Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2014-2015

Conference 9

9 November 2014

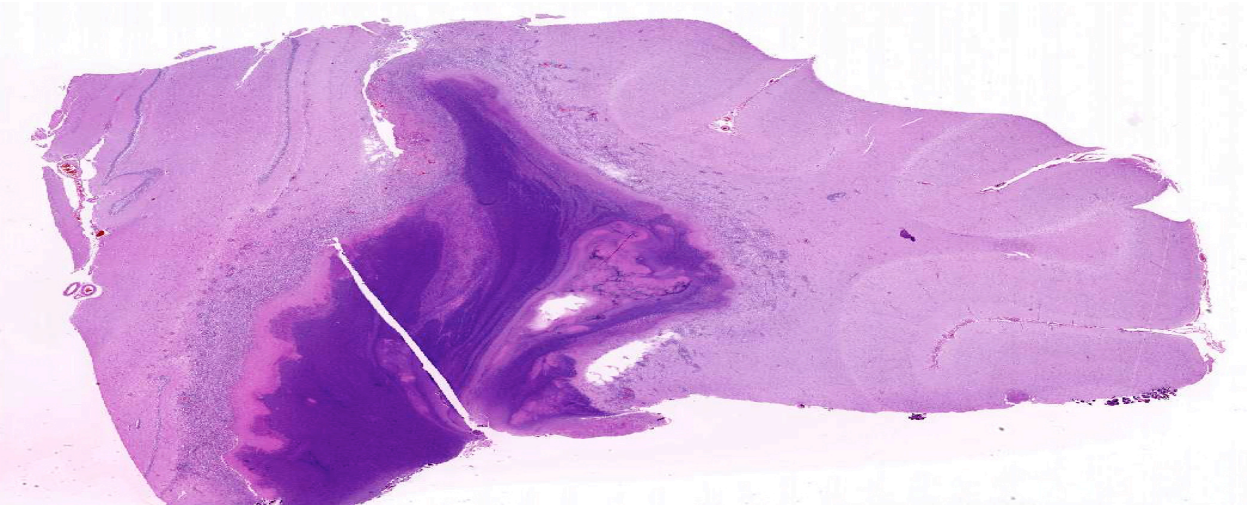
CASE I: AFIP-2 (JPC 4004343).

Signalment: Male white-tailed deer, age unknown, *Odocoileus virginianus*.

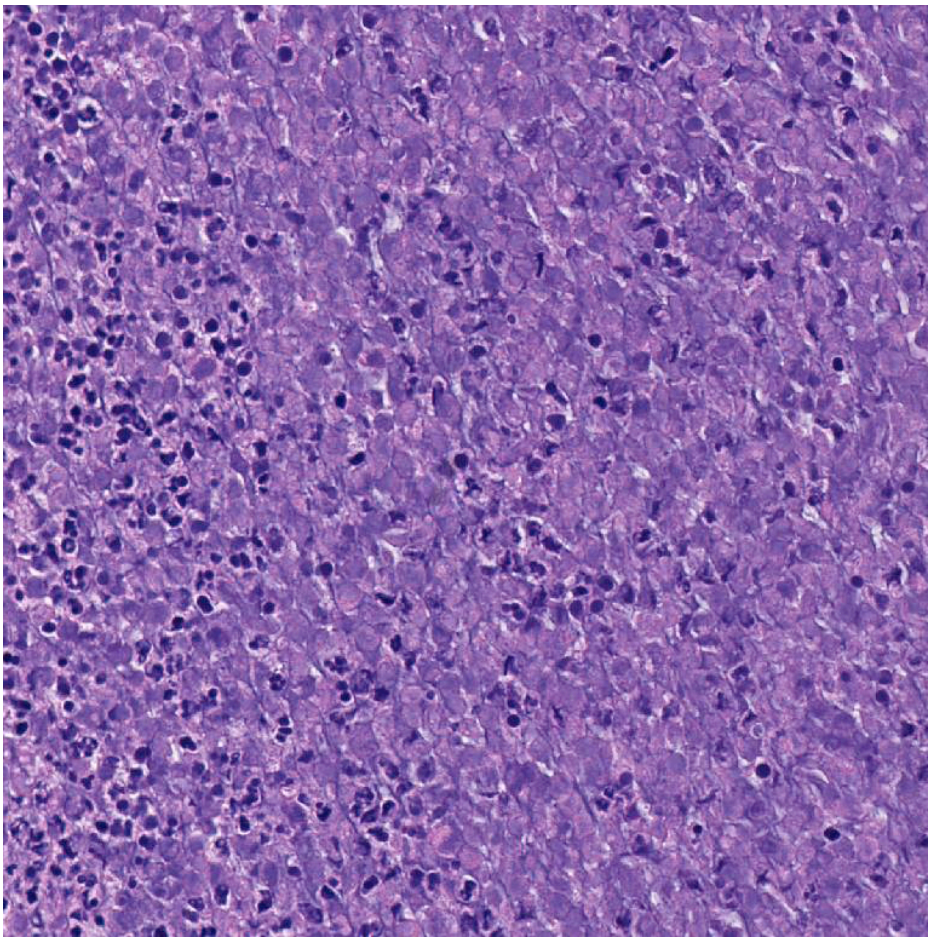
History: The animal has been submitted to necropsy with a history of CNS signs ("Disoriented, weak, antlers caught in shrub").

Gross Pathology: The gross pathology of the deer examined revealed inadequate body fat and 2.5 cm abscess filled with pus in the center of the left half of the cerebrum. No other gross lesions have been observed in the other organs of the deer examined.

Laboratory Results: The antigen ELISA for CWD was negative, *A. pyogenes* and *P. multocida* were isolated from the brain, and the result of fecal float showed rare strongyle-type ova and *Strongyloides*. No other ancillary tests have been performed on other organs.



1-1. Cerebrum, deer: Centrally within the section, there is a focal, well-demarcated cellular infiltrate. (HE 6.3X)



1-2. Cerebrum, deer: The cellular infiltrate is composed of degenerate neutrophils and abundant cellular debris, consistent with an abscess. (HE 196X)

Histopathologic Description: Brain (cerebrum): There is locally extensive necrosis of the nervous tissue containing cellular debris with bacterial colonization. The necrotic area is surrounded by a wide zone of inflammatory cells, mainly composed of neutrophils with fewer lymphocytes and macrophages. There is diffuse intravascular lymphocytic cuffing.

