OTHER DISEASES OF AMPHIBIANS

Amphibians have communicable and non-communicable diseases. The following pages provide information on some of these diseases. Chytridiomycosis, Ranaviruses, and mucormycosis are discussed separately.

Reviews of diseases are available in:

Berger L, Longcore J, Speare R, Hyatt A, Skerratt LF. 2009. Fungal Diseases in Amphibians. In: Amphibian Biology, Volume 8 Amphibian Decline: Disease, Parasites, Maladies, and Pollution. Edited by H Heatwole and JW Wilkinson, Surrey Beatty & Sons, NSW. Pp. 2986-3052. http://eprints.jcu.edu.au/11302/

Berger L and Green DE 2012 Amphibian Diseases. In Wildlife Pathology Short Course, Camden, Sydney. Pp 26-48. TCSA http://theregistry.org.au/amphibian-dz-homepage

Hadfield CA, Whitaker BR. 2005. Amphibian Emergency Medicine and Care. Seminars in Avian and Exotic Pet Medicine, 14(2): 79-89.

Hemingway V, Brunner J, Speare R, Berger L. 2009. Viral and Bacterial Diseases in Amphibians. In: Amphibian Biology, Volume 8 Amphibian Decline: Disease, Parasites, Maladies, and Pollution. Edited by H Heatwole and JW Wilkinson, Surrey Beatty & Sons, NSW. Pp. 2969-2986 http://eprints.jcu.edu.au/11279/

Pessier AP. 2003. An overview of amphibian skin disease. Seminars in Avian and Exotic Pet Medicine. 11: 162–174.

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CONTENTS

VIRAL DISEASES OF AMPHIBIANS

At least 6 groups of viruses other than ranaviruses have been reported from amphibians (Table 1). These viruses can cause diseases in amphibians, but their impact on wild populations has not been well studied apart from Frog Erythrocytic Virus (FEV) in Canada.

Table 1: Viruses, other than ranaviruses, reported from amphibians. Amphibians were free-ranging unless "captive" indicated.

Virus / virus group	**Amphibian host**	**Location**	**Effect on amphibians**	**Reference**
Guatapo virus 6 (GV6)	Cane toad *Bufo marinus*	Venezuela	Necrosis of haematopoeitic tissue	Hyatt et al 2000
Amphibian erythrocytic viruses	North American bullfrog *Rana catesbiana* Green frog *R. clamitans* *R. septentrionalis*	Canada	Anaemia; reduced survival	Gruia-Grey et al 1992
	Northern leopard frog *R. pipiens*	USA	Unknown	Bernard et al 1969
	Leptodactylus ocellatus	Brazil	Unknown	de Sousa & Weigl 1976
	Phrynohyas venulosa	Brazil	Unknown	de Matos et al 1995

	Cane toad (*Bufo marinus*)	Costa Rica	Unknown	Speare et al 1991
	Ptychadena anchieta	South Africa	Unknown	Alves de Matos & Paperna 1993
	Bufo gargarizans	China	Unknown	Werner 1993
	R. boulengeri	China	Unknown	Werner 1993
	R. nigromaculata	China	Unknown	Werner 1993
Amphibian leucocyte virus	*R. catesbiana*	Europe (captive source Mexico)	Lethargy, skin ulceration	Briggs & Burton 1973
Lucké tumor herpesvirus	*Rana pipiens*	USA	Lucké renal adenocarcinoma	Lucké 1938; McKinnell & Carlson 1979
Calicivirus	*Ceratophrys orata*	USA (captive)	Pneumonia, death	Smith et al 1986
Herpesvirus-like particles in skin	*Rana dalmatina*	Europe	Epidermal vesicles	Bennati et al 1994
Flaviviruses				
Sindbis virus	*Rana ridibunda*	Europe	Unknown	Kozuch et al 1978
West Nile virus	*Rana ridibunda*	Tadzhikistan	Unknown	Kostiukov et al 1985; 1986

FROG ERYTHROCYTIC VIRUS

FEV was discovered in wild populations of *Rana* spp. in Algonquin Park, Ontario Canada (Gruia-Grey et al 1989; Gruia-Grey and Desser 1992). Key details are:

1. FEV is a member of the viral family Iridoviridae.
2. FEV is a large (diameter up to 450 nm in diameter), enveloped, double strand DNA containing iridovirus of uncertain classification within the Iridovidae.
3. FEV is present in red blood cells.

