CASE I – 99-2047 (AFIP 2694661)

Signalment: Adult, female, South African Clawed frog (*Xenopus laevis*)

History: This adult female frog was found dead, floating in the tank (This individual frog died with clinical signs similar to those seen in a larger group of frogs involved in an outbreak in the animal facility). Investigators use these frogs for oocyte production. Frogs generally are taken from regular housing tanks, anesthetized, and oocytes are removed through a small abdominal incision. Following surgery, the frogs are kept for 1-2 days in antibiotic treated water before being returned to the regular housing tanks.

Gross Pathology: Gross lesions included scattered cutaneous petechiae, hyperemia of the cloacal aperture, marked subcutaneous edema, abdominal ascites, and copious amounts of opaque, orange/tan effusion in the coelomic cavity. Microscopic lesions consisted of inflammation within the interstitium and renal portal system, (ranging from mild to severe, depending on the sections) composed of moderately sized mononuclear cells that contained basophilic cytoplasmic organisms. These organisms were gram-negative and had thin rod-shaped morphology, yet more often, were slightly atypical for bacteria and appeared clumped or like round cytoplasmic granules. Similar inflammation was observed in the livers, pericardium and rarely in the lungs of some frogs.

Laboratory Results: *Flavobacterium meningosepticum* was cultured from the coelomic fluid. (The same organism was cultured from frogs from coelomic fluid, heart blood, brain, and liver during the previous outbreak).

Contributor’s Morphologic Diagnoses: 1. Kidney: Moderate, multifocal, subacute, granulomatous interstitial nephritis with myriad intracellular gram-negative bacteria (*Flavobacterium meningosepticum*).
2. Kidney: Moderate tubular nephrosis with single-cell cytoplasmic “inclusions” and tubular mineralization.

**Contributor’s Comment:** Flavobacteria are gram-negative, aerobic bacteria found in soil, water, raw meat, milk and various foodstuffs. These organisms are opportunistic zoonotic pathogens and may cause disease in a variety of species, including nosocomial infections, meningitis, and pneumonia in hospitalized infants and immunosuppressed adults. In our facility, factors such as increased colony population density and an increased number of frogs undergoing experimental manipulation appear to have played a role in the outbreak. Although environmental testing in our facility resulted in positive cultures in communal tanks, holding tanks and shared instruments, a point source for the infection was never found. This pathogen is difficult to eradicate: an outbreak of *F. oderans* in a colony of *Xenopus laevis* in a different research facility required depopulation and temporary closure and sanitation of the aquaculture housing to halt the outbreak. However, we were able to halt the outbreak by implementing sanitation and management changes, and educating investigators and animal care staff to the zoonotic potential of this organism. In addition to *Flavobacterium meningosepticum*, other causes of septicemia in South African Clawed frogs include *Aeromonas hydrophila* (Red Leg), and *Chlamydia psittaci*, and all can have similar clinical presentation and gross lesions. Amphibian tuberculosis presents more as a chronic wasting syndrome in *Xenopus laevis* (manuscript in preparation).

In addition to the inflammation in the kidney, these frogs almost always present with a variable degree of tubular changes, which have not been previously characterized. Tubular epithelial cells become markedly swollen, vacuolated, and often contain large oval eosinophilic cytoplasmic “inclusions” that stain positive with the PAS reaction. It appears that these swollen epithelial cells eventually rupture, and the released material undergoes mineralization within tubular lumina. Kidneys of frogs reared on spinach diets have been reported to develop macro- and microscopic renal calculi, but our frogs are not fed such a diet, and efforts are underway to further characterize this lesion.

**AFIP Diagnoses:**
2. Kidney: Tubules, vacuolar degeneration, multifocal, moderate, with eosinophilic to amphophilic irregular intracytoplasmic inclusions.

**Conference Comment:** A ubiquitous bacterium, *Flavobacterium meningosepticum* has recently been reclassified as *Chryseobacterium meningosepticum*. Although, infrequently reported in the veterinary literature, case reports include infection in a Barbour’s map turtle, leopard frog colony, and a cat. More frequently reported in
the human literature, cases include meningitis, septicemia, and pneumonia; less frequently, reports describe endocarditis, cellulitis, and infection following burns.