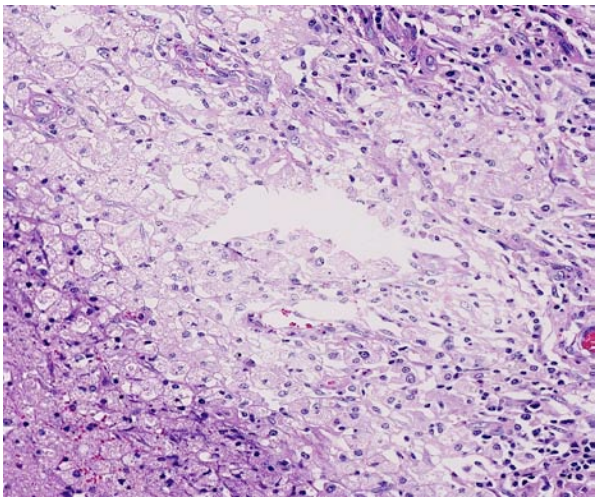
Contributor's Morphologic Diagnosis: Encephalitis, locally extensive, necrotizing, subacute with granulation tissue (capsule) formation and bacterial colonization.

Contributor's Comment: Brain abscess-related mortality is a growing concern of deer managers and biologists across the country. Brain abscesses are caused by a variety of bacteria (primarily *Arcanobacterium pyogenes*) that naturally inhabit the skin of deer, as well as other animals.[1,2,3,5] These bacteria typically enter the brain through lesions and skin abrasions associated with the antler pedicle (where the antler protrudes from the skull) or junctions between cranial bones that are referred to as "sutures". Mortality generally occurs from fall (following velvet shedding) through spring (shortly after antler casting). Thus, the period when bucks are developing antlers or when antlers have hardened is when they are most susceptible to this disease.[1]

Certain buck behaviors, such as aggressive sparring and fighting, can cause damage to the antler pedicle and/or other parts of the skull that can predispose them to brain abscesses.[1]

Different studies, from 1996-2008, showed that the intracranial abscesses were diagnosed as the cause of death or illness in white-tailed deer examined by the diagnostic laboratories across portions of the United States and Canada. The bacterium *Arcanobacterium pyogenes* was the primary cause of infection.[1,2,3,5]

The clinical signs include several behavioral characteristics associated with the neurological system, such as "circling" or loss of coordination. Often times, deer can become emaciated, which is characterized by excessive weight loss or having a "deteriorated" appearance. Bucks also may have a puss-like substance located around the antler pedicle that leaks through openings in the skull. It is not known whether this disease can be transmitted between deer or other animals through direct contact or other sources. Also, it is important to note that deer with brain abscesses are not recommended for consumption.[1]



1-3. Cerebrum, deer: Cerebral abscesses lack a capsule due to the lack of fibroblasts in the brain; there are areas of liquefactive necrosis infiltrated by large numbers of Gitter cells in the adjacent white matter. (HE 256X)

The clinical, gross examination and ancillary tests findings were compatible with the previous studies.

Note: Multiple blocks were used for the slides submission; therefore, not all the participants will get the same copy of the slides.

JPC Diagnosis: Brain, midbrain: Abscess, focally extensive, with gliosis, spongiosis, nonsuppurative perivascular cuffing, and numerous colonies of bacilli.

Conference Comment: The contributor highlights the disease pathogenesis in this case as associated with antler development and biologic behavior in this species. A second possible pathogenesis discussed by conference participants is a hematogenous route, likely subsequent to oral infection. Oral mucosal damage or severe dental disease could potentially lead to bacterial emboli seeding in the brain and inducing a lesion such as is observed in this case.

For a deer exhibiting neurologic symptoms, the differential of *Listeria monocytogenes* must also be considered. Lesions of listeriosis are typically smaller (microabscesses) and confined to the brainstem.

There is an increasing trend for hunters and property owners to allow bucks to reach a more mature age before harvesting to improve herd health and antler quality.[1] Associated with the trend is the unintended consequence of increased numbers of brain abscesses occurring in mature males. Recently, a study of 26 collared bucks over 2.5 years old identified brain abscess as the cause of death in 35% of cases, likely related to the increased competition among mature bucks.[5]

Arcanobacterium pyogenes is the most commonly cultured isolate from brain abscess, being the only isolate in almost 50% of cases.[2] The bacterium has been through a series of name changes, and has recently been reclassified as *Trueperella pyogenes* based on DNA sequencing and mass spectrometry.[4]

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http://www.sdstate.edu/vs/

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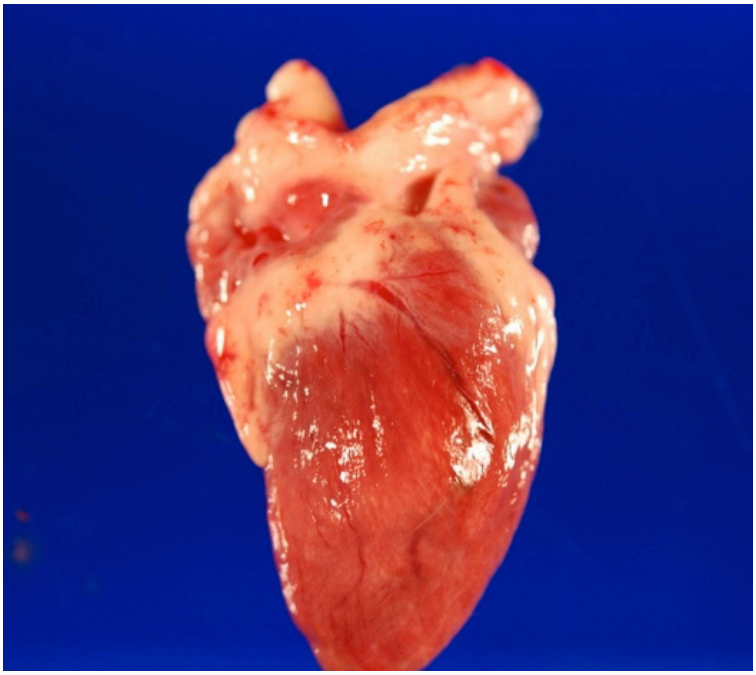
CASE II: 66400 (JPC 4048574).

Signalment: 8 year-old female African black-footed penguin.

History: This penguin has a history of intermittent seizures, mild anemia, severe leukocytosis and hyperglobulinemia. Recently the penguin presented with increasing frequency of seizures, acute facial swelling, lethargy, inappetance and weight loss (BCS 3/9). The penguin was treated with supportive care as well as doxycycline, enrofloxacin, itraconazole, terbinafine, and amphotericin B for suspected aspergillosis and avian malaria. The penguin did not respond to therapy and ultimately died.

