Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

Conference 10

20 November 2019

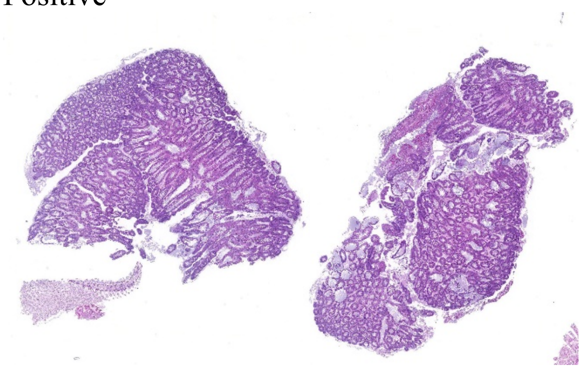
CASE I: R17104D (JPC 4135946).

Signalment: Caribbean spiny lobster (*Panulirus argus)* of unknown sex and age

History: In 2016, 14 Caribbean spiny lobsters (*Panulirus argus)* were collected off of Summerland Key, Florida to supplement the resident population at a large public aquarium. The lobsters were transported and placed in quarantine at a separate facility. After 5 months, lobsters began showing clinical signs of lethargy and dying in the molt. Samples of hemolymph were submitted to the Louisiana Aquatic Diagnostic Laboratory (LADL) at Louisiana Animal Disease Diagnostic Laboratory (LADDL) and the University of Florida for PCR for White Spot Syndrome Virus (WSSV) and *Panulirus argus* Virus 1 (PaV1), respectively. Upon obtaining the PCR results, the remaining lobsters were euthanized and gross necropsies were performed on site at the quarantine facility. Formalin-fixed tissues were submitted to LADL for histopathological evaluation.

Gross Pathology: There were no significant gross lesions in the lobsters examined.

Laboratory results:
PCR for White Spot Syndrome Virus (WSSV): Negative
PCR for *Panulirus argus* Virus 1 (PaV1): Positive



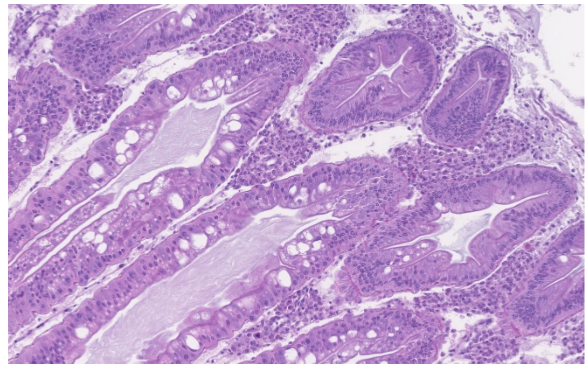
Hepatopancreas, lobster. Multiple sections of hepatopancreas are submitted. At low magnification, there is dilation of hepatopancreatic tubules which are filled with a bluish-grey material. (Photo courtesy of: Louisiana Animal Disease Diagnostic Laboratory (LADDL), School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html)

Microscopic Description: Hepatopancreas: Throughout the hepatopancreas, the hemal sinuses are variably dilated and increased in size in comparison to the adjacent tubules. The dilated sinuses are filled with a moderate to abundant amount of circulating hemocytes and proliferative spongy connective tissues. Fixed phagocytes surrounding the hepatopancreatic tubules are variably enlarged, with multifocal disruption of the typical rosette-like arrangement. Circulating hemocytes, fixed phagocytes, and spongy connective tissue cells often have enlarged, hypertrophic nuclei that contain large eosinophilic to amphophilic inclusion bodies, occasionally surrounded by a clear halo, with margination of the nuclear chromatin along the nuclear membrane (Cowdry-type A inclusion bodies). The cytoplasm of the affected cells often contain smaller, variably-sized, round eosinophilic globules. There are subjectively decreased numbers of reserve inclusion cells throughout the hepatopancreas. In few areas, the epithelial cells lining the hepatopancreatic tubules are variably decreased in size or completely absent, and the lumen contains a moderate amount of basophilic granular material and few individual sloughed epithelial cells.

In addition to the hepatopancreas, similar eosinophilic intranuclear inclusion bodies were observed within spongy connective tissue cells of the exoskeletal membrane (not included in submission).

Contributor's Morphologic Diagnosis: Hepatopancreas: Hepatopancreatitis, moderate, diffuse, chronic, with mild to moderate hepatopancreatic atrophy and numerous eosinophilic intranuclear inclusion bodies

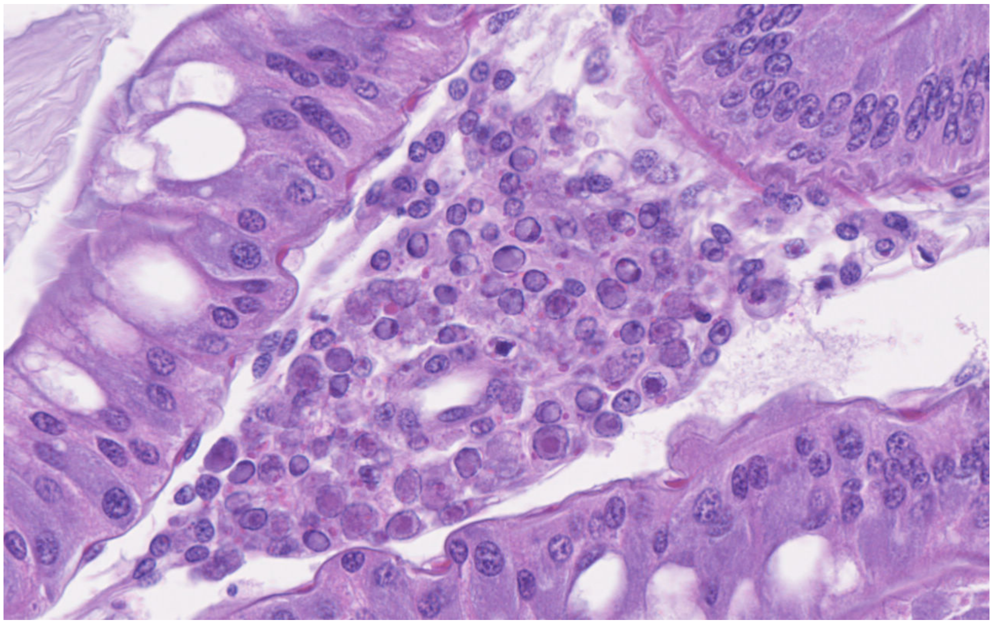
Contributor's Comment: As a brief overview, the normal hepatopancreas of



Hepatopancreas, lobster. H&E. 100X. The hemal sinuses are variably dilated and filled with variable numbers of circulating hemocytes and proliferative spongy connective tissue. (HE 100X)

most decapod crustaceans is a large compact paired glandular organ that surrounds the midgut and occupies a large portion of the cephalothorax.[2] The hepatopancreas is surrounded by a connective tissue membrane and each half consists of two or three lobes comprised of a complex network of blind-ending tubules, which are connected to the midgut gland through common absorptive ducts.[2,8] With exception of the distal closed end, each tubule is lined by a single cell layer of epithelial cells, which are divided into subtypes including the embryonic E-cells, fibrillar F-cells, resorptive or absorptive R-cells, and secretory B-cells.[2] The embryonic cells reside within the apical portion of the tubule, while the remaining cell types (B-, R-, and F-cells) reside within the digestion zone.[8] An outer basement membrane separates the tubules from the hemal sinuses, which contain an arteriole surrounded by a rosette-like structure of fixed phagocytes, which remove foreign material from the hemolymph, and reserve inclusion cells, which contain polysaccharides such as glycogen and proteins such as hemocyanin.[3,6,8]

Panulirus argus Virus 1 (PaV1) was discovered approximately 20 years ago as the first naturally occurring pathogenic virus of



Hepatopancreas, lobster. Fixed hemocytes within the hepatopancreas frequently have hypertrophic nuclei containing large eosinophilic Cowdry-type A inclusion bodies. (HE, 600X) (Photo courtesy of: Louisiana Animal Disease Diagnostic Laboratory (LADDL), School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html)

lobsters.[1,3-7] PaV1 was first detected in juvenile Caribbean spiny lobsters (*Panulirus argus*) from the Florida Keys, Florida, USA, but has since been detected throughout the Caribbean Sea, including reports from St. Croix, St. Kitts, Yucatan (Mexico), Belize, and Cuba.[1,3,6] At the time of submission, Caribbean spiny lobsters are the only reported affected host of the virus.[6] PaV1 is one of the most significant pathogens affecting spiny lobsters and is believed to be associated with a decline in the spiny lobster fishery in the Florida Keys.[6,7] Additionally, these lobsters are shipped internationally, which could play a role in the potential spread of the virus. The virus is not yet classified, but shares morphological characteristics with Herpesviridae and Iridoviridae; it is an unenveloped, icosahedral DNA virus with an approximately 182 nm nucleocapsid that develops within the infected host cells' nuclei.[6]

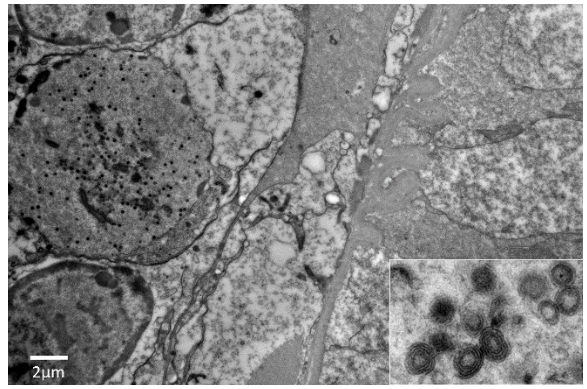
The virus is most prevalent within and nearly always lethal to the smallest juvenile lobsters, with a decrease in prevalence correlating with increase in the size of lobster; adults may harbor the virus, but do not typically exhibit signs of disease.[1,6] Healthy lobsters tend to avoid diseased lobsters, which may explain reduced disease transmission, but may also lead to increased shelter competition and increased predation on the infected lobsters.[1,6] Heavily infected lobsters become lethargic and sedentary, cease feeding, and eventually die of metabolic exhaustion.[1,6]

