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CASE I: ITPA BERN 2011/1 (JPC 4002421).

Signalment: 2 cichlids (order *Perciformes)*, male, *Neolamprologus multifasciatus*

History: Two moribund cichlids with poor general body condition were submitted to the Center of Fish and Wildlife Medicine (FIWI) Berne, Switzerland.

Gross Pathology: The main macroscopic findings at necropsy were:
Eyes: unilateral corneal opacification (ca. 3mm in diameter) and exophthalmos
Skin: 2 ulcerations on the head (ca. 2cm and 1mm in diameter)
Gallbladder: congestion
Spleen: severely enlarged, with round margins, scattered white pinpoints through the parenchyma
Intestine: low amount of whitish pasty content

Laboratory Results: Parasitology: negative
Bacteriology: mixed flora with *Aeromonas sobria* in kidney, spleen, liver, skin

Histopathologic Description: stomach, intestine, liver, spleen

Stomach, focally extensively, the mucosa is replaced by moderate amounts of cell and nuclear debris (necrosis), large amounts of macrophages with abundant foamy cytoplasm, lymphocytes and plasma cells intermixed with multiple protozoan parasites. These parasites are pear-shaped, 4x10 µm, with one to two apical nuclei and two visible flagella on the other site (interpreted as *Cryptobia iubilans*). The same flagellates are also located in groups of up to 10 in the lamina propria, submucosa and serosa surrounded by aforementioned inflammatory cells and necrotic material. There is moderate proliferation of fibroblasts.

Multifocally to coalescing, the lamina propria, the submucosa and the serosa are severely thickened due to accumulation of large amounts of macrophages and lymphocytes, few plasma cells and eosinophil-like cells. Multifocally, mainly in the submucosa, granulomas are formed with central necrosis, marginating epithelioid macrophages, lymphocytes and fibroblasts.

In the adjacent perivisceral fat tissue and the spleen there are the same granulomas formed.

In one section of liver, multifocally in the bile ducts, there are elongated structures with a basophilic membrane, granulated cytoplasm and multiple internal oval to pear shaped structures, 2x4 µm, with one to two nuclei (myxozoan plasmodia).

Contributor's Morphologic Diagnosis: Stomach, ulcerative and granulomatous gastritis, focally extensive, severe with intralesional flagellates.

Mesenteric fat tissue, granulomatous steatitis, multifocal, moderate

Liver, myxozoan plasmodia in bile ducts

Contributor's Comment: Histological examination of the affected fish revealed various degrees of granulomatous gastritis and many granulomas involving different organs, including the kidney, spleen, liver, mesentery, mesenteric fat, swim bladder, eye, heart and gonads. The etiology of these lesions is consistent with the flagellate *Cryptobia iubilans,* which is an important parasite of cichlids that typically induces granulomatous disease, primarily involving the stomach.

The ovoid or elongated body, 19x5 µm, has an anterior flagellum 1.5 to 2 times body length extending from the flagellar pocket, which is shielded by a mobile anterolateral protrusion of the anterior end, the rostrum, appearing as a small lobe and evidently supported by the microtubules of the preoral ridge.[1] It tends to be more elongated in early infection and more oval, tear-drop shaped or round in chronic infections.[2]

In transmission electron microscopy the flagellates are present in vacuoles (parasitophorous vacuole) in host cells. They are members of the order *Kinetoplastida* based on the detection of a kinetoplast, paraxial rod (lattice-like structure along the axoneme in the flagellum), and a cytoskeleton composed of microtubules lying beneath the body surface.[2]

Granulomatous gastritis caused by *Cryptobia iubilans* has previously been reported in 15 species of Old World and New World cichlids. As differential diagnoses for granuloma formation, mycobacteriosis, fungal, rickettsial, other parasitic (amoeba, nematodes) infection or foreign bodies could be considered. In this case, Ziehl-Neelsen special stain for acid-fast bacteria was negative. Flagellates commonly found in the intestine of many cichlid species are members of the order *Diplomonadida*, family *Hexamitidae*, including *Spironucleus* spp and *Hexamita* spp. They are typically found in the lumen of the intestinal tract and do not incite a granulomatous response.[2]

Morbidity and mortality rates in an infected population of fish appear to be linked to many environmental and biological variables such as water quality, the presence of other parasites or bacteria, diet, species, and age of fish. There are no effective clinically proven treatments against *C. iubilans* infection.