Gross Pathology: The animal is in poor body condition and there are minimal subcutaneous fat stores. A focally extensive, 5 x 5 cm focus of wet, gelatinous subcutaneous tissue (edema) is noted over the keel. Upon opening the coelomic cavity, approximately 100 ml of transudate primarily located within the pleura surrounding the heart and potentially cranial air sacs is noted. The heart is pale red with numerous tan streaks present throughout the myocardium. At the base of the heart on the endocardium there are multifocal to coalescing areas of petechial hemorrhage. Atrioventricular and semilunar valves are unremarkable.



2-1. Heart, penguin: The myocardium contains numerous pale streaks, and petechiae are distributed randomly at the heart base. (Photo courtesy of: Department of Molecular and Comparative Pathobiology, Johns Hopkins University, 733 N. Broadway, Suite 811, Baltimore, MD 21205)

Histopathology: Multifocal areas of degeneration, necrosis and inflammation centered on capillaries disrupt approximately 20 – 30% of the left and right ventricular myocardium. Areas of degeneration and necrosis are characterized by pale cardiomyocytes with extensive vacuolation of the sarcoplasm (degeneration), loss of cross striations, fragmented, hypereosinophilic fibers, pyknotic nuclei, scattered karyorrhectic nuclear debris (necrosis) and occasional degenerate heterophils. Multifocally, surrounding blood vessels are infiltrated by moderate numbers of macrophages, lymphocytes, plasma cells, and heterophils. Multifocally, within these areas of inflammation, there are numerous endothelial cells displaying abundant vacuolated cytoplasm often expanded by oval to round, 15 – 25 µm thin wall (1 µm) cysts (schizont) containing between 15 – 30 1 – 2 µ m, round, basophilic apicomplexans (merozoites). Some sections contain a focal, 1 – 2 mm, intravascular aggregate of basophilic to granular material surrounded by layers of fibroblasts, myoepithelial cells, fibrin, and scattered erythrocytes (thrombus), which occludes 80% of a medium caliber artery. Scattered lymphocytes are present within the endocardium. Multifocally separating muscle fascicles and in between blood vessels there are moderate amounts of edema, scattered hemorrhage and numerous small foci of fibrin localized in and around necrotic endothelial cells.

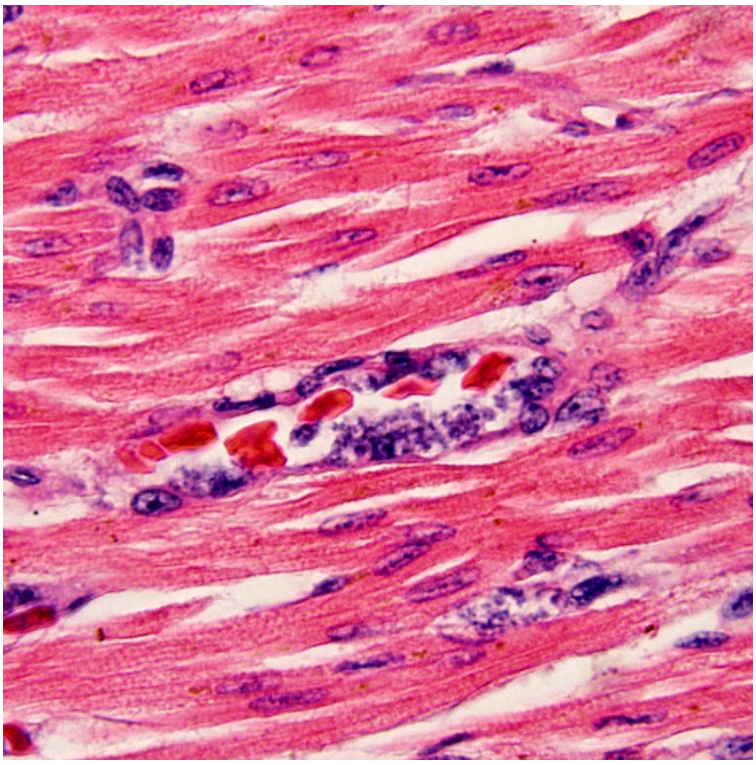
Morphologic Diagnosis: Heart, myocarditis, necrotizing, chronic, multifocal, moderate with lymphohistiocytic and heterophilic infiltrate, fibrin deposition, edema, hemorrhage, thrombosis and intra-endothelial, extra-erythrotic schizonts.

Contributor's Comments: Avian malaria is caused by single celled, intracellular parasite of the *Hemosporidia* phylum, which includes *Haemoproteus*, *Paraemoproteus*, *Leucocytozoon*, *Plasmodium*, and *Hepatocystis*.[1] *Plasmodium* spp. are one of the main hemoparasites affecting

captive bird species, including penguins. Development and transmission of *Plasmodium* species occur in mosquitoes of genera *Culex, Aedes*, and rarely *Anopheles*. Often wild bird species are able to control disease and serve as reservoirs for infection, however, symptomatic birds can present with a range of clinical symptoms including hemolysis and anemia. Most of the clinical symptoms are attributed as a result of severe anemia including anorexia, depression, vomiting, and dyspnea, which may all occur hours before death. Outbreaks in North America have been seen in anseriforms (ducks, geese, and swans), Passeriformes (perching birds, robins, blue jays, and etc.), and columbiforms (doves and pigeons).[7]

Avian malaria associated with *Plasmodium sp.* is a major cause of avian morbidity and mortality in zoos worldwide, most notably captive penguin populations. Several *Plasmodium* sp. have been implicated as causative agents of avian malaria in captive penguins, including *P. relictum*, *P. enlongatum*, *P. tejerai*, and *P. juxtanuclear*.[2,4,8,11,12] *Plasmodium* sp. have a complex life cycle involving both asexual and sexual reproductive stages, which take place in the host and vector. When an infected female mosquito takes a blood meal, sporozoites are released into the host's peripheral circulation. The sporozoites then invade cells of the reticuloendothelial system where they multiply and grow within parasitophorous vacuoles to produce schizonts, which can contain up to 10,000 – 30, 000 merozites (in the case of *Plasmodium falciparum*). Most commonly this development is in the liver, but they will also develop in other tissues such as the kidney, lungs, CNS, spleen, and heart. The schizonts produce thousands of merozoites, which are released into host cell-derived merosomes that are protected from host immunity. At this point individual merozoites are released into the circulation and infect erythrocytes. In the erythrocyte they develop into trophozoites (the feeding or ring stage).While feeding on hemoglobin they release hemoglobin pigments (hemozoin) which are a by-product of hemoglobin metabolism and a feature of the erythrocyte life cycle stage that can be appreciated on histopathology of the liver and lungs. Trophozoites then develop into a schizonts containing between 8-32 merozoites, which once released into the circulation can reinfect more erythrocytes recapitulating this stage of the infectious life cycle. After several cycles of invading erythrocytes, some of the merozoites transform into microgametocytes and macrogametocytes. Once a mosquito ingests erythrocytes containing these gametes, they further develop and fuse forming oocysts that yield the infective sporozoites.[1,6,7,10]

