Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 13

13 January 2016

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CASE I: N2015-5055 (JPC 4066453).

Signalment: Adult female cardinal tetra fish (*Paracheirodon axelrodi*)

History: This animal was one of 15 cardinal tetra (*Paracheirodon axelrodi*) in a 220 gallon heated freshwater tank. The tank contained a total of 29 species of tropical fish. Additional species included firehead tetra (*Hemigrammus bleheri*), rummy nose tetra (*Hemigrammus rhodostomus*), yellow phantom tetra (*Hyphessobrycon roseus*), serpae tetra (*Hyphessobrycon eques*), red phantom tetra (*Hyphessobrycon sweglesi*), bleeding heart tetra (*Hyphessobrycon erythrostigma*), Savanna tetra (*Hyphessobrycon stegemanii*), blue emperor tetra (*Inpaichthys kerri*), silver tetra (*Ctenobrycon spilurus*), white finned rainbowfish (*Bedotia leucopteron*), snakeskin barb (*Puntius rhomboocellatus*), cherry barb (*Puntius titteya*), three-striped glass catfish (*Eutropiellus debauwi*), glass catfish (*Kryptopterus bicirrhis*), diagonal-stripe catfish (*Corydoras melini*), slant bar catfish (*Corydoras loxozonus*), Napo catfish (*Corydoras napoensis*), eartheater (*Geophagus megasema*), Leopold's angelfish (*Pterophyllum leopoldi*), freshwater angelfish (*Pterophyllum scalare*), blue butterfly cichlid (*Mikrogeophagus ramirezi*), Bolivian ram (*Mikrogeophagus altispinosus*), slender hemiodus (*Hemiodopsis gracilis*), green knifefish (*Eigenmannia virescens*), festivum (*Mesonauta mirificus*), spotted platyfish (*Xiphophorus maculatus*), chessboard cichlid (*Crenicara filamentosa*) and panchax (*Pachypanchax spp*.).

Three adult cardinal tetra from the group of 15 were presented for clinical examination due to white foci noted on the skin surface. On examination each animal was alert, active and in good body condition with no evidence of fin fraying or increased opercular rate and the animals were reported to have no change in appetite. Numerous (between 10 and 20) 2 to 3 mm diameter transparent raised nodules on the skin surface that centrally contained a coiled to elongate approximately

Numerous 2- to 3mm diameter transparent raised nodules were present on the skin surface. Each contained a prominent coiled to elongate approximately 2 mm long and 0.1 mm diameter white vermiform structure. (Photo courtesy of: Wildlife Conservation Society, New York Aquarium, Zoological Health Program, Aquatic Animal Medicine & Pathology Department, http://www.wcs.org)

2 mm long and 0.1 diameter white vermiform structure were present on all three animals. Further investigation found 6 out of 15 cardinal tetra exhibited similar skin lesions, and the remaining 9 animals showed varying degrees of diffuse pallor with patchy to loss of their characteristic red and blue iridescent coloration. No other species in the tank exhibited similar lesions. Skin scrapings were performed on the 3 tetra presented for clinical examination and one representative of each of the other species present in the tank. Following skin scrape cytology results, two cardinal tetra were euthanized for histopathology and ancillary testing. The remaining 13 animals were removed from the tank and placed into isolation.

Gross Pathology: Similar lesions to those noted clinically were apparent on both of the euthanized animals. Numerous (between 10 and 20) 2 to 3 mm diameter transparent raised nodules were present on the skin surface. Each contained a prominent coiled to elongate approximately 2 mm long and 0.1 mm diameter white vermiform structure. Both animals were in otherwise good body condition and were reproductively active based on gonad development. Cytology findings are presented below.

Laboratory Results: Skin scrapings and wet mounts from both clinical and necropsy cases contained numerous elongate mesomycetozoal cysts that were approx.-imately 100 um diameter and tapered at one end to a 5 um diameter thin projection. The cysts were often ruptured at one end and were filled with innumerable approximately 5 to 7 um diameter spores. Individual spores had a prominent large central to eccentric refractile vacuole (refractile body). These findings were consistent with *Dermocys-tidium spp.* infection. Skin scrapings from the representative animals of each of the 28 other species in the tank were negative for mesomycetozoal cysts. One frozen tetra was submitted for 18s small subunit rRNA PCR to confirm the pathomorphologic diagnosis

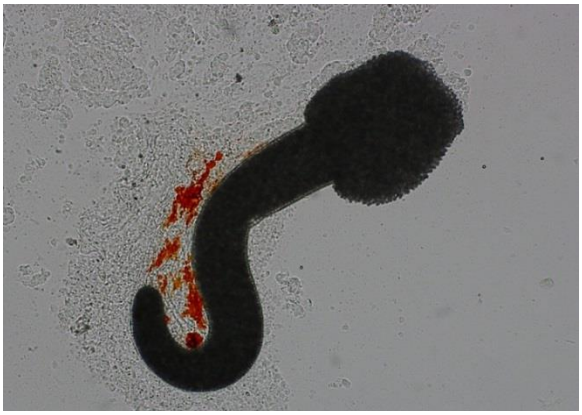
and provide species identification but results are not available at this time.

Histopathologic Description: Histologically, the dermis is multifocally expanded and the overlying scales are elevated in multiple locations by 100 to 200 um diameter by 1000 to 2000 um long tubular mesomycetozoal cysts that have a 1 to 2 um thick eosinophilic laminated wall and contain innumerable 3 to 7 um diameter round spores. Individual spores have a prominent large eosinophilic central to eccentric vacuole (retractile body) that is surrounded by a rim of weakly eosinophilic to clear cytoplasm containing a peripheralized deeply basophilic nucleus. There is a mild to moderate associated inflammatory infiltrate within the dermis composed primarily of granulocytes and macrophages. Cysts are occasionally captured as they penetrate the overlying epidermis and protrude from the epidermal surface. At the base of the anal fin, where the inflammatory cell infiltrate is most severe, moderate numbers of pyriform to ovoid holotrich ciliates (approximately 40 x 80 um) infiltrate the dermis and are admixed with the inflammatory cells and mesomycetozoal cysts and spores.

Contributor's Morphologic Diagnosis:

1. Skin, dorsum, ventrum, base of anal and caudal fins: Dermatitis, granulocytic, histiocytic, multifocal, subacute, mild to moderate with multiple intralesional mesomycetozoal cysts and luminal spores (*Dermocystidium* spp.)

2. Skin, base of anal fin: Dermatitis, granulocytic, histiocytic, focal, subacute, moderate with intralesional protozoa (*Tetrahymena* spp.)

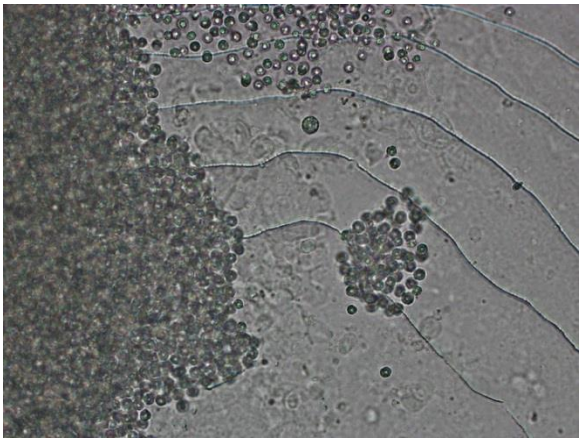


Skin scrapings and wet mounts from both clinical and necropsy cases contained numerous elongate mesomycetozoal cysts that were approximately 100 um diameter and tapered at one end to a 5 um diameter thin projection. (Photo courtesy of: Wildlife Conservation Society, New York Aquarium, Zoological Health Program, Aquatic Animal Medicine & Pathology Department, http://www.wcs.org)

Contributor's Comment: *Dermocystidium* is a member of the class *Mesomycetozoea* (previously known as the DRIP clade), which contains organisms that lie at the boundary between animals and fungi.

