AMPHIBIAN DISEASES

Dr Lee Berger
School of Public Health, Tropical Medicine and Rehabilitation Sciences,
James Cook University, Townsville, Queensland, Australia 4811

Dr D. Earl Green
U.S. Geological Survey, National Wildlife Health Center
Madison, Wisconsin, U.S.A.

General

General references on amphibian disease include:

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For detailed information including disease control, diagnosis of chytridiomycosis, disease bibliography and publications see the Amphibian Disease Home Page
http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm

Unpublished information in this presentation is based on necropsies of mostly free-living amphibians, conducted in the USA and Australia and since 1995.

Special anatomical considerations in amphibians

Anura, Anuran: The order of amphibians consisting of frogs & toads.
Caudata, Caudate: The order of amphibians consisting of salamanders (including sirens, amphiumas, newts, etc).
Bidder's organs: Remnants of ovaries at the anterior pole of each testis in male toads.
Heart: Two atria and 1 ventricle.
Endolymphatic system: These diverticula partially encircle the brain and spinal cord and store calcium carbonate; white symmetrical lateral pairs of out-pouchings along the spinal column are called endolymphatic ("lime") sacs.
Lymphatic sacs: The "subcutis" of post-metamorphic frogs and toads consists of an extensive network of large chambers (that are separated by thin septae) that drain fluids from the skin to lymph hearts.

Lymph hearts: Frogs & toads have multiple pairs of lymph hearts located just ventral to the urostyle and in the dorsal neck/pectoral girdle. Lymph hearts are very thin walled and contain leiomyocytes (not cardiomyocytes). The pelvic lymph hearts pump fluids into the interstitium of the mesonephroi.
Fat bodies: Frogs, toads & tadpoles have a pair of tuft-like fat bodies at the anterior poles of the mesonephroi within the body cavity; adipose tissue is sparse or absent from the "subcutis" and elsewhere in the body. Fat bodies of salamanders often are a pair of ribbon-like structures in the mid dorsal body cavity. Atrophied fat bodies appear as small cellular organs without lipids.
Gills: Aquatic larval (and neotenic) salamanders have external gills while tadpoles have internal gills. Within the internal gill chambers of tadpoles, the forelimbs form & then emerge during metamorphosis.
Lungs: Lungs develop early in larvae but may not become inflated for several weeks after hatching. Amphibian lungs are sac-like with a large central lumen. Some groups of terrestrial salamanders (plethodontids) lack lungs.
Kidneys: Larval amphibians have 2 pairs of "kidneys" at the time of hatching: the aglomerular pronephroi and the mesonephroi. Pronephroi disappear during metamorphosis. Mesonephroi contain intermixed "adrenal" cells (clusters of eosinophilic or basophilic granular cells), referred to as the inter-renals. Most terrestrial species excrete urea, aquatic species excrete ammonia.
Metamorph: This term has at least two meanings: an amphibian which is undergoing metamorphosis, or, an amphibian that recently has completed metamorphosis.
Recent Metamorph: An imprecise term for an amphibian that recently has completed metamorphosis.
Tadpole mouths: The **oral disc** of most tadpoles consists of the black **jaw sheaths**, black **tooth rows** and **labia**; these structures are lost during metamorphosis. Larval aquatic salamanders have an adult-like mouth.
Pigmented Macrophage Aggregates (PMA): These clusters of macrophages occur mostly in the liver and spleen; macrophages are laden with phagocytised debris, melanin, and multiple carotinoid pigments. Increase permanently during times of starvation.
Internal Iridophore Layers: Some species of tree or reed frogs (*Litoria chloris, Litoria gracilenta*, *Hyperolius* sp.) have a white opaque layer on the surface of internal organs that are seen histologically as refractile, khaki iridophores. Suggested purpose as added protection from UV radiation.
Salamander histology: Cells & nuclei of nearly all caudate organs are very large; nuclei of salamanders contain 10 to 100 times more DNA than other vertebrates. Be aware of magnification when examining tissue sections!
Substantia amorpha: Granular basophilic layer in dorsal dermis of some species. Composed of calcium and polysaccharides that may function to prevent water loss. Can appear similar to dystrophic calcification.
Yolk platelets: Mucosal epithelium of young tadpole intestine may be full of yolk platelets, as present in ova.
Immunology: Amphibians have a spleen, thymus, bone marrow and lymphocytes. Spleen has red and white pulp but no distinct germinal centres.

Microbial Cultures
As in fish, most aquatic amphibians autolyse rapidly. Consequently, bacterial and fungal cultures on viscera of amphibians that have been dead >2-4 hrs usually contain post-mortem invaders, foremost among these are *Aeromonas* spp., *Pseudomonas* spp., *Citrobacter* spp., *Flavobacterium* spp., enterococci, and numerous other gut flora and bacteria common in surface waters. As recommended by fish diagnosticians, submission of live sick amphibians is encouraged, especially if bacterial and fungal cultures are to be attempted.

VIRAL DISEASES

1. Ranaviruses (Family: Iridoviridae; Genus: Ranavirus) OIE list A disease, Notifiable

A. Bohle Iridovirus

Significance: Bohle iridovirus (BIV) is the only virus isolated from Australian frogs and has been isolated once - from metamorphs of the ornate burrowing frog (*Limnodynaste ornatus*) that died during metamorphosis in captivity. The frogs had been collected as tadpoles from a temporary pond at Bohle, a suburb of Townsville. BIV has since been experimentally transmitted to adults and tadpoles of *B. marinus*, juveniles and tadpoles of *L. terraereginae*, and juveniles of *Litoria latopalmata*,

as well as the barramundi (*Lates calcarifer*). Juvenile *L. terraereginae* and *L. latopalmata* were highly susceptible to BIV while larval *L. terraereginae* were less susceptible. Chronic infections may occur. Antibodies to unidentified ranaviruses were detected across the range of cane toads in Australia.

Clinical Signs and Gross Pathology: Lethargy, focal haemorrhage and ascites.

Histopathology: Typical pathology includes renal, pulmonary, hepatic, splenic and haemopoietic necroses and haemorrhages.

B. North American Ranaviruses

Significance: In North America ranaviruses are a major cause of massive mortality events in amphibian larvae and recent metamorphs. Onset typically is explosive. Hundreds or 1,000's of sick and dead larvae often are found. In North America, adult amphibians rarely suffer mortality. Carrier state in a low percentage of adult amphibians is suspected, but infected organs are unknown. Molecular work suggests there has been recent spread of ranaviruses.

Clinical Signs and Gross Pathology: Tadpoles & salamanders: Erythema, petechia and paintbrush haemorrhages in skin at base of hind limbs, around vent tube, around oral disc and scattered elsewhere on the ventral body, head and tail. Older tadpoles may have mild to severe clear or mildly fibrinous effusions in lymphatic sacs of thighs, ventral abdomen & gular area. Internally, miliary foci of necrosis in liver & spleen; occasional necrotic foci are haemorrhagic. Mesonephroi may have haemorrhagic glomeruli. Petechiae & ecchymoses may occur in muscles, GIT and many other organs. Hydrocoelom (clear or fibrinous) is common. **Adult frogs & toads**: Rarely affected in North America. Tree frogs may have multiple small irregular pigmented ulcers in dorsal skin. Oral (palatal) ulcers occasionally found in recently metamorphosed frogs.

Microscopic Pathology: Ranaviruses are pantropic, with predilection for small vessels, PMAs, spleen, liver & mesonephroi. Miliary fibrinocellular vasculitis (especially in gills, dermis, GIT, heart & lungs) with necrotising glomerulitis is common. Spleen and renal interstitial (or subcapsular hepatic) myeloid cells frequently show miliary or diffuse necrosis. Liver first shows necrosis of sinusoidal endothelium, then PMAs, and then hepatocellular necrosis; characteristic basophilic intracytoplasmic inclusion bodies are single or multiple and usually 25-50% of the size of the nucleus. Fortuitous sections of epidermis may show micro-vesicles, erosions and ulcers; occasional basophilic cytoplasmic inclusions are present in degenerating epidermal cells at margins of vesicles and ulcers. Secondary and opportunistic skin infections are common.

C. UK Ranavirus

Significance: Annual epidemic mortalities of common frogs (*Rana temporaria*) have occurred across much of Britain since the 1980's, caused by ranaviruses. The incidence of deaths peaks in July, August and September. Molecular studies suggest British viruses originate from North America.

Clinical Signs and Gross Pathology: Two main disease syndromes: one characterised by skin ulceration and one characterised by systemic haemorrhages.

Microscopic Pathology: Histological lesions included epidermal thickening, epidermal necrosis, and necrosis, granulocytic inflammation, congestion and haemorrhage in internal organs.

D. Diagnosis of Ranaviruses.

The current routine techniques for diagnosing ranaviruses in amphibians are histology, virus isolation from tissues, capture ELISA, and PCR. Low grade infections (carrier state) may only be detectable by PCR.

References

Cullen BR, Owens L, Whittington RJ. 1995. Experimental infection of Australian anurans (*Limnodynastes terraereginae* and *Litoria latopalmata*) with Bohle iridovirus. Dis Aquat Org 23: 83-92.

Cunningham AA, Hyatt AD, Russell P, Bennett PM. 2007. Emerging epidemic diseases of frogs in Britain are dependent on the source of ranavirus agent and the route of exposure. Epid Infect 135: 1200-1212.

