## MASTER CLASS: DISEASES OF AMPHIBIANS

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## ABSTRACT

A broad spectrum of infectious and noninfectious disease processes affect amphibians. These diseases will be reviewed with emphasis on pathology and diagnosis.

### Introduction

Wild amphibian populations worldwide are in decline, mostly due to habitat loss, but also due to disease, particularly certain mycotic, viral and parasitic diseases. Amphibians are commonly exhibited in zoos, and are increasingly popular in the pet and hobby trade. Individual and population medicine, diagnosis and disease intervention are important for veterinarians that work with amphibians.[1] This review will cover the common diseases encountered in amphibians, with emphasis on pathology and disease diagnosis. For population problems, it is best to emphasize that full necropsy of an affected and well preserved animal is more expedient and cost effective than antemortem clinical testing for establishing a treatment or management plan.

### Noninfectious Disease

Cardiovascular Disease

*Anasarca*

Anasarca refers to generalized lymph sac and coelomic fluid accumulation and is common in amphibians. Lymph sacs and lymph hearts are unique and large subcutaneous spaces connected with the lymphatic system and appear to function in fluid homeostasis. Lymph flow is aided by lymph hearts in anterior and posterior portions of the body. Unfortunately, this connected fluid system also facilitates the spread of disease throughout the body. Differentials for anasarca include bacterial or viral infection; lymphatic heart failure (infectious, hypocalcemia, congenital); renal, hepatic or cardiac failure; osmotic imbalances (environmental water with low solutes). The diagnostic workup should include signalment, hemogram/serum chemistries, fluid analysis (cytology and culture), dietary history, evidence of metabolic bone disease (derangements in calcium metabolism, renal disease), and full necropsy.

Degenerative Disease

Many amphibian species can have long lives in captivity, and some species can liver up to 15 or more years. In this regard, it is not uncommon to see degenerative disease processes as part of

the pathologic review. The most commonly encountered degenerative diseases are osteoarthritis, cataracts, cirrhosis and chronic renal disease. These conditions may be encountered incidentally, or can be significant contributing factors to the animal's quality of life. Previous insults to these systems can accelerate the age related degenerative processes.

Deposition Disorders

As in birds and reptiles, deposition disorders are common in amphibians and are important pathologic processes that affect quality of life. Important conditions include urolithiasis, Pseudogout, mineralization, Xanthomatosis, melanosis and hepatic lipidosis.

Mineral deposition disorders are most common and include 4 different pathologic presentations. Calcinosis circumscripta (tumoral calcinosis, pseudogout) refers to deposition of calcium hydroxyapatite deposition in soft tissues adjacent to joints, and is usually not associated with other forms of mineralization. This may be a form of dystrophic mineralization that occurs following localized tissue damage.

Endolymphatic sacs are specialized calcium storage sacs adjacent to the ears and vertebral column. They likely function in calcium homeostasis, especially during periods of active reproductive status. These sacs can be quite prominently distended with mineral, and are radiopaque. When symmetrical or encountered as a incidental finding, their contribution to disease should be interpreted conservatively. Asymmetry, displacement of other tissues or discoloration (dark red or purple) may be associated with disease such as hypercalcemia, mycobacteriosis or mycotic infection.

Metastatic calcification is most commonly seen with hypervitaminosis D, imbalances in dietary calcium and phosphorus, or underlying renal disease. Less commonly it is seen with parathyroid, lymphoid or thymic neoplasia or chronic inflammatory disorders. Dehydration in face of active folliculogenesis may also predispose to metastatic mineralization. Commonly affected sites are great vessels, kidney, lung, gut and skin. Metastatic mineral has a tropism for basement membranes which impairs epithelial adherence and subsequently, mucosal or epidermal surfaces bleb or become ulcerated. Mineralization of the muscle causes contractility problems with subsequent disuse of legs, cardiac contractility failure, and gut stasis with subsequent microbial or parasitic overgrowth.

Urolithiasis is common in some species, especially tree frogs, and the pathogenesis is not well understood and may be multifactorial. Extensive stone analysis has not been done. Affected animals often have concurrent renal disease. Large stones in the urinary bladder can be associated with significant mechanical displacement of visceral, secondary bacterial infections/sepsis, and emaciation. Most amphibians secrete ammonia or urea as urinary waste products, but some species such as *Phyllomedusa* spp. (tree frogs) secrete uric acid and can develop gout or urate stones.

Xanthomatosis

Xanthomatosis in common in amphibians and implies deposition of cholesterol crystals and associated granulomatous inflammation. Common sites are cornea, visceral surfaces and meninges. Xanthomatosis is usually associated with hypercholesterolemia, either due to high dietary fat, or due to derangements in cholesterol metabolism due to underlying hepatic or renal disease. Females with active reproductive status may be predisposed, especially if concurrent yolk coelomitis is present.