During early stages of infection, the virus has an apparent predilection for the fixed phagocytes of the hepatopancreas, which later lyse and infect proliferating spongy connective tissue cells and circulating hemocytes, including hyalinocytes and semi-granulocytes.[1,3,5] As mentioned above, the fixed phagocytes and circulating hemocytes play an important role in phagocytosing foreign materials from the hemolymph.[6,8] The circulating hemocytes affected include hyalinocytes and semigranulocytes; granulocytes, as well as fibrous connective tissue cells are not affected.[1,3] All affected cell types are derived from the mesoderm, suggesting that the virus may have a tropism for this developmental germ layer.[6] In heavier infections, virtually all spongy connective tissue cells are infected and hepatopancreatic tubules become atrophied, with depletion of reserve inclusion cells, leading to metabolic wasting and death.[6]

The hemolymph of affected lobsters has a characteristic white, milky appearance and fails to clot.[6,7] Histopathologic lesions can be observed within 10-15 days of infection and include alterations to the fixed phagocytes and spongy connective tissue with enlarged nuclei containing Cowdry-like inclusion bodies.[8] Molecular diagnostics including polymerase chain reaction (PCR) and fluorescent *in situ* hybridization (FISH) have been utilized for disease confirmation.[4-6]

In this case, the abundance of intranuclear eosinophilic inclusion bodies coupled with the positive PCR result were diagnostic for *Panulirus argus* Virus 1 (PaV1). In addition to the intranuclear inclusions, eosinophilic globular material was observed within the cytoplasm of the infected cells. The origin of this material has yet-to-be determined, but based on electron microscopy, we believe this material could be virus-induced particles within the cytoplasm. As another alternative, the histologic appearance is somewhat similar to the eosinophilic globular aggregates associated with reserve inclusion cells and therefore, these globules may represent polysaccharide aggregates such as glycogen. The areas with loss of hepatopancreatic epithelium and intraluminal basophilic granular material are believed to be euthanasia-induced artifacts, though virally-induced change cannot be ruled out. However, PaV1 has not been reported to affect hepatopancreatic epithelium, so this is considered less likely.

Contributing Institution:
Louisiana Animal Disease Diagnostic Laboratory (LADDL), School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html)



Hepatopancreas, lobster. An electron micrograph of the intracytoplasmic globular material within reserve inclusions cells contains potential virus-induced particles. (Photo courtesy of: Louisiana Animal Disease Diagnostic Laboratory (LADDL), School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html)

JPC Diagnosis: 1. Hepatopancreas: Hepatopancreatitis, interstitial, hemolytic, diffuse, moderate, with numerous eosinophilic intranuclear inclusion bodies.
2. Hepatopancreas, gill, midgut; circulating hemocytes: Eosinophilic intranuclear inclusion bodies.
3. Hepatopancreas: Atrophy, tubular, diffuse, severe.

JPC Comment: The contributor has done an outstanding job of describing the pertinent anatomy of the decapod hepatopancreas as well as illustrating this important disease of spiny lobsters. Many pathologists are likely unfamiliar with the anatomy of the lobster, and are directed to reference 8 below, an excellent review of the gross and histologic anatomy of the lobster at: https://scholarworks.wm.edu/cgi/viewcontent.cgi?article=1005&context=reports (reference 8)

Many pathologists (as well as the majority of conference participants) are largely unaware of the diseases of spiny lobsters. In addition to Panulirus argulus virus-1, spiny lobsters are host to a number of infectious, parasitic, and syndromal conditions of interest.

At this time, PaV1, appears to be the only natural viral infection of spiny lobsters, although it appears they may be natural hosts for the virus that causeswhite spot syndrome virus, a viral disease clinically seen in shrimp.[6]

Spiny lobsters are host however, to a number of bacterial pathogens. *Aerococcus viridans* is a gram-positive bacillus that is most common in holding facilities but has been identified in Cuban spiny lobsters. This agent results in a reddish disocloration of the shell, pink hemolymph and coagulopathies when introduced through a broken exoskeleton. “Shell disease” is a condition caused by a numer of chitinoclistic gram-negative bacterial, including bacteria from the genera *Vibrio, Aeromonas, Pseudomonas, and Shewanella*. This multifoal erosion of the shell may occur randomly on the exoskeleton, or in spiny lobsters, affect the tail fan. In addition, *Vibrio* sp. may cause disseminated disease in spiny lobsters, especially larvae, but also may be cultured from hemolymph in apparently normal individuals of this species. Fouling bacteria, such as *Leucothrix mucor* damage eggs and larvae of lobsters in culture. Several species of microsporidia are also seen in the spiny labsters, and may affect the meat quality of infected animals.[6]

A number of fungi infect spiny lobsters,. Oomycetes and phycomycetes usually infect the eggs and larvae, and are more common when water quality is poor. *Fusarium solani* results in black shell disease, melanization which is often seen in a variety of diseases affecting the exoskeleton in this species. Cilites are often found as commensals on the exterior of lobsters particular in cultures.[6]

A number of helminth parasites are described in spiny lobsers, to include digenetic trematodes, which utilitze them as intermediate hosts and encyst as metacercariae in the msuculature. Connective tissues of metacestodes may encyst in connective tissues. Rotifers, copepods, and amphipods are often symbionts or commensals in spiny lobsters or their egg clutches.[6]

Disease syndromes in lobsters generally are associated with interesting names, even if their etiology is unknown. “Turgid lobster syndrome” occurs in spiny lobsters in New Zealand and Australia, is characterized by swelling of the arthrodial membranes, and a causative agent is unknown. Australia is also host to “pink lobster syndrome” in which the meat turns pink to yellow and is unpalatable. Mass mortality evens have been documented by habitat alteration such as hypoxia events, algal blooms, and mass strandings.[6]

The moderator reviewed the anatomy and histology of the lobster. In discussing the most appropriate morphologic diagnosis for this case, the moderator discussed a preference for the term “hemocytic” rather than granulocytic due to the difficulties in distinguishing the two on routine light microscopy. Atrophy of the hepatopancreas in this case is especially profound as normal hepatopancreas is filled with lipid vacuoles, and few remained in this particular specimen, but it was added as a separate morphologic diagnosis as it was difficult to establish whther it resulted from the viral infection, or simply diminished nutritional status as a consequence of captivity.

References:

1. Behringer DC, Butler MJt, Shields JD, Moss J. Review of *Panulirus argus* virus 1--a decade after its discovery. *Dis Aquat Organ*. 2011;94**:** 153-160.

2. Gibson R, Barker PL. The Decapod Hepatopancreas. *Oceanogr Mar Biol Ann Rev*. 1979;17**:** 285-346.

3. Li C, Shields JD, Ratzlaff RE, Butler MJ. Pathology and hematology of the Caribbean spiny lobster experimentally infected with Panulirus argus virus 1 (PaV1). *Virus Res*. 2008;132**:** 104-113.
4. Li C, Shields JD, Small HJ, et al. Detection of Panulirus argus Virus 1 (PaVI) in the Caribbean spiny lobster using fluorescence in situ hybridization (FISH). *Dis Aquat Organ*. 2006;72**:** 185-192.
5. Montgomery-Fullerton MM, Cooper RA, Kauffman KM, Shields JD, Ratzlaff RE. Detection of Panulirus argus Virus 1 in Caribbean spiny lobsters. *Dis Aquat Organ*. 2007;76**:** 1-6.
6. Shields JD. Diseases of spiny lobsters: a review. *J Invertebr Pathol*. 2011;106**:** 79-91.
7. Shields JD, Behringer DC, Jr. A new pathogenic virus in the Caribbean spiny lobster Panulirus argus from the Florida Keys. *Dis Aquat Organ*. 2004;59**:** 109-118.
8. Shields JD, Boyd RA: Atlas of Lobster Anatomy and Histology. *In*: Special Papers in Marine Science. Virginia Institute of Marine Science, College of William and Mary, 2014

CASE II: N18-0170 (JPC 4132732).

Signalment: Adult (≥4 years) female Oscar, cichlid (*Astronotus ocellatus)*

History: On August 9, 2018 the patient presented for rapid gilling. Upon physical examination the gill filaments were thickened, erythematous and mottled red to light pink. A black spot on the gill filaments of the first right gill arch was seen, and a trematode was observed on a gill clip. The patient was treated with 10 ppt NaCl baths, ceftazidime and praziquantel injections but no improvement was seen. Due to quality of life concerns, euthanasia with MS-222 was elected on August 19, 2018.

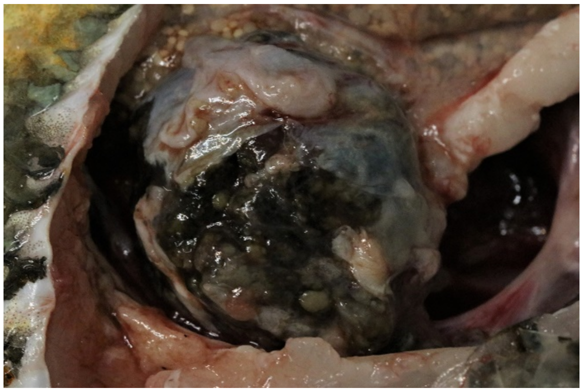
Gross Pathology: On gross examination there is abundant mucus coating the entire fish. The caudal pole of the kidney is expanded by a dark, mottled, red-brown to tan, 3.5 x 2.5 x 2.5 cm multinodular mass.

Viscera, oscar. Gross necropsy photo with coelomic wall retracted cranially. Orientation: Dorsal aspect is to the top of the photo, cranial aspect is to the left. a) liver b) ovary c) spleen d.) mass e) GI f) swim bladder. (Photo courtesy of: Smithsonian's National Zoo and Conservation Biology Institute, https://nationalzoo.si.edu/animals/veterinary-care)

The mass is partially encapsulated, soft, wet, friable, and located ventral to the swim bladder. The kidney is similarly mottled and attached by apparent renal tissue to the mass**.** No other abnormalities are seen.