In salmonids, the haemoflagellate *Cryptobia salmositica* causes a microcytic and hypochromic anaemia and the severity of the disease is directly related to the parasitemia.[3]

In salmonids susceptible to *Cryptobia salmositica,* a generalized inflammatory response with severe lesions in connective tissues and in the reticuloendothelial system, as well as

microlesions appearing in the liver, gills, and spleen at 1–2 weeks after infection, followed by endovasculitis with mononuclear cell and extravascular parasite infiltrations was described.[4]

JPC Diagnosis: Stomach: Gastritis, granulomatous, transmural, multifocal, severe, with numerous intracellular and extracellular flagellates.

Conference Comment: The multiple granulomas in the stomach are extensive and coalesce and replace over half the normal architecture in some sections. .In this case, the organisms are more readily apparent within the mucosal epithelium than within areas of inflammation. Often in severe infections that lead to death of the fish, no identifiable organisms are present at necropsy, as they have likely been killed and cleared by the inflammatory cells. It is akin to the pauci-bacterial lesions of *Mycobacteria* spp. in which much of the tissue destruction occurs due to the extensive inflammation long after the acid-fast organisms are removed from the tissue.

Cryptobia spp. infections in fish colonies have severe consequence for the aquarist, whose often only option to combat the disease is to depopulate, as there are no treatments yet clinically proven to be effective.[2] Relatively little is known about this recently reported parasite of African cichlids. While *C. iubilans* is considered to be pathogenic, some researchers consider it a facultative organism which capitalizes on stressful conditions such as poor water quality or other parasitism.[2] Granulomatous gastritis is the most commonly reported lesion, but granulomatous disease is often observed in multiple organ systems simultaneously. Within some sections in this case, granulomas are present in the spleen and liver, while pancreatic atrophy and testicular degeneration is also often apparent. The second parasite described by the contributor is a myxosporean, of which many have an evolved relationship with their host and thus do not result in disease. The myxozoan was not observed on most participants' slides.

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CASE II: 13-8143 (JPC 4034291).

Signalment: 20 whole barramundi fingerlings (*Lates calcerifer)* submitted. All approximately 60 days old and male. 5000 fingerlings at risk, 1-200 sick and 15-20 dead.

History: Following a recent large mortality event of up to 10,000 fish, the surviving fish were graded based on size. Three days prior to submission to the SVDL, a proportion of fingerlings began to lose interest in feed and die.

Gross Pathology: No visible gross lesions evident, other than mild darkening. Many showed flaring of mouth and gill covers

Laboratory Results: None

Histopathologic Description: Fish, gills; Bilaterally and diffusely, there is marked distortion of normal gill architecture with marked blunting and fusion of lamellae. Interlamellar, extensive epithelial hyperplasia is present in most filaments. Multifocally, discrete colonies of small, 0.5um punctate, basophilic organisms markedly expanded individual epithelial cells, surrounded by a clear halo and with margination of the epithelial cell nucleus.

Contributor's Morphologic Diagnosis: Gill; branchitis, proliferative, diffuse, marked with fusion and blunting of lamellae and discrete bacterial colonies.