4. FEV is transmitted between frogs by mosquitoes or midges, and is not transmitted by water, orally or by leeches.
5. Infection with FEV results in red blood cells changing shape from oval to spheroidal, and infected frogs can become anaemic.
6. Infection is more common in juveniles than adults.
7. Infection appears to contribute to mortality of juvenile frogs with more infected juveniles than FEV-free frogs disappearing from the population structure.
8. FEV has been reported only in Canada although similar large viruses have been discovered in red blood cells of amphibians in Costa Rica, Brazil, South Africa and USA (Table 2).

LUCKÉ TUMOR HERPESVIRUS

Lucké tumour herpesvirus (LTHV) has been reported only from the northern leopard frog, *Rana pipiens*, in USA (McKinnell and Carlson 1997). Recently LTHV has been referred to as Rana herpesvirus 1 (RaHV-1) (Davison et al 1999).

1. RaHV-1 is a member of the viral family Herpesviridae.
2. Genomic studies indicated that RaHV-1 belongs to the fish virus lineage of the herpesvirus family rather than to the lineage populated by mammalian and avian viruses (Davison et al 1999).
3. RaHV-1 induces renal adenocarcinoma in *R. pipiens* in USA.
4. The disease was described in 1934 (Lucké 1934) and its transmissible nature recognised in 1938 (Lucké 1938).
5. Clinical signs are bloating, lethargy and death, which occur when the tumour is large or has metastasised (Anver and Pond 1984). Single or multiple white nodules occur in the kidneys and grow into large masses. The tumour is an infiltrating and destructive adenocarcinoma, or less often it is more orderly and adenomatous (Lucké 1934).
6. Although the gross appearance of the tumour remains relatively unchanged, there are significant seasonal differences in the microscopic appearance. Winter tumours display cytopathic characteristics associated with the presence of virus (enlarged nuclei with eosinophilic inclusions) whereas summer tumours lack virus (McKinnell 1973).
7. Metastasis of the cancer depends on temperature with metastasis more common with higher temperatures (29°C vs 4°C).
8. Studies have shown that above 22°C virus replication does not occur and viral particles are not present in the tumour (Anver and Pond 1984).
9. Surveys of wild *R. pipiens* for the Lucké tumour have found prevalences up to 12.5% (McKinnell 1969). However, since 1977 the prevalence of Lucke renal adenocarcinoma appears to have declined in Minessota (McKinnell et al 1979). In retrospect this may have coincided with the arrival of the amphibian chytrid, *Batrachochytrium dendrobatidis*, a serious fungal pathogen of amphibians.
10. Neither RaHV-1 nor the disease it causes has been found in any species other than *R. pipiens* in USA.

OTHER VIRUSES PATHOGENIC TO AMPHIBIANS

Other viruses found associated with disease or pathologic changes in amphibians have been reported in single papers with no experimental work. Hence, their significance as pathogens of amphibians is largely unknown.

Herpes-like virus of skin: In Italy, up to 80% of a wild population of *R. dalmatina* had epidermal vesicles associated with a herpes-like virus, but dead frogs were not found (Bennati et al 1994).

Calicivirus: Calicivirus was isolated from two captive *Ceratophrys orata* found dead. Both had pneumonia, while one also had oedema and the other had lymphoid hyperplasia (Smith et al 1986).

Leucocyte viruses: Polyhedral cytoplasmic DNA virus was found in the cytoplasm white blood cells of a Mexican *R. catesbiana* that was lethargic and had small exudative ulcers (Briggs and Burton 1973). The large iridovirus found in red blood cells of *B. marinus* in Costa Rica also was found in the cytoplasm of reticular cells in the spleen (Speare et al 1991).

VIRUSES THAT CAN USE AMPHIBIANS AS RESERVOIR HOSTS

At least 2 arboviruses, West Nile virus and Sindbis virus, can infect amphibians and produce viraemias. West Nile virus in *Rana ribidunda* caused a viraemia capable of infecting mosquitoes (Kostiukov et al 1986; 1985). West Nile virus causes serious disease in humans, birds and horses, has appeared in USA in 1999 and spread extensively (Petersen and Roehrig 2001) Antibodies against other arboviruses including Japanese encephalitis virus have been found in sera of amphibians indicating infection (Doi et al 1983), but whether amphibians can develop viraemias capable of infecting mosquito vectors is unknown.