Phylogenetic analysis of the 18s small subunit rDNA genes indicates this class contains 10 different genera of parasitic and saprophytic microbes including: *Amoebidium, Anurofeca, Dermocystidium, Ichthyophonus, Pseudoperkinsus, Psoros-permium, Rhinosporidium, Sphaerosoma* and two currently unnamed agents "clone LKM51" and "rosette agent."[2]

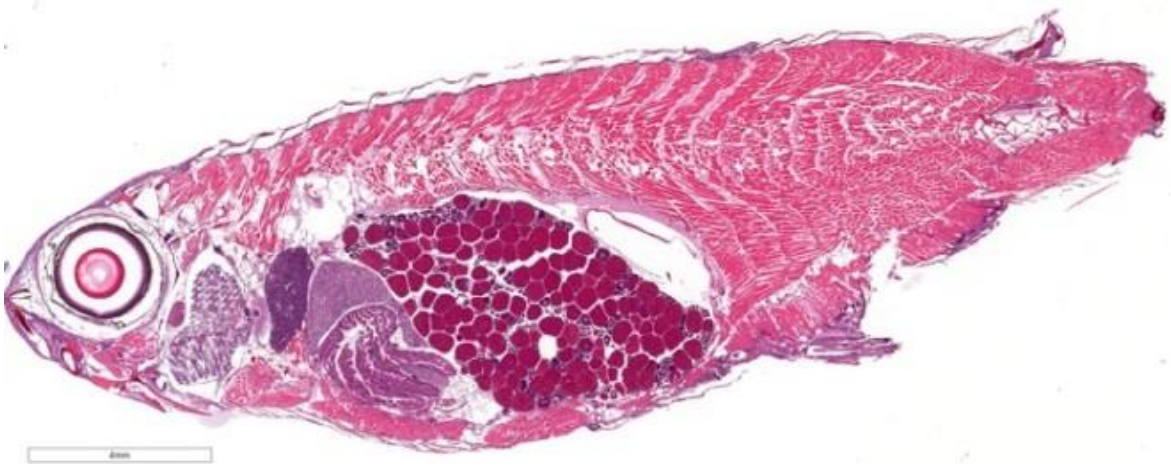
The majority of mesomycetozoea are pathogens of aquatic species, specifically fishes and invertebrates with *Rhinosporidium* a noteable exception. The life cycles of these organisms have not been completely documented. In vitro, both *Dermocystidium* and the rosette agent develop uniflagellated zoospores which could serve as a method of transmission and infection.[2] Waterborne transmission of *Dermocystidium* has been documented.[4]



The cysts were often ruptured at one end and were filled with innumerable approximately 5 to 7 um diameter spores. Individual spores had a prominent large central to eccentric refractile vacuole (refractile body). (Photo courtesy of: Wildlife Conservation Society, New York Aquarium, Zoological Health Program, Aquatic Animal Medicine & Pathology Department, http://www.wcs.org)

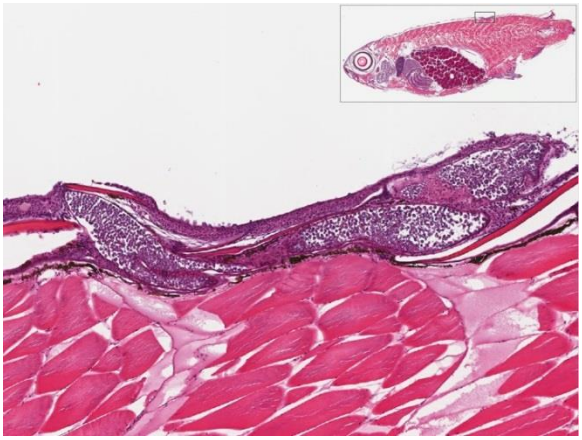
There are currently 14 recognized species of *Dermocystidium* which all cause pathogenic infection in fishes and aquatic invertebrates.[7] The skin and gills are the primary sites of infection, though visceral lesions have also been reported.[5] The most diagnostic feature on cytology and histology is the presence of cysts (sporocysts) containing the characteristic spherical spore (endospore) stage with a large central vacuole (refractile body).[3] There are four previous reports of *Dermocystidium* infection in *Paracheirodon* genus fishes (cardinal tetra, *Paracheirodon axelrodi* and neon tetra, *P. innesi*) with two providing histopathology similar to what is presented in this case. [1,6] In the two most recent reports the agent was confirmed by 18S rDNA PCR to be *Dermocystidium salmonis*, a pathogen previously reported in Pacific salmon species. Both reported lesions to be predominantly epidermal and most severe along the anterior body and fins.[1,6] The histologic lesions and distribution of the infection in the fish presented here are similar to these two previous reports, which suggests *Dermocystidium salmonis* infection in this group of cardinal tetra. One cardinal tetra from this group was submitted for 18S rDNA PCR and diagnostic confirmation; however, results were not available at the time of case submission. In recent case reports as well as in this case infection occurred in mixed species tanks, but only *Paracheirodon* genus fishes were affected. This may indicate a sensitivity of this genus to *Dermocystidium* infection. A source for infection was not clear in the current case. There were no recent changes in water source and there was no prior history of this infection in other tanks. There had been recent (6 weeks prior) addition of other genera of fishes to the impacted tank following 30 day quarantine, however there had not been a recent introduction of *Paracheirodon* spp. It is not clear from the current literature if a carrier state in other fishes is possible. The focal *Tetrahymena* infection in this case was believed to be opportunistic and incidental.

JPC Diagnosis: Skin: Dermatitis, ulcerative and granulocytic, subacute, multifocal, moderate with multiple mesomycetozoan cysts and ciliates.



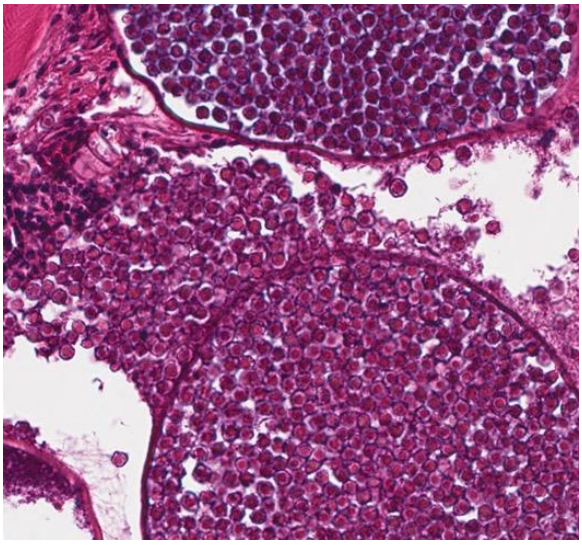
A sagittal section of an entire tetra was submitted for examination. (HE, 5X)

Conference Comment: The conference histologic description was aligned very closely with the contributor's description above. Participants commented on the excellent quality of the digital slide and parasagittal section. Participants were careful to point out the shape of the cysts located dorsally as being elongated, versus the cysts located ventrally which are round to ovoid; this is an important component to the description and can aid in identification of the organism. The contributor provided excellent gross images and the moderator led a discussion regarding the difficulty in identifying the mesomycetozoan tubular, opaque cysts grossly due to their resemblance to nematodes. Another important point raised by the moderator included recognizing the fish contains abundant eggs, which is indicative of good body condition and lack of debilitation.



Scaled skin, tetra. Multifocally, the dermis is expanded by elongate mesomycetozoal cysts. (HE, 200X)

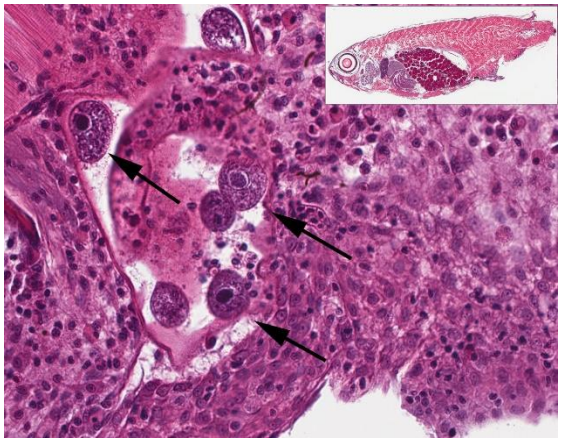
Some conference participants identified the ciliate as a trichodinid due to its similar size compared with a tetrahymenid, but as the moderator pointed out, *Trichodina* spp. don't penetrate the skin whereas tetrahyminids do. Trichodinids, while also ciliates, generally cause a relatively mild disease in healthy fish but can result in significant losses in young fish, particularly when secondary bacterial



Scaled skin, tetra. The cysts are filled with numerous round spores with a central to eccentric vacuole and a peripheralized nucleus. Occasional cysts are ruptured and spores are extruded into the surrounding dermis. (HE. 400X)

infections are present or in cases of debilitation due to other causes. Tetrahymenids are often opportunistic invaders, as seen in this case, but can also invade internal organs and result in lethal infections in severe cases. Infection has been referred to as "guppy disease" due to its preference for infecting guppies. *Tetrahymena* sp. can also cause disease in catfish, common carp, and rainbow trout secondary to skin damage and invasion of internal organs.[3]

Contributing Institution:
Wildlife Conservation Society - New York Aquarium
Zoological Health Program – Aquatic Animal Medicine & Pathology Department
www.wcs.org



Scaled skin, tetra. In areas of ulceration, several ciliates with prominent hyperchromatic nuclei have invaded the dermis. (HE, 400X)

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CASE II: 35139 (JPC 4068374).

