The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 25

14 May 2008

Moderator:

JoLynne Raymond, DVM, Diplomate ACVP

CASE I – 04L-1129 (AFIP 2937349).

Signalment: Discus fish (*Symphysodon a equifasciata*) adult female, in moderate body condition.

History: Zoo owned fish held in a community fish tank with other discus and tropical fish. This individual had been identified with an increased respiratory rate for approximately 18 months but problems with isolation of this fish meant that it was left on display. Other individuals in tank had a much lower respiration rate and were clinically normal. This female fish was submitted live for necropsy.

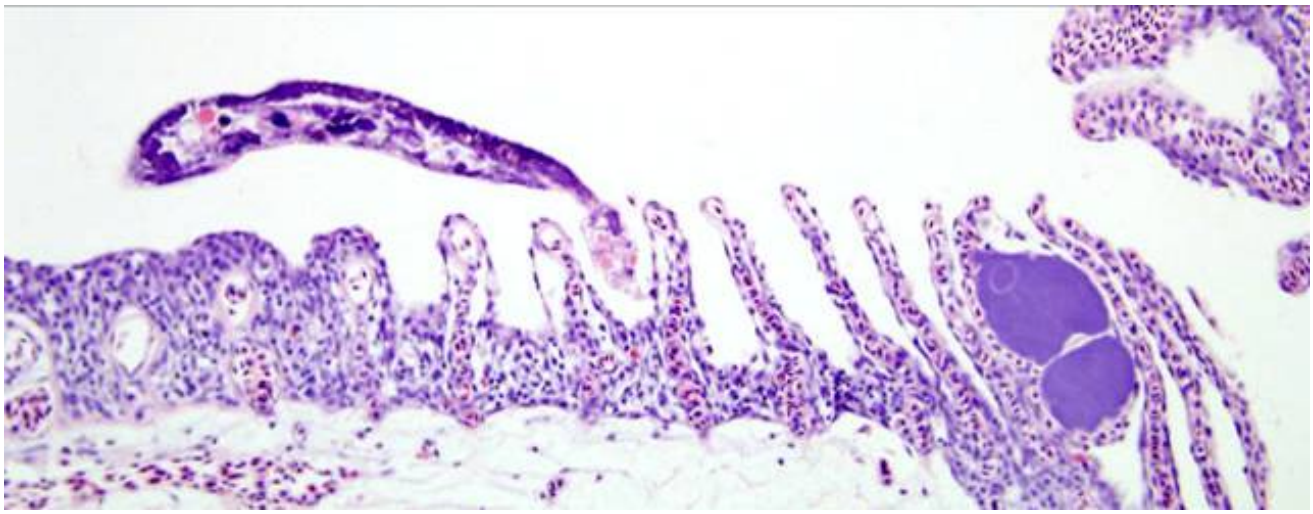
Gross P athology: No gross abnormalities identified at necropsy.

Laboratory Results: Wet preparation of gills: Numerous motile parasites attached to the gill epithelium. Appearance was consistent with *Dactylogyrus* species, monogenetic gill trematodes, due to its shape being flattened and leaf-like, with four anterior eyespots; a cephalic end which was scalloped and had an attachment organ (haptor).

Histopathologic D escription: Multifocally, the gills are moderately thickened by oedema and show mild hyperplasia with stunted, fused or complete lack of secondary lamellae. Gill interstitial tissue is multifocally infiltrated by moderate to severe numbers of inflammatory cells, predominantly lymphocytes with lesser numbers of eosinophilic granulocytes. There is multifocal moderate external haemorrhage and in between gill filaments are numerous (50 x 300um) multicellular parasites showing a thin (~2um) eosinophilic tegument, poorly discernable basophilic parenchyma and occasionally an oral sucker by which they are attached to the gill epithelium (trematodes). Multifocally, at the base of gill filaments, arterial vascular walls are moderately to severely thickened and show hyalinisation (fibrinoid necrosis) and infiltration by mild to moderate numbers of degenerate and viable leucocytes with much cell debris. Occasional clusters of basophilic, finely granular material are present in the secondary lamellae (bacterial colonies).

Contributor's Morphologic Diagnosis:
Gill – multifocal moderate to severe gill inflammation with oedema, mild hyperplasia, a leukocytoclastic fibrinonecrotic vasculitis and epithelial attached adult trematodes, Discus fish, aetiology consistent with *Dactylogyrus* sp.

