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Guest Moderator:
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CASE I: 2008 Case#2 (JPC 3106257)

SIGNALMENT: Captive adult female green iguana, *Iguana iguana*

HISTORY: Chronic ascites; suspected neoplastic or inflammatory infiltrate in the liver

LABORATORY RESULTS: None provided.

GROSS PATHOLOGIC FINDINGS: The liver lobes were diffusely enlarged, pale and rubbery, with a slight raised cobblestone appearance to the left lobe. There were numerous 0.3 to 1.5 cm diameter, occasionally pendulous fluid-filled cysts containing pale yellow, clear fluid in the capsule along margins of the left lobe. Approximately 200 ml of blood-tinged, watery fluid was present in the coelomic cavity.

HISTOPATHOLOGIC DESCRIPTION: Liver: The normal liver architecture is almost completely replaced by coalescing, variably dense aggregates of ductules and tubules separated by variably broad trabeculae of fibrous connective tissue in which are scattered capillaries. Spared are several small patches of short cords of hepatocytes. Ductules are lined by a single layer of cuboidal cells with light eosinophilic cytoplasm and central round to oval nuclei having uniformly granular chromatin, with 1-2 nucleoli. Mild anisocytosis is present, and mitotic figures are not seen. Golden-brown, granular pigment is present in the cytoplasm of some ductal epithelial cells, scattered macrophages and hepatocytes (hemosiderin). Scattered throughout the parenchyma are small foci of ducts with shrunken, hypereosinophilic cells, pyknotic nuclei and karyorrhectic debris (necrosis). There are sparse perivascular infiltrates of lymphocytes and heterophils. Segments of the capsule are mildly to moderately thickened by fibrous connective tissue.

CONTRIBUTOR'S MORPHOLOGIC DIAGNOSES: 1) Liver: Severe, diffuse, chronic, pseudocarcinomatous biliary hyperplasia with marked interstitial fibrosis. 2) Liver: Moderate biliary, hepatocellular and histiocytic iron accumulation.

CONTRIBUTOR'S COMMENT: In this case, the diagnosis of pseudocarcinomatous biliary hyperplasia (PBH) met the criteria discussed for two previously reported cases in female green iguanas.[19] Histologically, well-differentiated biliary ductules replace the majority of normal liver parenchyma, sparing small islands and clusters of hepatic cords. Mild cellular atypia, the absence of mitotic figures, and lack of invasion of basement membranes by ductule epithelial cells make

cholangiocarcinoma a less likely differential diagnosis.[6] In support of a benign process are the lack of gross or histologic evidence of metastasis, and the absence of a gross tumor.

Cholangiocarcinomas can have a massive or multilobular appearance, are often umbilicated and protrude from the liver capsule. Clinically, prolonged survival after initial diagnosis is also supportive of a diagnosis of PBH.

Pseudocarcinomatous biliary hyperplasia must also be differentiated from biliary hamartoma and cholangioma. In human and veterinary medicine, biliary hamartomas are rare and consist of ducts of varying caliber, unique cystic cavity formation and fibrosis.[15,17] In domestic animals, cholangiomas are usually solitary, well demarcated, round, cystic and solid masses, which grow by expansion and tend to bulge beyond the normal liver surface. In one retrospective study, 31% of all primary neoplasms in lizards affected the liver versus other organ systems,[16] with malignant biliary processes being the most frequent.

In veterinary medicine, biliary hyperplasia has been described in association with internal papillomatosis and chronic mycotoxicosis in avian species. Aflatoxins are excreted in the bile, causing periportal necrosis and inflammation in acute cases. With chronic exposure, there is bile duct hyperplasia and fibrosis, as described with chronic active hepatitis.[13] Severe biliary hyperplasia has been reported in an alpaca in association with parasitic ova of *Fasciola hepatica,*[8,14] and hepatic coccidiosis.[14] Experimental bile duct ligation or administration of alpha-naphthylisothiocyanate (ANIT), both producing obstructive cholestasis, or intravenous estradiol glucuronide, which causes non-obstructive cholestasis, have been shown to cause biliary epithelial cell hyperplasia in male Sprague-Dawley rats.[10]

In human medicine, the term pseudocarcinomatous hyperplasia is used to refer to marked epidermal proliferation with down growth into the dermis, associated with chronic granulomatous conditions of the underlying dermis or with keratinocyte atypia.[9] It has been described as hyperplastic glandular or ductular epithelium with a cribriform pattern, mimicking carcinoma, in different neoplastic and inflammatory processes. These include granular cell tumors,[2] anaplastic large cell lymphoma,[12] chronic osteomyelitis of the jaw and limbs,[18] oral syphilis infection,[1] and chronic salpingitis.[3] Pseudocarcinomatous urothelial hyperplasia of the urinary bladder has been associated with prior irradiation or chemotherapy and, recently, in several cases without such predisposing treatment.[11] Hyperplasia of biliary ducts in humans has been associated with biliary atresia, where the pathological changes include hyperplasia of canaliculi, inflammation, cholestasis and interstitial fibrosis of portal zones.[20]

The pathogenesis of pseudocarcinomatous hyperplasia (of the skin) has been related to epidermal growth factor (EGF) and transforming growth factor (TGF) elaborated by the primary tumor (e.g. lymphoma) or inflammatory cells.[4] Both of these growth factors have the same specific membrane receptor (EGFr) which has tyrosine kinase activity. The activation of this receptor can be involved in epithelial hyperplasia, wound healing and tumorigenesis.[7]

JPC Diagnosis: Liver: Biliary hyperplasia, diffuse, severe, with fibrosis.