Conference participants discussed the possibility that the cytoplasmic inclusions in the renal tubules may represent infection with ranavirus, an iridovirus of uncertain pathogenicity. Ultrastructural characterization may be beneficial.

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**CASE II – 95-495 (AFIP 2790771)**

**Signalment:** 7-year-old, female, garter snake (*Thamnophis sirtalis*)

**History:** One-month history of lethargy and anorexia mixed with convulsions and dyspnea

**Gross Pathology:** The snake has no body fat and is emaciated. A loose yellow feces exudes from the cloaca. The abdomen contains 5ml of clear fluid and the intestinal tract is empty except for a small amount of mucus. Both ovaries contain multiple, firm, cystic masses ranging in size from 1cm to 3cm.

**Laboratory Results:** None

**Contributor’s Morphologic Diagnosis:** Ovaries: Granulosa cell tumor, malignant

**Contributor’s Comment:** This ovarian mass consists of solid cords of granulosa cells or cysts lined by granulosa cells and surrounded by a thick hyalin, eosinophilic capsule, all within a dense collagenous stroma. The centers of the cysts contain one or more round to oval bodies of granular eosinophilic material. These globules
often contain a karyorrhectic nucleus and resemble ova. The granulosa cells do not appear malignant but foci of neoplastic cells are present in the kidney, which is seen on some slides submitted.

Neoplasia in snakes has been reported frequently and has involved all organ systems. Granulosa cell tumors have been reported in several species of snakes including the garter snake.

AFIP Diagnosis: Ovary: Granulosa cell tumor, garter snake (*Thamnophis sirtalis*), reptile.

Conference Comment: Conference discussion focused on the distinctive features of this neoplasm. Characteristic of reptilian granulosa cell tumors are the eosinophilic hyalinized bands surrounding follicular cells. These bands, which are strongly PAS positive, are reminiscent of the zona pellucida. Conference participants also commented on the occasional small gland-like structures with central eosinophilic material resembling Call-Exner bodies. Call-Exner bodies, which are considered diagnostic for granulosa cell tumors in domestic species, are much less distinct in this case.

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CASE III – M-1535-01 (AFIP 2789005)

Signalment: One-year-old, female, Rocky Mountain elk (*Cervus elaphus*)

History: The animal was found standing and swaying in a field.

Gross Pathology: The elk was in moderate condition and post mortem decomposition and had been killed by gunshot. Two slender light green nematodes were in the subdural space of the right cerebral hemisphere. Adequate plant fiber was within the rumen. Formed stool was in the descending colon and rectum. No additional significant macroscopic alterations were detected.

Laboratory Results: Nonpathogenic bacteria were cultured from samples of liver, spleen and intestine. The lung was overgrown with saprophytic microorganisms.
No parasite ova were detected in a sample of stool. Fluorescent antibody tests for epizootic hemorrhagic disease and blue tongue viruses were negative.

**Contributor’s Morphologic Diagnosis:** Cerebrum: Meningoencephalitis, verminous, subacute, multifocal, mild, with nematode-type larvae and ova.

**Etiology:** *Pneumostrongylus (Parelaphostrongylus) tenuis*

**Contributor’s Comment:** Moderate numbers of nematode larvae and ova are in the leptomeninges and in many cases the white and/or gray matter of the elk cerebrum. The leptomeninges are infiltrated with lymphocytes, plasma cells and low numbers of hemosiderin laden macrophages. Infiltrates are diffuse and form perivascular cuffs. Focal meningeal fibroplasia is occasionally present. Lesions within the underlying neuropil are variable and include small microcavitations with or without hemorrhage; variably sized foci of gliosis; swelling of axons; vacuolation of myelin sheaths; focal accumulation of macrophages; and cuffing of blood vessels by lymphocytes, plasma cells and in some cases, by eosinophils and macrophages containing hemosiderin granules. Larvae, when present, are in tunnels surrounded by compressed neuroparenchyma and are typically not associated with inflammatory cell infiltrates unless necrotic.