Laboratory results:
Renal mass cytology - Rafts of epithelial cells. Cells occasionally surround acellular material (possible mineral) in a tubule-like formation.

Microscopic Description: Preexisting renal tissue is identified by renal tubules and glomeruli. Greater than 90% of the normal renal tissue is expanded and obliterated within the original renal capsule by a neoplastic mass with lobules separated by a fibrovascular stroma. Neovascularization is noted throughout the mass within the interstitium. The mass is primarily cystic forming distinguishable lumina up to 1.8 mm in diameter containing viable and degenerate erythrocytes, indistinct cellular debris, and eosinophilic fluid. The cells lining these



Kidney, oscar. Renal mass in situ, connection with trunk kidney. (Photo courtesy of: Smithsonian's National Zoo and Conservation Biology Institute, https://nationalzoo.si.edu/animals/veterinary-care)

spaces are well-differentiated, cuboidal to occasionally columnar epithelium exhibiting mild anisocytosis and anisokaryosis. Each cell contains a moderate amount of eosinophilic occasionally vacuolated cytoplasm, and a single round, centrally to apically located nucleus. Nuclei have finely stippled to vesicular chromatin and 1-2 basophilic nucleoli. No mitotic figures are seen. The interstitium of the mass is moderately expanded by macrophages containing golden brown pigment (hemosiderin), lower numbers of lymphocytes and plasma cells and regions of neovascularization. Numerous coalescing organized granulomas are present within cystic lumina and the interstitium. They range from 0.1 mm solitary granulomas to 3 mm coalescing granulomas. The granulomas consist of a necrotic core of brightly colored eosinophilic cellular and karyorrhectic debris contained by an inner layer of concentric compressed epithelioid macrophages surrounded by a thicker layer of foamy macrophages. Hemosiderophages are prominent throughout the interstitium and occasionally within outer foamy layers of granulomas. Scattered mineralization is present within granulomas. There are areas within the mass where disorganized and coiled membranes of cuboidal epithelium are seen (likely handling artifact).

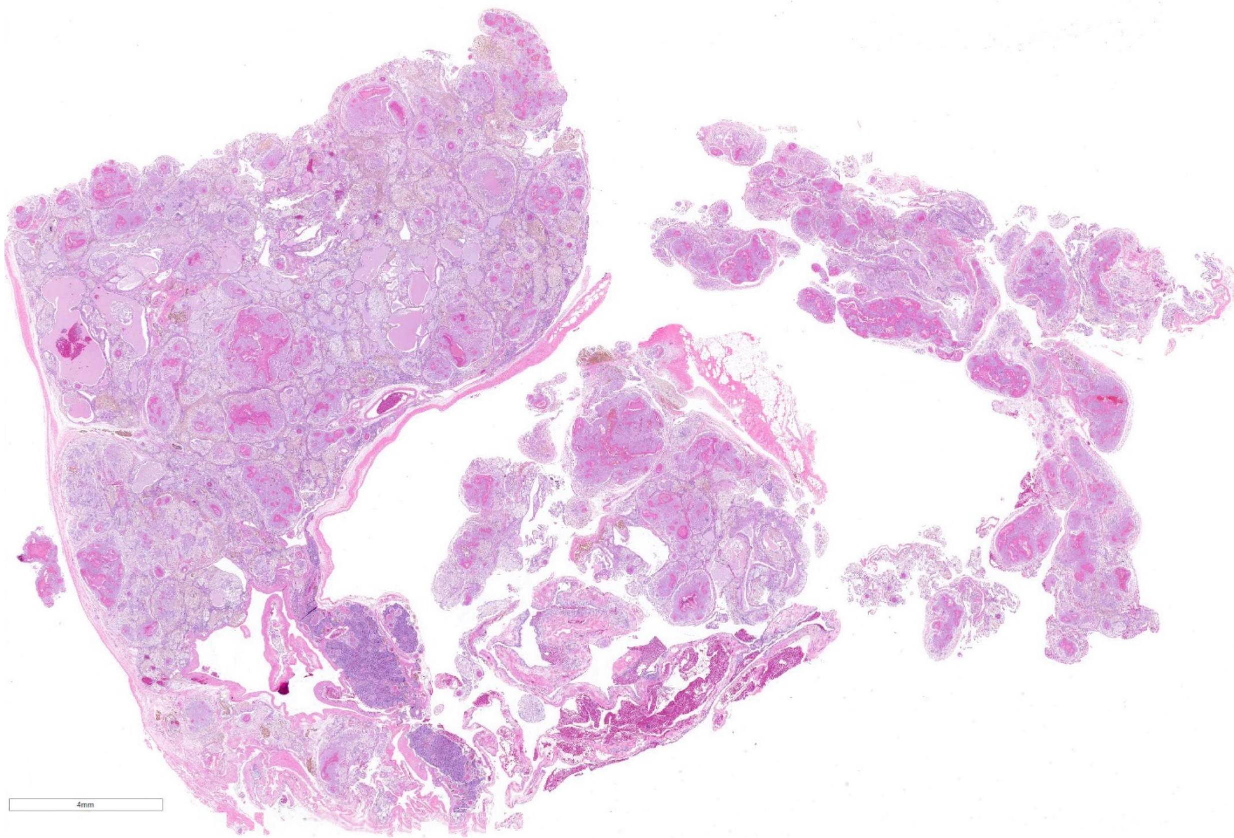
Slide N18-0170-2 Acid Fast –There are abundant intracellular and extracellular robust acid-fast rods (1 x 3 um) within granulomas.

Contributor Morphologic Diagnosis:
Kidney, posterior: Cystadenoma with concomitant multifocal, subacute to chronic, granulomatous nephritis with acid-fast bacilli

Contributor Comment: Renal tumors in teleosts (bony fish) are generally quite rare with the most common reports being nephroblastic tumors both in rainbow trout (associated with toxin exposure) and in Japanese eels (*Anguilla japonica*).[4,5] Most reports are of single cases, including a cystic adenocarcinoma in a European eel (*Anguilla Anguilla*), an adenoma in a brown bullhead catfish (*Ameiurus nebulosus*), and a papilliferous cystadenoma of the mesonephric duct in a Chinook salmon.[4,5] However, the tumor featured in this case is a well described tumor of this species and has been seen in multiple *A. ocellatus* specimens including three from the National Cancer Institute's Registry of Tumors in Lower Animals, one from North Carolina State

Kidney, oscar. The renal mass measures 5.7 x 2.5 x 2.7 cm. Numerous tan granulomas are visible on the service. (Photo courtesy of: Smithsonian's National Zoo and Conservation Biology Institute, https://nationalzoo.si.edu/animals/veterinary-care)

University College of Veterinary Medicine[10] and one from the University of Veterinary Medicine Veterinarplatz, Vienna Austria.[2] The clinical presentation was similar in all cases where recorded (abdominal distention and deteriorating condition despite medical therapy). While there were minor differences between reported tumor specimens (+/- granulomas, mineral crystals), all appeared cystic and originated from the posterior kidney and, with the exception of one case where there was invasion of surrounding tissue, all cases appeared benign.[7] In addition to the cases listed above, another oscar in the same aquarium this one died on August 1, 2018. That fish was also an adult (>4 years) female that presented with a smooth, firm, white tissue was protruding from the vent which was not able to be reduced. On necropsy a mass that was similar grossly to the mass in this case fish was found. Histologic examination identified it to be a renal cystadenoma but there were no granulomas in that case. Based on the number of specimens of the same species with similar tumors and lack of literature to indicate otherwise, it can be inferred that oscar cichlids are predisposed to renal cystadenomas. It is unknown whether this tumor occurs in wild individuals or if its development is due in part to low genetic diversity of the captive-bred population. Other potential etiologies of this tumor cannot be ruled out; it could be a unique species response to an unidentified environmental factor or an as-yet-unidentified microbe such as in proliferative kidney disease in goldfish caused by the myxozoan *Hoferellus carassii*.[7]



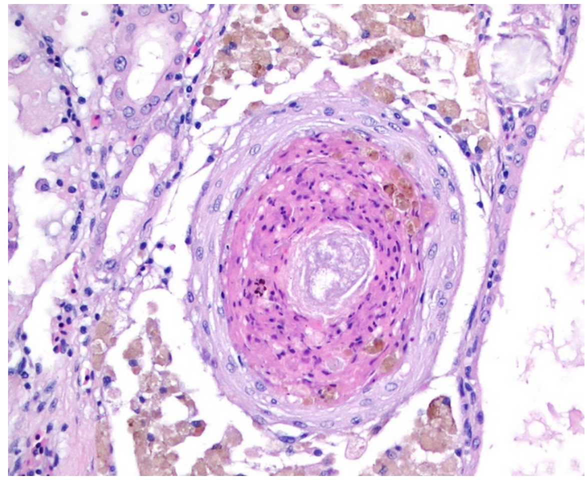
Kidney, oscar. A section of kidney is submitted. 95% of the kidney is effaced by a large cystic neoplasm studded with granulomas. Normal kidney is present in the section (arrows) (HE, 7X)

Clinical signs attributable to the tumor are nonspecific, though it can be presumed that the neoplasm (and in this case infection with mycobacteria) may have affected renal function and that large tumor size may have had an impact on buoyancy. This tumor's abnormal tissue likely provided a permissive environment predisposed to secondary infection. Smaller granulomas were noted in heart, liver, spleen, and ovary, and other animals in this tank had also been previously diagnosed with mycobacteriosis. In the case presented, opportunistic mycobacteriosis is an unsurprising, though florid, complication of both systemic and tissue-specific disease associated with the neoplasm.