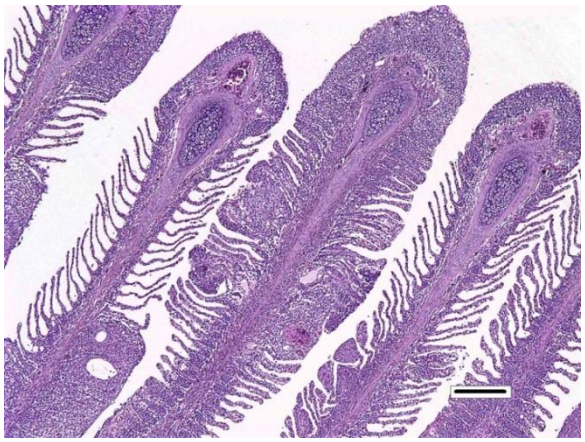
Signalment: Adult, unknown gender, Atlantic salmon (*Salmo salar*)

History: This case was submitted as part of a sample of 10 fish from a sea cage in which fish were presenting with rapid breathing and increased mortalities.

Gross Pathology: There were moderate numbers of poorly defined, multifocal to coalescing, white to grey, swollen plaques over the lamellae of all gill arches.

Laboratory Results: N/A

Histopathologic Description: These are sections of gill. There is mild multifocal telangiectasis (euthanasia artifact – expect slide variation). Approximately 60-80% of the filament surface has variably sized plaques of lamellar hyperplasia. These are characterized by interlamellar accumulation of haphazardly arranged cells (lamellar epithelial hyperplasia). There are occasional interlamellar spaces (lacunae) in areas of hyperplasia, and at the edge of these areas, there are frequent lamellar synechiae. Within these interlamellar spaces, as well as on the surface of the lamellae, there are variable (mostly small) numbers of amoebic organisms. These are 15-20um round, with a vacuolated cytoplasm, and a small nucleus containing a dense, basophilic aggregate. Gram stains did not reveal any Gram positive organism in any of the sections examined.



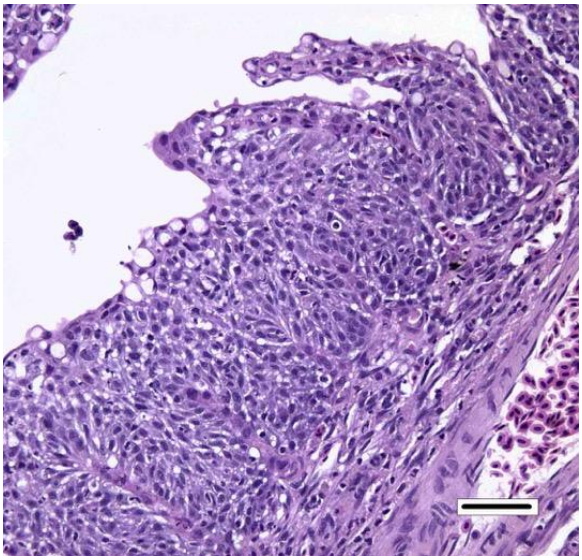
Gill, salmon. There is marked hyperplasia of lamellar epithelium, primarily at the tips of the gill filaments (HE, 40X)

Contributor's Morphologic Diagnosis: Gill: Moderate, multifocal to coalescing, lamellar hyperplasia with synechiae and lacunae formation, and intralesional amoebic organisms.

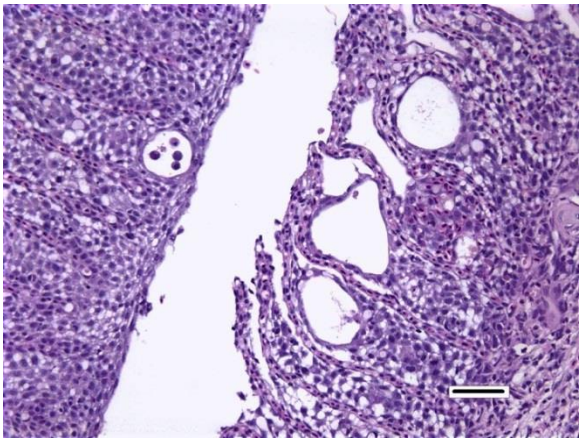
Contributor's Comment: The gross and histological presentation of this case is consistent with Amoebic gill disease (AGD). AGD is caused by *Neoparamoeba perurans*.[32] Koch's postulates have been recently confirmed by culture of *N. perurans* and challenge of naïve *Salmo salar*, [8] disproving the aetiological role previously ascribed to *Neoparamoeba pemaquidensis*.[21] Work on the virulence factors of *N. perurans* is ongoing, and a protease-like exotoxin has been associated with a cytotoxic effect of amoeba.[7] This may be associated with the epithelial necrosis noted ultrastructurally in AGD affected *S. salar* gills. [16] It is noteworthy that *N. perurans* has been reported to lose virulence in culture, and this has been postulated to occur due to lack of attachment and/or the absence of an extracellular product.[5]

AGD affects salmonids, turbot (*Scophthalmus maximus*), ayu (*Plecoglossus altivelis*), European seabass (*Dicentrarchus labrax*), sharpsnout seabream (*Diplodus puntazzo*), and ballan wrasse (*Labrus bergylta*).[12,21] In this review, we will focus on Atlantic salmon (*Salmo salar*), as this is the species in which this disease has the highest economic impact. In *S. salar*, the downstream effects of AGD include reduced growth, increased susceptibility to other pathogens and, if untreated, mortality. Its economic burden is large; in Tasmania (where AGD has been endemic since 1980) costs have been estimated at ~$1 AUS/kg salmon (not cited). Disease occurs at temperatures 12-20ºC and high salinity (35º/₀₀).[17] However, there is variability on this presentation and in Scotland outbreaks may happen as low as 8ºC.

Grossly, AGD presents as multifocal white to grey swollen areas on the gills, associated with excess of mucus.[17] Lesion distribution favours gill areas with lower water flow (i.e. at the dorsal aspect of the gill arches).[3] Several gross scoring systems have been developed, with diverse focus on aspects of the severity and distribution of AGD-associated lesions.[2,30] These scoring systems have been used to predict expected mortality levels of AGD affected fish if left untreated[30]. There is moderate to good agreement



Gill, salmon. Lamellar epithelium is hyperplastic to the extent that there is fusion and effacement of individual secondary lamellae. (HE, 100X)



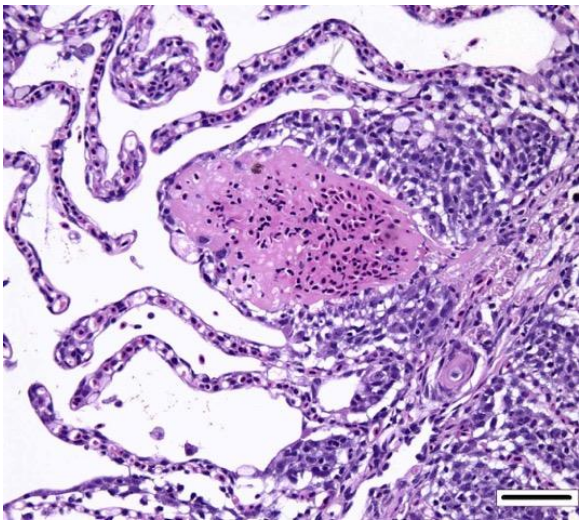
Gill, salmon. Focal fusion of adjacent lamellae (lamellar synechiae) results in the formation of dilated pseudocysts. (HE, 100X)