Contributor's Comment: Although monogenean gill flukes are commonly found on wild fish, they are rarely a direct cause of disease or death in free-ranging populations. In captivity, epidemics can occur with significant



1-1. Gill, Discus fish. Within secondary lamellae, there are few, up to 300 um diameter, microsporidian cysts (right) characterized by a thin, 1 um thick eosinophilic wall and filled with numerous lightly basophilic oval spores. Adjacent to the secondary lamellae there is a trematode (left) characterized by a thin tegument, spongy parenchyma, digestive tracts and reproductive organs. (HE 400X)

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morbidity and mortality especially in cultured fish with excessive parasite loads under conditions of overcrowding, inadequate sanitation and poor water quality.[1,2] Under these circumstances, the parasites rapidly multiply. Gill fluke infection affects many fresh and saltwater species across a variety of temperatures, but is especially common in carp, goldfish and discus fish.[4] There is a diverse range of fluke species, most are host- and site-specific, requiring only one host to complete an entire life cycle.[5]

Freshwater fish infected with heavy gill infestations result in respiratory disease. Clinical signs can include opaque mucus covering the gills, protrusion of the gill filaments from under the gill covers and gills may be swollen and pale. Infected fish are less tolerant of low oxygen conditions and have an increased respiratory rate with gulping of air at the water surface. Fish become anoxic with flaring of the gill opercula.[1] At a very advanced stage, the fish will isolate itself and spend long periods lying on the bottom with its fins clamped to its body. Acute infections are characterized by a short period of dyspnoea followed by sudden death.[1]

Most monogenean flukes are browsers, moving about the body surface and feeding on dermal mucus and gill debris. Monogeneans have a series of hooks that enable them to attach while feeding.[2] Flukes anchoring to the gills induce a variety of lesions, depending on the density and species of parasite. Lesions can range from excessive mucous secretions, hyperplasia of gill epithelium with fusion of secondary lamellae to the presence of haemorrhage, necrosis / ulceration and inflammation.[3,6] Secondary infection by bacteria and fungi are commonly established at damaged epithelial sites.[4]

In this case, there is a widespread arterial vasculitis, which we propose may be mediated by immune complexes. Other discus fish in the collection are almost certainly affected by flukes but the involvement of a vasculitis, in other cases, remains to be determined.

The Dactylogyrus gill fluke life cycle is direct, not requiring intermediate hosts. The adults are oviparous and produce eggs with long filaments. The eggs are usually attached to the gills and develop into a free-swimming onchomiricidium, which then locates and attaches to the fish within a few hours.[5]

AFIP Diagnosis: 1. Gill: Branchitis, lymphocytic and granulocytic, multifocal, moderate with blunting, fusion, and loss of lamellae, mild epithelial hyperplasia and adult trematodes **(Fig. 1- 1)**, Discus fish (*Symphysodon aequifasciata*), Pisces.
2. Gill: Vasculitis, necrotizing, multifocal, moderate with edema and hemorrhage **(Fig. 1-2)**.

Conference Comment: The contributor gives an excellent overview of *Dactylogyrus* sp. gill infections. Gills are composed of two sets of four holobranchs that are located on either side of the pharynx. Each holobranch is composed of two hemibranchs that project from the pos-

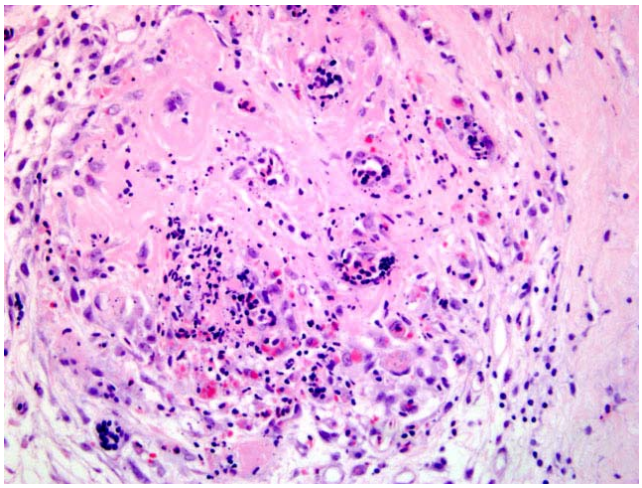
terior edge of the branchial arch. These hemibranchs contain numerous primary lamella and their secondary lamella.[4,8] Cells on the primary and secondary lamellae include melanocytes, lymphocytes, macrophages, endothelial cells, mucous cells, rodlet cells, and chloride cells.