Melanosis

Melanosis is common in amphibians and usually not associated with clinical disease. As with fish and reptiles, amphibians have melanin-producing cells of the mononuclear phagocyte system. These are most prominent as individual or aggregated cells in the liver, but also are in the spleen, ovary, kidney, lung, heart, skin, brain, etc. These cells or aggregates increase in number or size with season, gaining, emaciation, antigenic stimulation, and free radical detoxification. The black pigment instilled in the tissues as the result of melanosis is often overinterpreted as pathologic. In fact, the only significant pathologic disruption appears to occur in the liver, where these cells or cell aggregates can completely or near completely replace the normal hepatic architecture. This is seen mostly in old animals and likely is associated with some degree of hepatic insufficiency.

Hepatic Lipidosis

Hepatic lipidosis is the accumulation lipid vacuoles in the cytoplasm of hepatocytes. This condition may be seen with anorexia, obesity, malnutrition, endocrine or metabolic derangement, hepatotoxins, active reproductive status, and preparation for or emerging from brumation. Hepatic lipidosis is generally considered a reversible process, but when severe can be associated with significant clinical hepatopathy. Long-term hepatic lipidosis can lead to cirrhosis.

Developmental Anomalies

Developmental anomalies are common in amphibians and there is much attention to this now in wild populations, and how these anomalies may be sentinel changes for environmental contaminants. Amphibians seem to be particularly susceptible to mutagenic events during metamorphosis. Mutagens may include environmental toxins such as metals or organophosphates, ultraviolet light, and temperature extremes. An apparent emerging parasitic disease caused by trematodes of *Ribeiroia* spp. has also been implicated in causing anomalies. In wild populations, anomalies can be spectacular, and include multiple or missing toes and legs, gnathism, open fontanelles, micropthalmia or anopthalmia or combinations of these. A common form of anomaly occurring in captive anuran morphs is spindly leg, for which the etiology is not understood.

Nutritional Diseases

For captive populations, the vast diversity of the species precludes having a single formulation. Additionally few nutritional analyses of dry weight contents have been performed. The recent trend in zoos towards conservation and research provides a opportunity to make large contributions in the areas of amphibian nutrition and the pathogenesis of nutritional disease. Important disease processes in amphibians that may be related to nutritional problems include failure to thrive, reproductive failure, infectious diseases, edema syndromes, spindly leg syndrome, metabolic bone disease and tetany. Much of the problem in amphibian nutrition may relate to the diets fed to prey species such as crickets and meal worms. Cultured prey items may not have the same nutrient exposure as their wild counterparts, and thus may be deficient I minerals or vitamins, or may have excess fat or cholesterol. Thus it becomes paramount to understand the nutritional requirements and management of nutritional sources of the prey item.

Emaciation

Emaciation or suboptimal fat stores are commonly encountered in captive amphibians. Causes include conspecific competition, poor exhibit design, maladaptation, and underlying disease. Nutritional status can be difficult to assess, especially in small amphibians. Histologically, examination of the fat bodies, liver, pancreas, kidney and bone marrow are very helpful for assessing nutritional status.

Metabolic Bone Disease

Metabolic bone disease (MBD) is common in amphibians, especially anurans. Some causes include inverse C:P and low total calcium in insect-based diets, morphs raised in water that has low calcium hardness, excess phosphorus in tap water, inappropriate ultraviolet light for vitamin D metabolism, antagonism by other mineral s and vitamins. Clinically, affected animals may have tetany, fluid accumulation, gas in the gastrointestinal tract, bowed legs, spinal curvature, malocclusion and radiographic evidence of decreased bone density. Histologic changes include reduction in cortical bone, bowing of the bones, pathologic fractures and cartilaginous metaplasia of the metaphyseal regions of the long bones. Fibrous osteodystrophy is not a common concurrent or subsequent change.

Tetany

In most cases, tetany is due to hypocalcemia. Affected animal may have rigidity exacerbated by stress and may respond to calcium therapy. Tetany is commonly seen in conjunction with MBD.

Hypovitaminosis A

Hypovitaminosis A is mostly associated with squamous metaplasia. Affected tissues include the conjunctiva, tongue, esophagus, respiratory and urinary tracts, and skin. Short tongue syndrome, a common condition in a number of different captive species, including the endangered

Wyoming toad (*Anaxyrus baxteri*), Kihansi spray toad (*Nectophrynoides asperginus)* and Puerto Rican Crested Toad (*Peltophryne lemur*), is believed to be due at least in part to hypovitaminosis A. Hypovitaminosis A may also contribute to reduced hatchability and morph survival, infectious disease and renal disease.