Green DE, Converse KA and Schrader AK. 2002. Epizootiology of 64 amphibian morbidity and mortality events in the USA, 1996-2001. Annals NY Acad Sci 969: 323-339.

Speare, R., Smith JR. 1992. An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia. Dis Aquat Org 14: 51-57.

Wolf K, Bullock GL, Dunbar CE, Quimby MC. 1968. Tadpole oedema virus: a viscerotrophic pathogen for anuran amphibians. J Infect Dis. 118: 253-262.

Zupanovic Z, Lopez G, Hyatt AD, Green B, Bartran G, Parkes H, Whittington RJ, Speare R. 1998. Giant toads *Bufo marinus* in Australia and Venezuela have antibodies against "ranaviruses". Dis Aquat Org 32: 1-8.

2. Lucke Tumor Herpesvirus (ranid herpesvirus-1)
Significance: This was the first neoplasm proven to be caused by a virus. It induces renal adenocarcinomata in northern leopard frogs (*Rana pipiens*) in the US. Virus must be transmitted to eggs/embryos during breeding. Historically, prevalence varies greatly, but prevalence of renal neoplasms increases with age. Rarely seen in frogs <2 yrs old.

Gross Pathology: Renal neoplasms are multi-nodular, slightly pale, single or multiple, microscopic or enormous, and occasionally metastatic.

Microscopic pathology: Tumour morphology is consistent with a renal adenocarcinoma with one notable additional feature: in neoplasms from frogs recently emerged from hibernation, many neoplastic cells have large prominent intranuclear inclusions (typical of a herpesvirus). However, in those frogs maintained at room temperatures, no inclusions are present and viral particles cannot be found ultrastructurally. Viral replication is very temperature sensitive and occurs only at cold temperatures.

References
Lucké B. 1934. A neoplastic disease of the kidney of the frog, *Rana pipiens.* Am J Cancer 20: 352-379.

Lucké B. 1938. Carcinoma in the leopard frog: Its probable causation by a virus. J Exp Med 63: 457-468.

3. Frog Erythrocytic Virus (FEV, formerly *Toddia* spp. and *Pirhemocyton* spp.)
Significance: Considered an incidental finding in erythrocytes of adult frogs in Canada, Costa Rica, Brazil and South Africa. Virus has not been cultured but may belong in the family, Iridoviridae, though larger than ranaviruses. One report mentions an associated anaemia and that recapture of infected bull frogs was less than recapture rate of non-infected frogs. Transmitted by mosquitoes or midges.

Microscopic Pathology: Histopathology not reported. Intracytoplasmic viral inclusions are detected in stained blood smear slides. Three types of cytoplasmic inclusions are described: 1) most common are red or sometimes blue (in Giemsa stain) dense, usually spherical (but occasionally ovoid or pleomorphic) cytoplasmic inclusions are 1-3 microns diameter; these inclusions may be single or multiple; 2) occasionally, a single larger pale or clear, albuminoid, and vacuole-like inclusion is found; and 3) uncommonly, a single large dense elongate or crystalloid rectangular or trapezoidal membrane-bound inclusion is 10-20 microns long and often displaces the erythrocyte's nucleus and may cause cellular distortion. Up to 90% of erythrocytes may contain the former smaller inclusions, but more often 20-50% of erythrocytes are affected.

References
Gruia-Gray J, Desser SS. 1992. Cytopathological observations and epizootiology of frog erythrocytic virus in bullfrogs (*Rana catesbeiana*). J Wildl Dis 28: 34-41.

Speare R, Freeland WJ, Bolton SJ. 1991. A possible iridovirus in erythrocytes of *Bufo marinus* in Costa Rica. J Wildl Dis 27: 457-462.

Speare R. 1990. A review of the diseases of the cane toad, *Bufo marinus*, with comments on biological control. Aust Wildl Res 17: 387-410.

4. Adenoviruses of intestine
Significance: Enteric adenoviruses are an infrequent incidental histological finding in tadpoles, frogs and toads. Morbidity and mortality are not reported.

Microscopic Pathology: Typical basophilic intranuclear inclusions in intestinal epithelial cells.

5. Herpes-like virus of skin
Significance: In Italy, up to 80% of a wild population of *Rana dalmatina* had epidermal vesicles associated with a herpes-like virus, but dead frogs were not found.

Reference
Bennati R, Bonetti M, Lavazza A, Gelmetti D. 1994. Skin lesions associated with herpesvirus-like particles in frogs (*Rana dalmatina*). Vet Rec 135: 625-626.

6. Calicivirus
Significance: Calicivirus was isolated from two captive *Ceratophrys orata* found dead. Both had pneumonia, while one also had oedema and the other had lymphoid hyperplasia.

Reference
Smith AW, Anderson MP, Skilling DE, Barlough JE, Ensley PK. 1986. First isolation of calicivirus from reptiles and amphibians. Am J Vet Res 47: 1718-1721.

BACTERIAL DISEASES

Contrary to pre-1995 literature, bacterial infections of free-living amphibians are rare, seldom cause morbidity or mortality, and when detected, usually are secondary or opportunistic invaders. However outbreaks do occur in captivity.

1. "Red Leg Syndrome"
Significance: Primarily Gram-negative bacilli are implicated in this classic syndrome. *Aeromonas* spp, *Flavobacterium* spp., *Klebsiella* spp., *Acinetobacter* sp. and others are mentioned in older publications. Clinical signs of classically described "red leg syndrome" are suspiciously indistinguishable from ranaviral infection. With few exceptions, virus cultures were not attempted in published reports of "red leg syndrome" prior to 1995, and reports of mass mortality in wild frogs are likely due to Ranavirus or chytridiomycosis. High mortality rates occur in captivity due to bacterial septicaemia.

Gross Pathology: Same as for ranaviral infection with include pale skin, petechiation, haemorrhagic cutaneous ulcers, lethargy, anorexia, oedema, haemorrhages in internal organs, ascites and pale livers.

Microscopic Pathology: Similar to ranaviral infection. Mere presence of bacteria in blood vessels and sinuses of internal organs is not evidence of primary bacterial septicaemia (or red leg syndrome).

Bacteria need to be associated with necrotic cells, fibrin and inflammatory cells; bacteria also should be present intracellularly (phagocytised).

References

Taylor SK, Green DE, Wright KM, Whitaker BR. 2001. Bacterial diseases (Chapter 13). *In:* Wright K.M, and B.R. Whitaker (Eds): Amphibian Medicine and Captive Husbandry. Krieger Publishing, Malabar, Florida. Pp. 159-179.

2. Flavobacteriosis

Significance: Bacterial septicaemias caused by multiple species of flavobacteria are a threat to captive amphibian colonies and are rarely encountered in free-living amphibians. *F. meningosepticum*, *F. indologenes* and *F. oderans* are most often implicated. Mortality rates as high as 70% may occur.

Clinical Signs and Gross Pathology: In adult *Xenopus laevis*, signs and gross findings were hydrocoelom, anasarca, dyspnoea, marked lethargy, congestion of toe-webs and dermal petechiae. In recently metamorphosed *Rana pipiens*, findings included panophthalmitis, anisocoria, meningitis, otitis interna, head tilt, loss of righting reflex, abnormal posture (sternal recumbency), anorexia and emaciation. Olson et al. (1992) found weight loss, corneal oedema, effusions in the lymphatic sacs, marked haemorrhage in major muscle groups, serosanguinous hydrocoelom, purulent pericarditis, hepatosplenomegaly and distended gastrointestinal tract with viscous hemorrhagic contents.

Microscopic Pathology: Reports of histological changes are sketchy. May be infiltrates of macrophages, neutrophils and lymphocytes in the liver, spleen, mesonephroi, serosal surfaces and epicardium; macrophages contain Gram-negative bacilli. Neutrophils in the anterior chamber with retinal detachment and severe conjunctival and corneal oedema. Pericardium had a thick layer of fibrin, neutrophils and bacteria. Liver showed hepatocellular degeneration and coagulative necrosis.

References

Green SE, Bouley DM, Tolwani RJ, Waggie KS, Lifland BD, Otto GM, Ferrell Jr JE. 1999. Identification and management of an outbreak of *Flavobacterium meningosepticum* infection in a colony of South African clawed frogs (*Xenopus laevis*). J Am Vet Med Assoc 214: 1833-1838.

Olson ME, Gard S, Brown M, Hampton R, Morck DW. 1992. *Flavobacterium indologenes* infection in leopard frogs. J Am Vet Med Assoc 201: 1766-1770.

3. Mycobacteriosis

Significance: This disease is rare in free-living amphibians, however, certain mycobacteria can cause chronic problems in captive amphibian colonies. The more commonly identified species from amphibians include *M. chelonei, M. fortuitum, M. marinum, M. xenopi, M. szulgai, M. avium* and *M. liflandii*. The latter species is associated with ulcerative skin lesions in captive clawed frogs.

Gross Pathology: Granulomas may be irregular and poorly demarcated, or large and encapsulated. Granulomas may be solitary, miliary or prominent and coalescing. Liver, spleen, intestine and dermis are most often affected, but granulomas may be present in nearly any organ or tissue. *M liflandii* infection causes skin ulcers, hydrocoelom and inability to dive.

