The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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CONFERENCE 24
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Conference Moderator: Dr. Donald Nichols
Department of Pathology
Smithsonian National Zoological Park
Washington, DC 20008

CASE I – 8497 (AFIP 2685152)

Signalment: Female, Madagascan flat-tailed tortoise, *Acinixys planicauda*

History: Tortoises were imported from Madagascar to Japan in February 1998. After their arrival to Japan, the animals were housed separately in an air-conditioned animal room (about 28 C), fed a standard diet, and supplied tap water *ad libitum*. In May 1998, some tortoises suddenly showed anorexia, depression of activity, and died within several days. Many nematodes were isolated from the intestine of the dead animals. After that, this tortoise was sent to an animal hospital. Under the diagnosis of helmintic infection, the tortoises were immediately treated with metronidazole, fenbendazole, penicillamine, and enrofloxacin, with fluid replacement and forced feedings. However, the tortoise died within a month.

Gross Pathology: Macroscopic examination of the tortoise revealed multiple severe erosions and ulcers with greenish pseudomembrane of the colon. The colonic wall was thickened and edematous. Many nematodes were found in the colonic lumen. Duodenum and jejunum had no significant change. In the liver, multifocal greenish foci were disseminated throughout the all lobes. The size of foci ranged from pinhead to 5 mm.

Laboratory Results: Many nematodes (*Atractis sp*.) were found in the colonic lumen.

Contributor's Morphologic Diagnosis: Liver: Multifocal hepatocytic necrosis with ameba (*Entamoeba invadens*).

Contributor's Comment: Microscopically, the colonic wall had severe erosions and ulcers covered with a fibrinonecrotic pseudomembrane. Heterophils and eosinophils severely infiltrated this lesion. Many amebic trophozoites were detected in edematous submucosal areas rather than in apical portions of the ulceration. They usually invaded the lumen of blood vessels in the submucosa. This animal was infected with bacteria and fungus resulting in a pseudomembrane formation.

Amebiasis is considered one of the most significant protozoal diseases of reptiles, especially snakes and lizards. The amebic trophozoites in our cases were confirmed as *Entamoeba invadens* according to their size and morphological character. Some tortoises are known to serve as healthy reservoirs of *E. invadens.* It is uncertain whether this tortoise is a healthy reservoir or susceptible to this protozoa. In the ulcerative lesions of the colon in the present case, nematodes, bacteria, and fungus along with the ameba simultaneously invaded the mucosa. Therefore, it is unclear which pathogenic organism caused the primary mucosal lesion. However, it is unlikely that amebic invasion was secondary to the invasion of other pathogenic organisms because the ameba invaded into the submucosa despite only superficial mucosal invasion by the nematodes, bacteria, and fungus. These findings supported ameba as a primary pathogen in our case. From these considerations, our case was thought to be an epizootic amebiasis in Madagascan flat-tailed tortoise.

Possible routes of ameba transport from the colon to the liver were considered via the portal system and biliary system. Morphological evidence of blood vessel invasion by ameba in the colon and liver, along with no significant change in the biliary system and duodenum, strongly suggested a portal system route.

AFIP Diagnoses: 1. Liver: Hepatitis, random, necrotizing, acute, multifocal, moderate, with numerous amoeba, etiology consistent with *Entamoeba invadens*, Madagascan flat-tailed tortoise (*Acinixys planicauda*), reptile.
2. Liver: Nematode, portal tract.

Conference Comment: *Entamoeba* is an obligate parasite with a direct life cycle. Resistant quadrinucleate cysts are released in the feces, and upon subsequent ingestion by a suitable host develop into trophozoites where they may invade the intestinal wall. Dissemination to other visceral organs can occur via the bile ducts or portal circulation. *Entamoeba invadens* is 10-35 um in diameter and characterized by a small endosome and agranular cytoplasm at one pole. While *Entamoeba* has been reported as pathogenic in chelonids, it is typically considered to be a commensal. In this case predisposing conditions may be involved. In contrast, *Entamoeba invadens* is well known to be a significant pathogen of captive lizards and snakes.

The nematode present in this case is consistent with *Atractis* sp. and characterized by a thin smooth cuticle, very large lateral chords, platymyarian meromyarian musculature, and a large intestinal tract composed of many uninucleate cells.

The degree of glycogen type vacuolar change in the hepatocytes was discussed in conference. While this finding would be significant in most domestic species, the moderator commented that this degree of change was within normal limits and not an unusual finding in chelonids.

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CASE II – 961/1305/3970 (AFIP 2789365)

Signalment: Adult, two females and one male, Common Wombat (*Vombatus ursinus*)

History: Sudden deaths occurred in all 3 wombats housed in a large enclosure in a wildlife park, over a period of 3 weeks. At that time there were increased rodent (mice) numbers noticed at the park. Two of these wombats (female) were presented for necropsy. Six months later, 2 adult replacements were acquired, and one died suddenly within 5 days of arrival at the park. This wombat (male) was also presented for necropsy.