Hypovitaminosis E

Granulomatous and necrotizing steatitis with concurrent myopathy and cardiac degeneration is occasionally seen in amphibians. This triad is associated with hypovitaminosis E or selenium deficiency in mammals and birds, and may have similar pathogenesis in amphibians. From our files, it appears that the Solomon Island leaf frog (*Ceratobatrachus guentheri*) may be predisposed to this condition.

Neoplasia

Neoplasia is common in amphibians and should be a differential for any mass. The clinical assessment should include signalment, history, radiographs, aspirate cytology and biopsy. There do not appear to be many species predispositions to tumors in amphibians. Tumors of the skin include melanocyte hyperplasia, benign and malignant melanoma, squamous cell carcinoma and various forms of papilloma or adenomatous polyp. Hematopoietic malignancy is common, especially lymphoma. Leukemia and myeloproliferative disorders are rarely seen. Tumors of the reproductive tract are common and include ovarian and oviduct adenocarcinoma, ovarian dysgerminoma, and seminoma. The most common and important urinary tract tumor is Lucke's renal adenocarcinoma, a herpesvirus model of oncogenesis seen in leopard frogs (*Rana pipiens*). Alimentary tract tumors also are common, especially in the oral cavity, and include squamous cell carcinoma and odontogenic tumors. In the stomach and small intestine, adenocarcinomas arising from the mucosa, and smooth muscle tumors arising from the muscular tunics also are occasionally seen. Mesenchymal tumors of the musculoskeletal and connective tissues are occasionally encountered. Not enough of these tumors have been reported or fully characterized as to cell of origin, and prognosis often is based on histologic evaluation of tissue invasion or cellular anaplasia.

Cysts

Cysts are common in amphibians. Most cysts represent cystic dilatation of the rich lymphatic systems in the skin and mesentery. These may occur as the result of localized scarring due to previous or current episodes of trauma or infectious disease downstream from the cyst.

Trauma

Common traumatic problems in captive amphibians include suboptimal exhibit design, conspecific predation, prey item predation, handling, temperature extremes and foreign body ingestion. Intestinal obstruction, intussusception, and cloacal prolapse are also common events but may be associated with primary disease processes. Skin trauma is especially common and

likely is the source for most skin infections with few exceptions. Skin trauma occurs with rough handling, abrasive or caustic substrates, and cagemate aggression.

**Infectious Diseases**

Bacterial Infections

Although bacterial infection is common in amphibians, with few exceptions primary bacterial pathogens probably are not. Most bacterial infections are opportunistic or secondary, and associated with husbandry problems or other infectious agents.

Most bacterial infections in amphibians involve the skin, It is important to review the properties of amphibian skin to understand the pathogenesis of bacterial infection, and the subsequent event that contribute to clinical morbidity and mortality. Amphibian skin is highly permeable, a major regulator of water absorption and osmoregulation and also functions in respiration. Most species have a epidermal keratin layer, a thin epidermal layer (1-3 cell layers), a prominent lymphatic system, and many specialized secretory glands. Because the skin is so thin, it is sometimes difficult to detect gross lesions. Sometimes only slight reddening or increased mucus is noted, regardless of cause. The differential list is long for reddened skin and includes such diverse diseases as physiologic hyperemia, irritation due to chemical exposure, bacterial sepsis ("redleg"), Chlamydiosis, iridovirus, chytridiomycosis, protozoan infection, rhabditiform nematodes, and cutaneous capillariasis. The important thing is there are no pathognomonic skin lesions in amphibians; thus it is important to include skin as a routine component of the necropsy and histologic examination.

When assessing skin conditions, a thorough review of the husbandry and history is essential. Evaluation should include the source of the animals and history of illness at the source. The source of water and water quality parameters must also be addressed. The tank setup and substrates must be appropriate for the species and include all seasonal requirements. Cagemates and cagemate medical history should be known. Type of disinfection and cleaning intervals should be assessed.

The physical examination of the skin should begin with visual inspection of all cutaneous surfaces, and magnification may be necessary for smaller species. Cytologic examination of the skin is very helpful and should be a component of all antemortem and postmortem skin examinations. Masses can be aspirated. Abnormal appearing skin can be scraped or swabbed. Squash preps of exfoliated skin can be examined stained or unstained. Saline (0.9% NaCl) wet mounts can also be helpful to distinguish some of the cutaneous protozoa and fungi. Popular cytologic stains are Dif-Quik/Wright -Giemsa (Romanowski) and Papanicolao. Gram and acid fast stains can also be useful and can be applied to Dif-Quik stained specimens, but it is better to prepare several slides in the event special stains are needed.

Histologic examination of skin should be a routine part of every necropsy, and is a very useful adjunct to the evaluation of skin disease in the live amphibian. Small amphibians are often

submitted whole in formalin, and adequately trained histology technicians know what parts to sample. For large amphibians necropsied on site, skin collection sites should include ventral chin, ventral coelom, medial thigh, and hind foot (preferably whole hind foot), dorsal snout, dorsum at about level of front legs or slightly caudal. These are target sites for a vast spectrum of infectious and noninfectious diseases.