(κ=0.52-0.74) between the gross signs of AGD and the histological presentation, and regions with gross lesions are those with histological signs.[1] However, this agreement is not ideal, with multiple instances of disagreement depending on the AGD stage, other pathogens, assessor, sampling method, histological technique and histological experience.[1]

Histologically, AGD presents with lamellar epithelial hyperplasia and fusion (with creation of small interlamellar vesicles/lacunae), which are variably associated with interbranchial lymphoid tissue area increase, filament fusion, changes in chloride cells, and haemorrhage.[4,17,22]. Gill epithelial hyperplasia is a stereotypical feature of gill damage, but in AGD it has been postulated that it may be a result of the effect of the cytotoxic protease of the amoeba.[7] Whether this is true or note, gill lamellar hyperplasia in AGD is associated with proliferating cell nuclear antigen (PCNA) increase [2], and p53 downregulation[18] in lamellar epithelial cells. Amoebic organisms are noted in close association with the epithelium of lamellae and filaments, and often around the margins of the hyperplastic plaques.[4] There is no description of epithelial invasion and these amoeba are considered ectoparasites.[16] In field studies, Adams et al. [2] first detected histological lesions at 13 week post transfer, with three distinct phases: (1) Primary attachment/interaction associated with extremely localized host cellular alterations, juxtaposed to amoebae, including epithelial desquamation and oedema; (2) Initial focal hyperplasia of undifferentiated epithelial cells; and (3) lesion expansion, squamation–stratification of epithelia at lesion surfaces and variable recruitment of mucous cells to these regions. A pattern of preferential colonization of amoebae at lesion margins was apparent during stage 3 of disease development.

There is a significant body of work on the systemic host response during AGD. Early work on innate immunity changes in *S. salar* recorded an increased chemotactic response of head kidney macrophages, with paradoxical reduction in head kidney phagocyte respiratory burst.[11] Overall, there is debate on the protective role of adaptive immunity in AGD.[26] There is evidence of anti- *N.perurans* antibodies circulating post exposure, but the response is considered poor.[10,31] A field study on the adaptive immune response to AGD revealed that even though there is increase of seropositivity against *N. perurans* (with predominance of carbohydrate epitopes over peptide epitopes), there was no evidence to support that these antibodies were protective.[29] Subsequently, Vincent et al.[31] noted reduced mortality in the second exposure which may support the existence of a protective acquired immune response. Interestingly this reduction in mortality was not associated with reduction in the severity of the gill lesions.

At the gill level, there is increased mRNA expression of IL-1β, TCR-alpha chain, CD8, CD4, MHC-IIα, MHC-I, IgM and IgT in AGD-challenged salmon at 10 days post- l,



Gill, salmon. There is multifocal aneurysmal dilation of lamellar capillaries; thrombosis of these vessels shows that it is not a postmortem change. (HE, 100X)

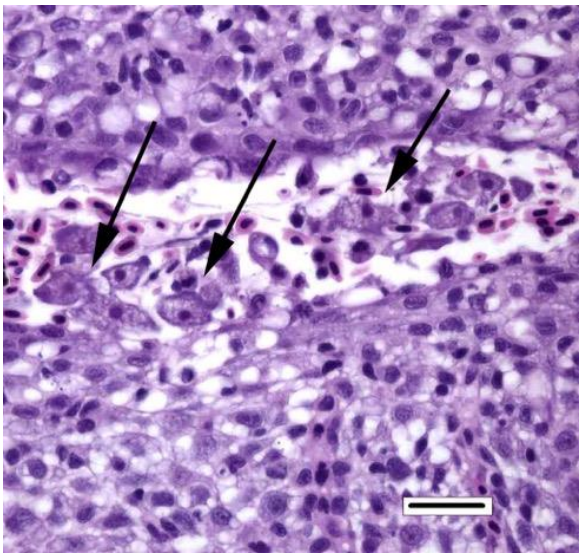
inoculation.[23] This suggests that an acquired immune response is present at the local levewhich is further supported by the presence of MHC class II+ cells within gill lesions.[19] An interesting pattern has been noted with the role of IL-1β in AGD, which is overexpressed in advanced AGD, despite an absence of marked inflammation.[6] This may be due to reduced transcription of the receptor IL-1R-I, although this effect is not complete, as IL1R-II is expressed at normal levels.[20]

The pathogenic effect of AGD on *S. salar* has also been studied, and includes cardiovascular compromise, osmoregulatory changes, and ion regulation changes. AGD results in cardiovascular compromise, which is noted even with light AGD lesions.[13,15] This may explain why mortalities can occur with light lesions, as it happens in the field, and could be related to individual variation in the ability to cope with acute and chronic increases in cardiovascular demand. In AGD, cardiovascular compromise may be the result of increased afterload, characterized by elevation of dorsal aortic pressure and systemic circulation resistance (systemic hypertension), without derangement of the renin-angiotensin system.[13,15,25] The increased afterload is coupled with cardiac morphological changes in chronic AGD. In a field study using gross lesion scores and number of freshwater baths required to assess clinical severity, *S. salar* with severe "AGD history" had increased ventricle length: axis length and ventricular width:axis length/height and thickened compact myocardium at the expense of the spongiosa, when compared with fish with "light AGD history".[27]

Very interestingly, the cumulative findings of several studies do not support respiratory failure as the main mechanism of AGD-induced mortality. This may be due to gill reserve capacity and reduction in mucus density, despite an increased amount of mucus.[26,28] AGD results in hyperplasia of the lamellar epithelium, with consequent reduction on respiratory-efficient gill surface area due to multifocal increase in blood water diffusion distance.[2] However, this is coupled with reduction on mucus density, which reduces the blood-water diffusion barrier resistance.[28] During AGD, there is increase in the rate and amplitude of opercular movements,[9] but this is not coupled with reduction in oxygen uptake.[26] Other respiratory changes are noted, however, and CO_2 plasma pressure is increased in AGD-affected fish, with reduced pH consistent with mild respiratory acidosis from 7dpi.[24], preceded by early alkalosis (48hpi), which could be the result of hyperventilation.[14]

JPC Diagnosis: Gill: Branchitis, proliferative, subacute, multifocal, moderate with fusion and adhesion of secondary lamella, dilation and thrombosis of lamellar capillaries and moderate numbers of intra- and extracellular amoeba.

Conference Comment: The contributor provides an excellent histopathologic



Gill, salmon. Low to moderate numbers of amebae with a prominent macronucleus are present within the interlamellar space. (arrows). (HE, 350X)

description and overview of amoebic gill disease (AGD). Conference participants described lamellar epithelial hyperplasia with fusion as a primary feature, as well as mild edema and goblet cell hyperplasia. The majority of organisms are present on surface areas of fused lamellar epithelium, and rarely are they seen within phagocytic cells. The moderator cautioned participants to avoid over-interpreting lamellar changes such as blunting, particularly when lamella are not oriented parallel to the plane of section. The moderator also explained a finding often termed "epithelial lifting" – it can represent lamellar edema, especially if the space beneath the epithelium contains proteinaceous or flocculent material, but it can also be a tissue handling (e.g., fixation) artifact. AGD can be a challenging diagnosis due to similarities in appearance between sloughed epithelial cells and the organisms. Organisms are most commonly seen in areas of lamellar fusion and, in some cases, may be small and shrunken. Geimsa stain is effective at staining the organisms purple on a blue background, aiding the diagnosis.

The moderator also discussed the differences between lamellar fusion and lamellar adhesion. In cases of lamellar fusion, there is filling of lamellar sulci from the bottom and it is most often associated with inflammation and epithelial proliferation. With lamellar adhesions, there is adherence of two or more lamella and there may be an absence of proliferation or inflammation. In this case, there are examples of both with lamellar fusion near the base of lamella and adhesions toward the top.

Dilated capillaries within secondary lamellae were described as either telangiectasia or aneurysmal dilation by conference participants. The moderator indicated this vascular change can be a perimortem event, or a non-specific change in certain diseases. In many cases, distinguishing between peri- and antemortem telangiectasis is difficult. However, in this case, the presence of organizing fibrin thrombi indicates the telangiectacic changes are antemortem.