The extra-erythrocytic pathway of replication predominates in penguins, explaining the generally low levels of parasitemia seen on blood smears.[4,11,12] Previous studies demonstrated the utility of leukocyte counts as another predictive factor of parasitism to combine with blood smear evaluation. In cases with detectable parasitemia, the severity of leukocytosis was often a predictive factor in disease severity.[11] In this animal, the relative leukocyte count was greater than 75,000 WBC/uL. Immune suppression associated with stress, other infectious agents, or immaturity have been consistently demonstrated to be a contributing factor in *Plasmodium* disease



2-2. Heart, penguin: Numerous capillary endothelial cells are distended, partially occluding the lumen, by a single intracytoplasmic 20 µm schizont containing numerous round basophilic merozoitse, consistent with Plasmodium sp. (HE 600X)

progression in captive penguins.[3] Penguin mortality is closely associated with the presence of disseminated extra-erythrocytic replication, including in the liver, lung, heart, kidney, and CNS.[4,8,11]

This penguin came from a colony previously known to be infected with both *Plasmodium relictum* and *P. elongatum*. Historically, disease surveillance of this colony via blood smear analysis has often yielded low to undetectable blood parasite levels, yet, necropsy of a small numbers of these animals over a 15-year period reveal that some of the animals suffered from severe multi-organ disease attributed to extra-erythrocytic replication of *Plasmodium sp*. Similar to previous cases, intra-endothelial protozoa with morphology consistent with *Plasmodium sp.* schizonts were noted in the liver, lung, spleen, kidney, meninges, brain, and heart. Repeated episodes of seizures in this animal are attributed to the development of meningoencephalitis. Interestingly, focal arterial thrombosis was noted in several sections of the heart from this animal. Coagulopathies in humans infected with *Plasmodium falciparum* are thought to be associated with the ability of parasitized erythrocytes to activate the coagulation cascade through recognition of numerous receptors and pathways.[5] Based on this finding it is a possibility that a similar phenomenon may exist with avian malaria.

JPC Diagnosis: Heart: Intraendothelial protozoa, etiology consistent with *Plasmodium* spp., with myofiber necrosis and edema.

Conference Comment: *Plasmodium* spp. has a complex, two-stage life cycle of which the contributor does an exceptional job of describing. Exoerythrocytic stages typically occur in the liver and spleen initially, while endothelial cell infection becomes especially prominent within the lung in more advanced cases. Tissue sections in this case represent the described exoerythrocytic stage where meronts are found within endothelial cells of the heart. Anemia is not usually a feature of this disease in penguins as erythrocyte infection rate is usually low while massive endothelial exoerythrocytic schizogony is quite characteristic.[1,4]

In the penguin alone, schizonts, merozoites and gametocytes may be detected simultaneosly. Schizonts are smaller than the host nucleus and there is little cytoplasm present. The merozoite stage is directly adjacent to the erythrocyte nucleus, also with little or no cytoplasm. Gametocytes are variably shaped, from irregular to spherical, and occasionally displace the host cell nucleus.[8]

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CASE III: L13 14765 (JPC 4041399).

Signalment: 2-year-old male inland bearded dragon, *Pogona vitticeps*.

History: A 2-year-old male bearded dragon was living in a glass terrarium with sand substrate, UVB light, and a basking bulb as a classroom pet. The animal presented with a 3-week history of multifocal raised nodules on its dorsum, ventrum, and head; several of which were exfoliating upon manual examination revealing areas of ulceration. Impression smears obtained from ulcerations on the dorsum, ventrum, neck, and above the left eye were suggestive of fungal infection with evidence of granulomatous inflammation. The owner elected euthanasia due to poor prognosis of medical treatment efficacy.

Gross Pathology: Post mortem examination identified bilaterally symmetrical, circular, oval areas of yellow discoloration on the ventral abdomen that each measured 3.5x3 cm, extending onto the proximal limbs, ventral neck, and head. Multifocally, there were also raised, irregular, firm lesions with thickened epidermis in the left periorbital region (1.5x1.5 cm), on the left caudal mandible (3x2.5x1 cm), on the right dorsal neck (2,5x2 cm), and on the right body wall caudal to the thoracic limb (2x1.5 cm). On cut surface, these raised lesions consisted of poorly defined, firm, whitish nodules, surrounded by pale yellow, soft tissue of gelatinous consistency. The skin also had multiple annular areas of ulceration: above the left eye (2x2 cm), left dorsum (2x2 cm), and ventrally between the thoracic limbs (1.5x1 cm). No other abnormalities were noted at necropsy.

Laboratory Results: Mycology culture: *Chrysosporium* sp. isolate.

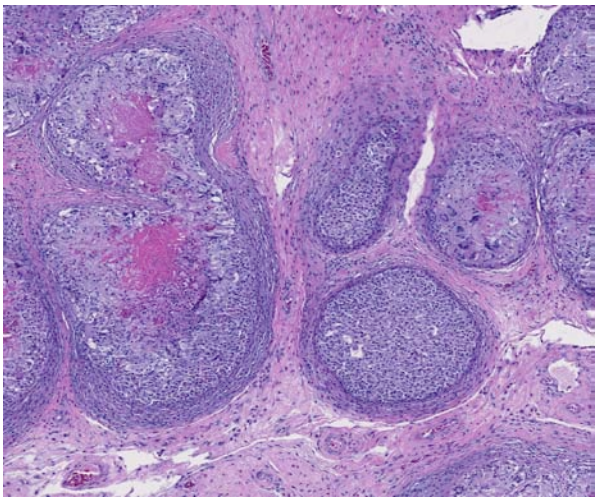
PCR: *Nannizziopsis guarroi* based on DNA sequence analysis of the ITS and D1/D2 regions (performed at the University of Texas Health Sciences Center).

