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CASE I – 2768804 (AFIP 2887158)

Signalment: 2 week old, male and female, mixed breed, porcine, *Sus scrofa domesticus*.

History: Sudden outbreak in nursery of piglets with inappetance, liquid yellow feces, dehydration, weight loss, mortality approaching 50% in young piglets. Gilts exhibited decreased appetites and occasional vomiting.

Gross Pathology: Stomachs filled with curdled milk. Lacteals within the mesentery were empty. The intestines were thin-walled, with yellow foamy fluid contents. Close examination of jejunum and ileum revealed atrophied mucosal lining.

Laboratory Results: Direct fluorescent antibody examination of intestines: positive for coronavirus, negative for rotavirus. PCR on feces strongly positive for coronavirus. Bacterial isolation of >100 cfu of *Escherichia coli* from intestines. PCR testing of *E. coli* isolates negative for the following pilus and toxin genes: F41, K88, 987p, F18, K99, Stx2, STa, STb, and LT.

Contributor's Morphologic Diagnosis: Jejunum and ileum: acute, severe, atrophic enteritis with villous blunting and fusion, and superficial epithelial attenuation.

Contributor's Comment: This is a fairly classic acute outbreak of Transmissible Gastroenteritis (TGE) due to coronavirus. Rapid spread, high mortality in young piglets, and illness in gilts indicates a herd with limited immunity and a case of epizootic TGE. Diarrhea and mortality typically affect piglets less than 3-5 weeks of age; with milder signs in older piglets, and only transient inappetance and vomiting in adults. The duodenum is typically spared by the TGE coronavirus, which replicates in and destroys the crypt enterocytes. The jejunum and to a lesser degree the ileum exhibit the most severe villous atrophy; in this case the reduction in villous to crypt ratio approaches 1:1.

Both FA and PCR are rapid means of confirming the diagnosis. More recently, a non-enteropathogenic variant of TGE known as Porcine Respiratory Coronavirus (PRCV) has been reported in Europe and North America. While TGE may be complicated by other enteric pathogens (rotavirus, *E. coli*, *Salmonella* sp.), this appears to be a relatively pure infection with TGE. *Escherichia coli* was isolated; however, no pilus or toxin genes were detected, nor was there microscopic evidence of bacterial colonization of the gut. In the face of an outbreak, supportive therapy and antibiotics are used to treat affected piglets, while intentional exposure of pregnant sows or vaccination can be utilized to limit further spread.[1]

AFIP Diagnosis: Small intestine: Villus blunting and fusion, segmental, with apical epithelial necrosis and multifocal regeneration, mixed breed, porcine.

Conference Comment: Conference attendees discussed the possible mechanisms for the age-dependent susceptibility to TGE virus. Neonates normally have tall villi (villus height to crypt depth is normally 7:1 to 9:1) with mature differentiated enterocytes and short inactive crypts of undifferentiated epithelium, resulting in a large population of susceptible villus cells and crypts that are slow to repair. A second mechanism may be associated with gastric secretions. Milk buffers gastric acid in neonates, so this acid-labile virus is better protected in the less acidic environment of the neonate's stomach. In addition to the above, neonates are inherently more susceptible to dehydration, electrolyte imbalances, and hypoglycemia, making them more susceptible to the effects of this virus.[1,2,3]

Although all ages of pigs may be affected in a susceptible herd, TGE is generally a disease of high morbidity and mortality in pigs younger than 10 days of age, causing vomiting and profuse diarrhea. Differential diagnosis for diarrhea in young pigs includes *E. coli*, rotavirus, *Clostridium perfringens* type C, hemagglutinating encephalomyelitis virus, and coccidiosis. Enteric colibacillosis is a common cause of profuse diarrhea, without vomiting, in piglets less than 10 days of age with peak incidence at 3 days of age. Rotavirus causes disease in suckling and weaned pigs (1-5 weeks of age) with less severe villus atrophy than seen in TGE. Clostridial enterotoxemia is a rapidly fatal disease of newborn piglets less than one week of age, causing bloody diarrhea. Hemagglutinating encephalomyelitis virus, another coronavirus, is the cause of vomiting and wasting disease. Vomiting and wasting disease affects pigs less than 10 days old and is characterized by vomiting and weight loss. Additionally, a number of affected pigs develop acute encephalomyelitis. Diarrhea may occur but, in contrast to TGE, is not severe. Coccidiosis causes diarrhea without blood in piglets 5-15 days of age, with peak incidence at 7-10 days of age.[4]

Grossly, TGE causes distension of the small intestine with gas and yellow frothy fluid and flaccid, thin, transparent intestinal walls. Of the differentials listed above, the gross lesions that most closely resemble TGE are those of *E. coli* and coccidiosis.[1,2,3]

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CASE II - Experimental Path Labs (AFIP 2890229)

Signalment: 2-year-old, female, Chinese Shar Pei, domestic dog (*Canis familiaris*).