Mycobacteria are slow growing, acid-fast staining bacilli that are natural occupants of water systems and opportunistic pathogens. They form biofilms within pipes and on surfaces of aquariums which makes them resistant to many forms of filtration and disinfectants. This makes eradication of mycobacteria from a system difficult. Opportunistic infections of fish within a contaminated system can occur when those fish are inflicted with external injuries or stress. Pathogenicity varies depending on the species of mycobacteria ranging from mild, chronic infections of a few fish to acute system wide infections with mortalities of 30-100%. Infection often results in granuloma formation, most notably within the liver and kidneys but diffuse disease can be seen, especially with the more pathogenic strains.[11] This case provides an example of environmental, host, tissue, and pathogen interactions resulting in dramatic pathology.

Contributing Institution:
https://nationalzoo.si.edu/animals/veterinary-care

JPC Diagnosis: 1. **Kidney**: Renal cystadenoma.



Viscera, oscar. Granuloma, composed of lamellated epithelioid macrophages and centered on a bacterial colony and associated cellular debris. (HE, 400X) (Photo courtesy of: Smithsonian's National Zoo and Conservation Biology Institute, https://nationalzoo.si.edu/animals/veterinary-care)

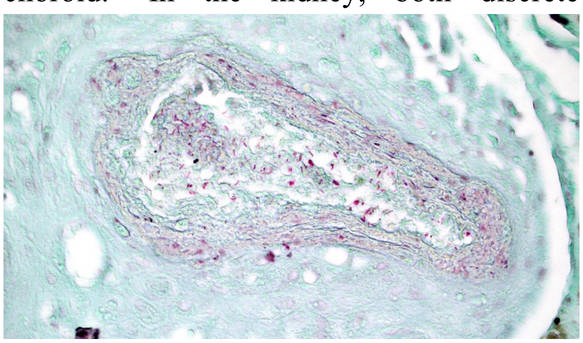
2. Kidney: Granulomas, numerous

JPC Comment: The contributor has done an excellent job of describing renal adenoma/cystadenoma in fishes. Benign renal neoplasms are rare tumors in animals, as they rarely cause clinical signs and are primarily diagnosed at autopsy. Many reports, as in fish, are single case reports, with large studies of renal neoplasms in dogs, where they amounted to 3% of cases overall, and in slaughtered cattle with an incidence of one in 13,500. Another survey of 6706 cattle at slaughter yielded no benign tumors. Due to the rarity of benign tumors in domestic species, definitive criteria of benignancy have not been well established.[8] As well-differentiated malignancies and benign tumors may overlap in histologic appearance, arbitrary criteria of 2cm diameter is often used, but tumors of this size may be problematic. Mitoses over 10 per 2.37mm suggest malignancy in the absence of more definitive criteria, including invasion and metastasis.[8]

A similar problem has been encountered in human medicine, in which metastasis has been documented repeatedly in tumors less than 3cm in diameter. Current human protocols define adenomas as tumors less than 0.5cm with densely packed tubules or papillary projections and bland nuclei with the low mitotic rate. Benign renal neoplasms are also encountered in higher incidence in cases of pyelonephritis and renal vascular disease and are reported in high frequency along with renal cysts in von-Hippel-Landau syndrome in children.[9]

Mycobacteriosis is a common, serious, and often lethal disease affecting fish and other poikilotherms in aquatic environment. As mentioned by the contributor, the ability for this species (and related species such as *Nocardia)* to survive in biofilms in recirculating systems, results in recurrent infections which are the bane of aquarists.[1]

This condition, which has appeared numerous times in poikilotherms in the WSC over the years, has played primary and secondary roles (as in this case.) The kidney appears to be one of the most common (if not the most common) organ affected along with the spleen, and granulomas are often seen as well in the liver, gills, mesentery, gonads, and choroid.[1] In the kidney, both discrete



Viscera, oscar. Acid fast stain revealing numerous acid fast positive rods 1x2 um consistent with Mycobacterium spp. (Acid-fast, 400X) (Photo courtesy of: Smithsonian's National Zoo and Conservation Biology Institute, https://nationalzoo.si.edu/animals/veterinary-care)

granulomas and diffuse granulomatous inflammation (as demonstrated in this slide) are seen.[1] The process of development of inflammatory lesions in affected fish has been studied in detail, characterized, and divided largely into temporal phases.[6] Subacute infections are characterized by diffuse infiltration of macrophages in infected organs with caseonecrotic centers. The chronic proliferative form is characterized by the formation of "hard" and "soft" granulomas, differentiated largely by the presence of fibrous connective tissue in the "hard" granulomas.[6] Calcification (as seen in this slide) occurs in more chronic lesions.[6]

A range of studies have demonstrated several paths of transmission in fish, including cutaneous wounds (common in bettas raised for export), transovarial transmission, and ingestion (demonstrated by feeding infected fish carcasses).[3] Commonly isolated species include *M. marinum, M. fortuitum, M. chelonae, M. peregrinum and M. abscessus.*[1] The moderator discussed additional differentials for the neoplasm in this case, to include polycystic kidney disease and obstructive cystic disease. The lack of epithelial attenuation within the large cysts tends to rule out obstruction as a cause, but polycystic kidney disease, remained a concern based solely on the histology. The moderator reviewed pertinent renal anatomy and histology in the fish, as well as general pathology of mycobacteriois, and a quick review of gross lesions in fish disease.

References:

1. Frasca S, Wolf JC, Kinsel MJ, Camus AC, Lombardini ED. Osteichthyes. *In* Terio KA, McAloose D, St. Leger J. Pathology of Wildlife and Zoo Animals. London, UK. Associated Press, pp. 964-966.

2. Hochwartner O, Loupal G, Wildgoose WH, Schmidt-Posthaus H. *Occurrence of spontaneous tumours of the renal proximal tubules in oscars Astronotus ocellatus*. Dis Aquat Organ. 2010. 89:185–189.
3. Jacobs JM, Stine CB, Baya Am, Kent ML. A review of mycobacteriosis in marine fish. *J Fish Dis 2009; 32(2): 119-130.*
4. Lee BC, Hendricks JD, Bailey GS. *Rare renal neoplasms in Salmo gairdneri exposed to MNNG (N-methyl-N'-nitro-N-nitrosoguanidine).* Diseases of Aquatic Organisms. 1989. 6:105-111.
5. Lombardini ED, Hard GC, Harshbarger JC. *Neoplasms of the Urinary Tract in Fish*. Veterinary Pathology. 2014. 51:1000–1012.
6. Lewis, S, Chinabut S. Mycobacteriosis and nocardiosis. *In:* Woo PTK, Bruno DW, eds. Fish Diseases and Disorders vol 3, ed. 2. Cambridge MA; CAB International 2011; pp. 397-423.
7. Noga EJ. *Fish Disease: Diagnosis and Treatment (2nd edition).* 2013. Wiley-Blackwell. 239-240.
8. Meuten DJ, Meuten TLK. *In* Meuten DJ, ed. Tumors in Domestic Animals, 5th ed., Ames Iowa, John Wiley and Sons, Inc., 2017; pp 634-638.
9. Murphy WM, Grignon DJ, Perlman EJ. *In*: AFIP Atlas of Tumor Pathology: Tumors of the kidney bladder and related urinary structures. Washington DC, ARP Press, Inc., 2004, pp.160-162.
10. Petervary N, Gillette DM, Lewbart GA, Harshbarger JC. *A spontaneous neoplasm of the renal collecting ducts in an Oscar, Astronotus ocellatus (Cuvier), with comments on similar cases in this species.* Journal of Fish Diseases. 1996. 19:279-281.
11. Racz A, Dwyer T, Killen SS. *Overview of a disease outbreak and introduction of a step by step protocol for the eradication of Mycobacterium haemophilum in a Zebrafish system.* Zebrafish. 2018. DOI: 10.1089/zeb.2018.1628.

CASE III: 19H4111 (JPC 4136173).

Signalment: Juvenile (approximately 2 months), unknown sex, bearded dragon (*Pogona vitticeps*)

History: A recently-adopted juvenile bearded dragon presented for 2-3 day history of lethargy and neurologic abnormalities (star-gazing behavior, intermittent head-tilt, inability to ambulate). Coelomic distention and a significant amount of intestinal contents were observed upon trans-illumination. Severe lethargy and depression persisted following administration of antibiotics, therefore euthanasia and post-mortem examination was elected.

Gross Pathology: The body was considered to be thin and exhibited mild autolysis. The liver was markedly enlarged, diffusely pale yellow to tan, and nodular.

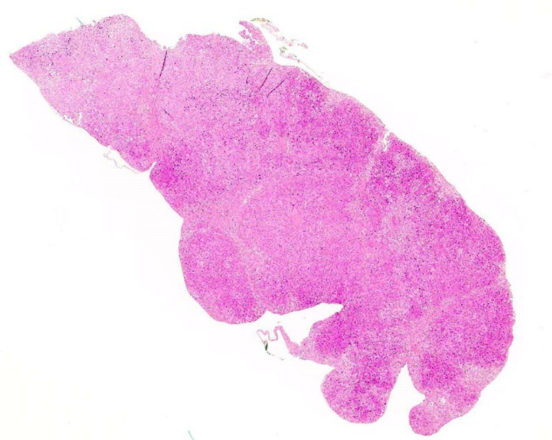
Viscera, bearded dragon. The liver is enlarged, yellow, and has a nodular appearance. (Photo courtesy of: Iowa State University, College of Veterinary Medicine, Department of Veterinary Pathology, Ames, IA 50010-1250, https://vetmed.iastate.edu/vpath)

Laboratory results:

Electron microscopy results revealed intranuclear icosahedral virions consistent with adenovirus, and smaller icosahedral virions suspected to be dependovirus. Whole-genome sequencing confirmed bearded dragon adenovirus in liver samples.