Histopathologic Description: Skin: Alterations in all sections of the examined raised skin lesions from different areas are similar and, thus, will be described collectively. There are extensive areas of ulceration flanked by epidermis with extensive chromatophore accumulation just beneath the epidermal basal lamina. The superficial epidermis has multifocal areas of degeneration, characterized by vacuoles filled with eosinophilic, proteinaceous cellular debris and overall loss of cellular stratification. At the interface of the ulcerated and intact epidermis, the stratum corneum is moderately thickened with no retention of nuclei (orthokeratotic hyperkeratosis). The dermis is moderately expanded by edema and inflammatory cell infiltrate, composed of heterophils and macrophages. Underlying the ulcerated regions, the dermis is markedly expanded by multifocal to coalescing granulomas within dense fibrous connective tissue. Granulomas are composed of necrotic centers, surrounded by concentrically arranged epithelioid

3-1. Scaled skin, bearded dragon: There are multiple firm areas of epidermal hyperplasia in the left periorbital region. (Photo courtesy of: Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, http://www1.vetmed.lsu.edu//PBS/index.html)

3-2. Scaled skin, bearded dragon: There are bilaterally symmetrical, irregularly round areas of yellow discoloration on the ventral abdomen which measure 3.5x3 cm each. (Photo courtesy of: Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, http://www1.vetmed.lsu.edu//PBS/index.html)

3-3. Scaled skin, bearded dragon: There was an annual area of ulceration on the left dorsum. (Photo courtesy of: Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, http://www1.vetmed.lsu.edu//PBS/index.html)



3-4. Scaled skin, bearded dragon: Underneath the ulcerated epidermis, there are numerous discrete granulomas within the dermis. (HE 47X)

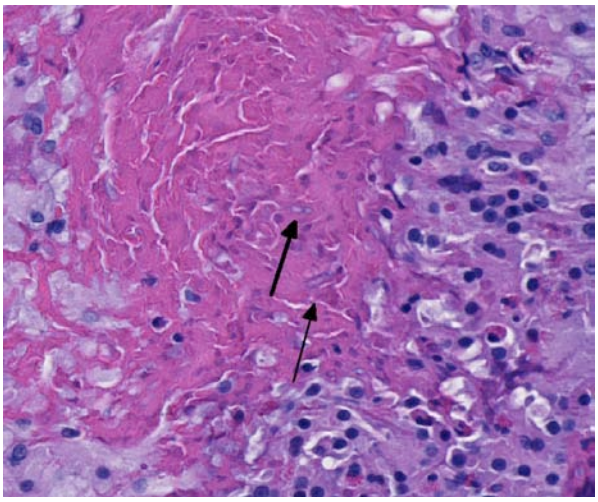
macrophages interspersed with heterophils and multinucleated giant cells, and encapsulated by fibroblasts. The dense connective tissue surrounding and separating the granulomas is markedly infiltrated by heterophils, macrophages, lymphocytes, and plasma cells. Overlying the granulomas and replacing the epidermis is extensive necrotic cellular debris admixed with hemorrhage, aggregates of dark yellow-to-brown pigment, and dense tufts of arthroconidia and hyphae embedded within dense layers of keratin. Occasional fungal organisms, similar to those described previously, are also identified within the granulomas and loosely within the dermal connective tissue. The subcutis contains mild inflammatory cellular infiltrates, consisting of heterophils, lymphocytes, plasma cells, and mast cells, with random foci of necrosis.

In sections of the ventral abdomen, deep layers of the epidermis contain multifocal, round, expansile aggregates of myriad fungal spores and hyphae. Fungal aggregates are primarily encased in dense keratin, and other times they are found clustered within the superficial stratum corneum. Fungal organisms are similar to those described above in the raised skin lesions. Commonly, arthroconidia are identified among the hyphae as well as free within the nearby epidermis. Epidermal changes surrounding the fungal aggregates include moderate dilation of intercellular spaces (spongiosis), ballooning degeneration, and mild transmigration of heterophils. Segmentally, in close proximity to the fungal organisms, the stratum corneum displays moderate orthokeratotic hyperkeratosis. In the underlying dermis, there is moderate infiltrate composed mainly of heterophils, lymphocytes, plasma cells and macrophages.

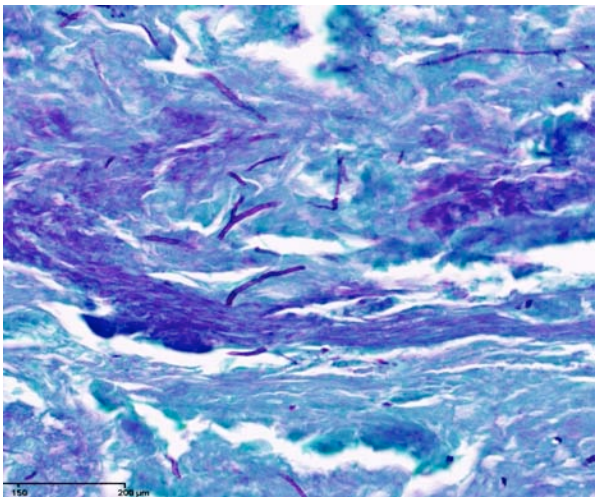
Contributor's Morphologic Diagnosis: Skin: Proliferative and ulcerative dermatitis, granulomatous, multifocal, chronic, severe, with myriad intralesional segmented hyphae and arthroconidia.

Contributor's Comment: While the majority of fungal organisms affecting reptiles are believed to be opportunistic, *Nannizziopsis* sp. have been suggested as obligate fungal pathogens of reptiles. An infectious model with veiled chameleons fulfilled Koch's postulate in transmitting what is now known to be *Nannizziopsis dermatitidis* via application of the fungus to intact and abraded skin, with subsequent development of the lesions observed naturally. *N. dermatitidis*. was also determined to be infectious via direct contact and/or through fomite transmission in the same study.[3]

The *Nannizziopsis* species seen most commonly in bearded dragons and green iguanas is *N. guarroi*.[1,2] The disease is often called Yellow Fungus Disease by hobbyists because the crusts found on bearded dragons tend to have yellow discoloration. Thus, crust formation, color change and necrosis are commonly seen in these cases. Lesions tend to be multifocal and may include the head, oral cavity, limbs, ventrum and dorsum. The infection is often aggressive, with extension into



3-5. Scaled skin, bearded dragon: Cross- and tangential sections of arthroconidia and pauciseptate fungal hyphae (arrows) may be seen within granulomas. (HE 356X)



3-6. Scaled skin, bearded dragon: A PAS stain on the overlying keratin crust demonstrates the morphology of numerous fungal hyphae and occasional arthroconidia. (Photo courtesy of: Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, http://www1.vetmed.lsu.edu//PBS/index.html)

the subcutaneous tissues, muscles, and bones.[3] In some cases there is also systemic spread to the lungs and liver after local cutaneous invasion.[3]

This inland bearded dragon had multifocal raised nodules on the skin, with multiple areas of ulceration and an irregular oval area of yellow discoloration on the ventral head, neck and abdomen, with no evidence of extension into underlying muscle and/or bone. Microscopically the raised nodules consisted of granulomatous proliferative dermatitis. Additionally, there was superficial ulceration with myriad intralesional segmented hyphae and arthroconidia, which by mycological culture and PCR were identified as *Nannizziopsis guarroi*.