History: A two year old, female, Chinese Shar Pei presented with vomiting and diarrhea. Blood work and urinalysis revealed an elevated BUN and creatinine and isosthenuria. The dog responded poorly to symptomatic treatment for renal failure and euthanasia was elected.

Gross Pathology: The kidneys had an irregular to finely granular surface and were diffusely pale (necropsy was limited to the kidneys).

Laboratory Results:
Blood Work:
BUN: 82.8
Creatinine: 8.35
Total Protein: 8.5
Cholesterol: 310

Urinalysis:
Specific Gravity: 1.011
Protein: Trace

Contributor's Morphologic Diagnosis: Kidneys: Renal amyloidosis, medullary and glomerular, severe.

Contributor's Comment: Familial renal amyloidosis has been described in the Chinese Shar Pei[1]. These dogs usually present at a relatively young age (average 4.1

years) and sometimes have a clinical history of intermittent fever and swelling of the tibiotarsal joints[1]. Unlike most forms of canine renal amyloidosis, in which the amyloid deposition is primarily glomerular, Shar Peis with this familial form typically develop medullary amyloid deposits sometimes leading to renal papillary necrosis[1]. However, over half of these dogs also have some degree of glomerular amyloid deposition as well as deposition in other organs including the spleen, liver, heart, prostate, pancreas, lymph nodes and intestine[1]. The amyloid in these familial cases is Congo Red positive and sensitive to potassium permanganate treatment indicating that it is of the AA (reactive) type[1].

AFIP Diagnoses:
1. Kidney: Amyloidosis, interstitial and glomerular, multifocal, moderate, Chinese Shar-Pei, canine.
2. Kidney: Nephritis, interstitial, lymphoplasmacytic, chronic, multifocal, moderate.

Conference Comment: The most common forms of amyloidosis are primary (immunocytic) and secondary (reactive). Primary amyloidosis is often seen in patients with plasma cell dyscrasias because AL (amyloid light chain) is composed of immunoglobulin light chains. In secondary amyloidosis, AA (amyloid-associated) is derived from serum amyloid-associated (SAA) protein, which is an acute phase protein produced in the liver as a result of chronic antigenic stimulation. Hereditary reactive amyloidosis is found in Chinese Shar-Peis, as demonstrated by this case, and is also reported in Abyssinian cats.[2,3,4]

Pulmonary arterial and renal vein thrombosis have been reported as sequelae in animals with glomerular amyloidosis. Glomerular amyloidosis results in loss of serum proteins leading to proteinuria and hypoalbuminemia. Renal loss of antithrombin III produces a hypercoagulable state that predisposes to thrombosis. Development of thrombi may also be exacerbated by stimulation of acute phase proteins, such as fibrinogen.[2]

In most animals Congo red is used to stain amyloid deposits, which shows green birefringence under polarized light. Congo red may not stain amyloid in cats. In these cases, thioflavin T may be used to demonstrate amyloid which fluoresces bright yellow when polarized.[2]

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CASE III – L02-7504 (AFIP 2889975)

Signalment: These tissues are from an adult, female, three-spined stickleback, *Gasterosteus aculeatus*.

History: A group of three-spined sticklebacks were caught in Alaska and introduced to a laboratory colony for genetic studies. These fish have subsequently developed white lumps on body surfaces. Affected fish have been culled, but new infected individuals continue to appear.

Gross Pathology: Ten spherical, creamy white, smooth nodules approximately 2 mm in diameter were distributed on the body surface from the opercula and extending caudally along the lateral and ventral body walls. These nodules oozed white fluid on section. An additional 6 nodules were identified in the oral cavity after euthanasia. A 16x7x1.5 mm, dorsoventrally flattened, segmented larval cestode filled the coelomic cavity.

Laboratory Results: Microscopic examination of a wet preparation from one surface nodule revealed myriad 2 x 5 micron, slightly elongated microsporidian spores each with a terminal vacuole (Fig. 3).

Contributor's Morphologic Diagnoses:
1. Body: Multiple xenomas, body wall and oral cavity, consistent with *Glugea* sp. (Microsporiasis).
2. Intestine and kidney: Mild to moderate protozoiasis, minimal tissue changes, suspect *Eimeria* sp.
3. Liver: Moderate, diffuse glycogen depletion.
4. Liver: Mild, diffuse hepatic lipidosis.
5. Ocular lens: Encysted trematode metacercaria, suspect *Diplostomum* sp. (only present in a small number of slides).
6. Coelomic larval cestode (plerocercoid) consistent with *Schistocephalus* sp. (gross diagnosis).