Contributing Institution:
The Royal (Dick) School of Veterinary Studies, Easter Bush Campus, Midlothian, UK

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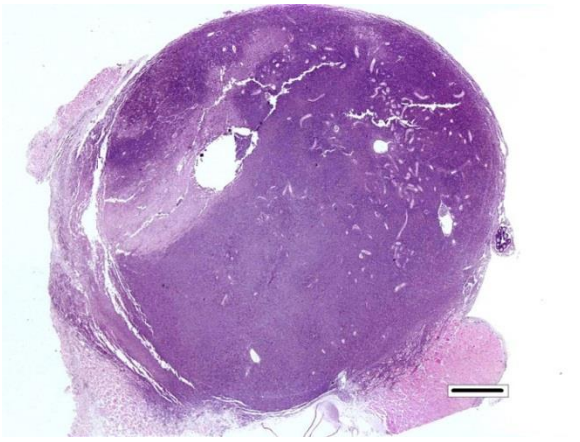
CASE III: 15-0816 (JPC 4067883).

Signalment: Approximately 6-month-old, gender unknown, lumpfish (*Cyclopterus lumpus*)

History: A corporate aquarium group undertook a captive breeding program to supply multiple aquaria within the group with juvenile lumpfish for display. All of the fish

were bred in a single facility, and after being grown, juvenile fish were dispersed to their new facilities at around 3 to 4 months. Over the course of the next few months, elevated mortality rates were observed in juvenile lumpfish in multiple locations. Typically, fish were either found dead or were observed to become lethargic, pale, and to develop buoyancy problems. In many fish, significant coelomic or generalized swelling was reported prior to death or euthanasia. Some cases displayed exophthalmos. Ongoing losses from affected groups proved unsustainable, and after a few months the entire originating group and dispersed populations were culled and restocked.



Kidney, lumpfish. The kidney is massively enlarged by an interstitial proliferation of lymphoid and phagocytic cells. (HE, 6X).

Gross Pathology: Postmortem examinations were typically undertaken at aquaria by aquarists, although some smaller fish were submitted whole in neutral buffered formalin to the laboratory. Typical gross findings included generalized or gill pallor, generalized "whole body" edema giving the fish a "jelly-like" consistency, marked intracelomic accumulations of clear watery fluid, exophthalmos and enlargement and mottling of the kidneys and occasionally spleen. Routine skin scrapes and gill presses undertaken on-site by aquarists were usually reported to be negative, with occasional identifications of amoebae from gills, or scuticociliates from skin.

Laboratory Results: In most cases, no clinicopathologic data was available. In one fish, air-dried celomic fluid smears and blood smears were submitted for cytological examination. The hematocrit was also measured in this fish, and was 14%. In this individual, aerobic cultures of coelomic fluid yielded a sparse growth of Bacillus species and light growth of alpha-hemolytic Streptococcus.

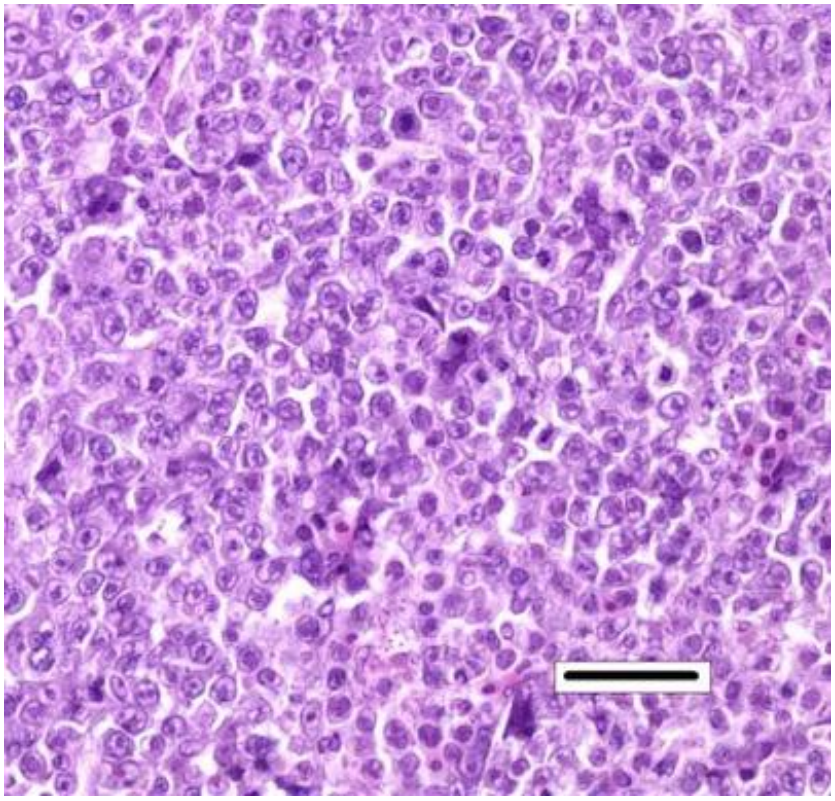
Cytologic description: Blood smear: Significant artifactual changes are present, resulting in poor cytoplasmic preservation of red cells, and abnormal chromatin staining. Assessment of red cell parameters is unreliable for this reason. This fish has a significant monocytosis, and lymphocytes also appear increased in number. In addition, significant numbers of the monocytic cells, and occasional presumptive lymphocytes, have intranuclear clusters of multiple (regularly 8 in some cells, occasionally more) elliptical refractile spores, consistent with intranuclear microsporidia.

Coelomic fluid: Moderately cellular smears in which mononuclear cell/macrophages and lymphocytes predominate. Cellular morphology is shrunken and nuclei are more condensed than in the blood smears. Intranuclear microsporidia are abundant within the mononuclear populations, and aggregates of similar spores within cytoplasmic fragments are also a feature

Histopathologic Description: Submitted material is derived from two different fish demonstrating similar histopathological changes.

Kidney: Hematopoietic kidney is massively expanded and normal architecture effaced by densely cellular sheets of mononuclear round cells, whose morphology is suggestive of

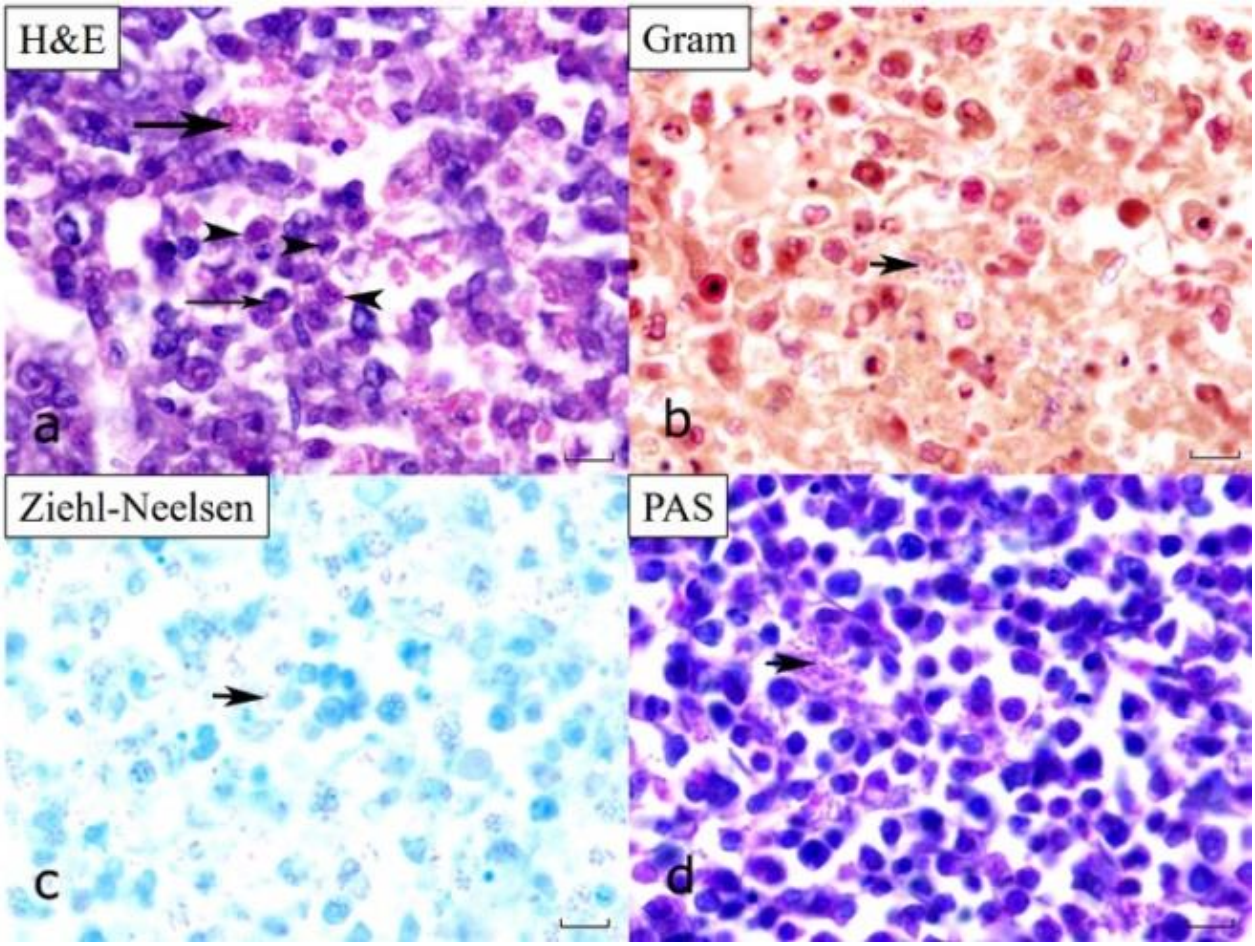
lymphoid/lymphoblastic origin. There is no intervening stroma, but small numbers of remnant tubules are present within the cell sheets. A subpopulation of cells have condensed aggregations of multiple (typically around 4 to 8) pale eosinophilic faintly refractile circular to ovoid spores (each approximately 1 x 3 µm) contained within their nuclear membranes (Figure 3a), and more numerous cells with intranuclear eosinophilic vacuoles/cytoplasmic folds are present. Varying numbers of spores are present in the extracellular milieu as a result of nuclear/cellular rupture, with phagocytosis by intermingled macrophages. Spores are most abundant in small multifocal areas of necrosis. There is extension of lymphoblastoid infiltrates into paravertebral striated muscle groups and connective tissue, spinal ganglia and spinal cord meninges. Similar cells are also present within the lumina of blood vessels. Spores stain weakly gram-positive, focally Ziehl-Neelsen positive and weakly PAS-positive.