Gross Pathology: Wombat 1 (female) – vague diffuse pallor of the myocardium, pulmonary congestion.
Wombat 2 (female) – focal pallor of the left ventricular myocardium, pulmonary congestion and oedema.
Wombat 3 (male) – distinct pale streaks through full thickness of ventricular myocardium, hepatic congestion.

Laboratory Results: Wombat 2 – Encephalomyocarditis (EMC) virus isolation positive on heart.

Wombat 3 – EMC negative on pooled tissues.

Contributor's Morphologic Diagnoses: 1. Necrotizing myocarditis, acute, diffuse in wombat 1. Virus isolation not attempted.
2. Necrotizing myocarditis, acute, multifocal in wombat 2. EMC virus infection confirmed in this animal.
3. Necrotizing myocarditis, subacute, multifocal, with fibrosis and calcification in wombat 3. EMC virus infection not confirmed in this animal.

Contributor's Comment: The lesions in the wombat hearts are similar to the necrotizing myocarditis seen in the hearts of young pigs with EMC virus infection. An outbreak of EMC virus mortalities was confirmed in a piggery (70 kilometers west of the nature park) around the time of the first wombat deaths. EMC virus is known to cause mortalities in a wide range of species in zoological collections associated with rodents having access to animal feed and/or litter.
This is the first report of EMC virus infection in wombats.

AFIP Diagnosis: Heart: Myocarditis, lymphohistiocytic, subacute to chronic, multifocal to coalescing, severe, with cardiomyocyte degeneration, necrosis, and mineralization, common wombat (*Vombatus ursinus*), marsupial.

Conference Comment: Encephalomyocarditis (EMC) is a ssRNA virus of the genus Cardiovirus, family Picornaviridae. EMC is a natural disease of rodents (particularly the genus *Rattus*), which is transmissible to, and able to cause disease in, domestic and exotic species and man. Of the domestic species, swine are most commonly affected. Infection in suckling to grower pigs results in myocarditis and encephalitis. The myocarditis is described as lymphoplasmacytic and histiocytic with myocardiocyte degeneration, necrosis, and occasionally mineralization. The encephalitis is described as nonsuppurative. Sudden death from cardiac failure with few, if any, clinical signs is typical. Gross findings include pallor in the myocardium, and peritoneal, pleural, and pericardial fluid. The differential diagnosis includes vitamin E/selenium deficiency and bacterial septicemia.

Clinical disease is often not apparent in adult pigs; although reproductive loss characterized by stillbirths and mummified fetuses does occur in infected gilts and sows.

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CASE III – 00-181-10 (AFIP 2790536)

Signalment: Eleven-year-old, female, red panda (Ailurus fulgens)

History: This female red panda had been housed in an outdoor exhibit with a male red panda for approximately 5 years. One morning this animal was noted to be lethargic and anorexic. That afternoon she was found down on the ground and was markedly dyspneic. Radiographs revealed bilateral pleural effue 0 Au2

HCT	24.5	%	30-50
MCV	44	/fL	39-52
MCH	12	pgm	12-18
MCHC	27	g/dL	29-37
WBC	28.1	k/uL	2.6-16.0
SEG	20.8	k/uL	0.7-8.7
BANDS	1.9	k/uL	0.0-0.1
LYMPH	4.5	k/uL	0.8-6.5
MONO	843	k/uL	0.0-1236
T. PROT	4.6	g/dL	5.0-8.8
ALBUMIN	1.8	g/dL	1.3-5.5
FIBRIN.	600	mg/dL	0-500

Bacterial cultures of the thoracic fluid and heart blood grew pure isolates of *Actinomyces*; the species of *Actinomyces* was not identified.

Contributor's Morphologic Diagnosis: Lung: pleuritis, fibrinosuppurative and hemorrhagic, diffuse, severe, with neovascularization, vascular thrombosis, and occasional bacterial colonies

Contributor's Comment: Fibrinosuppurative pleuritis and pyothorax without same cagemate for approximately 5 years. At gross necropsy, there were no lesions indicative of previous trauma.

AFIP Diagnosis: Lung: Pleuritis, fibrinosuppurative, chronic, diffuse, severe, with neovascularization, thrombosis, hemorrhage, and large bacterial colonies, red panda (*Ailurus fulgens*), procyonid.

Conference Comment: Actinomycetes are gram positive, non-acid-fast, branching filamentous rods. They are facultative anaerobes and normal inhabitants of the oral mucous membranes, tooth surfaces, and gastrointestinal tract. Infection is typically endogenous with introduction of the organism into susceptible tissues of its host.

In contrast, Nocardiae, which can be histologically indistinguishable from Actinomycetes, are common aerobic saprophytes. Mycolic acids present in the cell wall are responsible for their being variably acid-fast. Granules are rare, smaller, and lack the structure of Actinomycotic sulphur granules.