Contributor's Comment: "Epitheliocystis" is an intracellular infection of gills, and less commonly skin, caused by a genetically diverse group of gram-negative organisms belonging to the order *Chlamydiales*.[12,6] First recognized as a bacterial disease and named in 1969,[8] epitheliocystis has been reported in over 50 freshwater and marine species of teleosts,[12] elasmobranchs[3] and chondrosteans.[7] It is characterized by enlarged host cells, each containing a granular basophilic inclusion of organisms. Infections occur in skin, mouth and gill epithelial cells, including pavement cells,[10] chloride cells, goblet cells,[5] mucous cells, macrophages and pillar cells.[12] The organism's preference for chloride cells has been hypothesized to be related to its demand for energy. In contrast, it has also been suggested that the increased number of mitochondria in infected pavement cells could result in incorrect identification of pavement cells as chloride cells. On HE-stained sections, infected cells are morphologically obscured by the inclusion and identity is based on location in the gill.[10] The wide range of target cell types could be due to the specific preferences of the many different epitheliocystis-causing organisms and variations between host species.

Epitheliocystis is often considered an incidental finding.[5,1] However the prevalence, associated pathology and resultant mortality are highly variable among fish species and geographical locations. Risk factors associated with epitheliocystis infections include a higher morbidity and mortality in cultured fish, with losses up to 100%,[12] and seasonal variation related to water temperature.[12,13] Incidental epitheliocystis has been reported in Australian cultured barramundi.[1,11] A recent study of epitheliocystis in striped trumpeter *Latris lineate*, indicated a significant elevation of serum osmolality and lysozyme activity in infected fish, and this correlated also with the densities of infected cells in individuals. These findings indicate a measurable pathophysiological effect of epitheliocystis on the host. [10]

The etiological agents of epitheliocystis have not successfully been cultured in vitro, despite using a range of media and cell lines,[9] thus limiting our knowledge of this disease.[12, 10] Molecular

studies have identified a large variety of causative agents, however not all of these have included *in situ* methods for confirming the agents relation to the intracellular inclusions.[15] Agents identified by 16S rDNA include *Candidatus Piscichlamydia salmonis* in Atlantic salmon (*Salmo salar*)[4] and *Candidatus Parilichlamydia carangidicola* in Yellowtail kingfish (*Seriola lalandi*)[14], with sequences consistent with rRNA of *Chlamydiales* isolated from epitheliocystis in leafy seadragon (*Phycodurus eques*),[11] silver perch (*Bidyanus bidyanus*),[11] grass carp (*Ctenopharyngodon idella*)[8] and barramundi (*Lates calcarifer*)[11]. Mixed infections occurring in the same fish species have also been reported.[13]

A review by Nowak and associates (2006) revealed the most reliable test for epitheliocystis is histology. In some cases it may also be seen grossly or on wet preparations, however these techniques are not as sensitive.[12]

JPC Diagnosis: 1. Gills: Lamellar epithelial hyperplasia and hypertrophy, with multifocal lamellar fusion and numerous coccobacilli.
2. Skin, branchial cavity: Epithelial hyperplasia, diffuse, mild, with extracellular protozoans.

Conference Comment: This is a nice case of epitheliocystis, as the distinctive granular appearance of the bacterial colonies are well represented. The gill lamellae are often blunted, fused and thickened by a mixture of inflammatory cells and epithelial hyperplasia. Most participants believed the epithelial component predominated which is reflected in our diagnosis.

The specific cause of epitheliocystis remains elusive, and these bacterial colonies can often be observed without any other apparent pathology. A true causal relationship has not necessarily been demonstrated. Adding interest to this case, there are numerous flagellated protozoans along the skin surface and occasionally within the branchial cavity. These are most consistent with *Ichthyobodo necator*, an important parasite of hatcheries which is capable of producing significant pathology of the skin, gills and fins. This parasite is found in both fresh water and marine species and have been known to induce T cell and IgT lymphocyte depletion in the skin under experimental conditions.[2] Whether its presence here is related to the gill pathology was a point of conjecture among conference participants and is left for speculation among our readers.