Conference participants noted a microsporidian-like organism within some of the sections examined. Coinfections are not uncommon within compromised gill epithelium.[7]

Other diseases of importance that may affect the gills include the following list adapted from Moeller[4] and Wootten[8]

Bacterial
Flexibacter columnaris, *Flexibacter psychrophilus*, *Cytophaga p sychrophilia*, and *Flavobacterium* - prominent hyperplasia, clubbing adn fusion of lamella, necrosis of gill lamella

Fungal
Branchiomyces sa nguinis and *B. demi grans* (gill rot) - fungal disease of carp, trout and eels, prominent gill necrosis, with hyphae
Saprolegnia, *Achyla*, *Aphanomyces* (Saprolegniasis) - white to brown cotton-like growths on skin, fins, and gills



1-2. Gill, Discus fish. The tunica intima and media of small and medium-sized vessels are disrupted and replaced by eosinophilic fibrillar material admixed with numerous inflammatory cells and necrotic cellular debris (vasculitis) which occludes most of the lumen. Multifocally, within the occlusion there are small caliber, endothelial lined vascular spaces (recanalization). (HE 400X)

Protozoal
Ichthyophthirius multifiliis (Ich) - ciliated protozoan with horseshoe nucleus, hyperplasia with encysted trophozoites on skin and gills
Aurantiactinomyxo sp (myxosporidean) - hamburger gill disease, granulomatous inflammation and swelling of gills, with epithelial hyperplasia and gill necrosis surrounding cysts
Microsporidians (*Glugea*, *Pleistophora*, *Loma*) - cysts contain 1-2 um spores

Trematode
Diplostomum spathaceum - (eye fluke) - digenetic trematode, gulls/pelicans definitive host, metacercaria in the anterior chamber, vitreous body, and lens, snails 1st intermediate host, salmonids 2nd intermediate host
Gyrodactylus sp. - a monogenetic trematode that attaches to skin, fins, and gills
Uvulifer a mbloplitis (black spot disease) - digenetic fluke, numerous black to brown spots over skin, gills, and eyes, snails 1st intermediate host, fish 2nd intermediate host

Other
Argulus sp. - (fish louse) parasite of the skin and buccal cavity resulting in cutaneous ulcers, contains a retractile preoral stylet used to pierce the skin
Lernea sp. (at base of fins) & *Ergasilus* sp. (on the gills) - Copepod, invades the skin, forms ulcers that are slow to heal
Cryptosporidiosis - intracellular extracytoplasmic protozoan,

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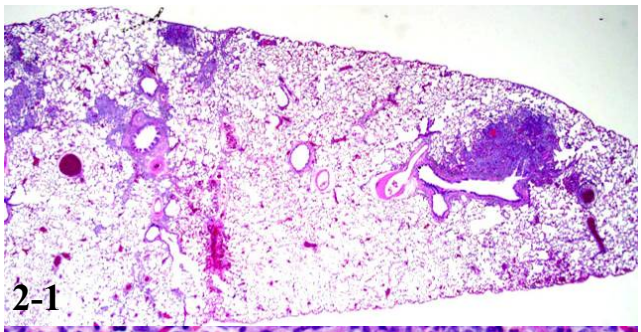

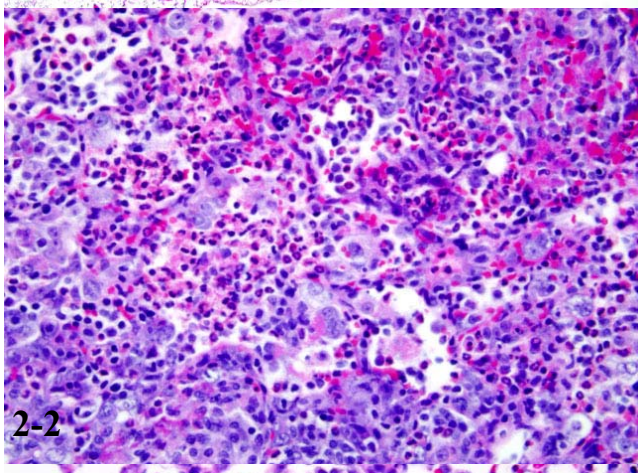

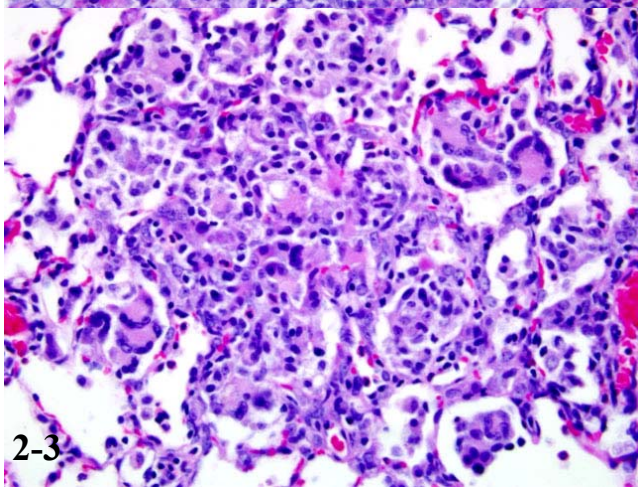

CASE II – 06-0768 (AFIP 3031295).

Signalment: One-year-old, male, Brown Norway rat

History: This animal came from a room where rats had a history of respiratory lesions. It was euthanized and submitted for pathologic examination.