Other useful diagnostic procedures for assessing skin disease in amphibians include evaluation of water quality parameters (pH, ammonia, chloride, total solids), bacterial and fungal culture, and PCR for chytrids and iridovirus.

Bacterial Septicemia (Redleg, Dermatosepticemia)

This is perhaps the most common of the cutaneous infectious processes in amphibians. This condition is essentially a cutaneous manifestation of sepsis, rather than a primary external insult. Although skin may be the source of the septic process, often other conditions are present such as enteric or pulmonary parasitism, bacterial nephritis, metastatic mineralization, chytridiomycosis, iridovirus infection, etc. *Aeromonas hyrophila* is the classic isolate from these lesions; however, many other bacteria can be isolated from these lesions, including *Pseudomonas* spp., various coliforms, *Flavobacterium* (*Chryseobacterium*), and gram-positive bacteria. Often, the implicated organisms are normal inhabitants of aquatic or enteric environments. Often there is high colony or group morbidity and mortality and the presentation can be acute or there may be a long history of similar problems and long duration of illness in some animals.

Diagnosis is achieved by reviewing clinical signs and history, culture of subcutaneous or coelomic fluid, heart blood and visceral organs, cytologic evaluation of lesions, and histologic examination (preferably comprehensive). While culture results may be helpful in guiding the treatment protocol, it is important to note that most of the bacteria isolated will be opportunists, and that other diseases may be present. Cultures and other diagnostic procedures should be assessed in conjunction with histopathology.

Mycobacteriosis

Mycobacteriosis is the most important primary infectious disease process in amphibians. M. marinum is a common isolate, but remarkably little is currently known about the different mycobacteria that can infect amphibians and how this disease is transmitted, and this area warrants further study. Affected animals present with macroscopic or microscopic cutaneous nodules, cutaneous ulcers, cutaneous and visceral abscesses/granulomas. The feet, legs and head seem to be predisposed skin sites. A rapid presumptive diagnosis can be achieved by aspiration or impress cytology. Typically the cell population will be macrophages and lymphocytes, and the macrophages may have intracytoplasmic clear silhouettes of bacilli which do not stain with the Romanowski stain. An acid fast stain can be applied to accentuate the presence of the organism.

Histopathology also is diagnostic for the condition, but for the uninitiated pathologist the presentation can be confused with neoplastic processes, especially lymphoma. Culture also is

helpful, but does not seem to be commonly performed. Molecular techniques are improving for the diagnosis of specific mycobacteria from paraffin blocks, and this is of great potential value regarding the epizootiology, zoonotic potential and control of the disease. Current control measures include identifying and eliminating risk factors, disinfection and depopulation.

Chlamydiosis

Chlamydiosis is somewhat common in amphibians, especially anurans. Infection with *Chlamydophila psittacii* and *C. pneumoniae* have been described, and the disease has been reported in African clawed frogs (*Xenopus* spp.), bullfrogs (*Lithobates catesbeiana*), and giant barred frog (*Mixophes iterates*). Chlamydiosis is a zoonotic disease. Grossly the disease can resemble dermatosepticemia, because it is basically a septic process. Histologically, inflammation and necrosis affect most of the viscera and skin. Inflammation is primarily histiocytic and these cells contain fine granular material corresponding to the organisms, which are magenta in Gimenez stained sections.

Chlamydiae also have distinct electron microscopic features which aid in distinguishing them from other obligate intracellular bacteria. Immunohistochemistry works well for confirming the presence of Chlamydia in lesions. PCR is also a useful adjunct procedure for establishing an antemortem or post mortem diagnosis. Chlamydiae have a direct and simple life cycle. Elementary bodies (EB) enter the cell cytoplasm and appear to have the ability to inhibit phagolyzosomal fusion. Elementary bodies differentiate to reticular bodies (RB) that multiply by binary fusion forming a “inclusion” in the cell cytoplasm, where the RB then differentiate to infectious EB. Via exocytosis or cell rupture, these EB are released to infect other cells. There may be delays in this intracellular growth cycle that allow for latent or subclinical infection.

Chlamydia-like Disease

There is an organism or several similar organisms that infected anurans, for which the genus and species have not been identified. These organisms resemble Chlamydia in size and intracytoplasmic location, as well as host response; however, their ultrastructural and tinctorial features are slightly different, and they have more tropism for bone marrow and bone than does Chlamydophila. Attempts to sequence this agent have failed to date.