Recently, morphologically similar isolates formerly referred to as members of the "CANV" complex, have been properly identified into three genera: *Nannizziopsis, Paranannizziopsis*, and *Ophidiomyces*, which are not particularly closely related within the Onygenales. The genus *Nannizziopsis* was split into 8 unique species: *N. vriesii, N. dermatitidis, N. crocodili, N. barbata, N. guarroi, N. infrequens, N. hominis*, and *N. obscura*. Benefits of this classification scheme include clarification of the range of susceptible hosts for each fungal species, monitoring trends of infection, determination of the prevalence of specific species in the environment, ability to evaluate species-specific antifungal efficacy, and development of specific strategies for disease prevention.[4]

N. guarroi is a keratinophilic ascomycetous fungus that has been associated with several cases of granulomatous dermatitis in toxicoferan squamates. Due to the inherent stress that may accompany classroom pets related to frequent handling and/or taunting, the role of stress cannot be ruled out in the susceptibility to the fungal infection in this case. *N. guarroi* do not grow at 40 °*C* and hospitalizing reptiles at increased temperatures during treatment may help the patient eliminate the fungus. Additionally, laboratories may be underreporting *N. guarroi* infections if culture protocols call for temperatures in excess of 37 °*C (false negatives), stressing the importance of communication with laboratories in suspected N. guarroi cases*.[2]

JPC Diagnosis: Skin: Ulcerative crusting dermatitis, with granulomatous inflammation and intralesional fungal elements, etiology consistent with *Nannizziopsis* guarroi.

Conference Comment: *N. guarroi* dermatomycosis is a contagious disease that can have severe consequences for reptile collections. Hyphal proliferation occurs initially within the superficial dead epidermal layers.[3] As with many skin diseases in reptiles, the scale fold becomes an excellent media for infection as keratin becomes impacted. From here, hyphae penetrate downward pushing through the basement membrane. The fungal invasion can continue beyond the dermis into the subjacent musculature.[3] Necrotizing

cutaneous infections can progress to systemic disease and may become fatal.[2]

Fungi have used a nomenclature inconsistent with the rest of biology. There are separate genus and species names for asexual anamorph stages and sexual teleomorph stages of the same organism, resulting in multiple species names and paraphyletic taxa. In 2011, it was decided by the Nomenclature Section meeting of the International Botanical Congress that teleomorph names should be used (Hawksworth, 2011). This is problematic for medicine, as nearly all names of systemic fungi routinely used are anamorph names (e.g. *Cryptococcus, Blastomyces, Histoplasma, Aspergillus*, etc.). The anamorph genus *Chrysosporium* is widespread across the tree of the fungal order Onygenales, and contains organisms in more than 15 teleomorph genera, including *Nannizziopsis*. Many *Chrysosporium* sp. infecting reptiles have been morphologically misidentified as "*Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV)"

Recent publications have identified *Nannizziopsis guarroi* as the most common cause of yellow skin disease in bearded dragons.[1,5] This correlates with PCR findings in this case, and further illustrates the inaccuracy of the term CANV for this entity.

Special thanks to Dr. Jim Wellehan, Asst. Professor at the University of Florida College of Veterinary Medicine for his contributions to this case

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CASE IV: S11-1330 (JPC 4019410).

Signalment: Adult male giant ditch frog, *Leptodactylus fallax*.

History: The frogs lived in the Zürich zoo and were put in quarantine for one week because of renovation of their terrarium. After putting the frogs back in the compound, some frogs suddenly died. These were sent to the Institute of Veterinary Pathology of Zürich for examination.

Gross Pathology: Nutrient status: The frog was severely emaciated.

Integument: The skin on the ventrum, as well as proximally on the hind legs, was diffusely reddened and, mainly on the ventrum, severely eroded. The right forelimb, distal from the elbow, was swollen and had a focal poorly demarcated thickening (1-2 mm) measuring 1 x 0.4 cm dorsally; this involved the subcutis and the underlying musculature which was diffusely discolored yellow (with multifocal yellow crumbly material deposits).

Coelomic cavity: the liver was interspersed with multiple, round, variably sized (0.5-2 mm in diameter) yellowish soft masses that had sometimes a creamy center. All other organs were macroscopically unremarkable.

Laboratory Results: Ziehl-Neelsen staining: No acid fast bacteria were visible.