Contributing institution: www.dpi.nsw.gov.au

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CASE III: 50646 (JPC 4048506).

Signalment: Adult, male, African bullfrog (*Pyxicephalus adspersus*)

History: Found dead. Animal caretakers noted some redness near the cloaca.

Gross Pathology: There were no relevant gross findings.

Laboratory Results: none

Histopathologic Description: The epidermis ranges from multifocally to diffusely hyperplastic with a thick layer of hyperkeratosis. The basal layers are multifocally disorganized with loss of orientation. The stratum corneum frequently contains multiple stages of fungal organisms (all stages may not be present in all sections). There are 10 micron zoosporangia containing numerous 2-3 micron diameter basophilic zoospores, and 5-15 micron thalli. Zoosporangia rarely have a funnel or discharge tube. Thalli include various forms: uninucleate thalli, larger multinucleated thalli, and empty thalli with fine internal septations (colonial thalli). There is multifocal widespread necrosis of the epidermis. There are few granulocytes within dermal glands. Within the superficial epidermis there is multifocal secondary invasion by bacteria and fungal hyphae.

Contributor's Morphologic Diagnosis: Skin: Severe chronic multifocal hyperkeratosis, hyperplasia, dyskeratosis, and necrosis with intralesional fungal thalli and luminal zoospores, and superficial secondary bacteria and fungal hyphae (etiology: *Batrachochytrium dendrobatidis*)

Contributor's Comment: *Batrachochytrium dendrobatidis* (Bd) is a type of chytrid fungus that is pathogenic to a wide range of amphibian species and is an important contributor to amphibian morbidity and mortality as well as loss of diversity. Chytrids are a large phylum of fungal organisms that are predominantly involved with degradation of plant and animal matter, and, unlike Bd, most chytrids are not pathogenic.[5]

Bd only affects keratinizing stratified squamous epithelium, which includes the mouthparts in tadpoles and the skin in adults. Clinical signs are variable and may include anorexia, lethargy, reluctance to place the ventrum on substrate, or even loss of righting reflex. Gross lesions most commonly occur on the ventrum and feet. Lesions include excessive skin shedding (dysecdysis), skin discoloration, and roughening of the skin. In tadpoles, depigmentation of the jaw sheath is commonly seen. Lack of gross lesions does not rule out chytridiomycosis, as was seen in this case.[5]

Histopathologic lesions of Bd include hyperplastic and hyperkeratotic epithelium containing the distinctive thalli within the keratin layers. There are 3 morphologic types of thalli which can range from 7 - 20 microns in diameter. Zoosporangia are the mature form, which contain 2 - 3 micron diameter basophilic zoospores and occasionally a flask-shaped discharge tube. Other types of thalli include a uninucleate stage with homogenous basophilic cytoplasm, and a multinucleated thallus with stippled to microvacuolated cytoplasm. Empty thalli with fine internal septations (colonial thalli) are zoosporangia that have discharged their zoospores and are a common finding. In many cases the empty thalli may be the only indication of Bd. Periodic acid-Schiff may be helpful in highlighting empty thalli, which could be interpreted to be an artifact in hematoxylin and eosin stained sections. Rhizoids are thin, root-like extensions from thalli and may be visible in silver-stained sections. Inflammation is usually minimal to absent. Secondary bacterial, fungal, and oomycete infections are common.[5]

The life cycle of Bd is well described. The zoospore is the infective stage and is released from a mature zoosporangium through the discharge tube. Transmission of the infectious motile flagellated zoospores occurs via direct animal contact or contact with water or substrates contaminated by affected animals. The zoospores become encysted in a new keratinocyte to

develop into a thallus. Cleavage of the thallus results in the development of new zoospores within a zoosporangium. All stages can be found in the keratin layers of an infected animal.[5]