Microscopic Pathology: Mycobacteria elicit granulomatous inflammation which in amphibians consists of epithelioid macrophages, small lymphocyte-like macrophages and true lymphocytes. Early granulomas are solidly cellular, poorly demarcated, and have been confused repeatedly for "lymphosarcoma". Multinucleated giant cells may be present in chronic granulomas but otherwise are uncommon. Caseation is variable and an unreliable feature. Mineralization and cavitation are not reported. *M liflandii* infection causes skin ulcers that contain acid fast bacilli but also widespread infection of the mucosal epithelium of gall bladder, oviduct, renal tubules, and ova. Acid fast staining (Ziehl-Neelsen) is recommended for all caseous and non-caseous granulomata of amphibians, especially those in long-term captive situations.

References
Asfari M. 1988. Mycobacterium-induced infectious granuloma in *Xenopus*: Histology and transmissibility. Cancer Res 48: 958-963.

Chai N, Deforges L, Sougakoff W, Truffot-Pernot C, DeLuze A, Demeneix B, Clement M, Bomsel MC. 2006. *Mycobacterium szulgai* infection in a captive population of African clawed frogs (*Xenopus tropicalis*). J Zoo Wildl Med 37: 55-58.

Suykerbuyk P, Vleminck K, Pasmans F, Stragier P, Ablordey A, Tran HT, Hermans K, Fleetwood M, Meyers WM, Portaels F. 2007. *Mycobacterium liflandii* infection in European colony of *Silurana tropicalis*. Emerg Infect Dis 13: 743-746.

4. Salmonellosis
Significance: *Salmonella* spp. are often isolated from amphibians at low elevations in neotropical sites and high loads can occur. Although not reported as a cause of morbidity or mortality in amphibians, it may be a serious contaminant of frog legs for human consumption. In Australia, Salmonella were isolated from 12.7% (19/150) of *B. marinus* collected from the wild and 9 serotypes were identified. All 9 had previously been isolated in Australia from humans and livestock. However, in the US only 9 (0.7%) isolates have been obtained from >1300 selective cultures for *Salmonella* spp. from free-living amphibians nationwide (DE Green, unpubl. data).

Reference
O'Shea P, Speare R, Thomas AD. 1990. Salmonellas from the cane toad, *Bufo marinus.* Aust Vet J 67: 310.

5. Streptococcosis
Significance: A non-haemolytic group B *Streptococcus* caused an outbreak killing 80% of about 100,000 farmed bull frogs (*R. catesbeiana*) in Brazil. The outbreak was associated with overcrowding and stress.

Clinical signs and Gross Pathology: Floating frogs. Enlarged spleens.

Microscopic Pathology: Septicaemia, necrotising splenitis and hepatitis with haemorrhages occurred in frogs. Cocci in blood smears and many organs. Viral cultures were negative.

Reference
Amborski RL, Snider TG, Culley DD. 1983. A non-haemolytic, group B Streptococcus infection of cultured bullfrogs, *Rana catesbiana,* in Brazil. J Wildl Dis 19: 180-184.

6. Spinal arthropathy associated with *Ochrobactrum anthropi*
Significance: Cane toads in Australia commonly have nodular proliferations at intervertebral joints.

Clinical signs and Gross Pathology: Proliferation often results in ankylosis.

Microscopic Pathology: Most lesions involved bone and cartilage proliferation, often with associated pyogranulomatous inflammation. *Ochrobactrum anthropi* was isolated from affected toads but not from controls. An interaction between degenerative and bacterial aetiologies was suggested.

Reference
Shilton CM, Brown GP, Benedict S, Shine R. 2008. Spinal arthropathy associated with *Ochrobactrum anthropi* in free-ranging cane toads (*Chaunus [Bufo] marinus*) in Australia. Vet Path 45: 85-94.

7. Chlamydiosis
Significance: *Chlamydophila* (formerly *Chlamydia*) *pneumoniae* was formerly a significant hazard to colonies of African clawed frogs (*Xenopus laevis*) being fed raw beef liver. Mortality rates of about 50% may occur in colonies of adult clawed frogs. A sick wild Giant barred frog (*Mixophyes iteratus*)

from New South Wales was found with a chronic pneumonia caused by a strain of *C. pneumoniae* that infects koalas. Recent surveys of amphibians in Switzerland and Russia by PCR suggest amphibians may be asymptomatic carriers of multiple spp of chlamydiae.

Gross Pathology: Emaciation, hepatosplenomegaly, hydrocoelom & lymphatic sac effusion in systemic infections.

Microscopic Pathology: Liver is organ of choice for tissue section. Disseminated to diffuse lymphohistiocytic hepatitis is found with scattered necrotic hepatocytes, fibrin in sinusoids, and intracytoplasmic granular basophilic inclusions. Irregular foci of purulent inflammation also may be present in liver and spleen. Granulomatous or pyogranulomatous inflammation in lungs (interstitial pneumonitis), heart (lymphohistiocytic myocarditis & epicarditis), spleen and mesonephroi (fibrinopurulent glomerulitis) tend to have fewer cytoplasmic inclusions.

References

Howerth EW. 1984. Pathology of naturally occurring chlamydiosis in African clawed frogs (*Xenopus laevis*). Vet Path 21: 28-32.

Berger L, Volp K, Mathews S, Speare R, Timms P. 1999. *Chlamydia pneumoniae* in a free-ranging giant barred frog (*Mixophyes iteratus*). Australian J Clin Micro 37: 2378-2380.

Reed KD, Ruth GR, Meyer J, Shukla SK. 2000. *Chlamydia pneumoniae* infection in a breeding colony of African clawed frogs (*Xenopus tropicalis*). Emerg Inf Dis 6: 196-199.

8. Rickettsiosis (*Aegyptianella ranarum*)

Significance: *Aegyptianella* (formerly *Cytamoeba*) *ranarum* is an anaplasma-like agent infecting erythrocytes of bullfrogs, green frogs and mink frogs in USA and Canada.

Microscopic Pathology: In blood smear slides, organisms are cytoplasmic, and measure 3 to 11 microns; organisms occasionally are densely packed and arranged in parallel.

References

Desser SS. 1987. *Aegyptianella ranarum* sp. n. (Rickettsiales, Anaplasmataceae): Ultrastructure and prevalence in frogs from Ontario. J Wildl Dis 23: 52-59.

FUNGAL DISEASES

1. Chytridiomycosis (*Batrachochytrium dendrobatidis* ["BD"]; Chytridiomycota) OIE List A Disease, Notifiable

Significance: Declining amphibian populations are reported on every continent where amphibians occur and about 30% of species are threatened. Worldwide the declines of 233 species have been attributed to habitat loss and degradation, invasive species or to overexploitation, but for 202 species the declines were labelled as "enigmatic" as there was no obvious cause. These frogs mostly occurred in high altitude, protected forested sites in the tropics of Central and South America and Northern Australia, although temperate species in Australia and North America have also been affected. From the pattern of the declines it is now clear that the introduction of BD was the cause of most of these. The "impact of chytridiomycosis on frogs is the most spectacular loss of vertebrate biodiversity due to disease in recorded history" (Skerratt et al. 2007).

In Queensland chytridiomycosis has probably caused the extinction of 6 species since 1979. In Victoria and New South Wales the Spotted tree frog (*Litoria spenceri*), Green and golden bell frog (*L. aurea*) and Corroboree frog (*Pseudophryne corroboree*) are endangered. In Queensland declines have spread from south to north between 1979 and 1994. Arid areas are too hot and dry for the fungus and it does not appear to have entered Cape York. However BD may still be spreading into naïve areas in Tasmania.

After arrival BD remains common in surviving populations, with seasonal outbreaks. A mark-recapture study showed infection reduced the monthly survival rate by about 38% in a population in south east Queensland, where BD had been present for around 30 years.

There are >1000 chytrid species (phylum Chytridiomycota) but BD is the only pathogen of vertebrates. The other species are saprobic or infect algae, fungi, insects, or plants. BD has a very wide amphibian host range although susceptibility varies among species. American bullfrogs (*Rana catesbeiana*) and the African clawed frog (*Xenopus laevis*) may be worldwide carriers.

Mortality rates (0-100%) and incubation times (about 10 to 70 days) vary with temperature, host species and age, and fungal dose and strain. BD infects superficial keratinizing cells of the adult skin and tadpole mouthparts. As healthy carriers are common, there is a high risk of introducing disease in imported frogs.

Clinical Signs and Gross Pathology: In tadpoles, infection of mouth parts is characterised by multifocal to diffuse loss of black colour on jaw sheaths ("beaks") and tooth rows. While tadpoles usually survive infection, they may have reduced growth rates and smaller size at metamorphosis. In captivity it is typical to see high mortality rates 2-3 weeks after metamorphosis, when the skin over the body become keratinized and infection spreads,

Signs in frogs include discoloured and reddened skin, inappetance, lethargy, loss of righting reflex and seizures. Sick frogs usually die within days. There may be accumulations of shed skin, thickened translucent-to-opaque moults, and occasionally paint-brush haemorrhages in ventral skin. Infections are most intense in ventral abdominal & thigh skin, toes-tips and toe webs.

In terminal stages electrolyte concentrations are greatly decreased, but other biochemical parameters are normal.

Microscopic Pathology: BD produces spherical or oval sporangia, not hyphae. Sporangia divide internally to form the infective, waterborne flagellated zoospores that are released via a discharge tube. BD as a parasite is intracellular infecting cells of the stratum granulosum and stratum corneum.