BACTERIAL DISEASES OF AMPHIBIANS

The range of bacteria reported as causing disease in amphibians is small. Bacterial septicaemia appears to be the only bacterial disease associated with significant mortality in wild amphibians, and it can cause significant disease in captivity. Infection with non-haemolytic group B *Streptococcus* and chlamydia have caused outbreaks in captive amphibians. Other bacteria have caused sporadic cases of disease with no epidemics reported. Another group of bacteria can be carried by amphibians with minimal effect and are potentially capable of causing infections in humans (zoonotic diseases). *Salmonella* and *Leptospira* are in this category and are a potential risk to humans, livestock and domestic pets.

BACTERIAL SEPTICAEMIA

Bacterial septicaemia is infection of the blood stream by bacteria with accompanying signs of disease.

There are many well-described accounts of large epidemics of bacterial septicaemia in captive amphibians. These are often caused by *A. hydrophila* and other gram negative bacteria or combinations of bacteria, including *Pseudomonas* spp., *Proteus* spp., *Flavobacterium indologenes* and *F. meningosepticum* (Hubbard, 1981; Taylor et al., 1993; Olson et al., 1992; Anver and Pond, 1984). A syndrome attributed to bacterial septicaemia was called “red leg” due to haemorrhages and erythema on hindlimb skin (Emerson and Norris, 1905). This descriptive term appears to have been misinterpreted by some zoologists and the general public to mean that any frog with reddening of the skin of the hind legs has “red leg” and hence bacterial septicaemia (Berger 2001).

In captive amphibians outbreaks of bacterial septicaemia have high mortality rates. Clinical signs include pale skin, petechiation, haemorrhagic cutaneous ulcers, lethargy, anorexia, oedema, haemorrhages in internal organs, ascites and pale livers. On histology, there may be degenerative myopathy and multiple foci of coagulative necrosis with clumps of bacteria. Variable results were obtained from transmission experiments - the disease usually required inoculation of the bacteria, or bath exposure and stress (Glorioso et al 1974; Dusi 1949; Somsiri et al 1997). *A. hydrophila* and many of the other bacteria causing infections in captive amphibians can be isolated from healthy animals and from the environment (Carr et al 1976; Hird et al 1981) suggesting that disease occurs secondary to stresses caused by poor husbandry such as overcrowding, dirty conditions, trauma, temperature changes, and also after transport (Hubbard 1981; Glorioso et al 1974).

These bacteria can also be cultured from cases of viral disease particularly when frogs are collected dead, and symptoms of “red leg” are similar to those caused by iridoviruses (Cunningham et al 1996a). Some mass die-offs in the wild have also been attributed to bacteria due to their presence in dying animals, but these diagnoses are dubious due to a lack of histopathological confirmation and since other agents, particularly viruses and the amphibian chytrid, were not looked for. Bacteria were implicated in die-offs in *Alytes obstetricans* in the Pyrenean Mountains in Spain (Marquez et al 1995), in *R. muscosa* in California (Bradford 1991) and in *B. boreas boreas* in Colorado (Carey 1993). An epidemic among tadpoles of *Rana sylvatica* in Rhode Island, USA was reported to be caused by *A. hydrophila* (Nyman 1986). Usually, deaths due to *Aeromonas* or *Pseudomonas* are secondary to environmental stressors or in captive conditions, poor management practices.

NON-HAEMOLYTIC GROUP B *STREPTOCOCCUS*

A non-haemolytic group B *Streptococcus* caused an outbreak killing 80% of about 100,000 farmed bull frogs (*R. catesbiana*) in Brazil (Amborski et al 1983). Septicaemia, necrotising splenitis and hepatitis with haemorrhages occurred in frogs. The outbreak was associated with overcrowding and stress. Mortality due to a similar streptococcus occurred in bullfrogs, *R. catesbiana,* being raised for consumption in Uruguay (Mazzoni 2001).

CHLAMYDIA

Chlamydia is a degenerate bacteria which lives intracellularly within the amphibian host. Outbreaks of chlamydiosis in captive amphibians resulted in fulminant, multisystemic infections with pyogranulomatus inflammation. Chlamydial infections have been reported in captive amphibians, causing moderate to high mortality rates in various species including *Xenopus laevis* in the USA, and *Ceratobatrachus guentheri* in Canada (Honeyman et al 1992; Howerth 1984; Newcomer et al 1982; Wilcke et al 1983). In these cases, the chlamydial species was either unknown or assumed to be *C. psittaci.* Recently, *C. pneumoniae* has been identified as the cause of disease in a wild frog, *Myxophyes iteratus* with chronic pneumonia in Australia (Berger et al 1999) and in a captive colony of *Xenopus tropicalis* in USA (Reed et al 2000). *C. pneumoniae* is an important human pathogen that had previously been found only in humans, koalas and a horse (Storey et al 1993).