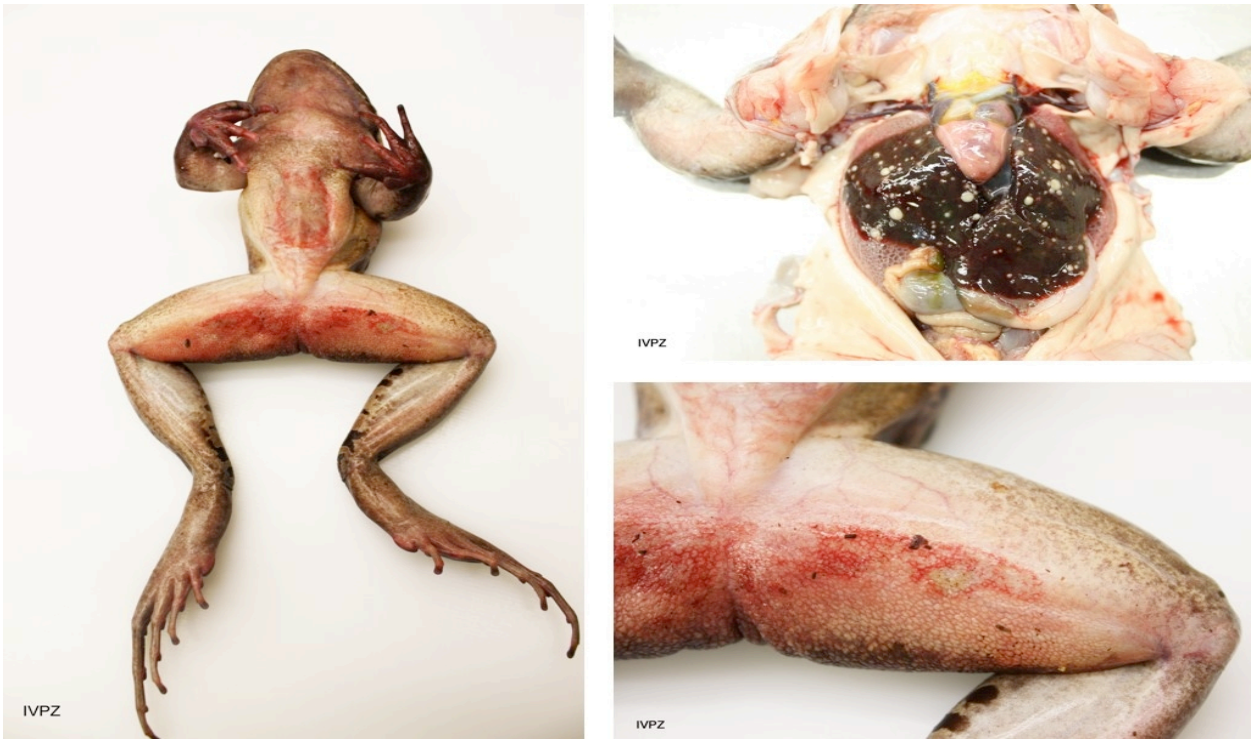
PAS staining: The fungal structures were positive but the brownish color obliterated the PAS staining.

A fungal culture was not performed and the PCR of paraffin embedded liver was negative for any fungus.

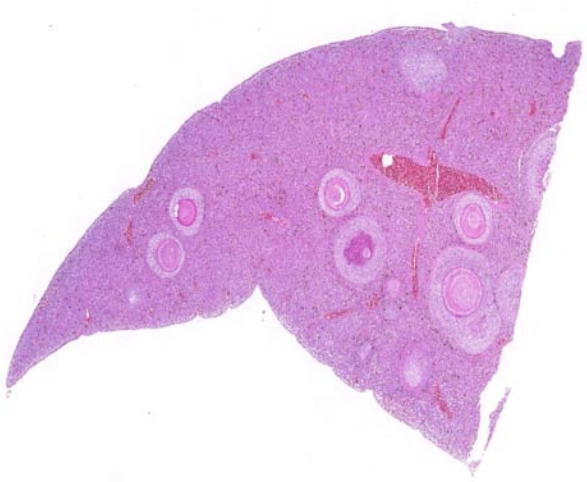
A parasitologic analysis of the feces was negative.

The bacteriological analysis of the coeloma (swab) demonstrated a mild content of *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter* sp., and *Acinetobacter* sp., all considered most likely as contaminants.

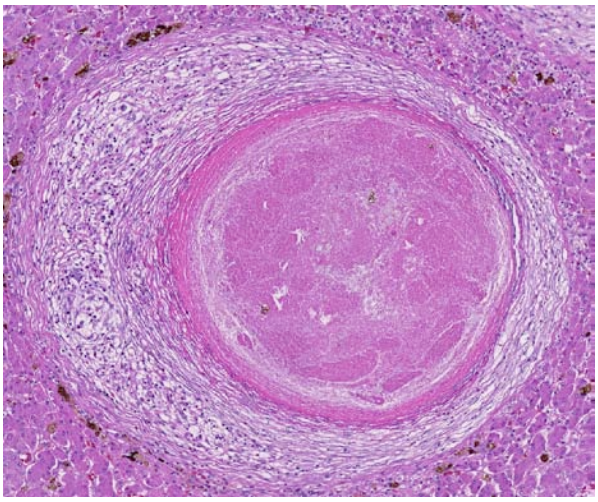
Histopathologic Description: Liver: Randomly distributed there are multiple 1 mm diameter granulomas consisting of central eosinophilic granular material (focal caseous necrosis) surrounded by macrophages and proliferating



4-1. Skin and coelom, frog: There is multifocal hyperemia and erosion of the skin of the abdomen and legs, as well as disseminated granulomas throughout the liver. (Photo courtesy of: University of Zürich, Institute for Veterinary Pathology, Vetsuisse Faculty, Winterthurerstrasse 268, CH-8057 Zürich, Switzerland)



4-2. Liver, frog: There are numerous discrete granulomas scattered randomly throughout the section. (HE 6.3X)



4-3. Liver, frog: Higher magnification of one of the hepatic granulomas. (HE 80X)

fibroblasts, forming a thin irregular to thick fibrous wall. Within the granulomas, multinucleated giant cells of the foreign body type (with all nuclei aligned circular at the border of the cells) and of the Langhans type (with randomly arranged nuclei), as well as lymphocytes, are visible. Mainly in the necrotic areas, but also in lesser numbers randomly distributed in the surrounding fibrous wall, there are roundish structures (10-15 µm in ø) that are sometimes centrally septated (binary fission) and sometimes grouped in clusters of 2-5. The wall of these structures is brownish-gold with a total thickness of about 1 µm (structures interpreted as fungal sclerotic cells = Medlar bodies). Hyphal structures were not found.

Kidneys (not submitted): Granulomas as described above were found.

Skeletal musculature (not submitted): Multifocally extensive muscular degeneration is present, characterized by loss of cross striations and hypereosinophilia of the cytoplasm. Within the degenerate musculature, small clusters of sclerotic bodies are present embedded within extensive granulomas.

Skin (not submitted): Multifocal extensive and deep ulcerations are observed with embedded pigmented fungi in small amounts.