Mycotic Infections

Mycotic disease is common in amphibians and there are several agents that appear to be primary pathogens or significant opportunists. Disease is frequently associated with environmental or husbandry factors, including temperature extremes, high aquatic organic load, poor water quality, and cutaneous injury. Important mycotic diseases include chytridiomycosis, chromomycosis/phaeohyphomycosis, Basidiobolus infection,

Chytridiomycosis, Saprolegniosis, and Fusariosis

*Chytridiomycosis*

Although once considered an emerging disease, chytridiomycosis is now probably endemic in most parts of its range, which is likely global. The disease was first diagnosed in U.S. zoological collections and wild populations in the late 1990's, where it continues to be a sporadic but serious problem. The agent of chytridiomycosis is *Batrachochytridium dendrobatidis*. Fungi in the phylum Chytridiomycota are ubiquitous in most soil or water, and the life cycle has a motile zoospore stage that facilitates transmission. A significant oyster pathogen, *Polycaryum laeve* is in this Phylum, but prior to the identification of chytrid pathogens in amphibians, there were no known vertebrate pathogens in this Phylum. Affected animals often are found dead with no pre-existing clinical signs and no gross lesions. Young metamorphs seem especially susceptible. Signs or gross lesions when present may include excessive shedding of skin, reddening of skin, postural or behavioral changes, and rarely neurologic signs. Diagnosis is made by cytologic examination of cutaneous wet mount or smear preparations in heavy infections, or by histopathology and PCR. Routine fungal culture is not helpful. Chytrids infect the squamous epithelial cells of the outer layers of epidermis, forming intracytoplasmic zoosporangia that contain developing zoospores. These motile flagellated zoospores exit the cell through a tube formed by the zoosporangia and move through the environment to another suitable host to complete the life cycle. Histologic changes in affected skin include mild to moderate epidermal hyperplasia and hyperkeratosis. The cause of death is not clear but most affected animals have renal tubular necrosis. Infection and associated histologic change likely causes disruption of cutaneous homeostasis, with subsequent disregulation of respiration, excretion, secretion and immune barriers. Secondary bacterial overgrowth in skin lesions is common and may also contribute to the pathogenesis of the disease. Toxin production by the organism may also be a contributing factor although this has not been proven. Koch's postulates have been fulfilled. Some species may be resistant to lethal infection, such as bull frogs and tiger salamanders (*Ambystoma tigrinum*). Successful forms of therapy in affected animals include itraconazole bath (oral solution diluted in amphibian Ringer's): 0.01% at 5 min/day $\times$ 10 days, formalin/malachite green soaks, and elevated environmental temperature (37°C $\times$ 16 hr). Itraconazole is apparently contraindicated in tadpoles. Most common disinfectants are effective against chytrids, including 1% bleach, benzalkonium, and quaternary ammonium. Complete drying of the exhibit and heat may also be useful. Ultraviolet light is ineffective.

*Saprolegniosis*

Saprolegniosis is common in aquatic and semi-aquatic amphibians. This disease is occurs when Oomycetes (water molds) colonize the skin. Common isolates are *Saprolegnia*, *Aphanomyces* and *Achyla* spp. Oomycetes have a complex life cycle that involves zoospores and hyphae, and it is generally the hyphae that are observed in the lesions of saprolegniosis. Although the pathogenesis is not entirely clear, the disease is almost always associated with suboptimal water quality or a history of skin trauma; thus, this disease is likely and opportunistic infection. Grossly, the lesions are white cotton-like growths on the skin. Histologically, broad, pale

staining, somewhat flattened and granular hyphae in mats colonize the epidermal surface and invade into the dermis. Because this process can occur after death, it is important to evaluate the affected skin histologically for an associated tissue reaction. Antemortem colonization is generally associated with epidermal hyperplasia, variably hyperkeratosis and a mild inflammatory infiltrate in the superficial dermis and epidermis. Concurrent bacterial overgrowth is common with saprolegniosis. Diagnosis is by gross, cytologic and histologic appearance of the lesion.

*Chromomycosis/Phaeohyphomycosis*

These terms imply infection with pigmented fungi or yeasts. Pigmented fungi that from sclerotic bodies in tissue include *Cladosporium, Phialophora* and *Fonsecaea* spp., and this disease is referred to as chromomycosis. Pigmented fungi that do not form sclerotic bodies in tissue are usually *Exophiala* spp. (in amphibians), and this disease is referred to phaeohyphomycosis. These agents are saprophytes, and it is thought that infection is due likely to traumatic introduction of the agent; however, these agents appear to be considerably pathogenic. Skin and visceral infections are common. Because these agents are pigmented, the gross lesions often have a green or black tint. The gross appearance of the lesions is not a reliable means of diagnosis, though. Cytologic or histologic examination is more accurate, and culture often is needed to determine the genus. Concurrent bacterial infection seems common in these animals, and occasionally concurrent mycobacteriosis is also seen. Control of pigmented fungal infections includes replacement of contaminated substrate, disinfection, elimination of predisposing factors. There is possibly some zoonotic potential for some of these agents, although the risk is probably low for healthy people.