MYCOBACTERIA

Mycobacterial infection of amphibians has been reported only in captivity and occurs mainly in immuno-compromised animals. Mortality rates are usually low. Natural resistance to mycobacteria, which are ubiquitous in aquatic environments, is higher than in homeotherms (Reichenbach-Klinke and Elkan 1965). *Mycobacterium marinum* was experimentally shown to cause a chronic granulomatous non-lethal disease in immunocompetent leopard frogs (*R. pipiens*) whereas frogs immunocompromised with hydrocortisone developed an acute lethal disease (Ramakrishnan et al 1997). Infections may primarily involve skin, respiratory tract or intestines. Frogs have been found with single large tumour-like masses or with disseminated nodules throughout internal organs. Organs such as liver, spleen, kidney or testes may become almost completely destroyed by the infection before the animal dies, usually with cachexia (Reichenbach-Klinke and Elkan 1965). Early granulomas are composed of mostly epithelioid macrophages, which may progress to form encapsulated foci with dry caseous centres. Granulomas typically contain large numbers of acid-fast bacilli.

M. marinum and *M. xenopi* have been isolated from amphibians showing a variety of lesions. *M. chelonei subsp abscessus* was isolated from 4/66 *B. marinus* and 2/86 *B. granulosus* in a survey of Amazonian amphibians (Mok and Carvalho 1984). None of these animals had histopathological lesions, although experimental intraperitoneal inoculation of 29 toads resulted in the death of five animals from mycobacteriosis.

SALMONELLA

Salmonella are members of the large bacterial family Enterobactericae, are usually found in the intestinal tract of clinically normal animals, are excreted in faeces, and can cause serious disease in humans and domestic animals. Salmonellae are a significant cause of “food poisoning”, causing gastroenteritis and more serious diseases.

Contamination can occur at any point in the processing of food. For humans salmonellosis is a notifiable disease due to its potential severity, but also because it is a marker of the microbiological safety of the human food chain. Water borne infections can occur, but are unusual (Taylor et al 2000).

Amphibians may carry pathogenic *Salmonella* species, but rarely are frogs reported to be showing signs of disease (Reichenbach-Klinke and Elkan 1965; Anver and Pond 1984). Prevalence of salmonellas isolated in surveys from clinically normal amphibians is generally greater than 10% and sometimes as high as 60% particularly if intestinal contents are sampled at several sites (Sharma et al 1974). Salmonella are generally not of significance to the amphibian host, but may infect humans and other animals. In Australia, *Salmonella* were isolated from 12.7% (19/150) of *B. marinus* collected from the wild and 9 serotypes were identified. All nine had previously been isolated in Australia from humans and livestock (O'Shea et al 1990). An outbreak of gastroenteritis in humans near Rockhampton was thought to have possibly arisen from green tree frogs (*Litoria caerulea*) contaminating drinking water in rainwater tanks (Taylor et al 2000). Sharma et al (1974) found high concentrations of bacteria in intestinal contents of toads and suggested that amphibians may be good reservoirs of salmonellae with a high potential to contaminate the environment. Some strains of salmonellae are cosmopolitan while others are not found in Australia, but could be imported.

Table 3. Prevalence of salmonellas in clinically normal amphibians.

Country	**Amphibian host**	**Prevalence**	**Reference**
Europe			
Canary Islands	*Rana perezei*	60%	Monzon Moreno et al 1995
Australia			
North Queensland	*Bufo marinus*	12.7% (19/150)	O'Shea et al 1990
	Litoria caerulea	One isolate	Taylor et al 2000
Asia			
India	*Bufo* spp.	7% (40/570)	Sahib Singh et al 1979
	Bufo spp.	36.7% (121/329)	Sharma et al 1974; Sharma 1979
Central America			
Trinidad, Grenada	*Bufo marinus* *Hyla minuta*	41.6% (25/60) 50% (1/2)	Everard et al 1979
South America			

Surinam	*Bufo marinus*	55.5% (15/27)	Bool et al 1958

LEPTOSPIRA

Leptospira are spirochaetal bacteria that usually invade the kidney of vertebrates and are excreted in the urine. Humans and domestic animals are susceptible to various strains of *Leptospira* usually from the species *Leptospira interrogans*. Serious acute and chronic disease occasionally with death can result. Workers in the banana industry in North Queensland have high rates of infection with leptospirosis, but the reason for this has yet to be determined (Smythe et al 1997). Serovars Zanoni and Australis are the most prevalent in banana workers.