Kidney, lumpfish. The interstitium is profoundly expanded by a monomorphic population of blastic lymphocytes with large nuclei, nucleoli, and a moderate mitotic rate. (HE, 400X).

Contributor's Morphologic Diagnosis: Lymphoproliferative and minimally necrotizing interstitial nephritis, diffuse, chronic, with intranuclear microsporidia (*Nucleospora cyclopteri)*, kidney, lumpfish (*Cyclopterus lumpus*).

Contributor's Comment: Intranuclear microsporidiosis has recently emerged in wild Icelandic fisheries of lumpfish (*Cyclopterus lumpus*, also known as lumpsuckers) and been described and speciated as a novel species, *Nucleospora cyclopteri*.[2] There are earlier clinical, histopathological and ultrastructural descriptions of intranuclear microsporidiosis from captive lumpfish in Canada.[6] Based on ultrastructural comparisons, it is suspected that the same parasite is implicated in these historical cases and the recent Icelandic cases.[3] Definitive confirmation of this awaits molecular studies of material from the Canadian cases.[2] The recent Icelandic cases were found in wild fish harvested for their eggs, which are a traditional caviar substitute. Although the prevalence of infection was relatively high (parasites were recognized at 12 out of 43 sites sampled around the Icelandic coast), and clinical disease was seen in 18 of 77 fish (presenting with enlarged pale kidneys), many



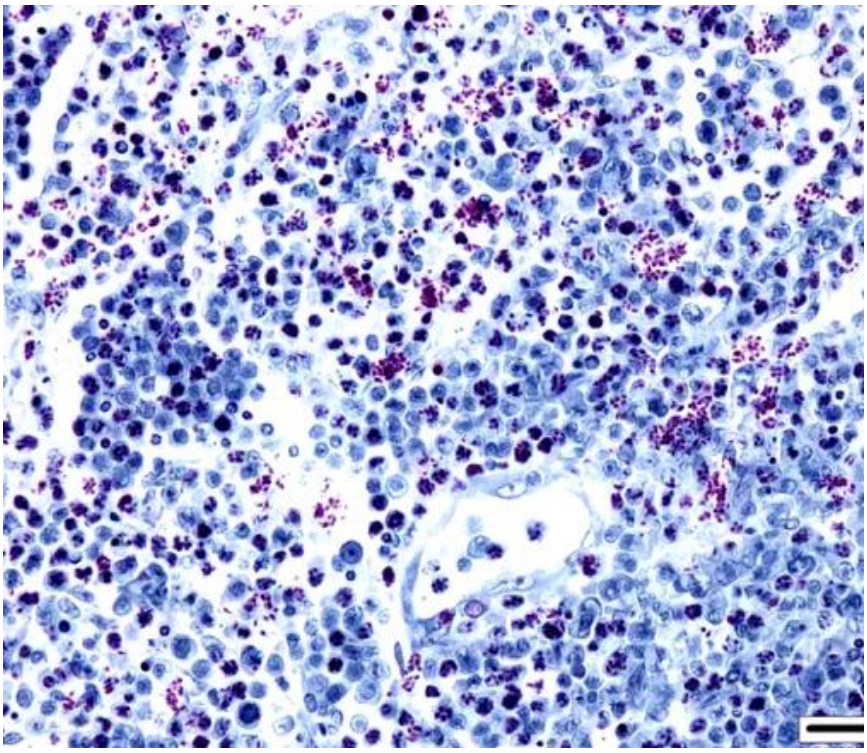
Kidney, lumpfish. Spores stain weakly gram-positive, focally Ziehl-Nielsen positive, and weakly PAS-positive. (Photo courtesy of: International Zoo Veterinary Group, Pathology Station House, Parkwood Street, Keighley, West Yorkshire, BD21 4NQ, United Kingdom, http://www.izvg.co.uk)

other fish were clinically unaffected.[2] A very recent report describes co-infection by *Nucleospora cyclopteri* and *Kudoa islandica* in farmed lumpfish in Norway.[1]

In the last 2 years in the United Kingdom we have recognized this infection as a significant cause of mortality in aquarium-bred colonies of lumpfish destined for public aquaria, and in research colonies used for experimental studies into lumpfish husbandry. Along with wrasse, lumpfish are increasingly employed as cleaner fish for the control of sea lice in Atlantic salmon aquaculture, and there is an increasing interest in their susceptibility to diseases, particularly those to which salmonids may potentially be susceptible.

On cytological examination, cells in the coelomic fluid and monocytes/lymphocytes within blood smears contain numerous distinct intranuclear microsporidial spores. They are easier to see in this context than in the more shrunken nuclei of histological sections. Enhanced visualization may have been possible with a Peterson-Luna stain.[8] In histological sections, infiltrates of proliferating lymphocyte-like cells with intranuclear organisms expand the hematopoietic kidney early in the course of the disease, before disseminating (consistent with the presence of spores in circulating monocytes in the blood smears) into multiple organs (including in advanced cases gastrointestinal tract, meninges, spleen,

gonads and coelomic and cutaneous connective tissues). Sheets of infiltrating cells contain foci of necrosis and there is extracellular liberation of spores, which are frequently phagocytosed by admixed populations of reactive macrophages. The identity of the proliferating cells is difficult to determine with certainty based on morphology alone, but the most recent publication suggests that they are lymphocytes and monocytic precursor cells[2, 3] and the earlier report describes the affected cells as lymphocyte-like.[6]



Kidney, lumpfish. A Luna stain demonstrates the microsporidian spores admirably. (Luna, 100X).

Although formerly classified as protozoans, microsporidia are now considered to be more closely related to fungi. They are obligate intracellular spore-forming organisms with a pan-taxonomic distribution, and commonly infect fish, typically causing xenomas in a range of tissues depending on host and parasite[6]. However, intranuclear microsporidia are also recognised in fish, in particular a closely-related parasite, *Nucleospora (*formerly *Enterocytozoon) salmonis*, which is distributed worldwide, and has been recorded in several salmon species, causing anemia and lymphoblastosis.[4] The precise mode of transmission of *N. cyclopteri* remains uncertain, but many microsporidia, including *N. salmonis*, have a direct life cycle. The possibility of vertical transmission has also been discussed.[2] In fish, intranuclear microsporidia have also been described infecting enterocytes in Tanzanian killifish (*Nothobranchius rubripinnis)* and, most recently, infecting enterocytes leading to a wasting syndrome in farmed gilthead sea bream (*Sparus aurata).*[7]

JPC Diagnosis: Kidney, hematopoetic tissue: Necrosis, diffuse with marked hyperplasia, multifocal infarction, and intranuclear, intracytoplasmic and free microsporidia.