In histological sections, BD is spherical or oval with occasional discharge papillae seen projecting from the skin surface. The contents of zoosporangia vary and four developmental stages can be identified: (1) Dark basophilic, rather homogenous mass. (2) Sporangia become multinucleate. (3) Cytoplasm divides to form distinct zoospores. (4) Once the zoospores are released via the discharge papilla, the empty zoosporangia remain. In some empty colonial stages, thin septa are visible dividing the sporangium into 2, 4 or more internal compartments. The empty sporangium may collapse into an irregular shape. During this terminal stage the empty shell sometimes becomes colonised by bacteria. Empty sporangia are the most common stage present, particularly in the sloughing surface layer. Immunohistochemistry, PAS or silver stains are useful for confirming suspect cases.

Focal hyperkeratosis, erosions and disorganised cell layers are common in infected areas. Epidermis may be thicker or thinner than normal. Ulcers occur in some species. Inflammatory reaction is minimal, but may be marked if secondary bacterial (and other fungal) infections develop.

Distinctive lesions are not seen in internal organs. The cause of death appears related to loss of electrolytes across the epidermis.

Wet mounts can be made from shedding skin, whole skin pieces or tadpole mouths. The round to oval intracellular sporangia (5 - 13 µm) occur in clumps. Old empty sporangia are the most prevalent stage in shedding skin, although sporangia containing zoospores are commonly found. Discharge tubes usually point perpendicularly to the skin's surface and thus appear as small circles, which can be difficult to discern. The observation of internal septa within sporangia increases confidence in the diagnosis. Epidermal cell nuclei are of similar size to sporangia but can be differentiated by their irregular, indistinct membranes and flat, granular, grey appearance. Congo red, cotton blue and DipQuick stains can be used if desired.

Diagnosis: Chytridiomycosis can only be diagnosed by laboratory tests. The clinical signs of chytridiomycosis are non-specific.

In ill amphibians, all diagnostic tests (including histology, wet skin mounts) are accurate. However for screening healthy animals, swabbing for standard or real-time PCR is much more sensitive. Culture requires special techniques and is insensitive for diagnosis.

Sample from ventral surfaces of adults. Sampling can be done by collecting skin scrapings or smears, excising pieces of skin, or by swabbing. Toe-webbing, and/or toes can be excised from live or dead frogs, and strips of skin from the inguinal area (pelvic patch) can be collected from dead animals.

Tadpoles need to be sacrificed if microscopy is to be done. Whole tadpoles can be collected and preserved and sectioned through the mouthparts to include the dark brown keratinized jaw sheaths or tooth rows. Tail stumps in metamorphs are a sensitive site to sample. In live tadpoles the mouthparts can be swabbed for PCR. The prevalence of chytridiomycosis in tadpoles may be high in some populations with long-lived tadpoles, and sampling tadpoles may be a sensitive way of assessing whether *B. dendrobatidis* is in a water body.

A survey protocol that maximises the chance of detection has been devised (Skerratt et al 2008).

References

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2. Oomycosis

Significance: Water moulds (family Saprolegniaceae, phylum Oomycota) are ubiquitous in surface water. The term, saprolegniasis, may be used for infections by multiple genera (e.g., Leptolegnia, Achlya, Aphanomyces). Oomycetes are not true fungi but are in the kingdom Stramenopila. They are principally saprophytes that invade dead or infertile eggs and larval skin wounds, but some species appear to be pathogenic. Secondary infections of aquatic amphibians often are associated with anchorworms (Lernaea sp.), leech bite wounds, other wounds, gangrene of extremities subsequent to gas bubble disease, and ranaviral skin ulcers.

High levels of infection with *Saprolegnia ferax* caused mortality of Western toad (*Bufo boreas*) egg masses in north western United States and were sufficient to affect local populations. May be associated with fish stocking or environmental cofactors.

Two epidemics of *Aphanomyce*s sp. in cane toad tadpoles in Queensland have been described with no associated underlying disease. Tufts of hyphal fungi attached to the nostrils, mouthparts, other parts of the head, and occasionally to the hind legs and tail.

Studies on the effects of silt and used motor oil on the growth and mortality of larval mole salamanders (*Ambystoma* spp.) found that exposed animals became infected with *Saprolegnia parasitica*.

Gross Pathology: White, pale tan, or pale grey fuzzy cottony growths from the skin or egg capsule. Infection may be focal or generalised. Best observed in the immersed specimen, since filaments collapse out of water and become very difficult to detect lying on the skin.

Microscopic Pathology: Filaments of most water moulds are aseptate, sparingly branching and occasionally stain poorly. Epidermal erosions and ulcers often are present. Extent of inflammation varies from negligible to moderate infiltrates of mononuclear cells in the dermis. Deeper invasion into dermis and muscles is variable.

In the cane toad tadpoles, hyphae did not invade past the dermis which was focally distended and inflamed. The epidermis was ulcerated where the mycelium attached.

Diagnosis: Living material is needed for isolations. Hyphal strands from infected eggs or tadpoles are placed on nutrient agar in culture plates. To culture oomycetes so that taxonomic features may be observed, split, boiled hemp seeds are placed around the edge of colonies on nutrient agar. Hyphae grow into the seeds, which are then placed into sterile, distilled water where asexual and sexual reproductive stages develop that allow the oomycete to be identified. However even when all the pertinent features can be seen, identifying species by following keys is often difficult.

References

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3. **Mucormycosis (*Mucor amphibiorum*)**

Significance: Widely distributed zygomycete in Australia. Occasional chronic cases were found in 0.7% cane toads and in Green tree frogs (*L. caerulea*), White-lipped tree frogs (*Litoria infrafrenata*) and Striped marsh frog (*Limnodynastes peronii*) in the wild in Qld, NT and NSW. Mucormycoses has also caused sub acute outbreaks in captivity in slender tree frogs (*Litoria adelensis*) (80% mortality rate), green tree frogs and dendrobatid frogs in Australia and Germany. It was first reported as a cause of death in captive anurans in Germany and the original source in the collection may have been specimens of *L. caerulea* from Australia. Has not been found in wild amphibians outside Australia. *M.*

amphibiorum is also a pathogen of free-living platypus in Tasmania, although isolates from frogs and amphibians differ genetically. The fungus can be isolated from soil and faeces of infected toads and may primarily be a saprobe. Ingestion may be a common route of transmission,

Clinical Signs and Gross Pathology: Signs include lethargy, emaciation, dehydration and occasional large cutaneous papules. However 74% of infected toads were found without clinical signs. Liver contains small pale nodules, up to about 5 mm in diameter, usually in massive numbers. These nodules can also be seen in other organs such as the kidney, lung, mesentery, urinary bladder, subcutaneous sinuses and skin.

Microscopic Pathology: Fungal sphaerules incite granuloma formation in most organs. In the amphibian host the fungus is a distinctive yeast-like spherical structure, called a sphaerule. *M. amphibiorum* forms daughter sphaerules inside the mother sphaerule, and these can be seen in histological sections or on wet mounts of infected tissue. Sphaerules range in size from 5 to 36 microns. In toads encapsulated granulomas contained multinucleate giant cells, macrophages, lymphocytes and eosinophils, and a less common lesion was more suppurative. In the captive *L. adelensis*, all frogs had more acute necrotising granulomas.

Mild, localised infections are found as incidental findings.

Diagnosis: Observation of sphaerules in sections is usually sufficient for diagnosis. PAS and other fungal stains may be useful. *M. amphibiorum* is dimorphic and in culture or soil it forms hyphae. It can be cultured on Saboraud's agar where it forms a mycelium. Two mating types exist, and when these meet they form resistant structures called zygospores

References

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4. Chromomycosis

Significance: Chromomycosis refers to infection by a range of pigmented, septate fungi from the phylum Ascomycota. Pigmented fungi, including *Fonsecaea pedrosi*, *F. dermatitidis*, *Cladosporium* sp. *Scolecobasidium* sp. and *Phialophora* sp., have been isolated from lesions in a range of captive and wild frogs. These organisms have also been isolated from tanks housing captive frogs. Transmission experiments had variable results and poor husbandry appears to be a cofactor.

Clinical Signs and Gross Pathology: Clinical signs are of chronic debilitating disease, and papules and ulceration may occur. Frogs died 1-6 months after first showing signs of infection. Multiple grey nodules occurred in liver, kidney, heart, lung, skeletal muscle, meninges, bone marrow and other organs.

Microscopic Pathology: Nodules are fibrous granulomas with mononuclear cells, epithelioid cells and multinucleate giant cells around pigmented, septate fungi or spherical chlamydospores.

References
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DISEASES CAUSED BY MESOMYCETOZOA & ALVEOLATES

Mesomycetozoa are a newly erected clade of protistan organisms, principally pathogens of shellfish, fish and amphibians. Alveolates include the newly recognised but unnamed pathogen of larval amphibians called a *Perkinsus*-like organism.

1. *Ichthyophonus* sp.

Significance: Usually an incidental finding in tadpoles, frogs and some aquatic salamanders but may cause morbidity and mortality in red-spotted newts, and rarely in bullfrogs, green frogs and wood frogs. Relationship of the amphibian pathogen to those found in marine fish is uncertain. Occasionally mis-identified as *Histocystidium*, *Ichthyophthirius* and adiaspiromycosis. Life cycle and infective stage are unknown. In amphibians, organism is known only from states and provinces bordering the Atlantic Coast and Great Lakes (and West Virginia & Vermont).