Histopathologic D escription: Multifocally there are numerous, variably sized, occasionally coalescing nodules **(Fig. 2-1)** composed of numerous epitheliod macrophages and fewer multinucleated giant cells admixed with numerous eosinophils and fewer neutrophils and lymphocytes **(Fig. 2-2, 2-3)**. Often, granulomas contain areas with many degenerate inflammatory cells, fibrin, and necrotic debris. Multifocally there is a perivascular infiltrate of low numbers of eosinophils and neutrophils.

Contributor's Morphologic Diagnosis: Lung: Inflammation, granulomatous and eosinophilic, multifocal, moderate to marked.

Contributor's Comme nt: The pulmonary immune re-

2-1. Lung, Brown Norway rat. Multifocal, often peribronchiolar and perivascular, variably-sized aggregates of cellular infiltrate. (HE 20X)
2-2. Lung, Brown Norway rat. The cellular infiltrate is characterized by numerous eosinophils admixed with moderate numbers of histiocytes, lymphocytes, fewer neutrophils and plasma cells. (HE 400X)
2-3. Lung, Brown Norway rat. Multifocally, within the alveoli and often admixed with other inflammatory cells, there are multinucleated giant cells. (HE 400X)

sponse of Brown Norway (BN) rats is unique and often differs from other strains. Following ovalbumin challenge, BN rats develop airway hyperresponsiveness where as Sprague-Dawley rats do not. In addition, Sprague-Dawley rats develop only a neutrophilic inflammation, while BN rats develop neutrophilic and eosinophilic inflammation.[1] In a separate experiment, ovalbumin sensitization produced marked eosinophil infiltration into the lungs of BN rats while no pulmonary inflammation was observed in Lewis or Fisher rats.[10] When fed hexachlorobenzene for at least 21 days, BN rats develop extensive eosinophilic and granulomatous lung inflammation that correlated with airway hyperresponsiveness.[5]

Because of this unique immunologic response, the Brown Norway (BN) rat has served as a model for asthma in humans. BN rats also tend to develop a spontaneous eosinophilic and granulomatous pneumonia that is most frequently observed in young females. The lesion consists of epitheliod cells and numerous multinucleated giant cells admixed with an eosinophilic inflammatory infiltrate. To date the pathogenesis had not been discovered and no infectious agent has been identified.[9]

AFIP Diagnosis: Lung: Pneumonia, granulomatous and eosinophilic, multifocal, moderate, Brown Norway rat (*Rattus norvegicus*), rodent.

Conference Comment: The Brown Norway rat strain is widely used as an animal model for allergic asthma because they share many immunological and physical responses seen in human asthma, such as high production of IgE antibody, contraction of airway smooth muscle, airway hyperresponsiveness, involvement of leukotrienes in lung reactions, and infiltration of eosinophils and lymphocytes into the airway.[8] Untreated Brown Norway rats have also been reported to have a high incidence of eosinophilic granulomatous pneumonia.[6] These lesions are usually seen after 7 weeks of age and do not appear to be related to foreign material, fungi, or bacteria.[4,7]

In the Brown Norway strain, submucosal glands are larger and more numerous, particularly in the middle and lower trachea, as compared to F344, but the histochemical nature of the mucin produced by these cells is the same. The relationship between the quantitative difference in the number of submucosal glands and the Brown Norway rat's predisposition for hyperresponsiveness to allergens is still under investigation.[6] It has been proposed that mucosal IgA and interleukin-8 stimulate the activity of eosinophils.[2,11] Following exposure to 1% ovalbumin (OVA) solution, Brown Norway rats exhibited increased levels of Th1- (IFN-γ and IL-2) and Th2-related cytokines (IL-4 and IL-5) and chemokines (eotaxin and MCP-1) as compared to exposed F344 rats.[8] Alveolar macrophages have been shown to produce significantly more nitric oxide, IL-10 and TNF when exposed to ovalbumin than similarly challenged Sprague-Dawley rats, indicating that alveolar macrophages have a role in expanding the Th2 response in allergic inflammation.[1]

Other strain-specific responses of the Brown Norway rat include a high susceptibility to Th2-mediated autoimmune disease such as mercury ($HgCl_2$)-induced autoimmune glomerulonephritis. They have a vigorous Th2 immune response, producing cytokines IL-4, IL-6, and IL-10, along with antibody isotypes IgG1 and IgE on antigenic stimulation.[3]

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http://wrair-www.army.mil/
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CASE III – 03-27 (AFIP 2936161).

Signalment: A wild-caught, male, owl monkey (*Aotus vociferans*)

History: This owl monkey was found dead in its cage. The animal was involved in a malarial study. A splenectomy had been performed and the monkey had received two inoculations of *Plasmodium falciparum* two weeks apart approximately one month before its death.