Contributor's Morphologic Diagnosis:

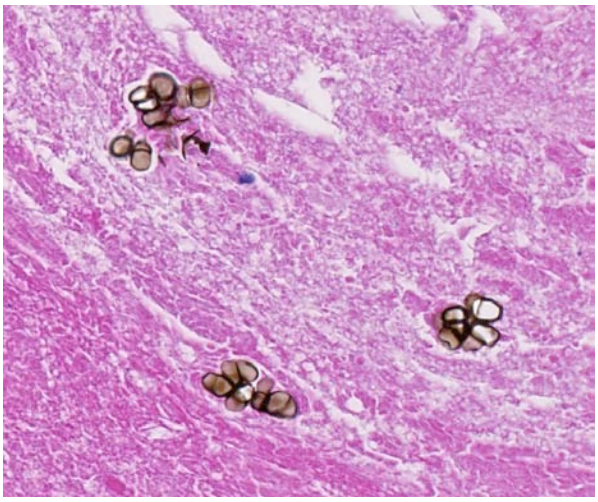
Liver: Hepatitis severe, multifocal, (necrotizing and) granulomatous, with presence of fungal pigmented sclerotic bodies and multinucleated giant cells (foreign and Langhans type)

Kidney, coeloma and musculature: Interstitial nephritis, coelomitis and myositis, severe, multifocal, (necrotizing) and granulomatous, with presence of fungal pigmented sclerotic bodies and multinucleated giant cells (foreign and Langhans type)

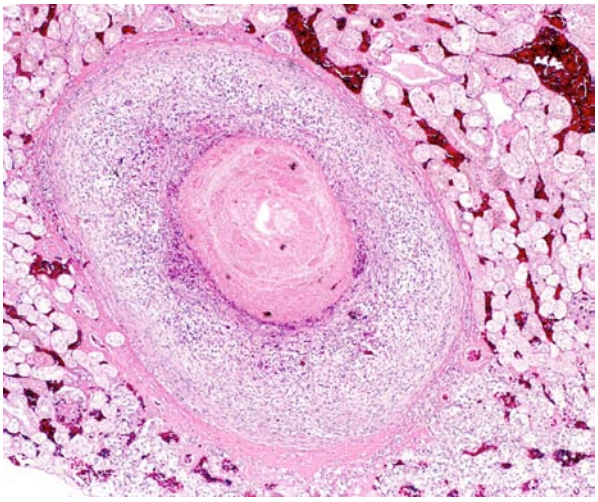
Skin: Dermatitis, severe, ulcerative and necrotizing, multifocal to confluent, with presence of pigmented fungal structures and secondary severe bacterial colonisation

Musculature: Hyaline muscle fiber degeneration, dystrophic calcification and necrosis, mild to moderate and multifocal

Contributor's Comment: Chromoblastomycosis is a worldwide distributed zoonotic skin disease that most commonly presents in the tropical regions and is caused by several dematiaceous (pigmented) saprophytic fungi belonging to the division of the ascomycota. The most important etiologic agents for this disease are *Fonsecae* (*F. pedrosoi and F. compacta*), *Phialophora verrucosa* and *Cladosporium carionii*. *Fonseca pedrosoi* is the most common agent found in the tropical forests (Amazon) and in temperate regions in Latin America; *Cladophialophora*



4-4. Liver, frog: Within the necrotic center of the granuloma, there are clusters of pigmented sclerotic bodies which are septate as a result of ongoing binary fission. (HE 80X)



4-5. Kidney, frog: Granuloma within the kidney. (Photo courtesy of: University of Zürich, Institute for Veterinary Pathology, Vetsuisse Faculty, Winterthurerstrasse 268, CH-8057 Zürich, Switzerland)

carrionii is the most common agent in dry countries and desert regions (Australia, South Africa and Cuba).[1]

These ubiquitous fungi are mainly inoculated by trauma. In warm blooded animals (most often humans and rarely other mammals like cats, horses and dogs), the subcutaneous skin is focally affected, growing slowly to warts or verrucous plaques; only seldom and very late in the disease they may disseminate into lymphatics and blood vessels. The most important complications are damage of the lymphatic system and malignant transformation of the epidermis in the affected regions. The pathogenesis of this disease in cold blooded animals differs from the mammalian cases and is usually systemic, involving mainly skin and internal organs; it has been observed mainly in frogs[6] and toads[2,7] but is also present in other amphibians and in reptiles like marine radiate tortoises.[8] Predisposing factors include any kind of stress in animals as, for example, removal from their natural habitat or competition for food with subsequent bite wounds.

Macroscopically, usually ulcerating and non-ulcerating gray to yellow nodules (up to 5mm) are present in the skin (mainly belly, head and legs) and other organs such as liver, kidneys, heart, lungs, body fat, ovaries, brain, spleen, bone marrow. Histologically, these nodules present as granulomas, with central coagulative to caseous necrosis, many multinucleated giant cells, epithelioid cells and dark brown roundish and thick walled fungal structures, also called sclerotic cells, or Medlar bodies, that frequently undergo equatorial septation (replication by binary fission) and lie extracellularly in small clusters or are phagocytosed by giant cells; more seldom, septated hyphae and pseudohyphae are found. In some cases (e.g. the madagascan radiate tortoise[7]), the lesion presents histologically as an abscess with a central core of heterophils and many dark brown sclerotic bodies surrounded by giant cells, lymphocytes and fibroblasts.

The detection of septate sclerotic bodies is pathognomonic for chromoblastomycosis. A phaeohyphomycosis, also caused by dematiaceous fungi, can be excluded morphologically because they form broad septate hyphae.

Different therapy protocols exist in humans depending on the fungal agent, location and extent of the lesion. In amphibians and reptiles, the best protocol seems to be amputation. It is only possible if performed during early stages when the infection is limited to the skin; other treatments like antifungal drugs have been reported to be ineffective.[2,6] Because of its zoonotic potential, careful handling of affected animals is advised.

JPC Diagnosis: Liver: Granulomas, multiple, with sclerotic bodies.

Conference Comment: This is a nice case of one of the darkly pigmented dematiaceous fungi which, in most cases, derive their characteristic appearance from the production of melanin.[3]

Melanin is used by many pathogens as a virulence factor aiding in colonization and evasion of the immune system by virtue of its antioxidant properties and ability to disrupt antibody-mediated phagocytosis, counteract antifungal agents and bind iron.[5]

Other dematiaceous fungi include those which cause phaeohyphomycosis. These typically exist in tissue as hyphae rather than the distinct sclerotic bodies of chromoblastomycosis species. Sclerotic bodies are the result of cell division by binary fission, in contrast to most fungal pathogens that replicate through budding.[3]

Important differentials to consider for granulomas in frogs include *Mycobacterium marinum* and *Brucella* spp., as both may have some overlap with histopathology. Chromoblastomycosis is a relatively common condition in amphibians and can result in severe systemic disease and frequently death, with the most often targeted organs being the skin, liver, lungs and kidneys. Encephalitis and meningitis has also been reported in these species.[2] Chromoblastomycosis can cause outbreaks in stressed colonies and strict quarantine guidelines must be adhered to due to its transmissibility between animals and humans.[6]

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