Clinical signs and Gross Pathology: Five forms of infection are reported.

a. Newts have subtle to moderate swellings of the dorsal axial, rump, thigh and tail muscles; overlying skin usually is intact.
b. Recently metamorphosed ranid frogs may present with mild to marked swelling of the rump (around urostyle).
c. Adult frogs with massive infections usually are lethargic and emaciated. On cut surface, skeletal muscle is tannish-brown in subacute and chronic infections.
d. Clinically silent infections are common and mild, probably incidental, infections are occasionally detected histologically.
e. Massive acute lethal infections with numerous mortalities occur infrequently in ranid larvae (D. Green & M. Gahl, unpubl. data)

Microscopic Pathology: Organisms are rhabdomyotropic and initially intracellular. Pre-myocytic stages have not been described in amphibians. Organism is weakly basophilic with lacy cytoplasm and numerous uniformly dispersed minute eosinophilic micro-nuclei. A prominent hyaline wall is present with an artefactual clear space separating wall from cytoplasm. Organisms are circular in cross section but may be ovoid to elongate and up to 700 microns long. Initial intracellular infections lack inflammatory cells but subacute and chronic infections elicit intense granulomatous inflammation. Both forms (no inflammation and intense granulomatous myositis) often are present in an animal.

References
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2. *Amphibiothecum* (formerly Dermosporidium) *penneri*; *Amphibiocystidium* spp. in European amphibians

Significance: A sporadic dermal infection of adult toads in Europe and America. Infection develops in autumn and matures during hibernation; toads emerge from hibernation with pustules and granulomas filled with spherical microscopic spores. Spores are discharged during spring breeding and then pustules are mostly healed by mid-summer. Route of infection, infective stage and intermediate hosts are unknown.

Gross Pathology: Pustules occur principally in ventral skin and around vent, are 1 to 5 mm diameter and often are coalescing. Overlying epidermis is ulcerated (umbilicated) and the caseous discharge is white, pale grey or light tannish-yellow; discharge consists entirely of spores. Within the dermis, the nodules consist of serpentine, entwined white tubules that occasionally are present in the lymphatic sacs.

Microscopic Pathology: Encapsulated organisms may elicit slight to extensive granulomatous inflammation. An indistinct or thin wall is present and organisms at the periphery usually are loosely packed, polygonal and separated by thin indistinct septae into small packets. Centrally, organisms are more mature, individualized, spherical and 8-15 microns diameter.

References

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Jay JM, Pohley WJ. 1981. *Dermosporidium penneri* sp. n. from the skin of the American toad, *Bufo americanus* (Amphibia: Bufonidae). J Parasitol 67: 108-110.

3. *Perkinsus*-like organism

Significance: A major cause of mortality events in amphibians in the US. Occurs predominantly in ranid larvae (*Rana* spp.) and may cause mortality rates of 80-99% in a pond over the course of 2-6 weeks. Like "dermo" in oysters, the amphibian pathogen may be temperature dependent. Route of transmission and method of infection are unknown, but cannibalism among tadpoles may be a factor. Very thick walls of the organism suggest the spores probably are resistant to drying. Predominantly occurs in Atlantic and Gulf Coast States with pockets of infected populations in Minnesota, Oregon and Alaska.

Clinical Signs and Gross Pathology: Weakly swimming, bloated and floating tadpoles are found. Tadpoles dead/dying of this infection consistently have very distended abdomen. Abdominal distension is due to massively enlarged (2 to 10x normal size) pale (white or light yellow) liver. Spleen, pronephroi and mesonephroi also are pale and exceptionally enlarged. White paintbrush streaking of intestinal wall may be seen. In acute (early) infections, only mild hepatosplenomegaly may be detected.

Microscopic Pathology: In early infections, liver has clumps of 5-8 microns diameter spherical basophilic organisms, probably associated with initial infection of macrophages in PMAs. In advanced lethal infections, liver is completely replaced by solid masses of organisms; it is difficult to identify the organ or find normal hepatocytes. In pronephroi and mesonephroi, organisms initially are multifocal clusters in interstitium and then become diffuse and replace most tubules. Tiny (0.5 to 2 microns diameter) eosinophilic spherical pre-spores may be detected within hepatocellular cytoplasm in early stages of infection.

References

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PROTOZOAL DISEASES

1. Trypanosomiasis

Significance: Over 60 species have been reported in anurans but the taxonomy is confused. Most infections are non-pathogenic. Trypanosomes are common in frogs from Queensland, but none has been associated with disease. Transmission is by the bite of leeches.

Gross Pathology: Experimental infections of European green frogs with *Trypanosoma inopinatum* caused haemorrhages, swollen lymph glands and anaemia. *T. rotatorium* can be pathogenic in tadpoles or in heavy infections, with trypanosomes accumulating in the kidneys.

Microscopic Pathology: Typical trypanosomes on stained blood smear slides or tissue squash-smears. Live organisms are readily detected in a fresh whole-mount unstained drop of blood.

References

Bardsley JE, Harmsen R.1973. The Trypanosomes of Anura. Adv Parasit 11: 1-73.

Delvinquier BLJ, Freeland WJ. 1989. On some trypanosomes of the Australian anura. Proc Roy Soc Qld 100: 79-87.

2. Coccidiosis

Significance: Fairly common but usually innocuous infection of larvae and adult amphibians in North America and in Australian and African tadpoles. May cause disease in tadpoles - reduced growth (runting) and thickened intestinal wall have been observed in one free-living population of leopard frogs (*Rana pipiens*). Australian parasites are likely to be *Goussia* spp.

Gross Pathology: In heavy infections only, runting and thickened intestinal wall observed in free-living tadpoles.

Microscopic Pathology: Slight to massive numbers of oocysts are present in enteric epithelium; inflammation is variable. Infections in tadpoles expire at metamorphosis.

References

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Paperna I, Ogara W, Schein M. 1997. *Goussia hyperolisi* n. sp.: a coccidian infection in reed frog *Hyperolis viridiflavus* tadpoles which expires towards metamorphosis. Dis Aquat Org 31: 79-88.

Upton SJ, McAllister CT. 1988. The coccidia (Apicomplexa: Eimeridae) of Anura, with descriptions of four new species. J Parasitol 56: 572-574.

3. Amoebiasis

Significance: An infrequent entero-invasive infection of amphibians in zoos and low elevation neotropical locations. Subclinical non-invasive infections may be more common. Valentine and Stoskopf (1984) reported amoebic mesonephritis in one captive giant (cane) toad (*Bufo marinus*) but failed to find intestinal infection. Multiple free-living Cuban treefrogs (*Osteopilus septentrionalis*) and two giant toads from 2 Caribbean Islands had mild entero-invasive amoebae in their cloacas (D Green & P Burrowes, unpub.).

References

Valentine BA, Stoskopf MK. 1984. Amoebiasis in a neotropical toad. J Am Vet Med Assoc 185: 1418-1419.

ECTOPARASITES

Trichodina & Epistylis

Significance: In the US, occasional larval amphibian populations may have slight to massive numbers of these innocuous protozoa. Trichodina-like protozoa are free-living and glide across the surface of skin, gills, gill chambers, and occasionally into the vent (cloaca) and urinary bladder. Epistylis-like protozoa are tulip-like in shape and attach to the surface epidermis by a thin stalk; oral disc, gills, ventral skin, ventral fin around vent opening and toe-tips are frequent sites of attachment.

Gross Pathology: Organisms are most easily detected in the anesthetized, submerged amphibian.

Microscopic Pathology: *Trichodina* spp. have characteristic pinwheel rotors and are distinctly hemispherical in shape. Epistylis-like protozoa are basophilic and pleomorphic in tissue sections and commonly found in/on the oral disc, pharynx, gill chamber, ventral skin and skin of tail; attachment stalks are seen rarely in fortuitous sections.

MYXOZOAN & MICROSPORIDIAL DISEASES

1. *Myxidium* spp.

Significance: Two species of *Myxidium* have been identified in Australian frogs as emerging pathogens. The earliest case detected is from 1966. Brain and liver genotypes occur in tadpoles and adults of native frogs (including green and golden bell frogs) and cane toads. The lifecycles are unknown but may involve invertebrate hosts.

Clinical Signs and Gross Pathology: Infection may result in lethargy and emaciation. The brain genotype can cause circling and paralysis. Within the gall bladder large trophozoites (1-5 mm) may present as white flocculent material.

Microscopic Pathology: Large trophozoites containing myxospores occur in lumen of gall bladder. Stages may be found in liver adjacent to or within bile ducts. In Australia moderate to severe biliary duct proliferation, lymphoplasmacytic hepatitis and periportal fibrosis occurs.

The brain genotype also infects CNS and root ganglia with extrasporogonic developmental stages occurring within myelinated axons. CNS stages are 5-25 µm in diameter and consist of numerous small cells within a primary cell wall. Infection may cause multifocal non-suppurative meningoencephalitis in the CNS. Light infections not associated with lesions have also been observed.

References

Delvinquier BLJ, Freeland WJ. 1988. Protozoan parasites of the cane toad, *Bufo marinus*, in Australia. Aust J Zool 36: 301-316.