Contributor's Comment: In multiple coronal sections of whole fish, the most significant changes are the multiple (11 profiles in the slide examined) roughly spherical masses (xenomas) measuring 1 to 1.5 mm diameter and distributed in the superficial body wall and oral cavity (Fig. 1). The xenomas are delineated by a prominent hyaline membrane and contain myriad approximately 2 x 5 micron bodies. Amphophilic to

basophilic cell debris is distributed within these xenomas, especially near the periphery. One xenoma has ruptured into the peritoneal cavity and another has ruptured into the subcutaneous tissue of the operculum. Skeletal muscle degeneration is associated with some body wall xenomas.

All microsporidians infect host cells, but some, such as *Glugea*, induce severe cellular hypertrophy that, in conjunction with the parasite, forms a "xenoparasitic complex" or xenoma (Fig. 2 - xenoma in abdominal cavity of a stickleback)[1]. *Glugea anomala* was first described in sticklebacks in 1887 [2]. This organism causes a chronic infection characterized by the production of tumor-like masses that may have little effect on the host unless vital organs are affected. No intermediate host is required; spores released from xenomas can directly infect new tissues. Infection occurs via a polar filament that anchors the spore to the host while the sporoplasm is extruded through the everted filament.

Microsporidians are common in the environment, including water supplies, and infect all the major animal groups. These organisms are opportunistic pathogens of humans; eight genera of microsporidians have been identified in human infections[3]. Recent phylogenetic analysis suggests microsporidians are closely related to fungi. Diagnosis of microsporidian infection can be made based on identification within target tissues of spores that are 2 to 10 microns in length, egg-shaped to elliptical and have a prominent posterior vacuole (Fig 3)[1]. Ribosomal RNA sequences are useful as more specific diagnostic tools. Diagnosis to the species level in the present case was not pursued.

All wild fish are likely to have a significant parasite load. The encysted trematode and the suspect protozoal organisms in the intestine and kidneys are considered incidental findings. Hepatic glycogen depletion and lipidosis likely reflect poor body condition secondary to debilitation and/or inadequate food intake.

AFIP Diagnoses:

1. Retroperitoneal and subepithelial tissues: Multiple xenomas, three-spined stickleback (*Gasterosteus aculeatus*), piscine.
2. Intestine, mucosa: Small number of protozoal zoites.
3. Kidney, tubules: Small number of protozoal zoites.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant for veterinary parasitology. Microsporidia are obligate intracellular parasites with a direct life cycle. A characteristic feature of all microsporidia is the extrusion apparatus. This is composed of a polar tube attached to the anterior end of the spore by an anchoring disc, which contains 4-30 coils. The polar tube everts and "injects" sporoplasm into the host cell cytoplasm. All stages of this protozoa are gram positive, a unique feature of *Microspora*. Mature spores are acid-fast, have a PAS-positive polar tube, and are anisotropic.[3,4,5]

Another microsporidium of veterinary importance is *Encephalitozoon cuniculi*. This protozoa infects a wide variety of mammals and causes lesions predominantly in the brain, kidney, and vascular endothelium.[6] Several microsporidia are common enteric pathogens in immunocompromised, HIV-infected patients. Such pathogens include *Enterocytozoon bienueusi* (also reported in SIV-infected macaques), *Vittaforma corneae* (*Nosema corneum*), and *Trachipleistophra hominis*.[4,5]

There are coccidia present in the sections examined by conference attendees. Dr. Gardiner identified sporulated oocysts in the tissue. By definition, *Eimeria* does not sporulate in tissue; however, we were unable to further classify these organisms.

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CASE IV - 00-0369 (AFIP 2741057)

Signalment: 13-year-old male owl monkey (*Aotus trivirgatus*).

History: This 13-year-old male owl monkey was previously on several malaria protocols, but has been in the issue pool for the last three years. Early radiographic signs of cardiac enlargement were noted in May 1999; however, the animal remained asymptomatic. Significant weight loss was recorded in February 2000. In the evening on 27 Mar 00 he was found sitting in the bottom of his cage (owl monkeys typically stay high in the cage on limbs or perches). At this time he was weak, but bright, alert and responsive, had a good appetite and normal respiratory rate and heart rate. He was found dead the next morning.