The diagnosis of *Pneumonstrongylus tenuis* infection was based on histologic alterations within sections of cerebrum, the presence of nematode larvae in the leptomeninges and brain and the location and morphology of the mature nematodes (one male and female) detected at necropsy.

*Pneumonstrongylus tenuis* is a neurotropic nematode of white tailed deer that uses a variety of terrestrial gastropods as intermediate hosts. Ingested larvae migrate from the gastrointestinal tract into the dorsal horns of the spinal cord, at all levels, and mature in approximately thirty days. They then migrate into the meningeal spaces. In white-tailed deer, lesions in the spinal cord and brain caused by adults and larval migration are mild and generally not accompanied by clinical signs, although an occasional animal may develop mild, transient posterior weakness and/or ataxia. In elk, other cervids, sheep and llamas, however, *P tenuis* causes severe, progressive, often fatal neurologic disease. Lesions are most severe in sections of spinal cord, but also occur in brain. Variably sized microcavitated malacic foci are typically associated with gliosis, macrophage infiltration, swelling of axons, dilation of myelin sheaths and perivascular cuffs. Migration tracts appear as empty cavities with ragged edges or cavities filled with blood and/or low numbers of macrophages. Inflammatory cell infiltrates vary from predominately eosinophilic to lymphocytic/plasmacytic and occasionally granulomatous. Mineralization of dead larvae has also been described.

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Rocky Mountain elk were recently reintroduced into the mountainous portions of eastern Kentucky. Because elk habitat overlaps that of white-tailed deer, meningeal worm infection in translocated elk and its affect on the establishment of healthy stable elk populations was a concern. Twenty-six of forty-seven elk necropsied since January of 1998 have had histologic evidence of meningeal worm infection suggesting *P. tenuis* has an affect on elk populations in Kentucky, but may not interfere with the establishment of elk populations in the state.

**AFIP Diagnosis:** Cerebrum: Meningoencephalitis, nonsuppurative, multifocal, moderate, with nematode larvae and eggs, Rocky Mountain elk (*Cervus elaphus*), cervid.

**Conference Comment:** Verminous encephalitis can result from errant migration of a parasite within its usual host, or parasitic infestation in an aberrant host. *Parelaphostrongylus tenuis* infection in domestic livestock and wild ungulates (e.g. moose, reindeer, caribou, and in this case elk) is an example of the latter condition.

In white-tailed deer, ingestion of the 3rd stage larvae results in migration from the abomasum to the spinal cord, a process which takes approximately ten days. The larvae migrate and develop in the dorsal horn of the spinal cord; exiting the spinal cord and entering the subdural space in approximately 30 days as subadults. This process as well as the subsequent migration to the cranium generally results in minimal lesions and/or clinical signs. Once in the cranium, preferred sites include the tentorium cerebelli, falx cerebri, and diaphragm sellae. Here, eggs are deposited within venous sinuses without invasion into the neural parenchyma.

In aberrant hosts, 3rd stage larvae follow a similar path from the abomasum to the spinal cord, but for unknown reasons often fail to exit the spinal cord within the normal 40 days. By this time larvae have reached the adult stage, are much larger, unusually active, coil upon themselves, invade the ependymal canal, and cause extensive damage. Often these adults are unable to complete their life cycle. Adult worms that do reach the subdural space of the spinal cord or the cranium occasionally re-invade the neural tissue to deposit eggs.

Other metazoan parasites reported to cause encephalitis and their aberrant hosts include: *Elaphostrongylus rangiferi* in sheep and goats; *Setaria digitata* in horses, goats, sheep, and camels; *Taenia multiceps* in sheep; and *Cysticercus cellulosae* in pigs and humans.

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CASE IV – 93-809-6 (AFIP 2790568)  

Signalment: 1.5-year-old, female, Inca tern (*Larosterna inca*)  

History: Cagemates initially traumatized this bird. Keepers brought it in and the Department of Animal Health (DAH) stabilized it with fluids, steroids and chloramphenicol therapy and topical Gentacin above the eye. The bird returned to DAH (10-27-93) and blood work indicated a parasitic anemia with presumptive diagnosis of malaria. The bird died overnight.  