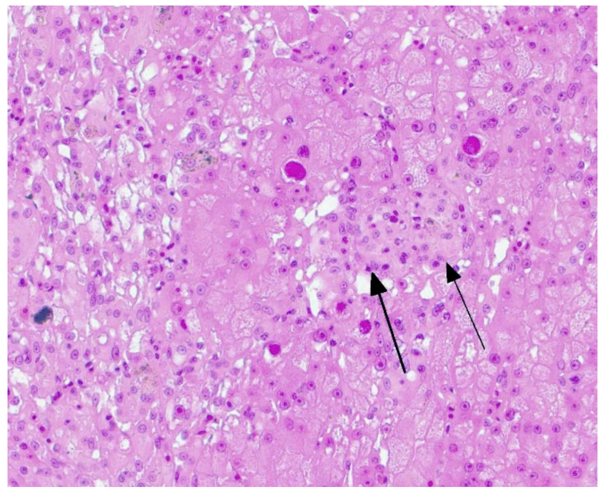
Microscopic Description: Liver: There is hepatocellular disintegration and loss with mild fibrosis and few discernable portal tracts. Diffusely hepatocytes display varying degrees of cytoplasmic microvesicular lipid type vacuolar change. Occasionally, individual or groups of hepatocytes are hypereosinophilic with pyknotic nuclei. In some areas, hepatic bile ducts are proliferative. Frequently, the hepatocyte nuclei are markedly enlarged (karyomegaly) with deeply basophilic round to irregularly-shaped intranuclear inclusions that occasionally displace the chromatin to the periphery. There are multifocal areas of hemorrhage and infiltrates of inflammatory cells consisting of heterophils, lymphocytes, plasma cells, and macrophages.

Other findings: (Not included in slide submission)



Liver, bearded dragon. The liver has a nodular appearance as a result of bands of fibrosis bridging portal areas. In addition, there are multiple areas of subcapsular hepatocellular loss and fibrosis which give the capsule and undulant appearance. (HE, 19X)

Intestine: There are moderate numbers of apicomplexan organisms in various stages of development within the intestinal mucosa. Mucosal enterocytes show intraepithelial microgametes and macrogametes. Some enterocytes appear degenerate and vacuolated with karyomegalic intranuclear basophilic inclusions. The lamina propria is infiltrated by a small number of lymphocytes, plasma cells, and heterophils.



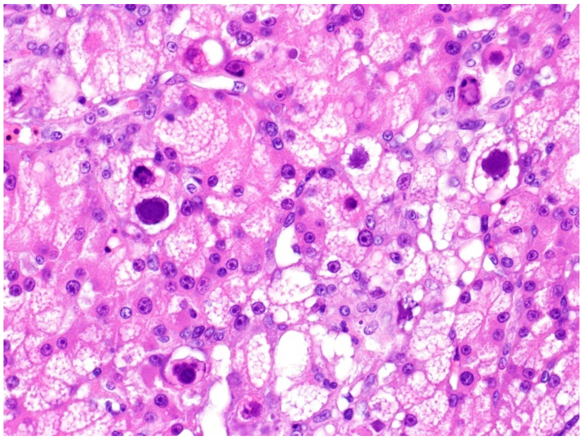
Liver, bearded dragon. At the periphery of attenuated nodules, hepatocytes contain a large intranuclear adenoviral inclusion, may be eosinophilic and rounded up, and there are nodular aggregates of macrophages (arrows). At left, hepatocytes contain small amounts of lipofuscin and melanin. (HE, 400X)

Lungs: Occasional, pneumocytes have karyomegalic nuclei with intranuclear, basophilic viral inclusions.

Pancreas: Pancreatic acinar cells occasionally have karyomegalic nuclei with intranuclear, basophilic viral inclusions.
Kidney: Renal tubular epithelium occasionally have karyomegalic nuclei with intranuclear, basophilic viral inclusions.

Contributor Morphologic Diagnosis:

Liver: Hepatitis, necrotizing, lymphocytic, random, multifocal with biliary hyperplasia, hepatocellular fatty degeneration, karyomegaly and basophilic intranuclear



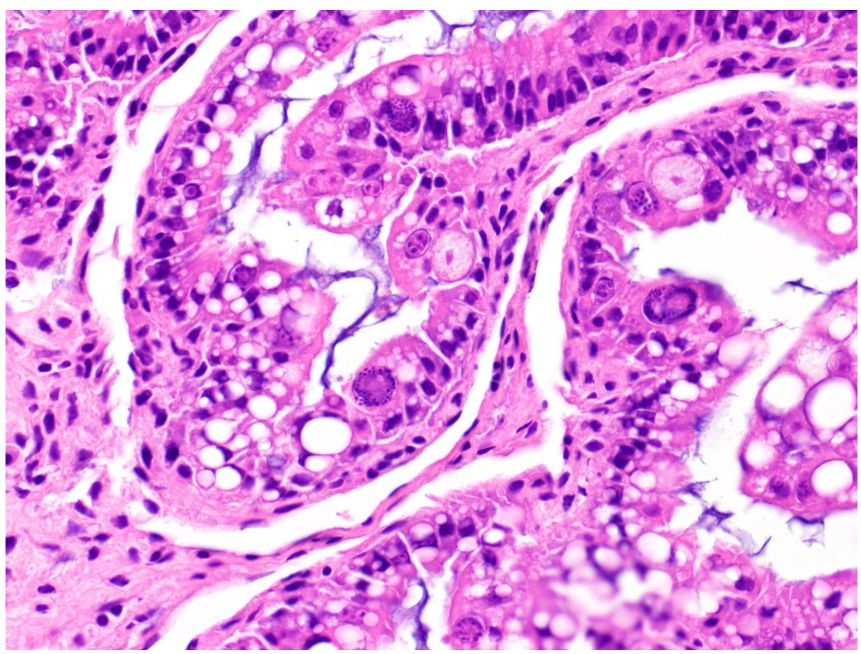
Liver, bearded dragon. Higher magnification of hepatocytes displaying adenoviral intranuclear inclusions and profound microvesicular lipidosis of hepatocytes. (HE, 400X) (Photo courtesy of: Iowa State University, College of Veterinary Medicine, Department of Veterinary Pathology, Ames, IA 50010-1250, https://vetmed.iastate.edu/vpath).

inclusion bodies, (consistent with adenovirus)

Intestine: Enteritis, lymphoplasmacytic, multifocal, moderate, with intraepithelial apicomplexans (coccidiosis) and intranuclear, basophilic inclusion bodies (consistent with adenovirus)

Contributor Comment: Adenoviruses are non-enveloped, linear, dsDNA viruses with an icosahedral nucleocapsid ranging in size from 80-110 nm. Agamid adenovirus-1 belongs to the *Atadenovirus* genera, one of five accepted *Adenoviridae* genera. It is the most widespread of atadenoviruses and has been described in other reptiles such as chelonians, snakes, crocodiles, and chameleons.[7,9] Genomic sequencing techniques have facilitated the characterization of numerous other squamate viruses within this genera, including chameleonid adenovirus 1 in chameleons, eublepharid adenovirus 1 in leopard geckos and fat-tailed geckos, helodermatid adenovirus 1 in Gila monsters and Bearded lizards, scincid adenovirus 1 in blue-tongued skinks, and snake adenovirus 1 and 2 in various snake species.[14]

Agamid adenovirus-1 is a common infection of bearded dragons (*Pogona vitticeps*), as well as other squamates. Clinical signs vary from asymptomatic or mild ill-thrift (e.g., weight loss, lethargy), to severe enterohepatic (e.g., diarrhea, weakness, anorexia) and neurological signs (e.g., head tilt, circling, opisthotonus) as well as death.[1-3,7-11] Disease is thought to manifest from stress-associated viremia, afflicting juveniles more so than adults. Agamid adenovirus-1 is most prevalent among captive breeding colonies though has been isolated from free-

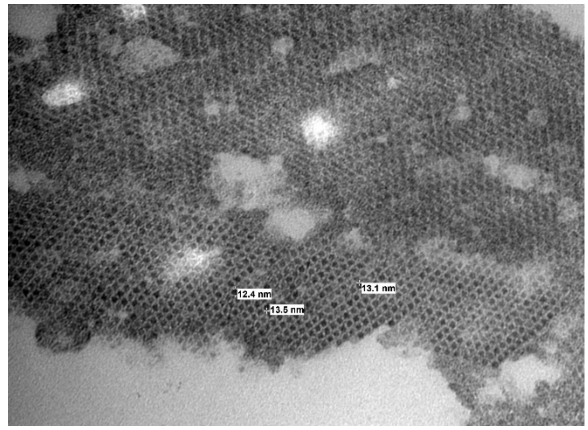


Intestine, bearded dragon. Coccidial schizonts and gametocytes are present within intestinal epithelium. (HE, 400X) (Photo courtesy of: Iowa State University, College of Veterinary Medicine Department of Veterinary Pathology, Ames, IA 50010-1250, https://vetmed.iastate.edu/vpath).

range bearded dragons, which are native to Australia.[3,6,9]

Disease caused by adenoviruses in various species is typically enterohepatic or respiratory in nature.[7,9] There are, however, exceptions to this generalization (see Table 1). Many species do not show outward clinical signs of adenovirus except in the presence of immunosuppression or another primary infection. Numerous cases of agamid adenovirus-1 are reported with concurrent coccidiosis (*Isospora* or *Eimeria* spp.) which also tend to be incidental, except in severe infestations or otherwise compromised bearded dragons.[5,8,11] Other reported comorbidities include *Cryptosporidium* spp., fungal infections, and other viruses (e.g., dependovirus).[7,12] Transmission is primarily fecal-oral, therefore, prevention is aimed at maintaining a hygienic environment and quarantining new colony additions.[9,11]

Antemortem diagnosis of agamid adenovirus-1 can be made via PCR on choanal-cloacal swabs at select diagnostic laboratories, which is preferred to serology due to lower sensitivity.[3,4,6] Postmortem diagnosis is based on presumptive karyomegalic, basophilic, intranuclear adenoviral inclusions and necrosis, particularly within the liver. Viral inclusions may be visualized within hepatocytes, enterocytes, esophageal epithelium, myocardium, endocardium, lung, renal tubular epithelium, brain glial and epithelial cells, and/or within the pancreas.[3,7,11] Other molecular techniques such as electron microscopy, in situ hybridization, and next gen sequencing are useful in obtaining a definitive diagnosis or further characterizing adenoviruses.[1,3,4,8,14] Despite this virus being typically host-specific, phylogenetic evidence supports some cross-over and viral adaptation through co-evolution in novel host species.[1,2,14]



Liver, bearded dragon. Icosahedral virions measuring 12.4 nm to 16.3 nm are seen intracellularly, compatible with dependovirus. (Photo courtesy of: Iowa State University, College of Veterinary Medicine, Department of Veterinary Pathology, Ames, IA 50010-1250, https://vetmed.iastate.edu/vpath).