Infection with chytrid fungi lead to morbidity and mortality through the disruption of both innate and cell-mediated immunity and loss of the physiologic function of the skin. The normal skin flora, including *Janthinobacterium lividum,* produce the antifungal toxin, violacein, which may be important for innate immunity.[2] Cell-mediated immunity may also be affected by toxins released from the fungus that inhibit T and B lymphocyte proliferation and cause lymphocyte apoptosis.[1] The major contributing factor to death in affected animals is disruption of normal cutaneous function. The skin of frogs is important for water absorption, osmoregulation, and respiration. In experimental infections, frogs became hyponatremic, hypokalemic, hypomagnesemic, and hypochloremic compared to controls. Disruption of cutaneous electrolyte transport is considered responsible.[6] As in this animal, secondary fungal and bacterial infections leading to epidermal necrosis may also contribute to death.

Other methods for the diagnosis of chytridiomycosis include cytology and real-time polymerase chain reaction (PCR). Cytology may be useful in identification of thalli or zoospores in shed skin or a skin scraping; however, differentiation from yeasts and oomycetes can be difficult. Confirmation by histopathology or PCR may be necessary. PCR is most commonly used for screening animals in quarantine or prior to translocation, surveying captive and free-ranging populations, and confirming a positive result on wet mount or cytology. PCR is highly sensitive and may help identify subclinical cases.[5]

In addition to the lesion presented in this case, sections also demonstrate the Eberth-Kastschenko (EK) layer that is characteristic of many frog species. The EK layer is a deeply basophilic layer of calcium deposits within the dermis. The function of this layer is unclear but speculated to be involved with protection from dessication, storage and mobilization of calcium, or the interchange of substances between the animal's internal and external environment.[3]

JPC Diagnosis: Skin, epidermis: Necrosis, hyperplasia and hyperkeratosis, multifocal to coalescing, moderate, with numerous fungal thalli and zoospores.

Conference Comment: This is an excellent example of chytridiomycosis, the all-important fungus associated with the declining population of amphibians. The keratinized epidermal crust is especially fragile and easily lost during processing of amphibian skin, and yet it is required to make this diagnosis. *B. dendrobatidis* is only found within this layer, illustrating the importance of delicate tissue handling during necropsy and tissue trimming. The additional presence of fungal hyphae seems to be most associated with an area of coagulative epidermal necrosis, indicating these are likely secondary invaders and we elected not to include them into our morphologic diagnosis.

The contributor provided extensive detail regarding the clinical presentation and pathogenesis of this entity. The discussed electrolyte disturbances induced by the loss of skin function often results in death due to cardiac arrest.[4] Interestingly, some species appear to be resistant to infection, including the well-studied *Xenopus laevis* which has contributed significantly to the understanding of the immune system's role in evoking this resistance. The immunoglobulins IgG and IgY secreted within the mucus from the skin appear to play a prominent role in providing

protection. Their innate immune system also prevents colonization through the release of antimicrobial peptides, whose secretion is dramatically increased during periods of stress.[4]

Contributing institution: Wildlife Disease Laboratories, Institute for Conservation Research, San Diego Zoo Global; http://www.sandiegozooglobal.org

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CASE IV: 09N1672 T2 (JPC 3166542).