Conference Comment: This is a very unusual lesion, and sparked not only lively debate but a number of post-conference special stains. This is a rarely reported case in iguanas, although it has been suggested through by previous WSC moderators that we have consulted with on this

case, it occurs commonly. Conference participants discussed at length several aspects surrounding this case. The acini, which completely replace hepatic parenchyma in most sections, certainly appear to be biliary ducts and in our view, lack malignant characteristics as mentioned by the contributor. Conference participants noted that certain hepatocellular neoplasms can form acinar structures also, however, further testing for hepatocyte antigen and pancytokeratin were negative, which proves these are all biliary epithelial cells. Additional debate centered on the composition of the abundant eosinophilic fibrillar material between acini, which proved to be collagen, based on staining properties with Masson's trichrome and a lack of fluorescence with Congo red.

Biliary hyperplasia is a nonspecific response to a variety of liver insults,[5] many of which are mentioned by the contributor. It is typically regarded as a result of long-standing hepatic injury, particularly after diseases which result in the obstruction of normal bile drainage.[5] It may also develop secondary to portal inflammation and fibrosis.[14] Ductular reaction is a term utilized when the progenitor cells with potential to differentiate into either biliary epithelium or hepatocytes proliferate, as also may occur in severe hepatic injury.[5] See the conference comments from WSC 2011-12, conference 3, case 2 for a detailed discussion of ductular reaction and its pathogenesis.

Diffuse hepatic fibrosis also corresponds with repeated toxic hepatic injury; however, this typically is followed by nodular regeneration as observed in a cirrhotic liver. When a single event induces widespread hepatocellular necrosis, fibrosis and condensation of preexisting connective tissue often occurs in the absence of regeneration and is termed postnecrotic scarring.[5] This is because the normal reticulin network of type III collagen, hepatic stellate cells, and nerves which occupy the space of Disse collapses, allowing portal triads to converge, giving rise to irregular bands of scar tissue.[14] Postnecrotic fibrosis, which develops around hepatic venules, is termed periacinar fibrosis and occurs commonly in cases of chronic passive congestion or pyrrolizidine alkaloid toxicity.[14]

In this case, the gross description and histopathologic findings best correlate with a diffuse, chronic hepatic insult. The presence of ascites is consistent with two previously reported cases,[18] and it would be interesting to compare clinical pathologic findings in this case to those previously reported to assist in determining whether the abdominal fluid is related to the hepatic lesion.

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References:

1. Barrett AW, Dorrego MV, Hodgson TA, Porter SR, Hopper C, Argiriadou AS, Speight PM: The Histopathology of Syphilis of the Oral Mucosa. *J Oral Pathol Med* **33**:286-291, 2004
2. Brannon RB, Anand PM: Oral Granular Cell Tumors: An Analysis of 10 New Pediatric and Adolescent Cases and a Review of the Literature. J *Clin Pediatr Dent* **29**(1):69-74, 2004

3. Cheung ANY, Young RH, Scully RE: Pseudocarcinomatous Hyperplasia of the Fallopian Tube Associated with Salpingitis. Am J Surg Pathol **8**(11):1125-1130, 1994
4. Courville P, Wechsler J, Thomine E, Vergier B, Fonck Y, Souteyrand P, Beylot-Barry M, Bagot M, Joly P, and The French Study Group On Cutaneous Lymphoma: Brit J Dermatol **140**:421-426, 1999
5. Cullen JM, Brown DL. Hepatobiliary system and exocrine pancreas. In: Zachary JF, McGavin MD, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Elsevier Mosby; 2012:415-418.
6. Cullen JM, Popp JA: Tumors of the Liver and Gall Bladder. *In*: Tumors in Domestic Animals, ed Meuten DJ, 4th ed., pp 483-508. Blackwell Publishing, Ames IA 2002
7. De Boer WI, Houtsmuller AB, Izadifar V, Muscatelli-Groux B, Van Der Kwast TH, Chopin DK: Expression and Functions of EGF, FGF and TGF beta-Growth-Factor Family Members and Their Receptors In Invasive Human Transitional Cell Carcinoma Cells. Int J Cancer 71:284-291, 1997
8. Hamir AN, Smith BB: Severe Biliary Hyperplasia Associated with Liver Fluke Infection in an Adult Alpaca. Vet Pathol **39**:592-594, 2002
9. KawachiY, Taguchi S, Fijisawa Y, Furuta J, Nakamura Y, Ishii Y, Takahashi T, Otsuka F: Epidermal Pseudocarcinomatous Hyperplasia With Underlying Epidermal Growth Factor-Producing Cutaneous CD30-Positive Lymphoproliferative Disorder. *J Eur Acad Derm Venereology*, Letter to the Editor, 2008
10. Kossor DC, Meunier PC, Dulik DM, Leonard TB, Goldstein RS: Bile Duct Obstruction Is Not A Prerequisite for Type I Biliary Epithelial Cell Hyperplasia. *Tox and Appl Pharmacol* **152**:327-338, 1998
11. Lane Z, Epstein JI: Pseudocarcinomatous Epithelial Hyperplasia in the Bladder Unassociated With Prior Irradiation or Chemotherapy. *Am J Surg Path* **32:**92-97, 2008
12. Lin J, Lee JY: Primary Cutaneous CD30+ Anaplastic Large Cell Lymphoma With Keratoacanthoma-Like Pseudocarcinomatous Hyperplasia and Marked Eosinophilia and Neutrophilia. J Cutan Pathol **32:**458-461, 2004
13. Schmidt RE, Reavill DR, Phalen DN: Liver. *In*: Pathology of Pet and Aviary Birds, 1st ed., pp 67-93. Blackwell Publishing, Ames, IA, 2003
14. Stalker MJ, Hayes MA: Liver and Biliary System. *In*: Pathology of Domestic Animals, ed Maxie MG, 5th ed., pp 298-387. Elsevier Saunders, Philadelphia, PA, 2007
15. Starost MF: Solitary Biliary Hamartoma with Cholelithiasis in a Domestic Rabbit (*Oryctolagus cuniculus*). Vet Pathol **44**:92-95, 2007
16. Sykes IV JM, Trupkiewicz JG: Reptile Neoplasia at the Philadelphia Zoological Garden 1901-2002. J Zoo Wildlife Med **37**(1):11-19, 2006
17. Tohmé-Noun C, Cazals D, Noun R, Menassa L, Valla D, Vilgrain V: Multiple Biliary Hamartomas: Magnetic Resonance Features With Histopathologic Correlation. Eur Radiol **18**:493-499, 2008
18. Warter A, Walter P, Meyer C, Barriére P, Galatir L, Wilk A: Mandibular Pseudocarcinomatous Hyperplasia. Histopathology **37**:115-117, 2000
19. Wilson GH, Fontenot DK, Brown CA, Kling MA, Stedman N, Greenacre CB: Pseudocarcinomatous Biliary Hyperplasia in Two Green Iguanas, *Iguana iguana*. J Herpetological Med & Surg 14(4):12-18, 2004
20. Zheng S, Luo Y, Xiao X: Analysis of the Pathomorphology of the Intra- and Extrahepatic Biliary System in Biliary Atresia. Eur J Pediatr Surg 18:98-102, 2008