Gross Pathology: The abdominal cavity contained 2mL of clear yellow fluid. The left and right kidneys revealed a pale tan surface. The left kidney was small (2.42g) and had a bumpy cortical surface compared to the right kidney (5.87g). *The average weight of a normal owl monkey kidney is 4-5g*.[1] All other gross findings were within normal limits.

Laboratory Results: Abdominal cavity fluid specific gravity: 0g/dl

Contributor's Morphologic Diagnoses:
1) Kidney, nephritis, interstitial **(Fig. 3-1)**, lymphocytic, multifocal, severe, owl monkey (*Aotus vociferans*), nonhuman primate.
2) Kidney, tubular ectasia **(Fig. 3-2)**, diffuse, severe with glomerulosclerosis and membranous glomerulopathy with mesangial proliferation **(Fig. 3-3)**, multifocal, moderate.

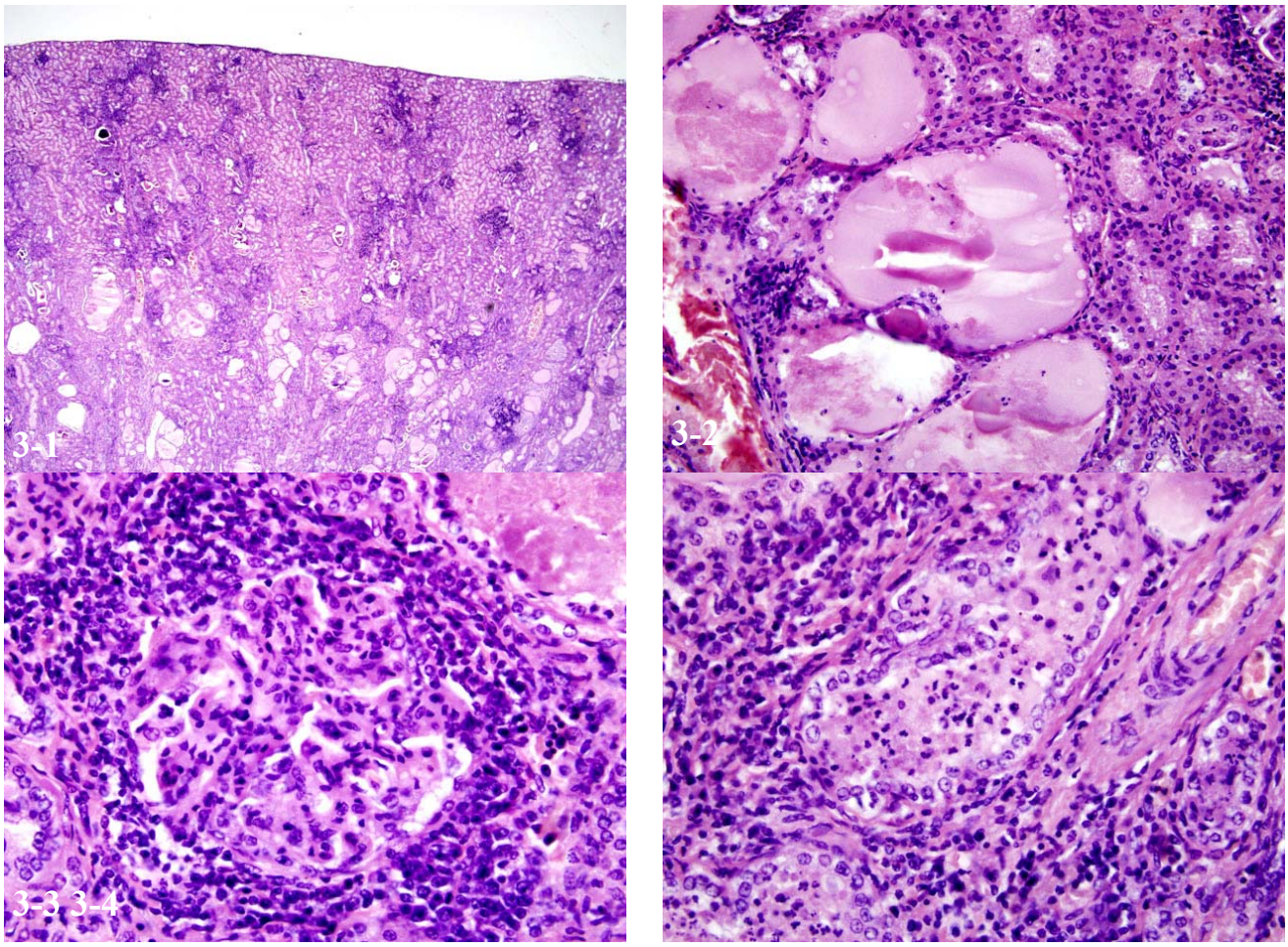
Contributor's Comment: Malarial infection continues to be a leading cause of morbidity and mortality in the world today. The importance of this disease has led to a great amount of research within the field. A murine model using a rodent malarial protozoa, *Plasmodium berghei*, was once used as an animal model for human malarial studies, but there was lack of evidence for similarity to the human disease.[1] The owl monkey is now the gold standard animal model for malarial studies. There are four species of human malarial parasites: *Plasmodium falciparum, P. vivax, P. malariae, and P. ovale*.[2] The splenectomized *Aotus* monkey is susceptible to all but the latter and is used to test new antimalarial drugs and vaccines and to study mechanisms of immunity and immunopathology of the disease.[1]