Little detailed work has been done on the occurrence of *Leptospira* in amphibians, and on the significance of amphibians as reservoir hosts for leptospirosis in humans and domestic animals. The most comprehensive studies appear to have been done on Barbados. A series of papers reported that toads (*Bufo marinus*) and frogs (*Eleutherodactylus johnstonei*) could act as reservoirs for serovars of *Leptospira* pathogenic to humans (Gravekamp et al 1991; Everard et al 1990; Everard et al 1988). In addition, a new serovar, bajan, within the Autumnalis serogroup was identified. Prevalences of *Leptospira* isolated from both species of amphibians was about 4%, but serological evidence of past infection was much higher (>21%). Toads and frogs on Barbados were potentially significant sources of environmental contamination for human infections. A new serovar “C3” was isolated from a clinically normal toad (*Bufo marinus*) in the Philippines (Babudieri et al 1973). Experimentally this isolate was pathogenic to mammals. No studies appear to have been done on leptospires in amphibians in Australia.

FUNGAL DISEASES OF AMPHIBIANS

FUNGI DISCUSSED ELSEWHERE

Amphibian chytridiomycosis is the most formidable disease of amphibians globally (Speare et al 2001) and is the most serious fungal disease. It is discussed elsewhere at this site. Another fungus that is a significant pathogen in Australia, *Mucor amphibiorum*, is also discussed elsewhere at this site.

BASIDIOBOLUS RANARUM

Basidiobolus ranarum is a zygomycete that can be frequently isolated from the intestines of healthy amphibians and lizards (Reichenbach-Klinke and Elkan 1965). *B. ranarum* was reported to cause an epizootic of cutaneous mycosis in captive *Hymenochirus curtipes* in America (Groff et al 1991), but the identification of the fungus involved appears doubtful and *B. ranarum* may have been cultured as a contaminant. The morphology of the organism in the skin was different to the

appearance of cultured *B. ranarum* and more typical of *B. dendrobatidis* since it occurred as a spherical form in the skin with no hyphae typical of *B. ranarum*. Experimental transmission could not be achieved using cultured *B. ranarum*, but the disease was transmitted when healthy frogs were exposed to sick frogs (Groff et al 1991).

SAPROLEGNIASIS

Saprolegniasis in captive amphibians is similar to the disease in fish with pale tufts of fungus growing on the skin. The disease mainly effects aquatic species and life-stages and usually occurs secondary to epidermal damage. *Saprolegnia ferax* and *S. parasitica* are the most common species isolated (Anver and Pond 1984). *S. ferax* was found to be responsible for high mortality rates in eggs of *B. boreas* in the wild in northwest USA (Blaustein et al 1994).

APHANOMYCES IN TADPOLES

Two outbreaks of fungal disease were described in tadpoles of *B. marinus* near Townsville (Berger et al 2001). The disease had an unusual appearance with tufts of fungi growing primarily on the head of tadpoles. When the disease was encountered a second time, more detailed investigations were undertaken. Although a range of fungi were cultured, all were considered to be secondary infections or contaminants except for an *Aphanomyces* sp. that is likely to be the primary agent. Mortality rate could not be determined, but the high prevalence (37%) and debilitating lesions show the disease would have impacted on the tadpole population. However, this would be unlikely to affect the adult population size as toads are phenomenally fecund. Also in normal circumstances tadpole survival has been reported to be very low (5%) due to density dependent predation (Calef 1973).