Signalment: 2.5-year-old male Lined Seahorse, *Hippocampus erectus*

History: This seahorse initially presented for a 3 week history of multiple bubbles on the tail. No changes in behavior or appetite were noted. The three other seahorses in the aquarium were unaffected. All four seahorses were kept in an indoor 40 gallon glass hexagonal tank with 60 lbs of live rock, crushed coral and sand substrate, and sea grass. The water temperature was kept at 67^{o} F. Tap water was used to make salt water. Water quality was maintained by a large filter, protein skimmer and a 20-30% water change every 2 weeks. The aeration unit was a pump with a submerged airstone. The diet was composed of frozen shrimp. On physical exam, the seahorse had inappropriate orientation with its tail floating above the head. At the distal half of the tail, there were five randomly scattered regions where the skin was lifted above a gas-filled space (bubble). Two cavities were aspirated for culture and the rest were reduced with a #28 needle. Bacterial culture from one aspirate was negative and from the other aspirate, small numbers of mixed growth grew including fastidious gram-negative bacteria. No bacteria or protozoa were seen on skin scrapes. A diagnosis of internal gas bubble disease was made and the patient was discharged on ceftazadine (20mg/kg, IM, every three days). Two weeks after the initial presentation, the seahorse was reevaluated. At this time there were more and larger bubbles on the tail, the seahorse was floating upside down, and was no longer feeding. One of the other seahorses in the tank had begun to show similar signs. The seahorse and 3 cohorts were treated with ceftazadine (20mg/kg, IM, every three days). A week later the seahorse was euthanized at home by the owner.

Gross pathologic findings: The adult male

lined seahorse weighed 20.7 g, was in good post-mortem condition and had adequate adipose stores. Five subcutaneous gas-filled bubbles were present on the ventral aspect of the distal half of the tail, measuring (cranial to caudal): 0.4 x .03 x 0.2 cm, 0.6 x 0.5 x 0.4 cm, 0.5 x 0.4 x 0.3 cm, 0.5 x 0.5 x 0.4 cm, and 1.1 x 0.6 x 0.5 cm. The swim bladder was severely distended and displaced the esophagus and stomach ventrally and compressed the kidneys dorsally. The intestine was markedly distended and displaced the liver ventrally and to the left.

Laboratory results: No bacterial organisms were seen on direct smear nor cultured from a coelomic cavity swab or liver sample.

Histopathologic description: In the examined longitudinal section of the tip of the tail, multiple 1 to 5mm clear spaces surrounded by variable amount of inflammation expand and compress the dermis, subcutaneous tissue and skeletal muscle. The clear spaces are variably lined by flattened elongated cells (presumed fibroblasts) or a combination of histiocytes, lymphocytes and infrequent multinucleate giant cells. Most spaces are empty. Less than 10% of the spaces contain scattered, radiating aggregates of eosinophilic, thin (<1μm diameter) up to 10 μm in length, beaded, filamentous bacteria that are surrounded by a 2-5 cell-thick rim of macrophages. In the deep dermis and subcutis between the clear spaces, there are dense, similar inflammatory cells that surround dilated blood vessels and are intermixed with haphazardly arranged plump fibroblasts. Focally, the inflammation extends to a central fragment of vertebral bone that has an irregular, scalloped surface lined by osteoclasts (bone resorption). Multifocally the epidermis is hyperplastic.

Contributor's morphologic diagnosis:
1. Tail (dermis, subcutis, skeletal muscle): Multifocal gas bubbles with chronic granulomatous cellulitis, and focal boney remodeling.
2. Subcutis: Granulomatous cellulitis with intalesional gram-positive filamentous bacteria

Contributor's Comment: Gas bubble disease (GBD) is a traumatic condition that can generically be referred to as a gas entrapment disorder.[3] The obvious gross lesions, gas-filled bubbles in the tissue, give the entity its name. In Syngnathids and sea horses in particular, gas bubble disease is a classic and frequently recognized entity.[1,2] In a 2005 review of syngnathids in public aquaria, *H. procerus, H. ingens, H. subelongatus* and *H. fisheri* were reported to be unusually susceptible to developing gas bubble disease.[2] Though susceptibility is more likely defined by housing and husbandry conditions rather than species susceptibility, *H. fisheri* is considered not suited for long-term display in aquaria because its particular susceptibility may be due to a need for access to deeper waters in order to maintain homeostasis.[2] Common presentations include gas entrapment in the brood pouch of male seahorses, subcutaneous emphysema, over-inflation of the swim bladder, and/or abnormal swimming, body posture or loss of neutral buoyancy. Subcutaneous emphysema most commonly presents as grossly visible bubbles in the tail and along with brood pouch over-inflation, are the most frequently observed gas entrapment problems.[1,2] Treatment of this disorder includes direct aspiration of the bubbles and administration of a carbonic anhydrase inhibitor, acetazolamide, and/or an antibiotic, ceftazidime.[1] Some institutions have had temporary success in reducing gas bubbles by increasing the barometric pressure (dropping the seahorses to a depth of 4 meters).[1] To date, the association between gas super-saturation and/or infectious agents and the development of gas bubble disease in seahorses has not been proven.[1,2]