*Fusariosis*

Cutaneous infections caused by *Fusarium* spp. are common in amphibians, especially toads. This disease has been a formidable problem in the recovery programs for The Puerto Rican crested toad and Wyoming toad. *Fusarium* spp. are saprophytic, and disease is likely an opportunistic event. It is believed that high humidity and suboptimal substrate may be important predisposing factors. Gross lesions included reddened skin, especially of the ventrum, toes and ventral aspect of the hind legs. Histologically, the organisms are present usually in large numbers in surface crusts, epidermis and superficial dermis, associated with a mixed to granulomatous inflammatory response, epidermal hyperplasia and hyperkeratosis, and sometimes ulceration or necrosis of the epidermis. The fungus also tends to invade and destroy the superficial dermal secretory glands, and it is unclear if the glandular secretions released by this process are a source of self envenomation.

Other Fungal Infections

Mycotic diseases less commonly seen in amphibians include zygomycosis (*Mucor, rhizopus*) and Basidiobolomycosis. *Basidiobolus* spp. has been a problem in free ranging Wyoming toad populations. This fungus has hyphenated and spore forms, is primarily a cutaneous pathogen, and

depending on its stage of development and the quality of the sections, can be confused histologically with chytrids. histologic lesions are similar to those of fusariosis, with more inflammation and crusting than is seen with chytridiomycosis.

Organisms Divergent from Fungi

Currently there are a few infectious disease processes in amphibians (and fish) that are not well characterized to the molecular level, but in the past were thought of as fungi or protozoa. These agents are tentatively classified as mesomycetozoa because of the morphologic similarities to some of the mesomycetozoan agents seen in invertebrates, especially shell fish. *Dermocystidium* spp. and *Ichthyophonus* spp. cause granulomatous nodules in the skin or skeletal muscle of frogs and newts. These organisms typically have a very thick outer capsule surrounding a granular sporoplasm. These lesions are unsightly but tend to be incidental. Life cycles are not understood.

Viral Infections in Amphibians

Viral diseases are not common in most captive populations, but a few important viral infections have been well documented, and it is likely that many more viral diseases are present in amphibian populations that are poorly documented or have not been recognized.

Lucké Herpesvirus/renal Adenocarcinoma

The Lucké herpesvirus causes renal tubular adenocarcinomas in the leopard frog. It is a model for viral oncogenesis. Virus and viral inclusions are seen in neoplastic cells during tumor development at low environmental temperature, as occurs during winter months. Tumors that are found in frogs during summer months do not have productive virus or inclusions. This condition is apparently unique to leopard frogs.

Iridovirus (*Ranavirus*)

Iridovirus infection is common in anurans, and the type species is *Ranavirus* 3, first isolated in 1966. This virus is likely nonpathogenic in most species. Recently, iridoviruses have become significant pathogens in wild and captive populations of amphibians, and have been considered important causes of large mortality events. Wild spotted (*Ambystoma maculatum*) and tiger (*A. tigrinum*) salamanders have been particularly affected in the U.S. The viruses isolated or sequenced from these outbreaks are sometimes similar to the *Ranavirus* 3 or are distinctly different, indicating that several different iridoviruses exist in captive and wild populations of amphibians. Mortality events seem more numerous in the late summer and early fall. Survivors may become asymptomatic carriers. Die offs seem to be associated with increased population density and decreased genetic diversity. Transmission is likely direct, through food, feces and water. Convincing evidence of immunosuppression due to exogenous factors is not documented. Larvae and juvenile are most severely affected. Clinical signs and gross lesions include reddened or ulcerated skin, lethargy, anorexia, gastrointestinal hemorrhage, and multicentric visceral petechiae or purpurae. Histologically, the disease is characterized by hemorrhage and necrosis in a variety of tissues, especially gut mucosa, spleen, and skin. Vessels in these tissues sometimes

have endothelial cell necrosis or transmural fibrinoid necrosis. Epithelial and endothelial cells in these lesions rarely have intracytoplasmic eosinophilic or amphophilic inclusions. The inclusions may be small and are easily confused with phagocytized cell debris, or hypertrophied organelles. Diagnosis requires a combination of history, gross and histologic lesions, electron microscopy, PCR, virus isolation and tissue inoculation into susceptible hosts. There is much still to learn about iridovirus infection in amphibians, especially regarding pathogenic strains and rapid diagnostic techniques.

Herpesvirus

Fatal systemic herpesvirus infection has been observed in Mexican giant tree frogs (*Pachymedusa dachnicolor*) and likely occurs in other species as well. Systemic herpesviruses have not be well characterized from a molecular perspective, and diagnosis has been based on characteristic histologic appearance and electron microscopic confirmation. This disease is characterized by widespread tissue necrosis, especially in the liver and spleen, with formation of syncytial cells and characteristic intranuclear amphophilic or eosinophilic viral inclusions.