The relationship between malaria and renal disease has been long understood and was first described in a malarial patient with edema in 1905.[1] There are two major renal syndromes associated with malarial infection. The first is a chronic, progressive glomerulopathy most common in children in Africa with *P. malariae* infection and the other an acute renal failure associated with falciparum malaria in Southeast Asia, India, and sub-Saharan Africa.[2] Falciparum malaria can also be associated with a severe disease in humans characterized by intravascular hemolysis, hemoglobinurea, acute tubular necrosis, and renal failure and is termed blackwater fever.

Although there are differences between *P. malariae* and *P. falciparum* infection in humans, the histological changes within the kidney of experimentally infected owl monkeys can be similar. Histological features include glomerular hypercellularity, infiltration of polymorphonuclear leukocytes, and thickening of the glomerular basement membrane and mesangium.[1,5,6] Immunofluorescent microscopy reveals IgG, IgA, IgM, and C3 within the mesangium and along the basement membrane and *Plasmodium sp.* antigens and corresponding antibodies.[1,5,6] Interstitial inflammation is a common finding in inoculated monkeys.

A condition exists that complicates studying malarial associated renal disease and nephrotic syndrome in owl monkeys. Owl monkeys commonly have spontaneous renal disease.[1,3,8] Renal disease such as glomerulonephropathies and interstitial nephritis are some of the most common causes of death in owl monkeys. The cause is unknown, but it has been speculated that it may be due to immune responses to infectious agents in their natural habitat of Central and South America. Histologically, lesions are characterized by increased mesangium, glomerulosclerosis, and interstitial nephritis with lymphocytic, eosinophilic, and plasma cell infiltration.[1,3]

AFIP Diagnosis: Kidney: Glomerulonephritis, mem-



3-1. Kidney, Owl monkey. Multifocal interstitial nephritis and tubular ectasia. (HE 20X)
3-2. Kidney, Owl monkey. Tubules are ectatic, lined by attenuated epithelium and contain eosinophilic homogenous material (proteinosis). (HE 400X)
3-3. Kidney, Owl monkey. Diffusely, glomeruli are often surrounded by high numbers of lymphocytes, few histiocytes and neutrophils; glomerular tufts are hypercellular with increased mesangium. (HE 400X)
3-4. Kidney, Owl monkey. Multifocally, tubular epithelium is often disrupted and necrotic characterized by shrunken, eosinophilic cytoplasm with pyknotic nuclei. (HE 400X)

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branoproliferative, global, diffuse, severe, with multifocal tubular degeneration and necrosis **(Fig. 3-4)**, suppurative tubulitis, tubular ectasia and proteinosis, and lymphoplasmacytic interstitial nephritis, owl monkey (*Aotus vociferans*), primate.

Conference Comment: The contributor gives an excellent review of *Plasmodium* infection in owl monkeys and the confounding lesions seen in that particular animal model. *Plasmodium* are intracellular protozoan parasites in the family of Plasmodiidae that can affect a wide rage of mammals, birds, and reptiles.[7,10] *Hemoproteus*, *Leucocytozoon*, and *Hepatocystis* are other members of the family Plasmodiidae.

Diagnosis may be made on a blood smear stained with Giemsa or Wright-Giemsa stains. Hemazoin, a brownish malarial pigment formed from the incomplete catabolism of hemoglobin by the parasite[7], can be seen in Kupffer cells of the liver, within macrophages in the bone marrow, and in the red pulp of the spleen.[10]

The life cycle[4, 9] consists of an infected mosquito feeding on a susceptible host injecting sporozoites into the host

circulation. These sporozoites invade hepatocytes by binding hepatocyte receptors for the serum proteins thrombospondin and properdin.[9] They develop in hepatocytes to form schizonts that release up to 30,000 merozoites when the hepatocytes rupture. Merozoites enter erythrocytes by binding a parasite lectinlike molecule to sialic residues on glycophorin molecules on the surface of red blood cells.[9] There, they reside within a parasitophorous vacuole, until it enlarges into a trophozoite stage, divides into schizonts containing 24 or more merozoites, and lyses the erythrocyte to then infect other RBCs. A few merozoites will develop into gametocytes (both micro- and macrogametocytes) which are taken up by a mosquito during a blood meal. Within the mosquito, the gametocytes develop into gametes, forming a motile zygote. Through repeated nuclear divisions, the oocyst ruptures releasing sporozoites into the hemolymph, where they then migrate to the salivary gland for injection into a susceptible vertebrate host.