CHROMOMYCOSIS

Chromomycosis refers to infection with a range of pigmented, septate fungi from the phylum Ascomycota. Many reports mention difficulties in identifying fungal species due to a lack of sporulation in tissues and in culture. Pigmented fungi including *Fonsecaea pedrosi, F. dermatitidis, Cladosporium* sp*. Scolecobasidium* sp. and *Phialophora* sp. have been isolated from lesions in a range of captive amphibians including *B. marinus, R. pipiens*, *R. catesbiana, Hyla caerulea, Phyllobates trinitatis, Ceratophrys ornata, Rhacophorus* sp*., H. septentrionalis* (Beneke 1978; Cicmanec et al 1973; Elkan and Philpot 1973; Rush et al 1974; Miller et al 1992) and wild *B. melanostrictus* (Dhaliwal and Griffiths 1963). These organisms have also been isolated from tanks housing captive frogs. 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. These were fibrous granulomas with mononuclear cells, epithelioid cells and multinucleate giant cells

around pigmented, septate fungi or spherical chlamydospores. The granulomas coalesced and replaced much of the parenchyma. Central caseation occurred in very large granulomas. A haematogenous spread was suspected due to the multi-organ infections. Transmission experiments had variable results. Rush et al (1974) transmitted disease in healthy, unstressed frogs whereas Elkan and Philpot (1973) could not infect healthy frogs by intraperitoneal inoculation. Cicmanec et al (1973) transmitted the disease by intracoeloemic injection only if toads were stressed by refrigeration, monthly feeding, or limited water.

DERMOCYSTIDIUM AND *DERMOSPORIDIUM*

Dermocystidium spp. and *Dermosporidium* spp. grow as large spore-filled cysts in subcutaneous tissue or the dermis and can cause inflammation and ulcerations (Broz and Privora 1951; Jay and Pohley 1981). Infections have been found in Europe and America, and can occur at high prevalence in a population (Reichenbach-Klinke and Elkan 1965). Sequencing of small-subunit rRNA genes from *Dermocystidium* spp. showed this genus to be part of a clade of protistan parasites near the animal-fungal divergence (Ragan et al 1996).

PARASITIC DISEASES OF AMPHIBIANS

PATHOGENIC PROTOZOA

Amphibians have a large range of protozoa, many of which appear to be commensals in the gastrointestinal tract. Only the potentially pathogenic protozoa are mentioned in this section. Most research has been done on taxonomy of the protozoa and very little on pathogenicity and biology.

MICROSPORIDIA

The microsporidian, *Pleistophora myotrophica*, caused high mortality rates in captive *B. bufo* (Canning et al 1964). This parasite infected all striated muscles resulting in atrophy and emaciation. White streaks between muscle fibres were obvious grossly, and microscopically these were spaces in the muscle fibres packed with microsporidian spores. Muscle regeneration occurred with long chains of sarcoblasts adjacent to damaged muscle. Experimental infections were achieved by feeding toads infected muscle. Tadpoles did not become infected experimentally but their development was arrested. Only one of 12 experimental *R. temporaria* became infected and had spores in the tongue, whereas 100% of *B. bufo* were infected.

A captive, wild-caught *Phyllomedusa bicolor* was successfully treated for an ulcerative dermatitis that was associated with a variety of infective agents including microsporidia (Graczyk et al 1996). The microsporidia were not identified.

MYXIDIUM

Two new myxosporean species, *Cystodiscus axonis* sp. n. and *Cystodiscus australis* sp. n., have found in eastern Australia and causing disease in a range of Australian frogs and tadpoles including the introduced Cane toad (*Rhinella marina*).

For information about these Myxozoans, please refer to:

Hartigan A, Fiala I, Dyková I, Rose K, Phalen DN, Šlapeta J. 2012. New species of Myxosporea from frogs and resurrection of the genus Cystodiscus Lutz, 1889 for species with myxospores in gallbladders of amphibians. Parasitology 139(4):478-96. doi: 10.1017/S0031182011002149.

Hartigan A, Phalen DN, and Šlapeta J. 2010. Museum material reveals a frog parasite emergence after the invasion of the cane toad in Australia. Parasit Vectors. 3: 50. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2901343/pdf/1756-3305-3-50.pdf

Hartigan A, Sangster C, Rose K, Phalen DN, Šlapeta J. 2012. Myxozoan parasite in brain of critically endangered frog [letter]. Emerg Infect Dis. http://dx.doi.org/10.3201/eid1804.111606

Hartigan A, Fiala I, Dyková I, Jirků 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 6(4): e18871. doi:10.1371/journal.pone.0018871