Genus	Virus(es)	Reported Disease/Syndrome
Atadenovirus	Agamid adenovirus 1 Duck adenovirus 1 Cervine adenovirus 1 Caprine adenovirus 1 Ovine adenovirus 7	Hepatitis, enteritis, encephalitis Egg drop syndrome Vasculitis, hemorrhagic disease, pulmonary edema None to mild respiratory disease None to mild respiratory disease
Aviadenovirus	Fowl adenovirus 2, 8, 11 Fowl adenovirus 4 Fowl adenovirus 1 Avian adenovirus 1 Turkey adenovirus 1 & 2	Inclusion body hepatitis Hepatitis-hydropericardium syndrome virus Gizzard erosion Quail bronchitis virus Decreased egg production

	Duck adenovirus 2	Hepatitis (rarely)
Ichtadenovirus	Sturgeon ichtadenovirus A	
Mastadenovirus	Canine adenovirus 1 Canine adenovirus 2 Bovine adenovirus Equine adenovirus 1 & 2 Porcine adenoviruses 1-5 Guinea pig Rabbit adenovirus 1 Goat adenovirus 2 Ovine adenovirus 1-5 Caprine adenovirus	Infectious canine hepatitis Infectious canine tracheobronchitis Pneumonia and enteritis (secondary pathogen) Bronchopneumonia in immunocompromised (SCID) Mild respiratory disease, enteritis, encephalitis Asymptomatic, or pneumonia (high mortality) Diarrhea Severe respiratory and enteric disease in lambs None to mild respiratory disease
Siadenovirus	Frog siadenovirus A Raptor siadenovirus A Psittacine adenovirus 2 Budgerigar adenovirus 1 Gouldian finch adenovirus 1 Silawesi tortoise adenovirus 1 Turkey adenovirus 3	Hemorrhagic enteritis (turkeys), marble spleen (pheasants), avian adenovirus splenomegaly (broiler chickens)

Table 1. Adenoviruses of importance in animal species

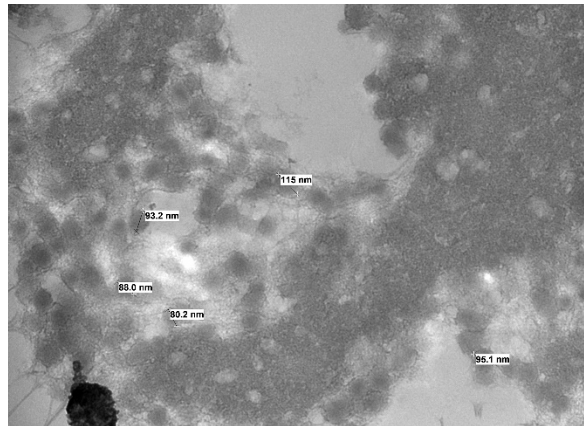
Contributing Institution:

Iowa State University
College of Veterinary Medicine
Department of Veterinary Pathology
Ames, IA 50010-1250
https://vetmed.iastate.edu/vpath

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal to coalescing, chronic, moderate with fibrosis and numerous hepatocellular intranuclear viral inclusions

JPC Comment: The contributors have done an excellent job discussing adenoviral infection in a wide range of species. Adenoviral hepatitis has made a number of appearances in the WSC over the years below. (Table 2).

In addition to the listing of infected lizards mentioned by the contributor, adenoviruses have also been documented in eastern bearded dragons, savannah and emerald monitors, Rankin's dragon lizard, central netted dragons, western striped tree dragons, green anoles, Jackson's and mountain chameleons, , common agamas, and Tokay geckos.[10] Most if not all adenoviral infections in lacertids focus primarily on the liver. Clinical signs may range from none to acute CNS signs to chronic wasting and gross findings are usually minimal. Histology in affected livers usually is that of a necrotizing hepatitis, and the biliary epithelium is also affected in some species. In addition to hepatocytes, intranuclear inclusions may be seen in a range of other cells, including enterocytes, endothelial cells, renal tubular epithelium, glomeruli, exocrine pancreas, and oral mucous membrane.[10] Proliferative changes have been reported (primarily affecting the tracheal and esophageal mucosa) in Jackson chameleons.[10] Coinfections are common, including dependoparvovirus (reported as a hepatic coinfection in bearded dragons with adenovirus)[7,10], ranavirus, iridovirus, and gastrointestinal parasites such as coccidia and helminths.[10] Nile crocodiles) also have an adenovirus which results in hepatitis as well.[7] While adenoviruses are well-known for their disease-causing effects in multiple species, including humans, adenoviruses are also on the forefront of human medicine, especially in the areas of gene therapy and vaccine delivery. As adenovirus DNA does not incorporate into the viral genome during transcription such as say retroviral DNA, they are an ideal vector for the delivery of genes or antigenic materials on a therapeutic basis.[13] An issue that faces adenovirus vectors, however, is the prevalence of antibodies to many common human adenoviruses in potential patients (arising from the mild infections of the respiratory, GI, and urinary tracts that adenovirus cause in immunocompetent individuals. The use of adenoviruses linked to animal, but not human, disease (such as chimpanzee adenoviruses) has been proposed to solve this issue, but concern about potential generation of severe cross-species disease has tempered its current usage.[13]



Liver, bearded dragon. Larger icosahedral virions measuring 80.2 nm to 115 nm are seen intranuclearly, compatible with adenovirus. (Photo courtesy of: Iowa State University, College of Veterinary Medicine, Department of Veterinary Pathology, Ames, IA 50010-1250, https://vetmed.iastate.edu/vpath).

WSC 2018-2019, Conf 21, Case 2 WSC 2013-2014, Conf 16, Case 4 WSC 1987 Conf 29, Case 2	Chicken
WSC 2018-2019, Conf 16, Case 1 WSC 2012-2014, Conf 1 Case 3 WSC 1996-1997, Conf 20, Case 4	Dog
WSC 2009-2010, Conf 9, Case 3 WSC 1995-1996, Conf 29, Case 2	Bearded Dragon
WSC 2007-2008, Conf 23, Case 3 WSC 1997-1998, Conf 29, Case 4	Falcon
WSC 1991, Conference 6, Case 2	Chimpanzee

Table 2. Adenoviral hepatitis in the WSC

The moderator reviewed the pertinent gross anatomy of the bearded dragon. The moderator gave a brief discussion of dependoviruses, which often are seen as coinfections with adenovirus, as they "depend" on the viral machinery of adenoviruses for replication.

References:

1. Bak E-J, Jho Y, Woo G-H. Detection and phylogenetic analysis of a new adenoviral polymerase gene in reptiles in Korea. *Arch Virol*. 2018: 1-7.
2. Benge SL, Hyndman TH, Funk RS, et al. Identification of helodermatid adenovirus 2 in a captive central bearded dragon (pogona vitticeps), wild gila monsters (heloderma suspectum), and a death adder (acanthophis antarcticus). *J Zoo Wildl Med*. 2019;50: 238-242.
3. Doneley R, Buckle K, Hulse L. Adenoviral infection in a collection of juvenile inland bearded dragons (Pogona vitticeps). *Aust Vet J*. 2014;92: 41-45.
4. Fredholm DV, Coleman JK, Childress AL, Wellehan Jr JF. Development and validation of a novel hydrolysis probe real-time polymerase chain reaction for agamid adenovirus 1 in the central bearded dragon (Pogona vitticeps). *J Vet Diang Invest.* 2015;27: 249-253.
5. Hallinger MJ, Taubert A, Hermosilla C, Mutschmann F. Captive Agamid lizards in Germany: Prevalence, pathogenicity and therapy of gastrointestinal protozoan and helminth infections. *Comp Immunol Microbiol Infect Dis*. 2019;63: 74-80.
6. Hyndman TH, Howard JG, Doneley RJ. Adenoviruses in free-ranging Australian bearded dragons (Pogona spp.). *Vet Microbio*. 2019;234: 72-76.
7. Jacobson ER. Viruses and viral diseases of reptiles. In: Infectious diseases and pathology of reptiles: color atlas and text. Boca Raton, FL: CRC Press; 2007:395-460.
8. Kim DY, Mitchell MA, Bauer RW, Poston R, Cho D-Y. An outbreak of adenoviral infection in inland bearded dragons (Pogona vitticeps) coinfected with dependovirus and coccidial protozoa (Isospora sp.). *J Vet Diang Invest*. 2002;14: 332-334.
9. Marschang RE. Viruses infecting reptiles. *Viruses*. 2011;3: 2087-2126.
10. Orrigi, F. Lacertilia. *In In* Terio KA, McAloose D, St. Leger J. Pathology of Wildlife and Zoo Animals. London, UK. Associated Press, pp. 875.
11. Schilliger L, Mentré V, Marschang RE, Nicolier A, Richter B. Triple infection with agamid adenovirus 1, Encephaliton cuniculi-like microsporidium and enteric coccidia in a bearded dragon (Pogona vitticeps). *Tierarztl Prax Ausg K*. 2016;5.
12. Schmidt-Ukaj S, Hochleithner M, Richter B, Hochleithner C, Brandstetter D, Knotek Z. A survey of diseases in captive bearded dragons: a retrospective study of 529 patients. *Vet Med (Praha)*. 2017;62: 508-515.
13. Tatsia N, Ertl HCJ. Adenoviruses as vaccine vectors. *Mol Therap* 2004. (10)4: 616-630
14. Wellehan JF, Johnson AJ, Harrach B, et al. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. *J Virol.* 2004;78: 13366-13369.