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CASE IV - 2005-126 (AFIP 3050843).

Signalment: A 2.8-year-old, female, pig-tailed macaque (*Macaca nemestrina*)

History: Tissue from a 2.8 year old female *M. nemestrina* which underwent experimental total body irradiation (TBI) and bone marrow reconstitution 11 months prior. The animal presented with mild dermal hyperemia in the axillary region. Over the subsequent 24 hours, lesions progressed to cover the trunk, face, and extremities. At physical examination, the animal was hyperthermic; oral ulcerations were noted. A skin scraping demonstrated suppurative inflammation. Serology was negative for Mumps, Measles, Monkey pox, Cercopithecine Herpes virus-1, and Simian Varicella Virus. Despite aggressive medical management, the clinical condition remained unchanged for the subsequent 48 hours. Ninety-six hours after initial presentation, the animal became lethargic and refused oral medications and food; euthanasia was elected.

Gross Pathology:
1. Diffuse, vesiculoulcerative and pustular dermatitis
2. Diffuse necroulcerative and hemorrhagic gastroenterocolitis
3. Multifocal splenic, hepatic, and renal necrosis and hemorrhage

Laboratory Results: Serology obtained at necropsy demonstrated antibodies to Simian Varicella Virus (SVV). Skin and pustule fluid were positive for SVV by PCR.

Histopathologic Description: Buccal skin and abdominal skin were submitted. The integrity of the epidermis is disrupted by multiple vesicles, pustules, and ulcerations. Multifocally, pustules and vesicles contain viable and

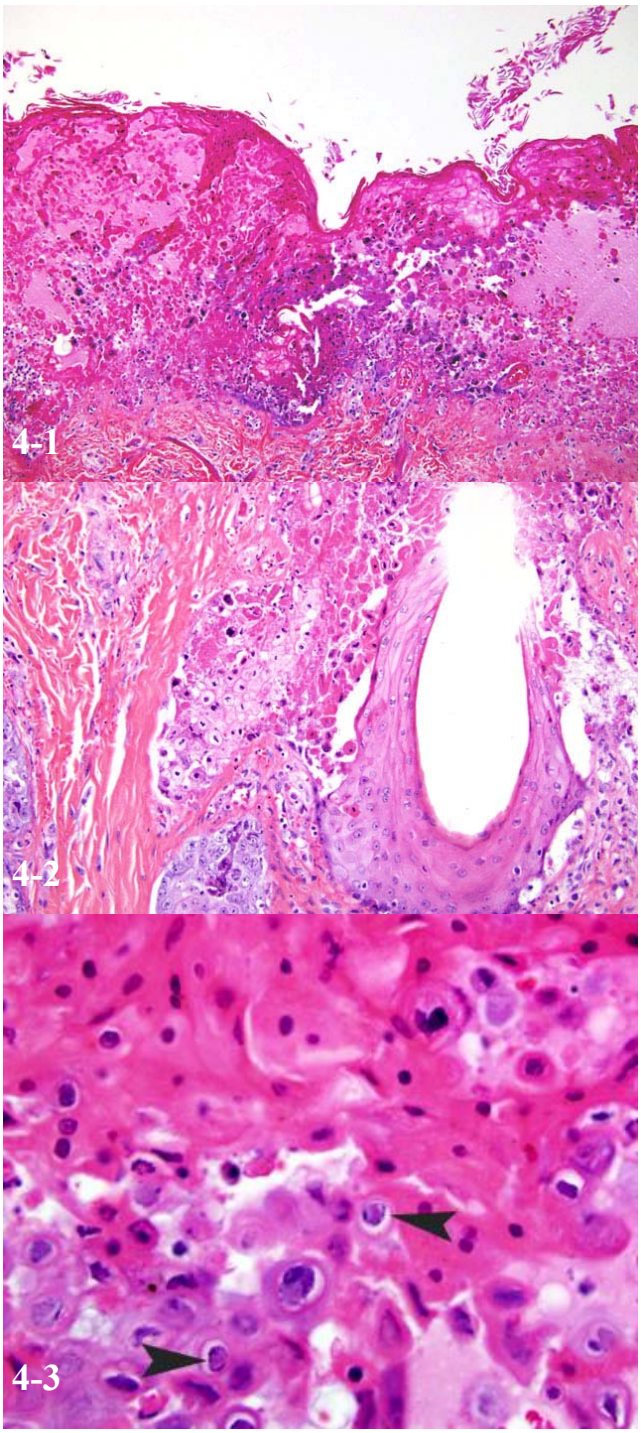
degenerate neutrophils, lymphocytes, plasma cells, and variable amounts of homogenous eosinophilic material, cellular and karyorrhectic debris. Occasional vesicles and pustules are intact; most vesicles have lost the surface epithelium (dermal ulcerations) and are covered by serosanguinous debris. Multifocally adjacent epithelial cells are swollen and contain abundant pale eosinophilic, homogenous, sometimes vacuolated, cytoplasm (intracellular edema), occasional "glassy" basophilic intranuclear inclusions, and are separated by clear space that accentuates intercellular bridges (intercellular edema; spongiosis).