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CASE IV – R00-138B (AFIP 2790820)

Signalment: Adult, male (weight 300g, snout-vent 20 cm), Cultured freshwater rainbow trout (*Oncorhynchus mykiss*)

History: At one cultured pond with a running water facility, a huge number of adult yearling trout were examined for clinical signs of anorexia and death in August 2000. A local veterinarian submitted moribund fish for pathological diagnosis.

Gross Pathology: There were multifocal white raised foci 1-2mm in diameter in the gill, liver, spleen, heart, kidney and digestive tract.

Laboratory Results: Gill: Compressed stamp smear: Many *Icthyophonus*-like spores were noted in the submitted gill tissue.

Contributor's Morphologic Diagnoses: 1. Heart: Various fungal organisms, with severe diffuse granulomatous pericarditis and myocarditis, rainbow trout (*Oncorhynchus mykiss*)
2. Skeletal muscle: Several fungal organisms, with mild multifocal granulomatous myositis
3. Liver: Various fungal organisms, with severe diffuse granulomatous hepatitis
4. Spleen: Numerous fungal organisms, within whole submitted spleen tissue
5. Kidney: Numerous fungal organisms, with severe diffuse granulomatous nephritis
6. Intestine: Numerous fungal organisms, with severe diffuse erosion and necrotizing granulomatous enteritis
7. Stomach: Various fungal organisms, with severe diffuse erosion and necrotizing granulomatous gastritis

Contributor's Comment: *Ichthyophonus* disease prevails in a wide variety of marine and freshwater fish. It is a systemic granulomatous infection due to the fungus *Ichthyophonus hoferi.* This internal fungal invader may damage several parts of the body, including the heart, skeletal muscle, liver, kidney, and digestive tract.

The life cycle of the organism is not thoroughly understood and may vary according to the species of fish affected. Herring may have ingested the spores, starting the cycle in which the organism germinated and penetrate the gut and new hyphal bodies or spores enter the blood stream for systemic distribution. Germination leads to spore production in new organs, and spores reach a new host after release to the external environment by ulceration or death and decay of original host.

Ingestion of infected viscera from feeds prepared from infected fish easily transmits the disease. Necrophagy of other poolmates is probably another important means of transmission.

Ichthyophonus produces a systemic granulomatous lesion clinically similar to mycobacteriosis. External clinical signs comprise emaciation, neurologic involvement, behavioral changes, curvature of the spine, and darkened skin. Other dermatologic effects include raised round areas approximately 1 mm in diameter, and similar black areas. The condition also produces ulcers of varying size, also called "sandpaper-like effect" and raised white sharply circumscribed nodules 1 to 3 mm in internal organs.

Squash preparations of infected lesions reveal the thick-walled "resting spores", which vary in size from 10 to 250 micrometers in diameter and possess a double wall. The outer wall thickness appears related to spore size, while the inner wall is consistently quite narrow. PAS and silver stains of both walls are positive and reveal the outer wall to be laminated and often rough on its outer contour. Almost immediately, usually 15 to 30 minutes after host death, the "resting spores" germinate to produce branching hyphae. These germinating spores are important in differentiating *Ichthyophonus* from other granulomatous diseases.

There is no known cure for *Ichthyophonus* disease. Normally by the time the fish displays signs of having the malady, the disease has progressed so far that treatments are futile.

AFIP Diagnosis: Multiple organs: Granulomatous inflammation, multifocal, mild to moderate, with numerous fungal spores and few hyphae, etiology consistent with *Ichthyophonus* sp., rainbow trout (*Onchorhynchus mykiss*), salmonid.

Conference Comment: *Ichthyophonus hoferi* is a true fungus, and as such has a complete lack of chlorophyll. Lacking photosynthetic pathways, fungi are obligate saprophytes or parasites. With respect to fish, parasitic fungi are grouped into three subdivisions: Mastigomycotina, Zygomycotina, and Deuteromycotina.

Mastigomycotina are characterized by a motile sexual stage and a motile or non-motile asexual stage. Mastigomycotina contains most of the significant fish fungal pathogens, which include the genera *Saprolegnia*, *Achlya*, *Aphanomyces*, *Branchiomyces*, and *Dermocystidium*. Zygomycotina includes the genera *Ichthyophonus* and *Basidiobolus* and are characterized by having no motile stages.

Ingested spores of *I. hoferi* form amoeboblasts capable of invading the gastrointestinal tract. Within the gastrointestinal tract, amoeboblasts form amoeboid embryos, breach the mucosa, enter the bloodstream, and spread to multiple organs to form new spores. The spore or resting stage is the most common developmental stage observed. Spores undergo germination characterized by budding elongated cytoplasm that protrudes through the thick outer spore wall but is still bound by the inner spore wall. The presence of the germinating spore is considered pathognomonic for *I. hoferi*. This bud will further develop forming non-septate macrohyphae and later new spores (hyphal bodies).

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