CASE IV: <u>19-33870 (JPC 4138045).</u>

Signalment: Adult, female Silvery Salamander *(Ambystoma platineum)*

History: This wild salamander was found dead in the water in late March at a local state park in central Illinois.

Gross Pathology: Examined is a 12.0 g adult female Silvery Salamander *(Ambystoma platineum)* in fair postmortem condition. There are numerous, discrete, 1-3 mm in diameter white, flat to slightly raised cutaneous

Skin, salamander. Multiple randomly arranged white nodules are present across the body surface. (Photo courtesy of: University of Illinois at Urbana-Champaign, Veterinary Diagnostic Laboratory http://vetmed.illinois.edu/vet-resources/veterinary-diagnostic-laboratory/)

foci scattered throughout the entire surface of the body (Figure 1). There are few discrete 1-2 mm in diameter white to tan flat foci within the subcapsular surface of the liver. The remaining viscera are unremarkable.

Laboratory results:
FV3 Ranavirus qPCR was negative.

Microscopic Description: Skin: Mostly confined within the epidermis and rarely expanding the dermis are large numbers of variably sized intraepidermal cysts (sporangia) packed with round, 5-10 µmin diameter, eosinophilic globular spores with scant crescent- shaped pale eosinophilic variably granulated cytoplasm. The sporangia mostly distend the mid to basal portions of the epidermis. There are moderate numbers of coccobacilli enmeshed in between the spores. There is minimal leukocytic reaction towards the cysts. The remaining epidermis is moderately hyperplastic with multiple segments of prominent superficial cornification.

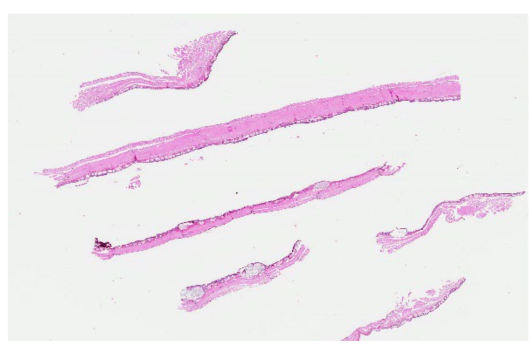
Special staining of sporangia within the liver with Grocott's Methenamine Silver (GMS) and Periodic acid-Schiff (PAS) is performed. The GMS stain highlights in black the majority of the spore's capsule and parts of the internal contents. Diffusely, the entire spore structure has strong reaction for PAS.

Other findings: (Not included in slide submission)
Intestine: There are moderate numbers of apicomplexan organisms in various stages of development within the intestinal mucosa. Mucosal enterocytes show intraepithelial microgametes and macrogametes. Some enterocytes appear degenerate and vacuolated with karyomegalic intranuclear basophilic inclusions. The lamina propria is infiltrated by a small number of lymphocytes, plasma cells, and heterophils.

Lungs: Occasional, pneumocytes have karyomegalic nuclei with intranuclear, basophilic viral inclusions.

Pancreas: Pancreatic acinar cells occasionally have karyomegalic nuclei with intranuclear, basophilic viral inclusions.

Kidney: Renal tubular epithelium occasionally have karyomegalic nuclei with intranuclear, basophilic viral inclusions.



Skin, salamander. Multiple sections of skin are submitted for examination. There are multiple irregularly round nodules which elevate the epidermis. (HE, 9X)

Contributor Morphologic Diagnosis: Skin (bodywide): Numerous intraepidermal Mesomycetozoan sporangia

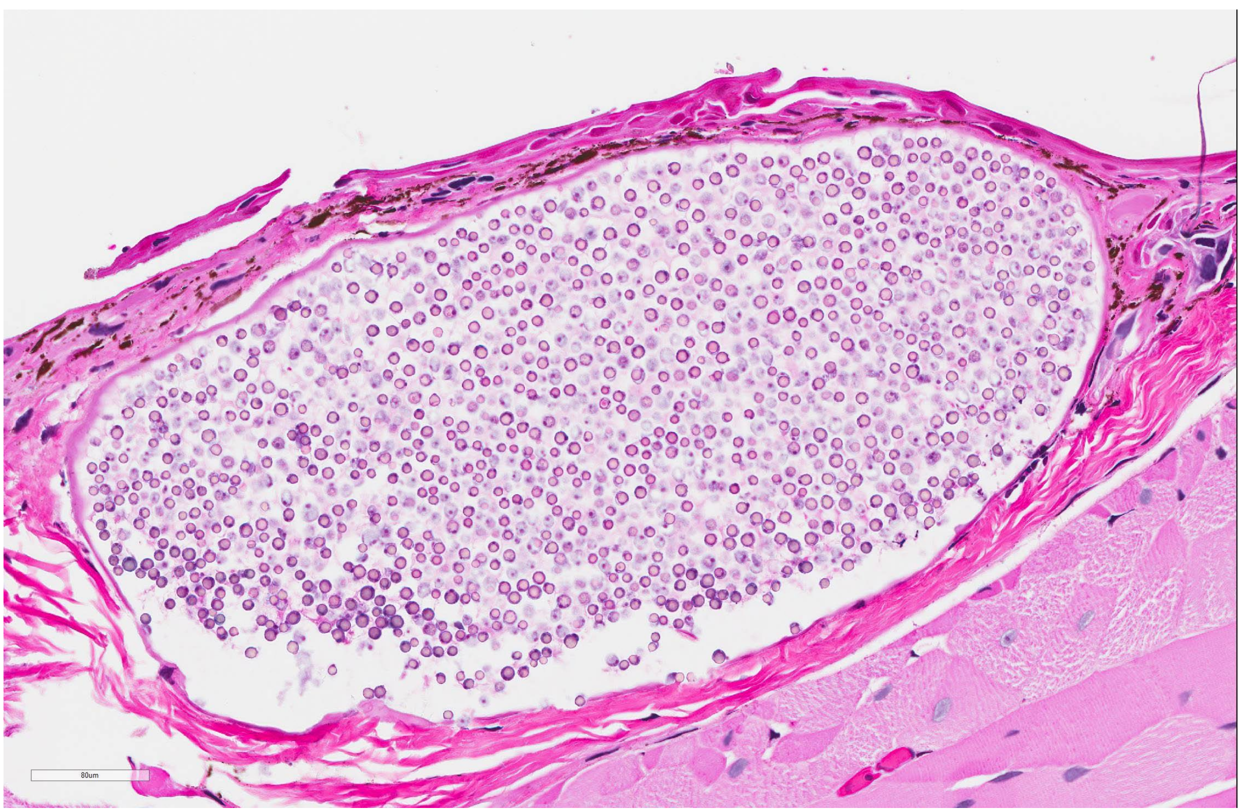
Contributor Etiologic diagnosis: Cutaneous amphiocystidiosis

Contributor Comment: The cause of death in this salamander is likely multifactorial. The presence of cutaneous amphiodiocystidiosis may be considered an incidental finding, however the concurrent visceral involvement (liver) in this case might suggest significant infection secondary to immune suppression or damaged defense mechanisms. A primary infectious etiology was not identified, so clinical deterioration due to environmental factors cannot be ruled out.

Amphibiocystidium is a genus of fungal-like parasites that cause characteristic skin nodules in amphibians of Europe and North and South America. A member of the class Mesomycetozoea, these protists arose at the animal-fungal divergence and were historically considered either protozoan or fungal.[7]

Mesomycetozoans are found in marine and freshwater environments as commensals or parasites to invertebrates, fish, amphibians, birds, and mammals.[8] *Amphibiocystidium* is closely related to the genuses *Dermocystidium,* which is a primary pathogen of salmonid fish, and *Rhinosporidium,* which causes clinical disease in birds and mammals, including humans.[7]

Grossly, *Amphibiocystidium* spp. cause multifocal, regular, 3-5 mm in diameter

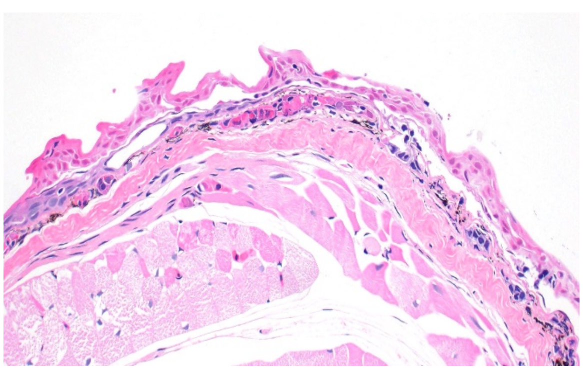


Skin, salamander. Cutaneous sporangia measure 0.3 to 0.6 mm, are bound by a thin hyaline wall, and contain numerous 10um endospores (HE, 270X)

often spherical, "C" or "U"-shaped skin nodules that are sometimes ulcerated. These lesions must be grossly differentiated from encysted parasites (particularly *Clinostomum* sp. trematodes) or granulomas caused by higher order bacteria or fungi.