Gross Pathology:
Brain: A red friable mass approximately 1.5 x 1.5 x 0.7 cm was present in the white matter of the temporal and occipital lobes of the right cerebral hemisphere. The mass compressed and distorted adjacent structures including the corpus callosum, lateral ventricles, thalamus, hippocampus and midbrain. The overlying gray matter was markedly thinned (Fig A - gross photo).
Kidneys: The kidneys were bilaterally small and diffusely pale. On cut section white, radiating streaks extended from the renal pelvis to the capsular surface and the corticomedullary junction was mildly obscured.
Heart: Thin white streaks were scattered diffusely throughout the myocardium.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Brain, cerebrum: Hemorrhage, acute, focally extensive, severe, with cerebral cavitation, owl monkey (*Aotus trivirgatus)*, primate.
2. Brain, cerebral arteries: Atherosclerosis, chronic, multifocal, moderate to severe, with thrombosis, aneurysmal dilatation and perivascular hemosiderin.

Other histologic findings [tissues not submitted for conference]:
Kidney: Nephritis, interstitial, eosinophilic and lymphoplasmacytic, chronic, multifocal, mild to moderate, with membranous glomerulonephritis and hyperplastic arteriolosclerosis.
Heart: 1. Fibrosis, multifocal, moderate with arteriosclerosis.
2. Perivasculitis, eosinophilic, multifocal, mild.

Contributor's Comment: The pathogenesis of atherosclerosis is complex and not fully understood. This case of naturally occurring atherosclerosis in an owl monkey is of interest in that it provides a framework in which to discuss this complex entity and the use of nonhuman primates as an animal model.

In the case submitted, meningeal arteries contain atheromatous plaques (Fig. 1), which are typically focal (not affecting the entire circumference of the artery) intimal lesions comprised of a fibrous cap and a central necrotic lipid rich core. In several areas this lesion is associated with aneurysm, thrombosis (Fig. 2) or both. In people, atherosclerosis most commonly leads to ischemic injury such as myocardial or cerebral infarction; it is also associated with aneurysms, typically in the aorta. In this case atherosclerosis is associated with cerebral hemorrhage, likely secondary to a ruptured aneurysm of a meningeal artery.

Current research suggests that atherosclerosis is a chronic inflammatory process initiated by endothelial injury (response to injury hypothesis). Factors thought to contribute to endothelial damage include hyperlipidemia (specifically elevated cholesterol-rich low-density lipoproteins (LDL)), hypertension, stress, cigarette smoking, diabetes mellitus, genetic alterations, elevated plasma homocysteine, and infectious agents. Ross[5], Woolf[7] and Schoen and Cotran[6] have recently reviewed the postulated

pathogeneses of these risk factors. In this case it is interesting to note that although systemic hypertension was not documented clinically, the presence of renal hyperplastic arteriolosclerosis or "onion skinning" (Figs. 3,4,5) is highly suggestive of hypertension. Additionally, multifocal myocardial fibrosis suggests previous ischemic episodes. Naturally occurring and experimentally induced atherosclerosis is well documented in a variety of nonhuman primates. According to April and Keith[1] the most commonly affected nonhuman primates are baboons, rhesus monkeys, squirrel monkeys, cynomolgus monkeys and African green monkeys. There is at least one report in the literature[4] of naturally occurring atherosclerosis in the owl monkey. The cynomolgus monkey is currently the most commonly used nonhuman primate model. Kaplan and Manuck[3] recently published an interesting study on the association of environment and behavior to development of atherosclerosis in cynomolgus monkeys.

AFIP Diagnoses:
1. Brain, cerebrum: Hemorrhage, acute, focally extensive, severe, with cerebral cavitation, owl monkey (*Aotus trivirgatus)*, primate.
2. Brain, cerebral arteries: Atherosclerosis, chronic, multifocal, moderate to severe, with thrombosis.

Conference Comment: Atherosclerosis is rare in animals. It has been reported as a sequela of hypothyroidism and diabetes mellitus in dogs. Miniature schnauzers have a breed predisposition for atherosclerosis that has been associated with idiopathic hyperlipoproteinemia.

Several animal models have been suggested for the study of atherosclerosis. Pigs are the only domestic animal that commonly develop atherosclerosis and may represent a natural animal model for this disease. Additionally, rabbits, chickens, pigs, and nonhuman primates fed high cholesterol diets also develop atherosclerosis.[8]

Two strains of rabbits commonly used as models of atherosclerosis are the Watanabe heritable hyperlipidemic rabbit and the St. Thomas's Hospital rabbit. The Watanabe rabbit was the first animal model of natural endogenous hypercholesterolemia, and is a well-established model with a deficiency of LDL receptors in the liver and other tissues. The St. Thomas's Hospital strain is named for the hospital in London where it was developed; these animals maintain hypercholesterolemia despite normal LDL receptors.[9,10]

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