CASE II: 11-0047D (JPC 4007417)

Signalment: Southern hairy nosed wombat, *Lasiorhinus latifrons*

History: Multiple free-ranging wild wombats reported by wombat conservation organization with alopecia, dermatitis and poor body condition. This wombat was in poor body condition and culled (bullet wound to the skull) for post mortem examination for a wombat health investigation study by the University of Adelaide.

Gross Pathology:

1. Moderate multifocal dorsal and lateral alopecia with mild seborrhoea and exudative dermatitis
2. Severe trauma to the head with comminuted fractures of the skull and jaw (as per method of euthanasia)
3. Poor body condition
4. Duodenal cestodiasis
5. Colonic helminthiasis

Laboratory Results: Not performed

Histopathologic Description: Lung: Diffusely there is thickening and hypercellularity of alveolar septa by increased macrophages, rare neutrophils and eosinophils and increased fibrocollagenous connective tissue. There are increased intra-alveolar macrophages, which have moderate to abundant foamy cytoplasm. Free within alveolar lumina or more commonly within multinucleated alveolar macrophages there are many large spherical organisms (yeasts). Yeasts measure 22 - 35 μm in diameter, have a thin 1-2 μm thick lightly basophilic translucent capsule, and internally comprise indistinct basophilic granular material. There are increased Goblet cells in the epithelium of large bronchioles and adjacent airways are filled with foamy basophilic mucoid secretion. Occasionally, subepithelial connective tissues of bronchioles are infiltrated by aggregates of foamy macrophages forming small granulomas with intralesional yeasts. There are infrequent subepithelial infiltrates of lymphocytes, plasma cells, and eosinophils; and there is focal exocytosis of eosinophils into bronchiolar epithelium. In some sections of lung, alveoli are filled by hemorrhage and alveolar septal capillaries are congested.

Contributor's Morphologic Diagnosis: Lung: Moderate histiocytic interstitial pneumonia and fibrosis with intralesional fungal elements (interpreted as *Emmonsia parva*). 2. Lung: Multifocal alveolar hemorrhage and congestion.

Contributor's Comment: Pulmonary adiaspiromycosis is caused by *Emmonsia crescens* or *E. parva*, dimorphic fungi which form thick-walled non-budding non-replicating adiaspores in tissue and elicit granulomatous inflammatory reactions in the host. *E. crescens* and *E. parva* are distinct but morphologically similar species; however, adiaspores of *E. crescens* generally form larger and sometimes multinucleated adiaspores (up to 500 μm in diameter), and *E. parva* monuonucleate adiaspores only 20-40 μm in diameter.[13] The two species also differ in their

geographical distribution, *E. parva* being more common throughout central Asia, Africa and parts of the Americas, and *E. crescens* mainly observed in Europe and the UK.[2,8,15]

Pulmonary adiaspiromycosis is known primarily to occur in small rodents, carnivores and mustelids. In Australia, *E. parva* is described as the cause of pulmonary adiaspiromycosis in wombats on the basis of fungal morphology, although results of genetic characterization and confirmation of organism identity is yet to be reported.[11,12] In New Zealand, *E. crescens* is the reported cause of pulmonary adiaspiromycosis in the brushtail possum (*Trichosurus vulpecula*),[9] most likely secondary to co-habitation of the opossum with introduced British mammals (otter, stoat, weasel, mole, red fox and pine martin) in which *E. crescens* is widespread in the UK.[3] Adiaspiromycosis in humans is rare; most human infections are attributed to *E. crescens* although *E. parva* may be observed in AIDs patients.[5,6] Rarely, fatal human infections have been described.[2]

Wombats are large herbivorous burrowing marsupials native to Australia, of which there are three extant species: the southern hairy nosed wombat (*Lasiorhinus latifrons*), the northern hairy nosed wombat (*Lasiorhinus krefftii*), and the common wombat (*Vombatus ursinis)*. The southern hairy nosed wombat is native to South Australia and it is estimated that up to 100,000 remain in the wild. The wombat presented in this case was culled and examined as part of a larger study examining skin disease and poor body condition in wombats in the Murrayland region of South Australia. Pulmonary adiaspiromycosis was observed in all wild wombats culled concurrently from this site. Gross lung lesions were not evident at post mortem. Previously reported gross findings in affected wombats have ranged from minimal change, to pale consolidation of ventral lung lobes with mucopurulent exudate in the bronchi and bronchioles.[11,12] The significance of pulmonary adiaspiromycosis in these wombats is uncertain; however, it may have contributed to poor body condition. Alternatively pulmonary fungal load and infection may have been exacerbated due to the presence of concurrent disease or immune suppression. Investigations into Southern hairy nosed wombat health in the region are continuing.