Contributor's Mor phologic Diagn osis: Multifocal to diffuse, severe, necroulcerative and vesicular suppurative dermatitis **(Fig. 4- 1, 4-2)** with intraepithelial herpetic inclusion bodies **(Fig. 4-3)**

Contributor's Comment: Submitted tissue is from one of two cases of SVV occurring in *M. nemestrina* at the WaNPRC in 2005. Both animals had similar signalments (TBI 11-15 months prior) and clinical presentations. Both cases were housed in same room for greater than 6 months prior to presentation. Four weeks prior to clinical presentation, animals from domestic quarantine were co-housed with the two clinical cases.

Simian Varicella Virus (SVV) is a naturally occurring herpes virus of Old World Primates characterized by fever, vesicular skin lesions, hemorrhagic ulceration throughout the gastrointestinal tract, and multifocal hemorrhagic necrosis of the liver, spleen, lymph nodes, and endocrine organs.[4,6] SVV is responsible for sporadic epizootics in biomedical research facilities.[1-3,5-8,10] Simian Varicella is antigenically related to Varicella Zoster Virus (VZV) in man; the two viruses must be distinguished from one another through PCR (serology from this animal was negative for VZV by PCR and nested PCR). Both SVV and VZV demonstrate a high incidence of asymptomatic or mild disease with seroconversion in immunocompetent animals. A colony survey performed at the WaNPRC demonstrated a 20% incidence of SVV antibodies within *M. nemestrina*.

The reason for overt clinical disease in these two animals



4-1. Lip, macaque. Within the epidermis there are areas of epithelial necrosis and degeneration. (HE 400X)
4-2. Lip, macaque. Multifocally, there is necrosis of the follicular epithelium and adjacent sebaceous glands. (HE 400 X)
4-3. Lip, macaque. Within areas of epithelial necrosis, there are oval, 4-6 micron, eosinophilic intranuclear inclusion bodies which often peripheralize the chromatin (arrowheads). (HE 400X)

remains unclear. Both VZV and SVV may induce symptomatic disease with immunosuppression; VZV is an important pathogen in man post TBI and chemotherapy. It is hypothesized that the stress of new animal introductions into an established housing room may have precipitated viral shedding in seropositive animal(s). Despite normal blood profiles, the TBI animals may not have been fully immunocompetent, and thus more susceptible to systemic disease.

AFIP Diagnosis: Haired skin and oral mucosa: Dermatitis and stomatitis, necroulcerative, neutrophilic and lymphoplasmacytic, multifocal, marked, with vesiculopustules, epithelial dyscohesion, syncytia, and intranuclear inclusion bodies, pig-tailed macaque (*Macaca ne mestrina*), primate.

Conference Comment: Simian varicella virus (SVV) is a naturally occurring disease in non-human primates that closely resembles human varicella (Varicella-zoster virus) infections. SVV is an alphaherpesvirus, classified as a single species (Cercopithecine herpesvirus 9) within the Varicellovirus genus.[9]

Natural transmission is primarily through inhalation and/or direct contact with infected skin lesions. The virus is disseminated throughout the body during a transient viremia that clears by day 11 post-infection. SVV DNA has been detected within B and T cells, but not monocytes.[4] The virus becomes latent in the neural ganglia, although infectious virus has not yet been recovered from these sites. This could indicate an unknown intermediate stage between acute infection and latency. SVV DNA has been identified in cervical, trigeminal, thoracic and lumbar ganglia, but not in the brain, liver or lung tissues of naturally infected monkeys.[4]

Clinical signs, which occur 10-15 days following inoculation, usually consist of a skin rash starting in the inguinal area and becoming generalized over the course of 48 hours, developing into macules, papules, and vesicles. Areas of the skin affected include the face, thorax, and abdomen, but not the palms or soles.[4] Symptoms begin to subside by day 14 post-infection. Lesions can range from mild and easily overlooked to severe infection resulting in pneumonia, hepatitis, and death. Reactivation following latency may occur spontaneously or in response to immune suppression or stress months to years after the initial infection.[4]

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