The rare published reports of the histopathologic correlates have described gas-filled pseudocysts surrounded by varying amounts of fibrosis and granulomatous to histiocytic inflammation.[2] (personal communication, A. Nyaoke) No infectious organisms, unlike in this case, were described in association with the lesions. In this case, we suggest that the filamentous bacteria were an opportunistic infection, potentially introduced during the needle-aspirate reduction of the gas filled spaces and may explain the success of using antibiotics as an adjunct therapy to gas-reducing methods. In support of this theory, the deeper gas filled spaces, such as those within the muscle, did not contain bacteria. The filamentous bacteria were not recovered on culture, likely due to sampling site (coelomic cavity and liver), and were gram-negative and Fites acid-fast negative.

In general, in teleost fish and other aquatic species, gas bubble disease is associated with supersaturation of the water with nitrogen or oxygen and can be caused by anything that alters the gas saturation of the water, such as leaks in the pump or valve systems, sudden temperature gradients, altitude changes during air transportation and/or rapid barometric changes most often associated with collection of specimens at depth.[3] The disease is particularly important in cultured fish and amphibians;[4,5] however, wild fish are occasionally affected with reported cases in several fresh and marine species.[3] Some of the cases in the wild have been associated with heavy macroalgal blooms that were considered responsible for excessive levels of dissolved gases and subsequent disease. Fish often die of GBD without overt clinical signs. Occlusion of the large branchial vessels by gas emboli with endothelial damage and thrombi formation is considered the principal cause of acute mortality.[3,6] When clinical signs are present in teleost fish, they range from bilateral exophthalmia, subcutaneous emphysema, gasping behavior, hemorrhages, and anomalous swimming or loss of neutral bouyancy.[3,5]

JPC Diagnosis: 1. Tail, skeletal muscle: Cavitary pseudocyst, consistent with gas bubbles in tissue. 2. Tail: Cellulitis, granulomatous, chronic, multifocal, with granulation tissue and numerous bacilli.

Conference Comment: This is an intriguing case that initiated the exchange of theories among conference participants regarding the origin of the cysts in this seahorse, as there seemed to be three varieties present. The first occurring most prominently are the empty lumens lined by a layer of inflammatory cells which most believed to be gas-filled pseudocysts within the subcutaneous tissue. Occasional cysts in most sections appeared to be lined by endothelial cells leading some to conclude they were gas-distended lymphatics. Finally, as the contributor mentioned, the inflammatory infiltrate surrounded filamentous bacteria and, in some sections, encompassed much of the cystic space lending credence to speculation these were either primary granulomas or secondary bacterial infections. The hypothesis proposed by the contributor is quite plausible given the history of aspiration in this case.

In rare slides, there is a section of bone likely representing the vertebrae of this animal. When present, the bone edges are scalloped and often lined by activated osteoclasts consistent with active bone remodeling. This seems to provide further evidence in support of a chronic bacterial infection, which may correlate with the initial positive bacterial culture of this area.

Considerable information has been derived from the lifecycle of seahorses following the development of commercial rearing facilities. They are peculiar creatures with the responsibility

of fertilization and incubation of the young taken on by the males following a monogamous relationship with a single female. In addition to vibriosis and mycobacteriosis, gas entrapment problems are a major health issue for seahorses in culture and their relationship with gas supersaturation and infectious agents is largely unresolved.[1]

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