Aleuriospores of *Emmonsia* are ubiquitous and soil borne, and on inhalation form thick-walled non-replicating adiaspores in host tissues which continue to increase in size. Infection of wombats is thought to occur when they are pouch young, and a linear increase in *Emmonsia* spherule size with increasing wombat age has been observed.[11] The habitat and burrowing habits of the wombat is thought to render them prone to infections.[10] Southern hairy nosed wombats spend up to three-quarters of their time underground, and have small home ranges centered around their clay/calcrete or calcrete warrens.[7]

Emmonsia adiaspores must be differentiated in tissue section from other dimorphic fungi forming yeasts in host tissues. Phylogenetic studies recently found isolates of *E. parva* to be closer to *Blastomyces dermatitidis* than *E. crescens*, and the authors further suggest that there may be little basis to maintain *Blastomyces* and *Emmonsia* as separate genera.[13] In tissue section, the yeasts may be distinguished as either budding yeasts (*B. dermatitidis* and *H. capsulatum)* or thick-walled, or non-budding adiaspores (*Emmonsia*). *Emmonsia* adiaspores also resemble *Coccidoides immitis* in tissue section, with the exception that *Emmonsia* lacks internal spores.[14]

JPC Diagnosis: Lung: Pneumonia, interstitial, granulomatous, diffuse, mild to moderate, with occasional intrahistiocytic adiaspores.

Conference Comment: This is a unique look at a rarely observed, but morphologically distinct fungus. Lesions are restricted to the lungs in reported cases and there is a tremendously broad host range.[3] Until recently, *Emmonsia* spp. were classified with *Chrysoporium* spp. which shares many similarities; though molecular genetics has clearly differentiated the two genera.[1] Conference participants briefly discussed whether to classify the pneumonia in this case as interstitial, as the described pathogenesis with this entity involves inhalation of the infectious organisms which typically corresponds with bronchopneumonia. Though the changes in this case were minimal, which was curious in itself when compared with the described poor body condition, they were largely confined to the interstitium as adequately described by the contributor. Additionally, it was not clear whether there was hemorrhage and congestion in the participant's sections due to the collapsed and often distorted tissue so we elected not to include this in our diagnosis.

With *Blastomyces dermatitidis* being a close relative of *Emmonsia* spp., it is curious how dramatically different the extent of disease is between the two species. *Emmonsia* spp. is more typically self-limiting and often human patients receive only supportive therapy,[1] which provides a stark contrast to *B. dermatitidis* which incites a more dramatic granulomatous reaction and is capable of spreading systemically.[4] It is suspected that *Emmonsia* spp. lacks the virulence factors identified with *B. dermatitidis* and the other dimorphic fungi which are well known to cause significant respiratory and often systemic disease in animals.

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References:

1. Anstead GM, Sutton DA, Graybill JR. Adiaspiromycosis causing respiratory failure and a review of human infections due to *Emmonsia* and *Chrysosporium* spp. *J Clin Microbiol.* 2012;50(4):1346-1354.
2. Barbas Filho JV, Amato MB, Deheinzelin D, Saldiva PH, de Carvalho CR: Respiratory failure caused by adiaspiromycosis. Chest **97:** 1171-1175, 1990
3. Borman AM, Simpson VR, Palmer MD, Linton CJ, Johnson EM: Adiaspiromycosis due to Emmonsia crescens is widespread in native British mammals. Mycopathologia **168:** 153-163, 2009
4. Caswell JL, Williams KJ. Respiratory system. In: Maxie, MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. Vol. 2. Philadelphia, PA: Elsevier Saunders; 2007:641-642.
5. Echavarria E, Cano EL, Restrepo A: Disseminated adiaspiromycosis in a patient with AIDS. J Med Vet Mycol **31:** 91-97, 1993
6. England DM, Hochholzer L: Adiaspiromycosis: an unusual fungal infection of the lung. Report of 11 cases. Am J Surg Pathol **17:** 876-886, 1993
7. Finlayson GR, Shimmin GA, Temple-Smith PD, Handasyde KA, Taggart DA: Burrow use and ranging behaviour of the southern hairy-nosed wombat (Lasiorhinus latifrons) in the Murraylands, South Australia. Journal of Zoology **265:** 189 - 200, 2005

8. Hubalek Z: Emmonsiosis of wild rodents and insectivores in Czechland. J Wildl Dis **35:** 243-249, 1999
9. Johnstone AC, Hussein HM, Woodgyer A: Adiaspiromycosis in suspected cases of pulmonary tuberculosis in the common brushtail possum (Trichosurus vulpecula). N Z Vet J **41:** 175-178, 1993
10. Ladds P: Pathology of Australian Native Wildlife, 1 ed. CSIRO Publishing, Melbourne, 2009
11. Mason RW, Gauhwin M: Adiaspiromycosis in south Australian hairy-nosed wombats (Lasiorhinus latifrons). J Wildl Dis **18:** 3-8, 1982
12. Nimmo J, Krockenberger M, Smith EF: Pulmonary adiasporomycosis in a Victorian wombat. *In*: Australian Society of Veterinary Pathology Annual Meeting, pp. 65-67. Attwood, Victoria 2007
13. Peterson SW, Sigler L: Molecular genetic variation in Emmonsia crescens and Emmonsia parva, etiologic agents of adiaspiromycosis, and their phylogenetic relationship to Blastomyces dermatitidis (Ajellomyces dermatitidis) and other systemic fungal pathogens. J Clin Microbiol **36:** 2918-2925, 1998
14. Sigler L: Agents of adiaspiromycosis. *In*: Topley & Wilson's microbiology and microbial infections, eds. Ajello LHay R, 9th ed., pp. 571-583. Arnold, London, 1999
15. Sigler L: Ajellomyces crescens sp. nov., taxonomy of Emmonsia spp., and relatedness with Blastomyces dermatitidis (teleomorph Ajellomyces dermatitidis). J Med Vet Mycol **34:** 303-314, 1996

