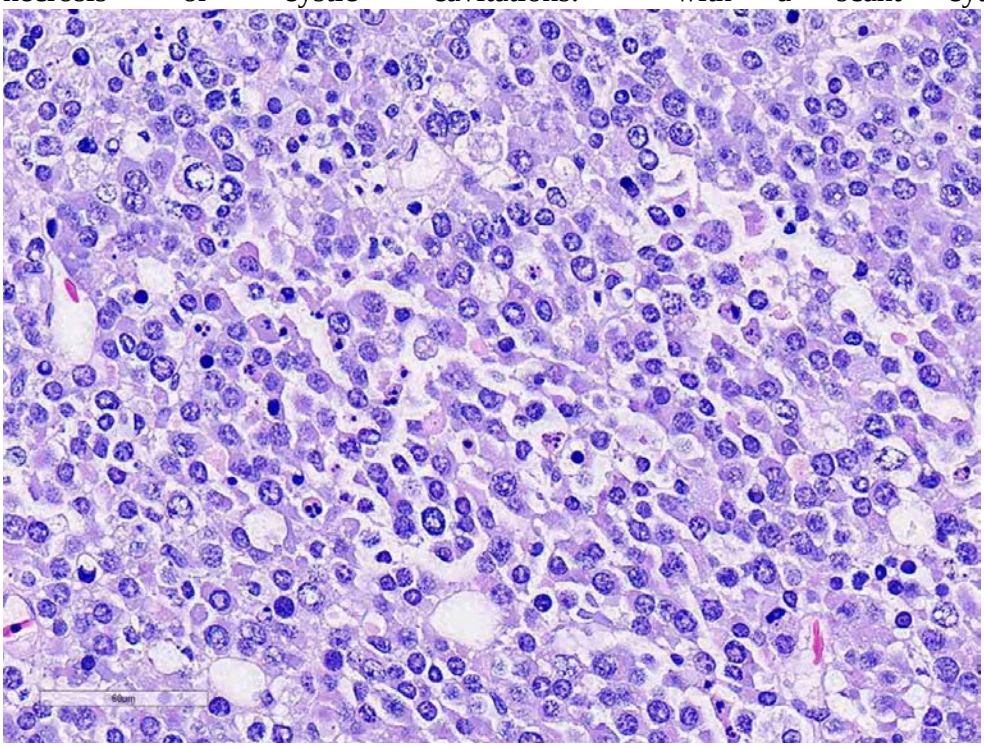
Conference Comment: Two types of germ cell tumors have been reported in female domestic animals: dysgerminomas and teratomas. Germ cells arise in the yolk sac, migrating to the gonadal ridge during differentiation, and associate with sex cords before formation of primary follicles.[1]

Dysgerminoma, a rare ovarian tumor, is most common in the bitch and queen, but have also



Ovary, turtle. Higher magnification of the mass demonstrates a nesting pattern that is unusual for a dysgerminoma. A mature vitellogenic follicle is at top. (HE, 40X)

been identified in the cow, mare, sow, and maned wolves. Dysgerminomas in fish may contain testicular elements. They correspond to seminomas in the testicle and occasionally are hormonally active, resulting in clinical signs of hyperestrogenism. Grossly, dysgerminomas are large, firm, white or gray homogenous tumors that elevate the ovarian capsule and can contain areas of hemorrhage, necrosis or cystic cavitations.



Ovary, turtle. Sheets of round cells with marked anisokaryosis and numerous red cytoplasmic granules composed the mass. There is a high mitotic rate as well as abundant cellular apoptosis. (HE, 200X)

Microscopically, they are densely cellular and composed of primitive germ cells arranged in sheets, cords, and nests separated by a thin connective tissue septa. Neoplastic cells have large vesicular nuclei with prominent nucleoli and scant eosinophilic cytoplasm giving them a blastic appearance. Multinucleated cells and bizarre mitotic figures are common as is metastasis to regional lymph nodes or explantation to adjacent tissues.

Teratomas are less common and are composed of at least two of the germinal layers (endoderm, mesoderm, or ectoderm).

A recent article[7] (discussed during the conference) identified a presumptive ovarian dysgerminoma in an orange-spot freshwater stingray which was composed of sheets of round cells arranged in solid and cystic areas with a scant cytoplasm, moderate anisokaryosis, multiple nucleoli and frequent mitotic figures. Additionally, the moderator shared an interesting case in a medaka in which there was spermatogenic progression within the dysgerminoma. Differentials discussed by conference participants included lymphoma, histiocytic tumor, and sex cord stromal tumor.

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http://vet.tufts.edu/foster-hospital-small-animals/departments-and-services/pathology-service/

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