Gross Pathology: The young adult, female Inca Tern has a prominent keel. Fat stores are low and orange-yellow, pectoral musculature is mildly reduced and evidence of dehydration is present. Spleen is enlarged, congested and dark brown-purple. The liver is also enlarged and dark purple-red. The lungs are pale pink-yellow and caudally, particularly the left lobe, edematous, dark red and firm. The gizzard contains seed material and the wall at the junction with the esophagus has a flaky, dark brown material at the surface. Intestine contains scant, pale brown paste. Pancreas is yellow and fatty. The peritoneal surfaces and the air sacs are moderately thickened with yellow fibrinous material. The kidneys are yellow-tan with some fibrin on the surface.  

Laboratory Results: Post-mortem cytologic impressions of the spleen, liver, and lung were positive for intra-erythrocytic malarial ring forms. Macrophages were frequently seen with phagocytosed red blood cells and malarial pigment. Heart blood culture was negative. *Plasmodium relictum* was confirmed by Memorial University of Newfoundland.
Contributor’s Morphologic Diagnoses: 1. Liver, hepatitis, lymphoplasmacytic, periportal
2. Liver, parasitosis with intraerythrocytic plasmodium and malarial pigment

Contributor’s Comment: Malaria occurs in birds, humans and other primates, and is caused by protozoal parasites in the genus *Plasmodium*. Sexual reproduction of this parasite occurs in many species of *Anopheles, Aedes* and *Culex* mosquitoes resulting in sporozoites that find their way to the insects’ salivary glands and saliva. Several sporozoites are injected into the vertebrate host at every blood meal. The parasites pass through two stages of asexual reproduction within the vertebrate host: exoerythrocytic and intraerythrocytic. Exoerythrocytic replication within the liver occurs first; the merozoites that are produced eventually break out of the hepatocytes and invade erythrocytes. Once in the cytoplasm of an erythrocyte, the merozoites progress through several morphologic stages. In the ‘ring’ stage, the protozoan contains a centrally located vacuole and a peripheralized, red nucleus. With development into the trophozoite stage, food vacuoles are formed from invagination and pinching off of the host cell’s cytoplasm. Digestion of this meal leaves the characteristic, birefringent pigment (hematoidin) associated with fulminant *Plasmodium* parasitism. As in the exoerythrocytic stage, the parasite replicates and forms merozoites which lyse the host red blood cell and enter other red blood cells. Serial infection of erythrocytes may occur indefinitely. Eventually, the intraerythrocytic merozoites develop into sexual gametes that are ingested by a mosquito during a blood meal. The incubation time of *Plasmodium* sp. (i.e. the period between initial infection of the vertebrate host by the sporozoites and the manifestation of disease) in birds has not been extensively studied. However, in humans infected with *Plasmodium vivax*, the incubation time can range between 12-14 days.

Individual *Plasmodium* species are not always species specific, but their infectivity and vector preference are usually limited. The human malarial pathogen, *P. falciparum* executes its sexual cycle only in *Anopheles* mosquitoes and infects only humans. A less selective species, *Plasmodium relictum* is carried by *Culex, Anopheles, Aedes* and *Culiseta* mosquitoes and is infective for many avian species including ducks, doves, and passerines.

A retrospective survey of the pathology records at the Smithsonian National Zoological Park (SNZP) revealed a high prevalence of malaria in Inca terns (*Larosterna inca*) housed at the zoo during the years 1986-2000. During that period, malaria was diagnosed in 50% (19/38) of the Inca terns that died. A study was undertaken to examine the epidemiology of the disease in this exquisitely susceptible avian species. Because the incubation period of *Plasmodium* is approximately 14 days, only birds that were greater than 14 days old were included in this study.
Manifestation of disease showed a variably seasonal occurrence. Most of the birds affected with malaria died in the summer and early fall with 74% (14/19) presented for necropsy between the months of June and October. A smaller, secondary peak of malaria mortality (5/19 or 26%) occurred in January and February. No Inca tern deaths due to malaria in occurred in the spring months of March, April and May or in November and December.

Birds affected with malaria had either a short clinical course or were found dead with no previous disease manifestations. When clinical signs were evident, they were vague and non-specific: lethargy and inactivity. The time between onset of clinical signs and death in these cases was commonly no greater than 24 hours.