TRYPANOSOMES

Over 60 species of trypanosomes have been reported in anurans but the taxonomy is confused (Bardsley and Harmsen 1973). Most infections are non-pathogenic. *Trypanosoma inopinatum* is the only trypanosome of anurans whose pathogenicity has been well studied. Experimental infections with the blood borne flagellate were lethal to European green frogs and caused haemorrhages, swollen lymph glands and anaemia (Brumpt 1924). Death resulted from destruction of the reticuloendothelial system. The lymph fluid was abundant and contained numerous trypanosomes agglutinated in rosettes with the flagellates in the centre (Brumpt 1924; Bardsley and Harmsen 1973). *T. rotatorium* can be pathogenic in tadpoles or in heavy infections, with trypanosomes accumulating in the kidneys (Bardsley and Harmsen 1973). *T. pipientis* causes spleen enlargement but rarely causes death (Flynn 1973). Trypanosomes are quite common in frogs from Queensland, but none has been associated with disease (Delvinquier and Freeland 1989).

MYXOBOLUS HYLAE

Myxobolus hylae was found in the reproductive organs of *L. aurea* from Sydney (Johnston and Bancroft 1918). Infected frogs appeared sickly and emaciated. The testes and vasa efferentia were infected in males and the oviducts were infected in females. High prevalences were observed with infections in 7/7 males and 2/~23

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 (Johnston and Bancroft 1918).

ICHTHYOPHONUS-LIKE ORGANISMS

Myositis associated with infection by *Ichthyophonus*-like organisms was reported in wild amphibians collected in Quebec, Canada, from 1959 to 1964 and 1992 to 1999 (Mikaelian et al 2000). Infection was diagnosed in 6 species (frogs *Rana clamitans*, *R. sylvatica*, *R. catesbeiana*, *R. palustris*, *Pseudacris crucifer*, and newts *Notophthalmus viridescens*). Spores of the organisms invaded striated muscle fibres and were associated with variable degrees of granulomatous and eosinophilic inflammation. Infection was considered fatal in 2 green frogs, 1 wood frog, and 1 red-spotted newt. It was considered potentially significant in 3 additional green frogs in which up to 100% of the fibres of some muscles were replaced by spores associated with a severe granulomatous reaction. This report shows that ichthyophonosis is enzootic in amphibians from Quebec.

PATHOGENIC HELMINTHS

Many helminth species infect amphibians, and some cause disease with heavy burdens. The pathogenic effects of trematodes, cestodes and nematodes are reviewed in detail by Flynn (1973). Disease is common in captivity but none has been reported to cause epidemics in the wild. Amphibians have a depauperate helminth fauna with low diversity and low infection levels compared to other vertebrates (Barton and Richards 1996). This may be due to host specificity of the parasites or to aspects of host biology (Barton and Richards 1996).

RHABDIAS

Rhabdias is a genus of parasitic nematode found in commonly in the lungs of anuran amphibians (Flynn 1973). Usually they are incidental findings but heavy experimental infections can cause disease (Tinsley 1995). Experimental transmission of *Rhabdias bufonis* to *B. bufo* resulted in a dose dependent decrease in growth rates, fitness and survival (Tinsley 1995). *B. marinus* were placed on a culture of infective larvae of *R. sphaerocephala* which rapidly burrowed through the skin and the toads died overnight (Williams 1960). The skin had numerous tufts of cast nematode skins. Hundreds of larvae were found internally including heart muscle, liver and eye (Williams 1960). Larvae may reach the lungs indirectly via the blood stream or by direct migration to the lungs. Some larvae do not reach the lungs and encyst in other organs. These aberrant migrating larvae incite granuloma formation that may affect the host (Reichenbach-Klinke and Elkan 1965). Only a small proportion of wild amphibians have heavy burdens (Tinsley 1995). In Australia *R. hylae* is the most widespread species (Barton 1994).

PSEUDOCAPILLAROIDES XENOPI

Pseudocapillaroides xenopi is a capillarioid nematode that burrows in the epidermis. Infection resulted in deaths in captive *X. laevis*. Bacterial and fungal opportunists contributed to the pathogenesis. Clinical signs developed over four months and included ulcers, sloughing of the epidermis, erythema and weight loss (Cunningham et al 1996b; Brayton 1992).

FILARIOIDS

Foleyella spp. can cause death due to heavy infections with microfilaria or adult worms (Reichenbach-Klinke and Elkan 1965). Infections may occur at high prevalence in a population and appear asymptomatic. *Foleyella confusa* and *Icosiella hoogstraali* were described from Philippine amphibians (Schmidt and Kuntz 1969), but no details about their pathology were given.