Hartigan A, Fiala I, Dyková I, Jirku M, Okimoto B, Rose K, Phalen DN, Šlapeta J. 2011. A suspected parasite spill-back of two novel *Myxidium* spp. (Myxosporea) causing disease in Australian endemic frogs found in the invasive Cane toad. PLoS ONE, e18871.

Hill BD, Green PE, Lucke HA. 1997. Hepatitis in the green tree frog (*Litoria caerulea*) associated with infection by a species of *Myxidium*. Aust Vet J 75: 910-911.

2. *Myxobolus hylae*

Significance: *Myxobolus hylae* was found in the reproductive organs of green and golden bell frogs (*L. aurea*) from Sydney and in green tree frogs (*L. caerulea*), a striped marsh frog (*Lit. peronii*), and a stony creek frog (*L. lesueuri*).

Clinical Signs and Gross Pathology: Infected *L. aurea* appeared sickly and emaciated. The testes and vasa efferentia were infected in males and the oviducts were infected in females. In cases of heavy infection, the whole testis was swollen and covered with white cysts up to 2-3 mm composed of myriads of spores.

Microscopic Pathology: Infection results in partial replacement of the gonads by large encapsulated cysts. Degenerate cysts are infiltrated with granulomatous inflammation.

References

Berger L. 2001. Diseases in Australian Frogs. PhD Thesis, James Cook University, Townsville, Australia.

Johnston TH, Bancroft MJ. 1918. A parasite, *Myxobolus hylae* sp. nov. of the reproductive organs of the golden swamp frog, *Hyla aurea*. Aust Zool 1: 170-175.

3. *Sphaerospora* (formerly Leptotheca) *ohlmacheri* (Myxozoan mesonephropathy)
Significance: In the US, fairly common but innocuous myxozoan infection of only mesonephroi in larval and post-metamorphic frogs and toads; occasionally seen in aquatic salamanders. Infective stage, route of infection and intermediate host(s) are unknown.

Gross Pathology: None.

Microscopic Pathology: Poorly-stained cellular-like debris within renal tubules (H&E) usually is this organism. In heavy infections, tubules are mildly to moderately dilated and partially filled with myxozoan spores. Plasmodia are very small. Giemsa stain demonstrates most myxozoan organisms, especially spores.

Reference
Eiras JC. 2005. An overview of the myxosporean parasites in amphibians and reptiles. Acta Parasitologica 50: 267-275.

4. *Alloglugea* sp. (Xenomas)
Significance: Alloglugea is a xenoma-producing microsporidia; described from cane toad tadpoles in the neotropics. Similar infections have been seen in anuran larvae in California. Some infections may resolve during metamorphosis.

Gross Pathology: Small (~1 mm) roughly spherical nodules in the intestine and occasionally other organs.

Microscopic Pathology: Typical rod-shaped microsporidial spores within cytoplasm. Inflammation may be absent in early infections or granulomatous in subacute and chronic infections.

References
Paperna I, Lainson R. 1995. *Alloglugea bufonis* nov. gen., nov. sp. (Microsporea: Glugeidae), a microsporidian of *Bufo marinus* tadpoles and metamorphosing toads (Amphibia: Anura) from Amazonian Brazil. Dis Aquat Org 23: 7-16.

5. *Pleistosphora* spp. (Microsporidian myositis)
Significance: Sporadic infection of tadpoles and adult anurans. Intense muscular infections may cause death. *Pleistophora myotrophica* caused high mortality rates in captive *Bufo bufo* and experimental infections were achieved by feeding toads infected muscle. Hepatic infections described from captive juveniles in Australia.

A captive, wild-caught *Phyllomedusa bicolor* was successfully treated for an ulcerative dermatitis that was associated with a variety of infective agents including microsporidia.

Clinical Signs and Gross Pathology: Lethargy, atrophy and emaciation. Inapparent or mild to intense white streaking of skeletal muscles.

Microscopic Pathology: Intracytoplasmic organisms within indistinct parasitophorous vacuoles; inflammation may be absent or mildly lymphohistiocytic. Muscle regeneration may occur with long chains of sarcoblasts adjacent to damaged muscle.

References
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Graczyk TK, Cranfield, MR, Bicknese EJ, Wisnieski AP. 1996. Progressive ulcerative dermatitis in a captive, wild-caught South American giant tree frog (*Phyllomedusa bicolor*) with microsporidial septicaemia. J Zoo Wildl Med 27: 522-527.

METAZOAN DISEASES

Trematodes & Monogenes

1. *Ribeiroia ondatrae* (metacercariae)
Significance: In the US infections by metacercariae of *Ribeiroia* cause 2 major problems: a variety of limb malformations (amelia, ectromelia, polymelia, polydactyly, etc) and acute death in young tadpoles. As few as 4 cercariae burrowing into skin of a tadpole may be lethal at young stages, whereas older tadpoles may acquire 100's of encysted metacercariae without ill affect. Experimental studies show tadpoles must be at specific stages of development (Gosner stages 26-28) for hind limb malformations to occur.

Gross Pathology: As early as Gosner stage 30, extra hind limb buds may be induced by encysted metacercaria. By metamorphosis, malformations of hind limbs may include amelia, polymelia, polydactyly, ectromelia, brachyphalangy, taumelia (folded long bones with bony triangles), and skin folds (across stifle or ankle). Encysted metacercariae in larvae usually are found in dermis at 1) base of hind limbs, 2) proximal ventral fin, 3) inguinal area, and 4) around vent tube. In post-metamorphic amphibians, encysted metacercariae (usually <0.2 mm diameter) occur in same pelvic area and in ventral dermis from tip of chin to vent, and in "subcutis" around urostyle.

Microscopic Pathology: Other than metacercarial size and location in host, the metacercariae have no known characteristic features to distinguish them from many other genera. Inflammatory reaction to encysted metacercariae is negligible.

References
Johnson PT, Lunde KB, Haight RW, Bowerman J, Blaustein AR. 2001*. Ribeiroia ondatrae* (Trematode: Digenea) infections induces severe limb malformations in western toads (*Bufo boreas*). Can J Zool 79: 370-379.

2. *Clinostomum* spp. (metacercariae)
Significance: An occasional cause of mild to severe skin lumps in tadpoles, frogs and salamanders in the US. Mortality not reported, even in heavy infections. As with all metacercarial & mesocercarial infections, the presence of the immature trematodes indicates a final host defecated in the pond and aquatic snails (1^{st} intermediate hosts) also are present in pond.

Gross Pathology: Encysted metacercariae are 1 per cyst, and are the largest North American metacercariae yet encountered. Each cyst is 1 to 3 mm diameter and often slightly yellowish; acutely encysted metacercariae may have blood in the cyst fluid. Excysted live metacercariae are 2 to 10 mm long.

Microscopic Pathology: Size of encysted metacercariae is best feature for identification; no characteristic histological features for the genus. Inflammatory reaction to cyst walls is negligible but may develop months after initial infection.

3. *Echinostoma* spp. (metacercariae)
Significance: Very common, minute, metacercariae of mesonephroi of larvae, frogs, toads and aquatic salamanders. Intensity of infection varies from <10 to >500. Metacercariae are present in glomeruli, tubules and interstitium. Clinical disease, even in heavy infections, is poorly described; usually considered innocuous & incidental.

Gross Pathology: Usually 0.05 to 0.2 mm diameter; requires dissecting microscope to detect at necropsy.

Microscopic Pathology: Initial infections lack inflammatory response and may have indistinct cyst wall. Dead metacercariae elicit caseo-granuomatous inflammation. Metacercariae have characteristic intensely eosinophilic spines on tegument.

4. ***Fibricola*** **sp. (metacercaria)**
Significance: Occur over the skin of tadpoles of various frog species in Australia. Can occur at high prevalence in a location. Adult stages may occur in the allied rat and the water rat, and the limpet (*Pettancylus assimilis*) can act as the first intermediate host.

Gross Pathology: Many smooth, round raised orange lumps < 1 mm occur over the tail, and dorsal and lateral body.

Microscopic Pathology: Cysts occur in the myxomatous tissue in the subcutis over the body and tail and muscles adjacent to the notochord. Cysts surrounding the larvae have rings of fibroblasts and fibrocytes with almost no inflammatory cells. There are occasional foci of mixed inflammation in the myxomatous tissue assumed to be the remains of degenerate cysts.

References
Barton DP. 1994. A checklist of helminth parasites of Australian amphibia. Records of the South Australian Museum 27: 13-30.

Berger L. 2001. Diseases in Australian Frogs. PhD Thesis, James Cook University, Townsville, Australia.

5. Lung flukes (*Haematoloechus* spp.)
Significance: Widely distributed adult parasite of post-metamorphic anurans in the US. Amphibians are final hosts; aquatic snails are first intermediate hosts, and aquatic insects (especially dragonfly naiads) are 2nd intermediate hosts; requires ingestion of insect by amphibian. Usually incidental intraluminal pulmonary infection, but occasionally associated with pleural cysts.

Gross Pathology: Adult flukes usually are present in central lumen of lungs or between caviae. Flukes often have striking tri-colour: orangish-tan, white and black.

Microscopic Pathology: In chronic infections, eosinophilic interstitial pneumonitis is disseminated or diffuse.