CASE III: AFIP 14 0454-93 C (JPC 4049379)

Signalment: Male boa constrictor imperator, *Boa constrictor imperator*

History: The boa was part of a reptile husbandry in which numerous animals were found to be in poor condition. Numerous petechiae were present on its ventral scales. Complete blood cell count revealed severe leukocytosis. These findings raised the suspicion for septicemia and, due to poor condition, the animal was euthanized. A necropsy was performed.

Gross Pathology: The animal was in poor body condition and showed severe cachexia. Numerous petechiae were present on ventral scales. The coelomic cavity contained about 15 mL of clear fluid. Serosa and organs were grossly unremarkable.

Laboratory Results: Antemortem blood cell count revealed severe leukocytosis with lymphocytosis (results not provided). Cytologic analysis was performed on coelomic effusion and revealed the presence of numerous bluish, homogenous, intracytoplasmic inclusions within leukocytes and red blood cells, leading to a diagnosis of Inclusion Body Disease (IBD). IBD was confirmed by PCR testing for IBD virus.

Histopathologic Description: The slide contains sections from the kidney, epididymis and nervous ganglia (including chromaffin cells). Numerous eosinophilic, round, variable-sized (2-10 μm) intracytoplasmic inclusion bodies are present in the renal tubular epithelium, neurons and

chromaffin cells, and epididymal epithelial cells. These inclusions are consistent with inclusions of IBD.

In the kidney, almost all glomeruli show abundant collagen deposition within the mesangium (glomerulosclerosis). Some glomeruli show cystic atrophy. Rare lymphocytes and heterophils infiltrate the interstitium. Numerous intensely eosinophilic and tightly packed 2-µm granules are present in nephrocytes of the distal convoluted tubules (sexual segment) whereas epithelium of the proximal convoluted tubules contain coarsely granular brownish pigments (see discussion).

Contributor's Morphologic Diagnosis:

Kidney:
1) Numerous intracytoplasmic eosinophilic inclusions within renal tubular epithelium, consistent with inclusions of Inclusion Body Disease.
2) Glomerulosclerosis, diffuse, severe with rare glomerular cystic atrophy.
3) Numerous brownish cytoplasmic pigments of unknown significance within epithelium of the proximal convoluted tubules.
4) Epididymis: Numerous intracytoplasmic eosinophilic inclusions within epididymal epithelium, consistent with inclusions of Inclusion Body Disease.
5) Nervous ganglia: Numerous intracytoplasmic eosinophilic inclusions with neurons and chromaffin cells, consistent with inclusions of Inclusion Body Disease.

Contributor's Comment: Inclusion body disease (IBD) is a well-known, worldwide and fatal disease of boids (boas and pythons), first described 30 years ago.[14] The disease is characterized by the presence of eosinophilic intracytoplasmic inclusions in cells of numerous tissues. The etiology of IBD remained uncertain for years. The discovery of virus-like particles by transmission electron microscopy in affected tissues raised the suspicion for a viral etiology. These particles had a diameter of 110 nm and a hexagonal capsid, resembling C-type retroviral particles.[14] However, the exact etiology of IBD was recently proven to be arenaviruses.[2,9,15] The bloodsucking snake mite *Ophionyssus natricis* may be a vector for IBD virus.[14]

Arenaviruses are negative single-stranded RNA viruses. Their genome contains two elements: small (S) and large (L). The S segment encodes the viral nucleocapsid protein and the glycoprotein while the L segment encodes the RNA-dependent RNA polymerase and a small ring-domain-containing protein.[3] The genus arenavirus is the only genus of the *Arenaviridae* family and comprises 25 species according to the International Committee on Taxonomy of Viruses (ICTV, 2014). Two major lineages of arenaviruses are described based on genetic differences and geographical distribution: Old World arenaviruses and New World arenaviruses.[3,5]

In humans, arenaviruses cause hemorrhagic fevers (Lassa, Junin, Machupo, Guanarito, Sabia and Chapare viruses) and lymphocytic choriomeningitis (LCM) due to LCM virus. Asymptomatic infections also occur.[6]

The LCM virus can also produce congenital malformations and has been recently described as an important cause of fatal infection in organ transplantations recipients and immunocompetent patients.[6] LCM virus has a wide range of hosts: humans, hamsters, guinea pigs, cotton rats, chinchillas, canids and primates.[11] *Mus musculus* is considered the natural reservoir host. In New

World primates of the *Callitrichidae* family (marmosets, tamarins), LCM virus causes callitrichid hepatitis.[11]

In boids, IBD does not manifest similarly between boas and pythons. Boas usually show intermittent regurgitation followed by anorexia. After a few weeks, neurologic signs appear and are characterized by head tremor, disorientation, ataxia, opisthotonos and behavioral changes.[14,16] Pneumonia and necrotizing stomatitis are common complications. After a few weeks or months of progression, death occurs.[13] Pythons do not display regurgitation but are often anorectic. Neurologic signs occur earlier in pythons than in boas, and are more severe. The progression is more rapid and death occurs after a few weeks.[4,14]