Kidney: Nephritis, histiocytic, diffuse, marked with tubular degeneration, necrosis and loss and intranuclear, intracytoplasmic and free microsporidia.

Conference Comment: Conference participants described the kidney as approximately 50% affected, with necrosis and hyperplasia of hematopoetic tissue being the most remarkable features. Participants also described mild, multifocal degeneration and necrosis of tubular epithelium with

aggregation of debris in tubule lumina (tubular casts) and tubular loss. The moderator pointed out glomeruli and tubule density varies between species and it is therefore useful to review a normal control animal before describing glomerular and tubular loss. There is slide variability with some sections having a focally extensive area of coagulative necrosis which participants postulated is an infarction. An excellent quality cytology image is provided by the contributor and participants commented on the unique intranuclear nature of this microsporidian.

The expansion of hematopoetic tissue was striking in this case, characterized by sheets of large blastic cells reminiscent of a lymphoproliferative neoplasm, admixed with a second population of smaller phagocytic round cells. Two additional alternate diagnoses in this case were discussed: lymphoproliferative neoplasia and *Exophiala* sp. infection. *Exophiala sp.* fungal infections can cause disease in both freshwater and marine species including channel catfish, lake and cutthroat trout as well as Atlantic salmon and cod among others. Lesions can be both cutaneous and visceral with areas of necrosis and chronic granulomatous inflammation, and in some cases infection has a predilection for the kidney with the presence of renomegaly and involvement of other visceral organs.[9] Fungal infection characteristically results in lesions with vasculitis, thrombosis, and infarction.

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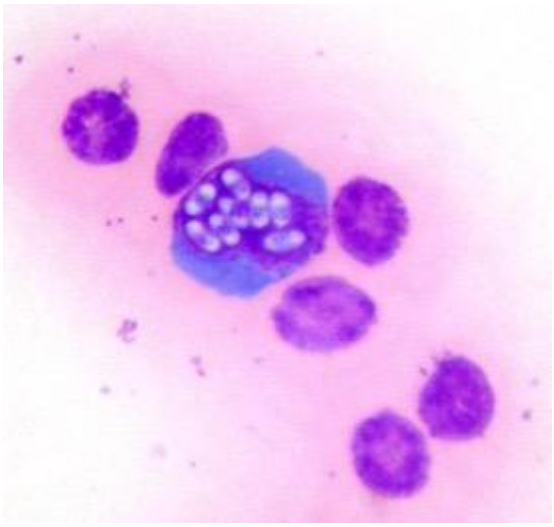

Coelomic fluid, lumpfish. Cells in the coelomic fluid and monocytes/lymphocytes within blood smears contain numerous distinct intranuclear microsporidial spores. (Photo courtesy of: International Zoo Veterinary Group, Pathology Station House, Parkwood Street, Keighley, West Yorkshire, BD21 4NQ, United Kingdom, http://www.izvg.co.uk)

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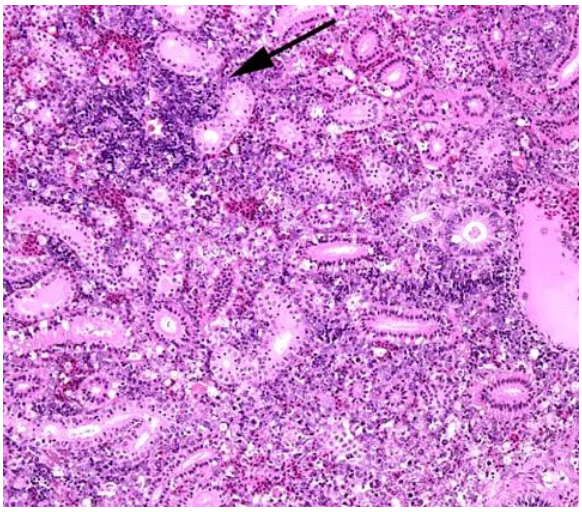
CASE IV: 10-9030 (JPC 4052876).

Signalment: Seven, 6- to 8-inch long goldfish (*Carassius auratus*)

History: In June 2010, a fish kill occurred at a lake in south east South Dakota. This pond's primary function is a storm sewer retention basin, and the kill occurred a couple days after a big rain event. The rain event was likely the stressor that started the die off. The die off involved a high percentage of goldfish, but dead bullheads and crappies were also seen. Mortality in these other species is common during the transition from spring to summer in our natural lakes and ponds. The goldfish first appeared in the lake about two years before the die off. The exact source of the goldfish is not known; however, it is assumed that someone released their pet fish. Dead goldfish were submitted to Animal Disease Research and Diagnostic Laboratory at South Dakota State University by the South Dakota Department of Game Fish and Parks
for diagnostic workup due to concern with possible viruses that attack species such as koi, carp, and goldfish.

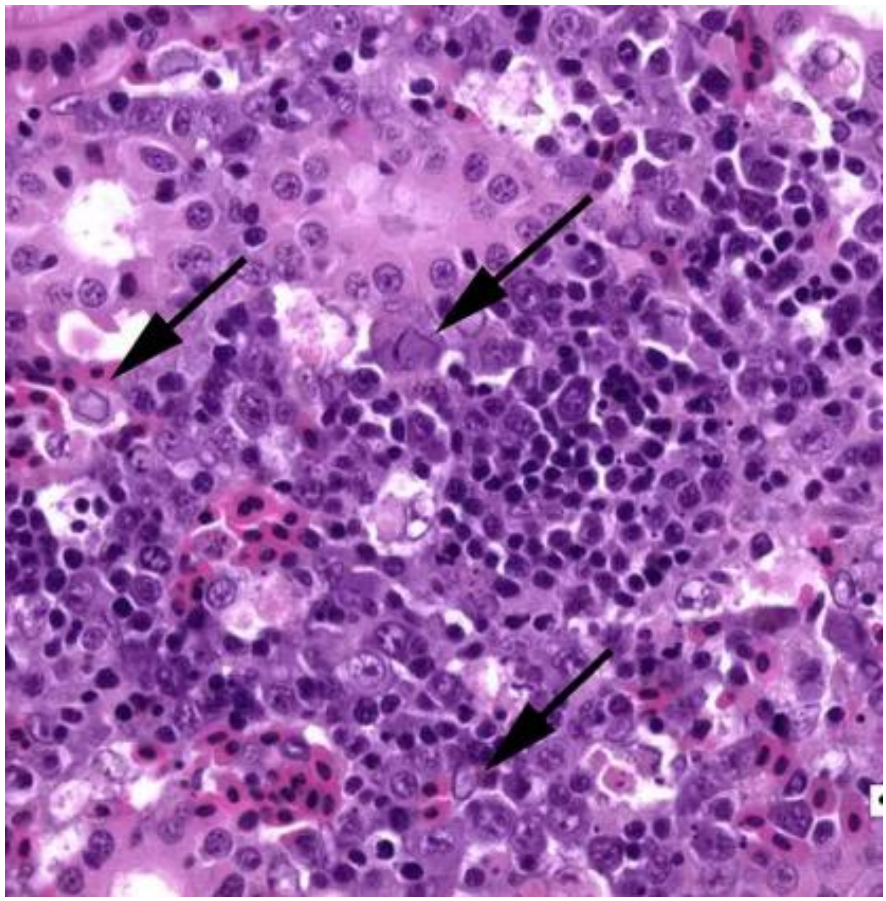


Kidney, goldfish. Nephrons are markedly separated by hyperplastic hematopoietic tissue. The pallor of this tissue is the result of diffuse necrosis; one focus of apparently normal tissue remains (arrow). (HE, 40X)

Gross Pathology: Some of the dead goldfish gills had limited multifocal hemorrhages and pale foci that appear to be necrosis. All fish had empty stomachs and full gallbladders. A couple of fish had edema and reddening of the lateral skin.