Papillomatosis

Cutaneous papillomatosis has been well described in the Japanese newt (*Cynops pyrrhogaster*). This condition has a season prevalence (autumn), and is capable of spontaneous regression. Herpesvirus-like particles have been observed in these tumors. Various forms of papilloma and adenomatous polyp are seen in the skin and cutaneous glands of amphibians, and florid presentations of multicentric cutaneous disease are sometimes encountered in captive animals. Remarkably, these presentations often are self limiting and spontaneously regress. Care is supportive. Only rarely do these lesions undergo malignant transformation, but the squamous papillomas can have inverted variant morphology that can be confused with squamous cell carcinoma. The nodular skin lesions of papillomatosis must be differentiated from those of other infectious processes, especially mycotic infections and mycobacteriosis, as well as noninfectious processes such as tumors and pseudogout.

Parasitic Diseases

Several different types of parasites infect or infest amphibians and can be associated with clinical disease.

Ectoparasites

*Acariasis*

The most commonly observed ectoparasites are mites and ticks. Maggots and leeches are also seen. Acariasis is primarily due to larval trombiculids, and 5 different Genera have been described. These organisms become embedded in the dermis, and are associated with a

granulomatous inflammatory reaction and varying degrees of epidermal hyperplasia and hyperkeratosis. The condition resembles “chiggers” in mammals.

*Leeches*

Leeches are annelid worms of the Class Hirudinea and are blood feeders. There are both ectoparasitic and endoparastic leeches: Ectoparasitic leeches attach to the skin or conjunctiva. Endo parasitic leeches attach to the oral mucosa or may reside in the lymphatics, heart or liver. They can be associated with localized trauma at the attachment site and may cause anemia if present for a long time or in large numbers. Leeches have also been implicated in the transmission of infectious agents.

*Nematodes*

Nematode infections are perhaps the most problematic form of parasitism in captive amphibians. Common sites of infection are gut, lung and skin. Visceral larval migrans can also be a cause for clinical disease.

*Psuedocapilloarides xenopi*

This is a cutaneous infestation seen only in *Xenopus* spp. Adults burro into the epidermis and superficial dermis where the complete life cycle occurs. Infestation causes much inflammation in the dermis and marked epidermal hyperplasia and hyperkeratosis. Affected animals have foci of thickened skin that may be pale and “flakey”. Heavy infestation can cause dysregulation of normal skin functions. Secondary bacterial and mycotic infections are also seen. Skin scraping and biopsy are diagnostic.

*Rhabdias* spp.

*Rhabdias* spp. lungworm larval and adult stages are important pulmonary pathogens particularly in anurans. Adults in the lung give birth to Larvated ova that coughed up, swallowed and expelled in the feces. The ova hatch in the environment, and larvae penetrate the skin, migrating back to the lung to become adults. The larval stages are especially immunogenic, invoking a severe granulomatous inflammatory response in all tissue locations. The reactive changes in the respiratory epithelium, inflammation in the faveoli and interstitium, and fibrosis in the interstitium likely result in ventilation perfusion mismatch and clinical morbidity. Secondary bacterial infection is common, and in treated animals, degenerative larvae in the lungs may be a source for the bacteria and for toxemia associated with necrosis of the parasite. Lung wash and fecal parasite exam are helpful in establishing a diagnosis of lungworm infection, although post mortem histopathologic diagnosis is the usual form of initial diagnosis in a collection. It is interesting to note that these frogs often are in good nutritional status at the time of death, underscoring the pathogenicity of the infection and suggesting that heavy infection may occur over a relatively short period of time.

*Strongyloides* spp.

*Strongyloides* spp. reside as adults in the small and large intestinal tract. Life cycle is direct and most of the damage is caused by the larvae as they migrate through the gut mucosa and visceral tissues. The parasite is associated with proliferative and inflammatory or ulcerative changes in the mucosa, emaciation and sepsis. In viscera other than gut, larvae may cause granulomatosis and tract formation, and can damage vessel walls causing thrombosis. Fecal parasite exam, skin biopsy and necropsy are typical forms of diagnosis.

*Filarid Worms*

Filarid infections are common in amphibians, especially anurans. Adults reside in the dermis and hypodermis, eye socket and coelomic cavity. Microfilaria reside in the peripheral blood. The parasites are usually incidental but unsightly findings. Occasionally, dead adults can invoke an inflammatory response in the dermis. Heavy microfilarial burdens can cause capillary clogging in the lungs and glomeruli.