Historically, the diagnosis of IBD relied on the demonstration of characteristic eosinophilic intracytoplasmic inclusions on histological sections. In pythons, these inclusions are mostly found in the neurons of the central nervous system.[4] In boa constrictors, inclusions are commonly seen, in addition to neurons and glial cells of the central nervous system, in esophageal tonsils (epithelium and lymphoid cells), gastrointestinal and respiratory epithelia, hepatocytes, pancreatic acinar cells and renal tubular epithelium.[4] Inclusions can also be demonstrated on blood smears and/or impression smears of organs (liver and kidney). Such smears can be stained with Wright-Giemsa stain but H&E stain can also be used and appears more sensitive.[4] On blood smears, inclusions can be demonstrated in erythrocytes, lymphocytes and heterophils.[4] Tissues for diagnosis can be obtained from necropsy samples but antemortem diagnosis is also possible through esophageal tonsil, liver or renal biopsies.[4] As the number and distribution of inclusions is variable, with boas having more inclusions than pythons, diagnosis relying on identification of inclusion bodies is not a very sensitive method. Furthermore, inclusions can be found in other diseases.[4] With the recent discovery of IBD virus, a definitive and more sensitive diagnosis is now possible through PCR testing.

In this case, inclusion bodies were found in a large number of tissues as well as in cells from coelomic effusion, allowing antemortem diagnosis. PCR testing confirmed infection by IBD virus. In the kidney, renal epithelial cells also contained variable-sized acidophilic granules and brownish pigments. The acidophilic granules are typical to adult males of some snake and lizard species. They are present in the distal convoluted tubules, referred to as the "sexual segment". The content of the granules is extruded into the urinary wastes and is believed to represent pheromones that are useful for sexual courtship and mating.[1] The brownish pigments are of unknown origin and significance. They were negative for Prussian blue stain, Schmorl's stain and PAS stain.

JPC Diagnosis: 1. Epithelial cells of renal tubules, ureter, and epididymis and neurons: Intracytoplasmic protein droplets, numerous. 2. Kidney: Glomerulosclerosis, diffuse, moderate. 3. Kidney, tubular epithelium: Brown pigment of unspecified origin. 4. Kidney, tubular epithelium: Intracytoplasmic apicomplexans, few.

Conference Comment: This case generated a lot of discussion, largely on the source of the unspecified brown inclusions within renal epithelium which are quite dramatic in most sections. The discussed differentials included protein, iron, copper, hemoglobin, melanin or lipofuscin. Unfortunately the contributor's stains and our additional stains did not aid in their characterization.

The contributor highlights the recent identification of an arenavirus as the cause of IBD. The characteristic cytoplasmic inclusions associated with IBD are dramatic and found in many tissues usually in the absence of inflammation.[4] Typically, viral inclusions are the result of viral nucleic acid templates liberated in the cytoplasm which are utilized by the host cell to produce aberrant production of viral proteins. These proteins are often produced in excess and subsequently accumulate as inclusion bodies. While most viral inclusions are composed of excess viral proteins and membranes with viral particles, some consist of mature viral particles (virions) arranged in lattice formations.[7] IBD inclusions are nonviral, composed exclusively of 68-KDa protein deposited by ribosomes[10] which may have hindered prompt identification of the virus.

The observation of viral protein inclusions and brown pigment within the tubular epithelium was further complicated by the prominent acidophilic granules common in male reptiles as discussed by the contributor. It is worth mentioning this snake was in its reproductive season at the time of necropsy as the granules are prominent and sperm production is abundant.

The presence of glomerulosclerosis is a common finding in older reptiles; and we chose to separate its diagnosis as most did not feel it was related to the viral infection. Glomerulosclerosis is characterized by shrunken and hyalinized mesangium, with an increase in fibrous connective tissue and a loss of capillaries. Tubular degeneration often occurs secondarily, as they receive their blood supply from the glomerular efferent arteriole which becomes compromised in these instances. Glomerulosclerosis is accelerated by inflammation, excessive dietary protein and increased glomerular capillary pressure.[12] Its widespread occurrence in reptiles is most often attributed to high protein diets.[8]

Rarely observed in a few sections and confined to one small area of renal tubules, there are few intraepithelial organisms closely resembling an undetermined species of coccidia which gave us a fourth diagnosis to contribute.

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References:

1. Aughey E, Frye FL. Urinary System. In: *Comparative Veterinary Histology with Clinical Correlates*. 1st edition. London: CRC Press; 2001:137-148.
2. Bodewes R, Kik MJL, Raj VS, *et al.* Detection of novel divergent arenaviruses in boid snakes with inclusion body disease in The Netherlands. *J Gen Virol.* 2013; **94**:1206–10.
3. Buchmeier M, de la Torre J, Peters C. Arenaviridae. In: Knipe DM, Howley PM, eds. *Fields Virology*. 5th edition. New York, NY: Lippincott Williams & Wilkins; 2006:1791–828.
4. Chang L-W, Jacobson ER. Inclusion Body Disease, A Worldwide Infectious Disease of Boid Snakes: A Review. *J Exotic Pet Med.* 2010; **19**:216–25.
5. Charrel RN, de Lamballerie X, Emonet S. Phylogeny of the genus Arenavirus. *Curr Opin Microbiol.* 2008; **11**:362–8.
6. Charrel RN, de Lamballerie X. Zoonotic aspects of arenavirus infections. *Vet Microbiol.* 2010; **140**:213–20.