Nematodes
6. Lungworms (*Rhabdias* spp.)
Significance: These rhabditoid lung worms are common in amphibians worldwide. Usually an incidental finding and only a small proportion of wild amphibians have heavy burdens. In Australia *R. hylae* is the most widespread species. *Rhabdias pseudosphaerocephala* occurs in cane toads in Australia and infection results in reduced growth rates. It is presumed to have been introduced to Australia with toads. Rhabdiasis is a potentially serious infection of captive amphibians because the parasite is parthenogenic and has a direct lifecycle. Hazard is from recently hatched larvae that actively burrow into skin of amphibians; large numbers of larvae may be fatal. Because of this parasite, prophylactic testing or anthelmintic treatments of newly arrived amphibians in captive colonies is warranted.

Gross Pathology: Adult nematodes are present in central lumen of lungs; individuals usually are black and white striped longitudinally. Tiny white larvae may be detected under dissecting microscope in body cavity.

Microscopic Pathology: Adults may elicit an eosinophilic interstitial pneumonitis in septae of caviae and pleura. Larvated ovoid eggs may be present in lumina of lung and GIT. Larvae may be detected as tiny, basophilic, 1-3 micron diameter, non-encysted nematodes in nearly any organ but especially dermis, intestinal villi and free in body cavity. Aberrant migrating larvae incite granuloma formation that may affect the host. The small larvae may be confused with fungal hyphae.

References
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Kelehear C, Brown GP, Shine R. 2011. Influence of lung parasites on the growth rates of free-ranging and captive adult cane toads. Oecologia 165: 585-592.

Reichenbach-Klinke H, Elkan E. 1965. Amphibia. In: The Principal Diseases of Lower Vertebrates. Academic Press, New York. Pp 209-384.

Tinsley RC. 1995. Parasitic disease in amphibians: control by the regulation of worm burdens. Parasitology. 111 Suppl: 153-178.

7. Skin worm (*Pseudocapillaroides xenopi*)
Significance: This intra-epidermal capillarid parasite occurs in captive colonies of African clawed frogs (*Xenopus laevis*). Parasite has a direct life-cycle, hence intensity of infections often steadily increase in colonies. May be associated with morbidity and mortality in captive adult clawed frogs, especially in husbandry situations where aquariums are not changed and disinfected regularly.

Gross Pathology: Reddened roughened thickened frayed skin with irregular patchy moulting occurs in heavy infections; epidermal erythema and ulcers may occur; mild infections may be clinically silent. Infects dorsal body skin.

Microscopic Pathology: The adult parasite occurs in epidermis and leaves burrow holes. Eggs are typically capillarid and embryonated (ovoid, brown with bipolar plugs). Inflammatory response is minimal but secondary bacterial and fungal infections may elicit intense dermal and epidermal mixed inflammatory cells. Worms and eggs seen on skin scrapings.

Reference
Cunningham AA, Sainsbury AW, Cooper JE. 1996. Diagnosis and treatment of a parasitic dermatitis in a laboratory colony of African clawed frogs (*Xenopus laevis*). Vet Rec 138: 640-642.

8. Filarioids
Significance: *Foleyella* spp. can cause death due to heavy infections with microfilaria or adult worms

Reference
Reichenbach-Klinke H, Elkan E. 1965. Amphibia. In: The Principal Diseases of Lower Vertebrates. Academic Press, New York. Pp 209-384.

9. Nasal nematodes (*Koerneria* sp. and *Rhabditis* sp.)
Significance: Nematodes (*Koerneria* sp. and *Rhabditis* sp.) were found in four captive Archey's frogs (*Leiopelma archeyi*) in New Zealand with hemorrhagic purulent nasal discharge and weight loss. Three frogs died before treatment could be commenced. One frog also had a ciliate infection (Tetrahymena) in the nasal cavity and was treated successfully with oral moxidectin at 0.4 mg/kg for the nematodes and topical metronidazole at 10 mg/kg for the protozoa.

Reference
Shaw S, Speare R, Lynn DH, Yeates G, Zhao Zeng, Berger L, Jakob-Hoff R. 2011. Nematode and ciliate nasal infection in captive Archey's frogs (*Leiopelma archeyi*). J Zoo Wildl Med 42: 473-479.

Cestodes
8. Sparganosis (*Spirometra erinacei)*
Significance: The adult stage of the cestode *Spirometra erinacei* inhabits the small intestine of carnivores such as the cat, dog, fox and dingo. The procercoid stage occurs in copepods and the plerocercoid stage (spargana) is found in amphibians, reptiles and mammals that ingest infected copepods or other vertebrates infected with spargana. *Spirometra erinacei* was probably introduced recently to Australia given that eutherian carnivores are the definitive hosts. *Spirometra erinacei* is also present in south-east Asia and is a public health problem, usually occurring as subcutaneous or intramuscular sparganosis in humans. Light infections are found in healthy frogs, including *Litoria* and *Limnodynastes* spp. Of 1000 *B. marinus* surveyed from Queensland, 3.7% were infected. However, heavy burdens e.g. >16 spargana appear to cause emaciation and death, particularly in White-lipped tree frogs *(L. infrafrenata*) from far north Queensland where severe, irreversible emaciation is a

common finding. Spargana were found in 12/243 (4.9%) sick wild frogs from southern New South Wales and northern Queensland from populated areas.

Gross Pathology: Frogs often in poor body condition or are emaciated with subcutaneous lumps over thighs and caudal belly. Spargana appeared as white flattened flexible structures with transverse wrinkles and are about 3 x 50mm. Infections occur in skeletal muscle and subcutis, predominantly of the thighs. With heavy burdens (e.g. 16-60 worms) infection may be occur within the coelomic cavity.

M**icroscopic Pathology:** Plerocercoids have features typical of cestodes including calcareous corpuscles, a thick cuticle and lacking a digestive tract. Spargana may be free or encapsulated within thin, membranous capsules or thicker fibrotic capsules. Usually negligible cellular reaction, though granulomatous inflammation can occur within capsules and in interstitial areas of muscle. Coagulative necrosis of individual muscle fibres or more extensive myonecrosis occurs. Concurrent infections are common.

References

Barton DP. 1994. A checklist of helminth parasites of Australian amphibia. Records of the South Australian Museum 27: 13-30.

Bennett LJ. 1978. The immunological responses of amphibia to Australian spargana. J Parasitol 64: 756-759.

Berger L, Skerratt LF, Zhu XQ, Young S, Speare R. 2009. Severe sparganosis in Australian tree frogs. J Wildl Dis 45: 921-929.

Young S, Skerratt LF, Mendez D, Speare R, Berger L, Steele M. 2012. Community surveillance for diseases of the white-lipped tree frog (*Litoria infrafrenata*) in Northern Australia. Dis Aquat Org (accepted)

Leeches

9. Leeches

Significance: The genera*, Batrachobdella, Macrobdella and Oligobdella*, are most often seen on amphibians. Two *Batrachobdella* sp. on a 1-5 g tadpole may cause lethal blood loss.

Gross Pathology: Many leeches leave a roughly circular hemorrhagic skin ulcer that is 1-6 mm diameter. Leeches may attach anywhere on head, body and tail. Bite wounds may become secondarily infected by bacteria and water moulds.

References

Boltz RS. 1997. The impact of the hematophorous leech *Batrachobdella picta* on wood frog (*Rana sylvatica*) larvae, or another reason wood frog tadpoles croak. Master of Science Thesis, Oakland University, Rochester, MI.

Copepods

10. Anchorworms (*Lernaea* sp.).

Significance: Infrequently encountered in the US in tadpoles that require permanent bodies of water (e.g. larval bullfrogs & green frogs).

Gross Pathology: Adult burrowing female anchorworms typically have exposed tail and pair of egg sacs. Most common site of infection is junction of body and tail, but also oral disc, gill chamber and body. Saprolegniasis occasionally develops at burrow hole of anchorworms and may obscure presence of the copepod's tail and egg sacs.

Microscopic Pathology: Inflammatory reaction to embedded portion of anchorworm is mild to intense. Composition of inflammatory cells varies greatly depending on presence or absence of secondary bacterial and fungal infections.

11. Fish lice (*Argulus* spp.)
Significance: Infrequently encountered in the US on the skin of larval amphibians, especially from permanent ponds/lakes with fish. *Argulus* have 2 suction cups and feed by inserting a sting organ which injects digestive enzymes into the body; liquidised body fluids are then sucked out with the proboscis organ. Secondary skin infections occur in fish, but apparently not reported in amphibians.

Gross Pathology: Attachment to epidermis of amphibian and feeding may cause skin erosions or ulcers and subsequent secondary water mould or bacterial infections.

12. Chiggers – larval Trombiculid mites (e.g. *Hannemania* spp.)
Significance: Amphibian chiggers occur in terrestrial stages of amphibians. Unlike chiggers of other vertebrates, amphibian chiggers burrow into the skin, encyst and remain more than 6 months. Mortality is not reported.

In Australia larvae of two mite species (*Vercammenia gloriosa* and *V. zweifelorum*) have been identified in a number of frog species from Queensland. Pathology in the skin of a wild tree frog, *Litoria wilcoxii*, infected with both mite species was described by Mendez et al (2010).

Gross Pathology: Raised orange or reddish-orange nodules (~ 0.5- 4 mm) are present on skin of body, limbs or toe webs. Lancing of cysts reveals orange mites.

Microscopic Pathology: Cysts occur as cavities in the dermis and elicit minimal inflammatory cell reaction with lymphocytes, macrophages and fibrosis.