*Trematodes*

Digenean infections are the most commonly seen forms of Trematodiasis in amphibians, although monogeneans can sometimes attach to skin or gills. Digeneans have complex life cycles with multiple host. When amphibians are the definitive host, adults are most commonly found in the gut. Encysted larval stages are found in the viscera and skin of amphibians that serve as intermediate hosts. Heavy intestinal burdens can cause emaciation, cloacal prolapse, or dysbiosis and bacterial enteritis. Diagnosis is by fecal parasite exam, and histopathology. Encysted larval stages sometimes are associated with secondary bacterial infection, especially in the skin, but in general are incidental. An important digenean is *Ribeiroia* spp. which have been associated with developmental anomalies in wild metamorphs, when the encysted larval stages cause mechanical disruption or displacement during development.

Monogeneans are flat worms similar to digeneans, but have direct life cycles and can adhere to the host at both anterior and posterior ends of the parasite. Monogeneans have anterior suckers and a posterior hooked apparatus termed a haptor. Larvae attach to the gills or skin of metamorphs, mature and produce eggs. From the eggs hatches a free living larval stage called a oncomiracidium that finds a new host to complete the cycle. Adults can reside in the urinary bladder, stomach or intestine. Except in heavy infections, these parasites cause little trouble, although method of attachment may affect pathogenicity. Diagnosis is by skin scrapings, gill clip or histopathology.

*Cestodiasis*

Cestode infections are common in amphibians, and several different species reside as adults in the gut of various amphibians. Enteric cestodes rarely are pathogenic, but sometimes can cause some mild proliferative change in the intestinal mucosa. Encysted larval stages (sparganums) in

the skin can be more problematic, especially if they degenerate or are traumatized. Secondary bacterial infection and sepsis are occasionally seen in association with the cutaneous larval stages.

*Microsporidia*

These parasites were formerly classified as protozoa but may more closely resemble fungi, and currently are being reclassified. This group of parasites has potential for causing disease in all Classes of animals. Microsporidiosis is somewhat common in anurans and caudate. Generally infection is associated with formation of microgranulomas in various tissues. Proliferative forms of tubulonephritis and enteritis are also seen. Spinal curvature may be seen in salamanders associated with microsporidial granulomas in the spinal skeletal muscle. Microsporidia have a direct life cycle and infection may be contracted via alimentary tract or respiratory tract. Infective spores evert a polar filament that is inserted through the cytoplasmic membrane of the host cell. Sporoplasm is injected into the host cytoplasm and develops into many more spores. The cell ruptures releasing the spores, which spread through the bloodstream to other sites. Spores are shed through the feces and urine. Microsporidia are further classified based on electron microscopic features and sequence analysis.

*Myxozoa*

Myxozoa were formerly classified as protozoa and currently are being reclassified. These organisms infect all classes of animals but are most common in fish. Most myxozoa have complex life cycles, involving plants and other invertebrates such as annelid worms. In amphibians they are most common in the kidney, testes, gall bladder and bile ducts, and usually are incidental findings. In some species, such as the Asian horn frog, *Chloromyxum* spp. cause tubulointerstitial nephritis and glomerulonephritis. Most myxozoan infections are diagnosed histologically. Myxozoa are further classified based on light and electron microscopic features, and sequence analysis.

*Coccidia*

*Eimeria* and *Isospora* infections are common the gut and kidney of amphibians but disease is uncommon. Young stressed animals and poor hygiene may predispose to disease associated with infection. Cryptosporidiosis is rarely seen in anurans, primarily in the stomach. Diagnosis is by fecal parasite exam and histopathology.

*Flagellates*

Flagellates are commonly encountered in wet mounts of feces from amphibians. They also are commonly observed in histologic section. Usually they are not associated with significant clinical disease or architectural alterations in the gut mucosa. Trypanosomes are occasionally encountered in blood films but do not cause disease.

*Ciliates*

Ciliates are commonly encountered in the gut of amphibians. We have not encountered any disease associated with these organisms in the gut of amphibians. We have seen pathologic changes in the gills of metamorphs that have branchial ciliate infections.

*Amoeba*

Amoeba are commonly encountered in the gut of amphibians. A few have been reported to be pathogenic in frogs and tadpoles, such as *Entamoeba ranarum.* We note that invasive amoebic infections of the gut are commonly associated with concurrent bacterial infection and nematodiasis, and it is difficult to determine if these are primary pathogens or opportunists. The organisms have potential for invasion of the gut wall and hematogenous spread to other tissues, with associated thrombosis, necrosis and inflammation. Because numerous commencal amoebae are seen in amphibians, interpretation of a positive fecal exam should be conservative, and judged with the clinical presentation. Clinical amoebiasis in amphibians reportedly is associated with decreased activity levels, weight loss, dehydration and anasarca.

*Hemoparasites*

Hemogregarine infections are common in amphibians but disease is rare. Inflammation associated with tissue phases in the lung may be associated with some degree of clinical morbidity. Lankesterella and related species of apicomplexan parasites have a histologic presentation similar to that of atoxoplasmosis in birds but are not as pathogenic.

## LITERATURE CITED

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