7. Cheville NF. Ultrastructural Pathology The Comparative Celullar Basis of Disease. 2nd ed. Ames, IA: Blackwell Publishing; 2009:851.
8. Hernandez-Divers S, Innis CJ. Renal disease in reptiles: diagnosis and clinical management. In: Mader DR, ed. *Reptile Medicine and Surgery*. 2nd ed. St. Louis, MO: Saunders Elsevier; 2006:879.
9. Hetzel U, Sironen T, Laurinmäki P, *et al.* Isolation, identification, and characterization of novel arenaviruses, the etiological agents of boid inclusion body disease. *J Virol.* 2013; **87**:10918–35.
10. Jacobson ER. Viruses and viral diseases of reptiles. In: Jacobson ER, ed. *Infectious Diseases and pathology of reptiles*. Boca Raton, FL: CRC Press; 2007:410-412.
11. Montali RJ, Connolly BM, Armstrong DL, Scanga CA, Holmes KV. Pathology and immunohistochemistry of callitrichid hepatitis, an emerging disease of captive New World primates caused by lymphocytic choriomeningitis virus. *Am J Pathol.* 1995; **147**:1441–9.
12. Newman SJ. The Urinary system. In: Zachary JF, McGavin MD, eds. *Pathologic Basis of Veterinary Disease.* St. Louis, MO: Elsevier Mosby; 2012:626-627.
13. Percy DH, Barthold SW. The mouse. In: *Pathology of Laboratory Rodents and Rabbits*. 3rd edition. Ames, Iowa: Wiley-Blackwell; 2007:3-124.
14. Schumacher J, Jacobson ER, Homer BL, Gaskin JM. Inclusion Body Disease in Boid Snakes. *J Zoo Wildl Med.* 1994; **25**:511–24.
15. Stenglein MD, Sanders C, Kistler AL, *et al.* Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease. *MBio.* 2012; **3**: e00180–00112.
16. Vancraeynest D, Pasmans F, Martel A, *et al.* Inclusion body disease in snakes: a review and description of three cases in boa constrictors in Belgium. *Vet Rec.* 2006; **158**:757–60.

CASE IV: E 5496/12 (JPC 4033372)

Signalment: Snowy owl of unknown age and gender, *Bubo scandiacus*

History: The snowy owl was found dead without any previous clinical signs.

Gross Pathology: Autopsy revealed numerous white spots of about 0.2 cm in diameter in the spleen and liver.

Laboratory Results: None

Histopathologic Description: Small intestine: Multifocally, the mucosa and submucosa are replaced by large areas of coagulation necrosis characterized by loss of cellular detail, karyorrhexis, karyopyknosis, karyolysis and the presence of numerous heterophils (mostly degenerated, partially viable), extravasated erythrocytes (hemorrhage), and deposition of fine fibrillar, pale eosinophilic material (fibrin). Adjacent to the necrotic areas are moderate infiltrates composed of macrophages and fewer lymphocytes. Occasionally, the necrosis and inflammatory cells extend through the tunica muscularis and to the serosa with multifocal mild serosal inflammation as described above. There are multifocal crypt abscesses characterized by attenuated epithelium and intraluminal accumulation of cellular debris, sloughed epithelial cells,

fibrin and few degenerate heterophils. In numerous epithelial cells, large (4-6 μm) intranuclear, eosinophilic inclusion bodies that almost fill the nucleus and marginate the chromatin are present. Some adventitial vessels show an increased number of erythrocytes (mild congestion).

Contributor's Morphologic Diagnosis: Small intestine: Enteritis, severe, acute, necrotizing and ulcerative, multifocal, with intranuclear eosinophilic inclusion bodies (Cowdry A type) consistent with strigid herpesvirus-1 infection

Microscopic Findings of Tissues not submitted: In the liver and spleen multifocal areas of acute necrosis with intralesional eosinophilic intranuclear inclusion bodies are present.

Contributor's Comment: Strigid herpesvirus-1 (StHV-1) belongs to the β-herpesvirinae[8] and is the cause of *hepatosplenitis infectiosa strigum*, a disease that affects only owls with a yellow or orange iris and was first described in 1936[7] and further classified in 1973.[1] It is closely related to falconid herpesvirus-1 (FHV-1) of falcons, eagles, and hawks, and columbid herpesvirus-1 (CoHV-1) of pigeons.[10] The three viruses were indistinguishable by serum neutralization and it was hypothesized they might represent the same virus.[8]

Sequence analyses revealed an almost 100% sequence identity between FHV-1, CoHV-1, and StHV-1 whereas there were considerable differences to other avian herpesviruses.[8] These results further supported the hypothesis that owls and falcons might get infected by the consumption of pigeons infected with CoHV-1.[6,8] Natural infections with StHV-1 have been observed in the eagle owl (*Bubo bubo L.*), long-eared owl (*Asio otus L.*) and snowy owl (*Bubo scandiacus*) as well as in great horned owls (*Bubo virginianus*) in the United States. [2,8]

The majority of infected owls are found dead without previous clinical signs. However, some show depression, anorexia, conjunctivitis, oral and pharyngeal ulcerations and respiratory symptoms as well as diarrhea.[2,6]

The main histologic feature of the disease is the presence of numerous necrotic foci in the liver, spleen and bone marrow.[6] Typically, in the liver, spleen and several additional organs, eosinophilic intranuclear inclusion bodies of Cowdry A type are present. Necrotic areas may appear in the pharynx and small intestine as well.[6]

JPC Diagnosis: Intestine: Enteritis, necrotizing, transmural, focally extensive, severe, with intranuclear viral inclusions.