Reference
Mendez D, Freeman AB, Spratt DM, Speare R. 2010. Pathology of cutaneous trombidiosis caused by larval trombiculid mites in a wild Lesueur's tree frog (*Litoria wilcoxii*). Aust Vet J 88: 328-330

13. Oligochaeta (*Dero* [*Allodera] hylae*)
Significance: Parasitic (or symbiotic) oligochaetes are darkly pigmented, elongate, segmented worm-like organisms within the lumina of the Wolffian ducts ("ureters") of treefrogs (*Hyla* spp. *Litoria* sp. & *Osteopilus* sp.) and rarely toads (*Bufo* sp.) in SE USA and Australia. Morbidity or mortality is not reported, even in heavy infections.

Gross Pathology: Multiple para-renal oligochaetes may cause marked dilation of the Wolffian ducts; in heavy infections, organisms may be present in urinary bladder.

References
Harman WJ, Lawler AR. 1975. *Dero* (*Allodera*) *hylae*, an oligochaete symbiont of hylid frogs in Mississippi. Trans Am Micro Soc 94: 38-42.

Hill BD, Green PE, Lucke HA. 1997. Hepatitis in the green tree frog (*Litoria caerulea*) associated with infection by a species of *Myxidium*. Aust Vet J 75: 910-911.

14. Miasis
Significance: Various fly species from the families Sarcophagidae, Calliphoridae and Chloropidae have larvae that can develop within amphibians. Some maggots stray into body cavity, death of host may occur when maggots emerge to pupate. In Europe the "toad fly" *Bufolucilia bufonivora* lays eggs in the nostrils of toads and the larvae destroy the epithelium and can penetrate deeper into the orbit or brain. Few toads survive an infection. Larvae of *Notochaeta bufonivora* parasitised wild *Atelopus varius* along a stream in Costa Rica. Frogs in early stages of miasis had a single small wound on the posterior surface of one thigh, and all hosts died within four days after they were found. Larvae of *Notochaeta* sp. infected farmed bullfrogs in Brazil. Larvae occurred in the mouth and caused necrotic perforations associated with a range of aerobic and anaerobic bacteria including *Clostridium* spp.

In Australia the genus *Batrachomyia* contains several species that have been found in 11 frog species. They inhabit the dorsal lymph sac with their posterior spiracles in or close to a hole in the frog's skin. When they are ready to pupate they leave the frog and drop to the ground. The number of maggots (1-5) is much less than seen with *Bufolucilia*, suggesting the eggs are not laid directly on

frog skin but are picked up from the soil. Frogs are reported to have survived infection and had little obvious tissue damage, although maggots have been associated with poor body condition, peritoneal perforation and death can result at the time of larval emergence.

Batrachomyia strigapes was detected in 5.1% *Uperoleia laevigata* in Sydney and infection was associated with reduced frog weight.

Gross Pathology: Typical deep skin ulcer with maggot's spiracle usually is present next to a lump in dorsal body, rump or base of a limb. White maggots are found the nasal cavity in infected European amphibians.

References

Baldassi L, Hipolito M, de Souza Junior F, de Souza C. 1995. *Clostridia* presence in bullfrog (*Rana catesbiana* Shaw, 1802) mouth myiasis lesions. B Inst Pesca 22: 95-102.

Bolek MG, Coggins JR. 2002. Observations on myiasis by the Calliphorid, *Bufolucilia silvarum*, in the eastern American toad (*Bufo americanus americanus*) from south-eastern Wisconsin. J Wildl Dis 38: 598-603.

Crump ML, Pounds JA. 1985. Lethal parasitism of an aposematic anuran (*Atelopus varius*) by *Notochaeta bufonivora*) (Diptera: Sarcophigidae). J Parasitol 7: 588-591.

Elkan E. 1965. Miasis in Australian frogs. Annals Trop Med Parasitol 59: 51-54.

Schell CB, Burgin S. 2001. *Batrachomyia strigapes* (Diptera) parasitism of *Uperoleia laevigata* (Anura). J Parasitol 87: 1215-1216

Vogelnest L. 1994. Miasis in a green tree frog (*Litoria caerulea*). Association of Reptile and Amphibian Veterinarians 4: 4.

NEOPLASTIC DISEASES

Compared with mammals, birds and fishes, reports of neoplasia in amphibians are uncommon. This may be due to neoplasms being over-looked and not reported, or alternatively, amphibians may have some resistance to neoplasms. However high prevalences occur with the infectious tumours (i.e. Lucké renal tumour and newt papilloma) where the same tumour occurs in many individuals of the same species, and incidence varies seasonally.

A wide range of tumours of amphibians have been described. In Australia published reports are of a wild adult Green tree frog with an adenocarcinoma and a captive adult White lipped tree frog with a squamous cell carcinoma.

References

Asashima M, Oinuma T, Meyer-Rochow B. 1987. Tumors in Amphibia. Zool Sci 4: 411-425.

Berger L, Speare R, Middleton D. 2004. A squamous cell carcinoma and an adenocarcinoma in Australian treefrogs. Aust Vet J 82: 96-98.

Balls M, Clothier RH. 1974. Spontaneous tumours in Amphibia. Oncology 29: 501-519.

Green DE, Harshbarger JC. 2001. Spontaneous neoplasia in Amphibia. *In*: Wright, K.M., and B.R. Whitaker (Eds). Amphibian Medicine & Captive Husbandry, Krieger Publishing Company, Malabar, Florida. Pp. 335-400.

NUTRITIONAL DISEASES

Nutritional bone disease is common in captivity. Juveniles develop spinal deformities and adults may be tetanic after activity. Other nutritional problems include obesity, impaction, iodine deficiency leading to large tadpoles that fail to metamorphose, and oxalate toxicity causing renal calculi from excess spinach.

References
Hulst F. 1999. Amphibian care and medical management. In: Wildlife Health in Australia, Healthcare and Management. Proceedings 327, Postgraduate Foundation in Veterinary Science, University of Sydney. Pp 119-146.

Raphael BL. 1993. Amphibians. Veterinary Clinics of North America; Small Animal Practice 23: 1271-1286.

SYNDROMES OF UNCERTAIN AETIOLOGY

1. Vacuolating and ulcerative dermatitis

Significance: In Australia amphibians were found active or ill with dermatitis ranging in severity from mild to severe with various lesions. The cause has not been determined and there may be more than one aetiology. Species affected include the red-eyed tree frog (*L. chloris*), cascade tree frog (*L. pearsoniana*), green tree frog (*L. caerulea*) and the cane toad (*B. marinus*).

Gross Pathology: Focal to extensive discolourations, erosions, and ulcers occurred mainly on the dorsal skin of the body and limbs.

Microscopic Pathology: Histological lesions included vacuolation and degeneration of epidermal cells progressing to vesicles and ulcerations, and breakdown of the basement membrane with pigment cells in the epidermis. Chronic lesions with fibrosis of ulcerated tissue were also seen. Some frogs had necrosis and inflammation in dermis, and degeneration or loss of dermal glands. Bacterial infections of skin and internal organs in a few frogs were suspected to be opportunistic infections. No pathogens were found despite special stains, cell culture, ranavirus PCR and electron microscopy. In two frogs, a few chytrids were found on examination of skin scrapings but were not seen on histology. Further work is required to determine the aetiology of this potentially important syndrome and if there is an association with chytridiomycosis.

Reference
Berger L. 2001. Diseases in Australian Frogs. PhD Thesis, James Cook University, Townsville, Australia.

2. Amphibian musculoskeletal malformations

Significance: Since 1995 there has been much attention on amphibian malformations in the US. Metacercarial infection by *Ribeiroia ondatrae* has been shown to be the principal cause of malformations of the skin and bones of amphibian limbs. The parasite induces partial limb atrophy, taumelia or polymelia/polydactyly in late stage tadpoles or post-metamorphic amphibians, but in order to produce malformations, the cercarial/metacercarial infection must occur at a precise, rather young, tadpole age.

Studies on hyperthermia in Japan with captive amphibians suggest bilateral hypoplasia of hind limb digits and distal extremities occur in larval toads that are raised in warm water at or near their maximum thermal tolerance. There is also evidence for malformations caused by ultraviolet light, DDT, heavy metals and other contaminants.

Diagnosis: Prior to metamorphosis, the “skeleton” of larval amphibians consists mostly of cartilage; hence, larvae are unsuitable for standard radiographs. At the completion of metamorphosis, the skeleton is nearly fully ossified, although some exceptionally small recent metamorphs (e.g. toads) may still have cartilaginous digits. Radiography is applicable to fully metamorphosed amphibians. Live amphibians may be radiographed as well as eviscerated fixed carcasses. It is vital that malformed amphibians intended for radiography be carefully fixed with their limbs and digits extended. Clearing-&-Staining is often used to examine vertebrate embryos, but also is useful for examining adult fish, amphibian larvae and adult amphibians. Fixed carcasses are cleared in potassium hydroxide and then two stains are applied which render bones and cartilage visible. This method is preferred to radiography, since cartilaginous structures become visible. Unfortunately, the clear-&-stain technique has been used mostly by biologists.

References

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Muto Y. 1969. Anomalies in the hindlimb skeletons of toad larvae reared at a high temperature. Senten Ijo Congenital Anomalies 9: 61-73.