Laboratory Results: Pools of gill, liver, and spleen were cultured and large growths of

Aeromonas hydrophilia were isolated from all pools. Viral examination included the collection of kidney, gill, and spleen from the goldfish, and after processing, inoculating EPC cells at 15°C for VHS (viral hemorrhagic septicemia); and EPC cells at 25°C for SVCV (spring viremia of carp virus), LMBV (largemouth bass virus), or KHV (koi herpes virus). After a week the EPC cells at 25°C had cytopathic effect (CPE). Supernatant from the cell culture was filtered and put on EPC cells again and CPE was present again at a week. These cells were harvested and polymerase chain reaction (PCR) for VHS and SVCV was run and was found to be negative. Kidney, spleen, and gill homogenate was sent to Veterinary Diagnostic Laboratory at the University of Minnesota; it was examined for koi herpes virus with PCR and found to be negative. Minnesota also put homogenate on KF-1 cells at 15°C and 25°C for 42 days being passed on day 14 and day 28 and the results were negative. Finally, pools of kidney, spleen, gill, liver, and intestine were sent to Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis; they were positive for Cyprinid Herpesvirus 2 (CHV-2) using PCR.



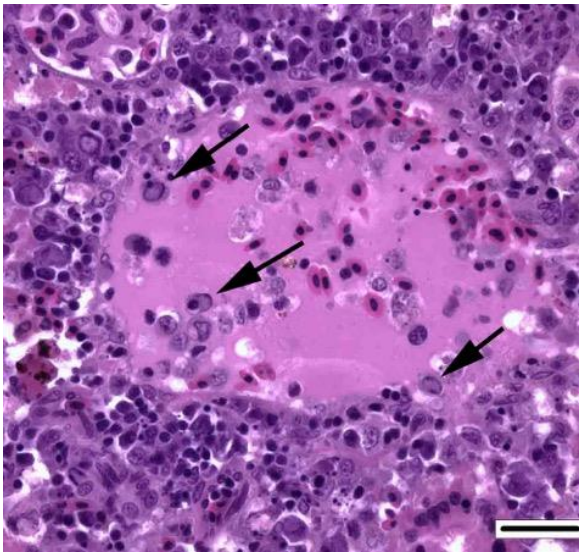
Kidney, goldfish. Nuclei of hematopoietic tissue are occasionally enlarged by a single glassy, amphophilic intranuclear viral inclusion. (HE, 360X)

Histopathologic Description: The gills have severe pleocellular inflammation with some proliferation and multifocal necrosis. There is severe multifocal meningoencephalitis. The inflammation is most extensive in the meninges and the ventricles. The head kidney, kidney, and spleen have severe multifocal to coalescing necrosis of hematopoietic tissue. Many intranuclear basophilic inclusion bodies are present.

Contributor's Morphologic Diagnosis: Submitted kidney slides: Acute severe multifocal to coalescing necrotizing interstitial nephritis with intranuclear inclusion bodies.

Etiology: Cyprinid Herpesvirus 2, confirmed by PCR.

Contributor's Comment: Herpesviral hematopoietic necrosis (HVHN) is a disease of goldfish *Carassius auratus auratus.* It is caused by Cyprinid herpesvirus 2 (CyHV-2), a member of the cyprinid herpesvirus group that includes carp pox (CyHV-1) and koi herpesvirus (CyHV-



Kidney, goldfish. Circulating leukocytes also contain intranuclear viral inclusions. (HE, 360X)

3). Cyprinid Herpesvirus 2 is found worldwide. Most goldfish populations carry the virus and disease outbreaks are sporadic and brought on by stress. Mortality associated with HVHN can approach 100 percent. The virus causes severe necrosis of hematopoietic tissue. Necrosis and inflammation is also found in the gills.[3,5]

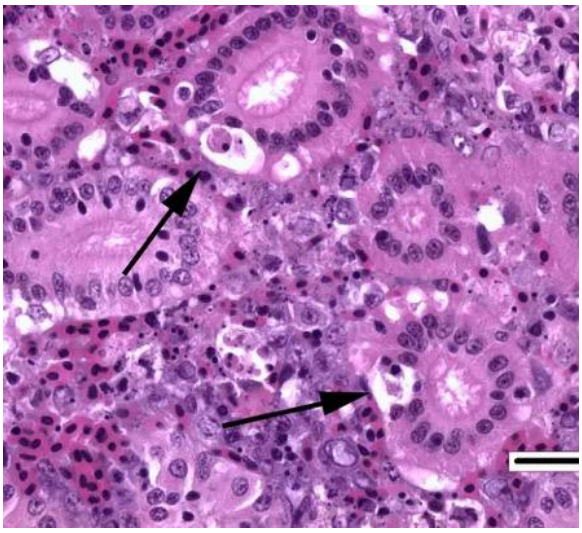
JPC Diagnoses: 1. Kidney, hematopoietic tissue: Necrosis, diffuse, with moderate hyperplasia and numerous intranuclear inclusion bodies.

2. Kidney, tubules: Degeneration, necrosis and loss, diffuse, with rare intranuclear inclusion bodies.

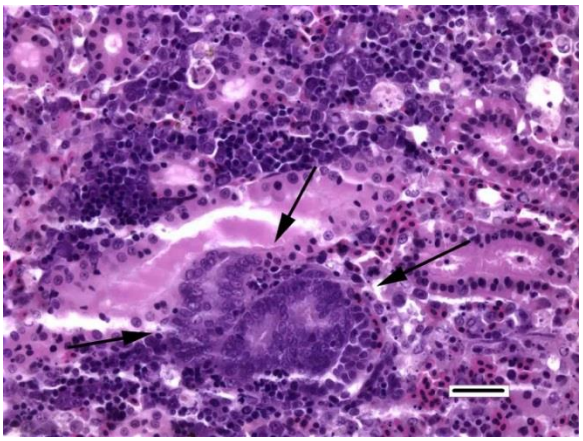
Conference Comment: Infection and goldfish mortality associated with CyHV-2 has been described throughout the world including in North America, Japan, Australia, the United Kingdom, Italy,[2] China and France; global fish trade is an important pathway for viral spread. Outbreaks are triggered by handling stress, transportation, and variations in water temperature.[1]

The three cyprinid herpesviruses are closely related to anguillid herpesvirus 1; all 4 viruses are grouped within the genus *Cyprinivirus*. Cyprinid herpesvirus-3 (CyHV-3) is highly contagious and is also termed koi herpesvirus disease. It is an OIE reportable disease and is also present throughout the world due to global fish trade; it can be devastating to the production of koi and common carp. Characteristic signs of disease include erratic swimming, gasping for air, poor appetite, discoloration and fin erosions. Histologic lesions include gill, liver and renal necrosis with intranuclear inclusion bodies.

Hyperplasia of gastric gland epithelium, intestinal villi, and respiratory cells may also be seen, resulting in lamellar fusion. Survivors of CyHV-3 outbreaks and other unaffected fish species can act as carriers. The virus is transmitted horizontally and fish density may play a role in severity and spread of infection in an outbreak. Cyprinid herpesvirus-1 causes papillomatous skin lesions in infected koi; the virus is lethal in young fish, but is generally not lethal in adult koi.[4]



Kidney, goldfish. Multifocally, there is necrosis of tubule epithelium. (HE, 400X)



Occasional regenerative tubules are scattered throughout the section. (HE, 200X)

Consistent histopathologic findings in CyHV-2 infection include necrosis of hematopoietic tissue in the spleen and/or kidney (necrosis may or may not be present in both locations), with or without the presence of characteristic herpesviral inclusion bodies. Other reported microscopic findings include: branchial epithelial hyperplasia, necrosis[1,2] and hypertrophy; necrosis and inflammation in the intestine; and lesions in the heart.[1] Viral DNA has been interstitium with eosinophilic cellular and demonstrated in subclinically affected animals with the presence of single cell necrosis in hematopoietic tissue, suggesting a possible role of latent infection in the pathogenesis of this disease.[2]

The most prominent lesion in these sections is the diffuse necrosis within the expanded renal hematopoietic tissue, and the presence of numerous prominent intranuclear inclusions within hematopoietic precursor cells and occasionally within renal tubule epithelium. The tunica media of few vessels is discontinuous and infiltrated by low numbers of inflammatory cells, however, the moderator pointed out that this is likely an "innocent bystander" lesion in areas of necrosis and not a true vasculitis. There is multifocal renal tubule epithelial degeneration, necrosis and rare regeneration. Herpesviral intranuclear inclusion bodies are also present within circulating leukocytes, which can be seen in the lumen of several small vessels. The moderator also discussed the presence of mild compensatory hematopoietic tissue hyperplasia and erythrophagocytosis.

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