The most significant gross pathologic changes in Inca terns with malaria at SNZP were enlargement and congestion of the liver and spleen. Intraerythrocytic and/or intrahepatocytic stages of *Plasmodium* protozoa were readily identified on cytologic impressions of these organs. Some birds had coincident systemic disease such as aspergillosis (2/19 or 11%) which may have been secondary to the *Plasmodium* infection.

Histologically, there was periportal lymphocytic and plasmacytic inflammation in the liver of affected Inca terns. Kupffer cells and macrophages within the liver contained varying amounts of malaria pigment. This pigment is birefringent and can be easily seen within macrophages and red blood cells when viewed under polarized light. Malaria pigment was also present within splenic histiocytes and was accompanied by moderate plasmacytosis. Protozoa could occasionally be seen within the red blood cells on histologic sections of various organs. However, tissue fixation and processing for histology causes shrinkage of blood cells, often making it difficult to detect the intraerythrocytic malarial organisms by this method.

Cytologic preparations of blood smears (ante mortem) or impressions of liver and spleen at necropsy were the most ideal methods for confirming the presence of malarial infections. Wright’s or Giemsa stains were used to visualize the intraerythrocytic and intrahepatocytic forms of the protozoa.

Speciation of *Plasmodium* organisms required examination of the shape of the meront and schizont stages and determining the degree of displacement of the host cell nucleus. Some researchers are examining the mitochondrial cytochrome b gene in the parasite for phylogeography. In some cases at SNZP, fixed blood smears and/or tissue imprints were sent to the International Reference Centre for Avian Haematozoa in Newfoundland or the University of Vermont for positive morphological identification. Two Inca terns were found to be infected with
Plasmodium relictum and one harbored P. cathemurium. Both these species are found in the common house sparrow.

The natural habitat of these birds may give us clues to their increased susceptibility to malaria. Inca terns are permanent residents of offshore islands and the rocky coasts of Peru and Chile. Mosquitoes require shallow, still water in a temperate or tropical environment for breeding. Cold coastal waters are not ideal for the insects’ life cycle. Thus, it is unlikely that these birds evolved with the presence of mosquitoes or malarial organisms; this may explain the increased susceptibility of these birds to malaria, compared to birds from more temperate climates. Black-footed penguins (Spheniscus demersus), like the species of birds that we studied, evolved in a marine environment which does not support the mosquito life cycle. Studies have shown that these penguins are highly susceptible to P. relictum and P. elongatum.

The increased susceptibility to malaria exhibited by these species warrants strenuous efforts on the part of zoos and wild animal parks aimed at mosquito control and detection and treatment of disease. Control programs may include removal of mosquito breeding areas, utilization of mosquito-feeding fish in standing ponds, mosquito trapping, and/or selective use of pesticides. Enclosed exhibits can minimize access of mosquitoes to malaria-susceptible bird species. In areas where mosquito control is inadequate or not feasible, cytologic screening of stained blood smears from susceptible species in summer and fall may identify affected animals. Early detection and treatment with chloroquine and/or pyrimethamine may minimize mortality due to Plasmodium infections.

AFIP Diagnoses: 1. Liver: Intraerythrocytic protozoal organisms with anisotropic brown/black malarial pigment, Inca tern (Larosterna inca), avian.  
2. Liver: Hepatitis, portal, lymphoplasmacytic, minimal.  

Conference Comment: The contributor has provided an excellent review of this entity. Plasmodium sp. are apicomplexan organisms of the order Eucoccidiorida, which is characterized by having both asexual and sexual phases. The differential diagnosis discussed in conference for similar intraerythrocytic organisms in avian species includes Haemoproteus sp. and Leucocytozoon sp. Relatively distinct morphology is helpful when differentiating these organisms on cytology or histology. In Plasmodium sp., merozoites and trophozoites tend to marginate the nucleus while not distorting it. In contrast, macrogametes and microgamonts of Haemoproteus sp. wrap around the nucleus keeping it central. Leucocytozoon sp.
have two distinct intraerythrocytic forms; elongate and round. These large
gametocytes often distort the erythrocyte while marginating and compressing the
nucleus.

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