Conference Comment: Herpesvirus infection in birds of prey is caused by a member of the subfamily *Alphaherpesvirinae*. The debate over whether owls and falcons are infected by a closely-related but genetically distinct herpesvirus, so named strigid herpesvirus-1 and falconid herpesvirus-1, seems to have been resolved with recent unequivocal evidence demonstrating all raptors being infected by a single herpesvirus, columbid herpesvirus-1 (CoHV-1).[6,8,11] CoHV-1 is harbored, often subclinically, within adult rock pigeons yet causes significant lesions in raptors,[6] akin to other host-adapted herpes infections such as ovine herpesvirus-2 in sheep. In squabs (nestling pigeons), its infection may manifest as observed in raptor species: hepatic necrosis, splenic necrosis, renal necrosis, and ulceration of the respiratory and gastrointestinal tracts.[2] Typically, raptors will prey on pigeons when more preferable options are scarce, as often

occurs in the early spring, which offers a reasonable mode of viral transmission. The number of great horned owls infected with *Trichomonas gallinae*, a protozoan parasite also harbored in rock pigeons, has been documented to be elevated during this time of year further supporting this theory.[11]

Herpesviruses are well-known, ubiquitous infectious agents across the entire animal kingdom, with new strains being discovered regularly, often which pose significant health threats to the affected population. Recent publications have described herpesviral necrotizing stomatitis in Eastern box turtles (terrapene herpesvirus-1),[12] significant mortality in koi and common carp (cyprinid herpesvirus-3),[5] and a sarcoma inducing herpesvirus in baboons (Kaposi's Sarcoma-associated herpesvirus).[13] Adding further interest to the subject are the peculiarities of pathogenesis and disease transmission among some herpes pathogens, such as the seasonal-dependent neoplastic growth or viral shedding of ranid herpesvirus-1 in leopard frogs[3] and the recent proposal of chelonid herpesvirus-5 "superspreaders" causing outbreaks of fibropapillomatosis in marine turtles.[14]

Central to the success of all herpesviruses is the interplay between latency and lytic modes of infection. Latency is characterized by restricted viral gene expression permitting the virus to evade the host immune system. Upon reactivation, a cascade of gene expression is initiated enabling its spread between cells and between hosts. In all subfamilies of human herpesviruses, the latent to lytic switch has been found to occur through expression of one or more microRNAs (miRNA).[4] MiRNAs are noncoding, or transcribed but not translated, single stranded RNAs which inhibit translation of messenger RNA through the action of the RNA-induced silencing complex (RISC). In this way, they are able to control cell growth, differentiation and survival. Their characteristics are now well understood regarding development of cancer and their expression is conserved among all eukaryotes, including microorganisms such has the numerous herpesviruses discussed here.[9]

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References:

1. Bürki F, Burtscher H, Sibalin M: Herpesvirus strigis: a new avian herpesvirus. I. Biological properties. Arch Gesamte Virusforsch. **43:** 14-24, 1973.
2. Burtscher H, Sibalin M: Herpesvirus strigis: host spectrum and distribution in infected owls. J Wildl Dis. **11:** 164-9, 1975.
3. Carlson DL, Sauerbier W, Rollins-Smith LA, McKinnell RG. The presence of DNA sequences of the Lucke herpesvirus in normal and neoplastic kidney tissue of Rana pipiens. *J Comp Pathol.* 1994;110:349-355.
4. Frapper L. Regulation of herpesvirus reactivation by host miRNAs. *J Virol.* 2014 Dec 24 pii: JVI.03413-14 [Epub ahead of print].
5. Fujioka H, Yamasaki K, Furusawa K. Prevalence and characteristics of Cyprinid herpesvirus 3 (CyHV-3) infection in common carp (Cyprinus carpio L.) inhabiting three rivers in Kochi Prefecture, Japan. *Vet Microbiol.* 2014 Dec 11. Pii: S0378-1135(14)005574 [Epub ahead of print]
6. Gailbreath KL, Oaks JL: Herpesviral inclusion body disease in owls and falcons is caused by the pigeon herpesvirus (columbid herpesvirus 1). J Wildl Dis. **44:** 427-33, 2008.

7. Green RG, Schillinger JE: A virus disease of owls. Am J Pathol. **12:** 405-410, 1936.
8. Kaleta EF: Herpesviruses of birds - a review. Avian Pathol. **19:** 193-211, 1990
9. Kumar V, Abbas AK, Aster JC. *Robbins and Cotran Pathologic Basis of Disease.* 9th ed. Philadelphia, PA: Elsevier Saunders; 2015:320.
10. Potgieter LN, Kocan AA, Kocan KM; Isolation of a herpesvirus from an American kestrel with inclusion body disease. J Wildl Dis. **15:** 143-9, 1979.
11. Rose N, Warren AL, Whiteside D, et. al. *Columbid herpesvirus-1* mortality in great horned owls (*Bubo virginianus)* from Calgary, Alberta. *Can Vet J.* 2012;53(3):265-268.
12. Sim RR, Norton TM, Bronson E, et. al. Identification of a novel herpesvirus in captive Eastern box turtles (Terrapene carolina carolina). *Vet Microbiol*. 2014 Dec 13. Pii: S0378-1135(14)005549. [Epub ahead of print]
13. Whitby D, Stossel A, Gamache C, et. al. Novel Kaposi's sarcoma-associated herpesvirus homolog in baboons. *J Virol*. 2003;77(14):8159-8165.
14. Work TM, Dagenais J, Balazs GH, Schettle N, Ackermann M. Dynamics of virus shedding and in situ confirmation of chelonid herpesvirus 5 in Hawaiian green turtles with fibropapillomatosis. *Vet Pathol.* 2014 Dec 1 DOI: 10.1177/03000985814560236 [Epub ahead of print].