Microscopically, studies report several 100-600 µm cysts (sporangia) containing myriad 2-6 µm endospores.[7] An inflammatory cell infiltrate composed primarily of lymphocytes and macrophages may be found in the vicinity of these cysts. The associated epidermis may be hyperplastic. Histologic lesions must be differentiated from unidentified alveolate protozoa observed in ranid frogs, which have been associated with mortality events in free-ranging ranid tadpoles in the United States.[8] Tissues affected by alveolate protozoa will be infiltrated by large numbers of 6-9 µm spherical basophilic spores in absence of a host inflammatory response, and spores are positive for periodic acid-Schiff (PAS), Grocott's methenamine silver (GMS), and Congo Red stains.[8]

The phylogeny of *Amphibiocystidium* is still unsettled; the genus was recently created to encompass members of the

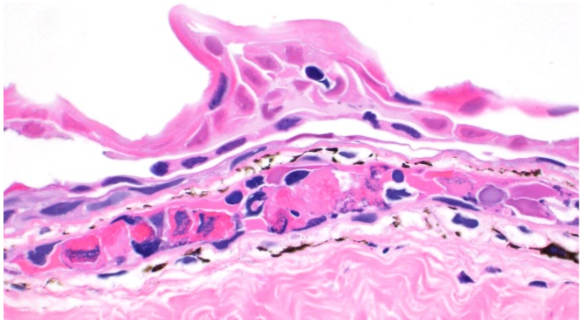


Skin, salamander. Multifocally, there are areas of epidermal necrosis in which the full thickness of the epidermis has lost differential staining and is separated from the underlying dermis. (HE, 100X)

Dermocystidium, Dermomycoides, and *Dermosporidium* spp. affecting amphibians, interpreted as monophyletic,[6] and may also include the related genus *Amphibiothecum,* a parasite of American toads *(Anaxyrus americanus).* Predominant species of *Amphibiocystidium* include A. *ranae, A. viridescens.* and A. *pusula.*[7]

Amphibiocystidium spp. have been reported to affect both anuran and caudate species in Europe, where it has been detected for over a century, and was more recently discovered in North America.[9] Although lethal infections have been documented, the large majority of reported cases are associated with low morbidity and mortality.[2] The life cycle and pathogenesis of *Amphibiocystidium* is largely unknown.[3] Although clinical challenge studies have yet to be performed, a 7-year survey of a natural population of Italian stream frogs *(Rana italica)* found no appreciable negative effects of *Amphibiocystidium* infection; however, ecological and host-specific factors influencing susceptibility were not assessed in this study.[2]

Primarily attributed to climate change and emerging pathogens such as chytrid fungus *(Batrachochytrium dendrobatidis)* and ranaviruses, the current massive decline in global amphibian populations



Skin, salamander. Higher magnification of an area of epithelial necrosis. The vessel in the dermis beneath is thrombosed and contains numerous granulocytes. (HE, 400X)

has emphasized the need to understand the myriad of other infectious agents affecting amphibian species, most of which have yet to be well-described and understood.[9,10] Some of these agents, such as *Amphibiocystidium,* have been found in amphibians co-infected with *Batrachochytrium dendrobatidis,* with several other reports implying but not confirming co-infection.[1] Many of these agents, such as *Amphibiocystidium* have unknown pathogeneses and have yet to undergo clinical studies.

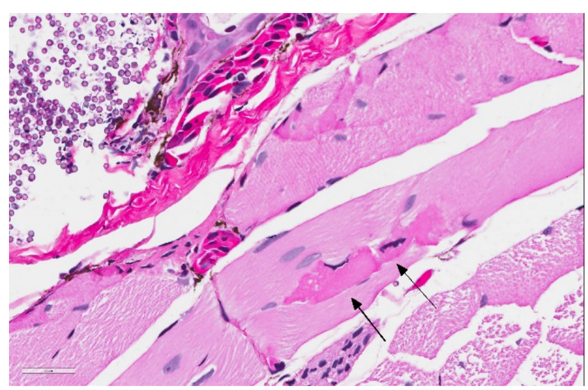
Contributing Institution:
University of Illinois at Urbana-Champaign, Veterinary Diagnostic Laboratory
http://vetmed.illinois.edu/vet-resources/veterinary-diagnostic-laboratory/

JPC Diagnosis: 1. Skin, epidermis: Numerous sporangia.
2. Skin, epidermis: Necrosis, multifocal, with vasculitis, dermatitis, and epidermal hyperplasia.

JPC Comment: The contributor has done an outstanding job in this review of *Amphibiocystidium*, an emerging mesomycetozoan pathogen. The Mesomycetozoae are a class of organisms that contain ten different genera of saprophytic and parasitic organisms (grouped by 18s ribosomal DNA analysis) which have changed in classification over the years, previously being referred to as both fungi and protists. Their grouping was initially called the "DRIP clade", an acronym composed of its initial members *Dermocystidium,* rosette agent, *Ichthyophonus* and *Psorospermium*. With the exception of *Rhinosporidium*, parasitic species primarily infect aquatic animals. Most of these agents have poorly documented life cycles.[5]

Rhinosporidium seeberi is the most well-known of the group, due to its propensity to infect higher vertebrates, including dogs, horses, and humans. It is thought that most infections arise via traumatized epithelium. Nasal polyps are the most common and identifiable lesions associated with infection and in humans (account for 70% of lesions; however, humans have demonstrated a range of other staging areas, including the palpebral and bulbar conjunctiva (15%), sclera, lacrimal sac, and genital mucous membranes. No immunity is developed against rhinosporidiosis, although rarely spontaneous regression has been noted.[4]

The term "rosette agent" refers to a unique species of mesomycetozoan (*Sphaerothecum*



Skin, salamander. There are rare necrotic myocytes scattered randomly through skeletal muscle. (HE, 340X)

destruens) which was first identified in China and appears to have been spread through the aquaculture trade to Europe and the US in association with another invasive species, its natural host the stone moroko (*Pseudorasbora parva).* It has been identified in over 4 species, including salmon, carp, and sea bass. This mesomycetozoan results in systemic and often fatal infection in aberrant hosts, but invading gills, liver, kidney, intestine, and gonads. The spores pass from infected fish in both urine and seminal fluid. The parasite has the ability to infect both salmonids and cyprinids, with great potential to impact global piscine biodiversity as well as have a profound impact on aquaculture and food security.[12]

Mesomycetozae have made a number of appearances in the Wednesday Slide Conference:
Rhinosporidium seeberi: (WSC 2017-2018 Conf 3, Case 3-horse, WSC 2016-2017 Conf 8, Case 1-horse, WSC 2010-2011 Conf 14, Case 3-horse, WSC 2002-2003 Conf 11, Case 3-dog, among others); *Ichthyophonus* (WSC 2001-2002 Conf 24, Case 4 – rainbow trout, WSC 2015-2016 Conf 13, Case 1 – red-spotted newt); *Dermocystidium* (WSC 2015-2016 Conf 13, Case 1-tetra, WSC 2010-2011 Conf 22, Case 3-koi); and *Perkinsus* (classified at the time on the shifting sands of the mesomycetozoans-but now classified as in the phylum Perkinsozoa) (WSC 2013-2014, Conf 12, Case 3 – abalone).
A pathologist from the National Zoo in attendance commented on the presence of myonecrosis in this slide and the frequency with which it is seen in fresh autopsies on amphibians, to the point that he considered these changes to be almost artifactual in this case. Several participants, including the moderator saw areas in which the epidermis was diffusely pink and without differential staining, and mildly separated with serum pooling. One area was associated with a focal area of granulocyte aggregation. Spirited discussion greeted this finding (which may not have been present on all slides), but its cause is unclear. The moderator also discussed the peculiar life cycle of this gynogenic species, in which all specimens are female, and mating with males of another species. The sperm of the male (usually blue-spotted or Jefferson salamanders) stimulates egg development, but does not contribute to the DNA of the offspring. The moderator also discussed the difficulty (per Dr. Alan Pessier of Washington State University) of definitively differentiating *Amphibiocystidium* from *Perkinsus* based on HE slides.

References:

1. Borteiro C, Cruz JC, Kolenc F, et al. Dermocystid-chytrid coinfection in the Neotropical frog Hypsiboas pulchellus (Anura: Hylidae). *J Wildl Dis*. 2014;50(1):150-153.
2. Fagotti A, Rossi R, Canestrelli D, et al. Longitudinal study of Amphibiocystidium sp. infection in a natural population of the Italian stream frog (Rana italica). *Parasitology.* 2019:1-8.
3. Gonzalez-Hernandez M, Denoel M, Duffus AJ, Garner TW, Cunningham AA, Acevedo-Whitehouse K. Dermocystid infection and associated skin lesions in free- living palmate newts (Lissotriton helveticus) from Southern France. *Parasitology International.* 2010;59(3):344-50.
4. John D, Selvin SST, Irodi A, Jacob P. Disseminated rhinosporidiosis with conjunctival involvement in an immunocompromised patient. *Mid East Afr J Ophthal* 2017; 24(1):51-53.
5. Mendoza L, Taylor JW, Ajello L. The class *Mesomycetozoea*: a heterogenous group of micro-organisms at the animal-fungal

boundary. *Ann Rev Microbiol* 2002; 56:315-244.

Pascolini R, Daszak P, Cunningham AA, et al. Parasitism by Dermocystidium ranae in a population of *Rana esculenta* complex in Central Italy and description of Amphibiocystidium n. gen. *Dis Aquat Org.* 2003;56(1):65-74.

6. Pereira CN, Di Rosa I, Fagotti A, Simoncelli F, Pascolini R, Mendoza L. The pathogen of frogs Amphibiocystidium ranae is a member of the order Dermocystida in the class Mesomycetozoea. *J Clin Microbial.* 2005;43(1):192-198
7. Pessier A. Amphibia. In: Terio, K. A., McAloose, D., & Leger, J. S, eds. *Pathology of Wildlife and Zoo Animals.* Academic Press; 2018:915-944.
8. Pessier AP. Hopping over red leg: The metamorphosis of amphibian pathology. *Vet Path*. 2017;54(3):355-357
9. Pessier AP. Management of disease as a threat to amphibian conservation. *International Zoo Yearbook*. 2008;42(1):30-39.
10. Raffel TR, Bommarito T, Barry DS, Witiak SM, Shackelton LA. Widespread infection of the Eastern red-spotted newt (Notophthalmus viridescens) by a new species of Amphibiocystidium, a genus of fungus-like mesomycetozoan parasites not previously reported in North America. *Parasitology.* 2008;135(2):203-215.
11. Sana S, Harodouin EA, Gozlan RE, Ercan D, Tarkan AS, Zhang T, Andreou D. Origin and invasion f the emerging infectious pathogen *Sphaerothecum destruens.*