SPARGANA

Spargana are the intermediate stage of cestodes (Order Pseudophyllidea) that occur in frogs worldwide (Flynn 1973). They are potentially pathogenic but their effects in frogs have not been well studied. The adult stage of the cestode, *Spirometra erinacei* (the only species of pseudophyllidean cestode known to occur in Australia), inhabits the small intestine of carnivores such as dog, cat, fox and dingo. The procercoid stage occurs in copepods and the plerocercoid stage (spargana) is found in tadpoles and adult frogs that ingest infected copepods (Sandars 1953).

In Australian amphibians, spargana have been reported in wild adults of *B. marinus*, *L. aurea*, *L. caerulea, L. nasuta* and *L. rubella* (Berger et al 2009; Barton 1994; Sastrawan 1978), and experimental infections were produced in adults of *L. latopalmata* and *Limnodynastes tasmaniensis* and tadpoles of *L. latopalmata, L. caerulea* and *L. tasmaniensis* (Bennett 1978; Sastrawan 1978; Sandars 1953). Sandars (1953) reported that about one quarter of the population of *L. caerulea* in the Brisbane area was infected with spargana. In a group of 1000 *B. marinus* from Ingham, Queensland, 37 (3.7%) were found with infections of spargana provisionally called *S. mansoni* (Bennett 1978). These toads had light infections, an average of 6.3 spargana per toad with 59% spargana found in thighs. There was a marked local inflammatory response in these toads and over half the spargana were dead. Immunodiffusion and immuno-electrophoretic tests in the toads revealed antibodies were produced to components of the spargana. Attempts to study the reactions in experimental *L. tasmaniensis* failed due to inconsistent infection rates and frequent deaths of infected frogs and tadpoles, which were thought to be due to the combined stress of parasitism and captive conditions (Bennett 1978). Growth of experimentally infected *L. latopalmata* tadpoles was inhibited (Sandars 1953). In a

survey of 948 Malaysian frogs, 11.8 % were found infected with spargana, 57% of which had bleeding and/or swelling at infection sites (Mastura et al 1996).

TREMATODE METACERCARIA

Metacercariae of various trematode species occur in tadpoles and frogs. The definitive host may be snakes, frogs, birds or mammals (Reichenbach-Klinke and Elkan 1965). Usually encysted larvae are not pathogenic although infections have been found in vital organs such as eyes, heart, liver, lung and CNS where they may cause disease. Metacercariae of *Neascus* group encysted in the dermis along the lateral line system of captive adults of *X. laevis*, leading to paralysis and death (Elkan and Murray 1951). *Diplostomulum xenopi* infected the pericardial cavity of *X. laevis* causing pericarditis, respiratory distress and death (Flynn 1973). Heavy experimental infections with *Cercaria ranae* caused bloat in tadpoles (Cort and Brackett 1938).

ACANTHOCEPHALA

The spines of acanthocephala inhabiting the stomach and intestine of frogs can cause perforation and death. *Acanthocephalus ranae* is a common species in Europe (Reichenbach-Klinke and Elkan 1965).

PATHOGENIC ARTHROPODS

DIPTERA

Various fly species from the families Sarcophagidae, Calliphoridae and Chloropidae have larvae that can develop within amphibians (Reichenbach-Klinke and Elkan 1965; Crump and Pounds 1985). 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 (Reichenbach-Klinke and Elkan 1965). Larvae of *Notochaeta bufonivora* parasitised wild *A. varius* along a stream in Costa Rica during the dry season. Frogs in early stages of myiasis had a single small wound on the posterior surface of one thigh, and all hosts died within four days after they were found. Female frogs were parasitised more often (Crump and Pounds 1985).

Larvae of *Notochaeta* sp. infected farmed *R. catesbiana* in Brazil. Larvae occurred in the mouth and caused necrotic perforations associated with a range of aerobic and anaerobic bacteria including *Clostridium* spp. (Baldassi et al 1995).

In Australia the genus *Batrachomyia* contains several species that have been found in 11 frog species (Elkan 1965). 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 *B. bufonivora*, 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 death can result at the time of larval emergence (Elkan 1965; Vogelnest 1994). Especially with small frogs, the hole left in the skin after escape of the maggot would be expected to seriously affect the frog. A *Pseudophryne bibronii* was found with a perforation of the peritoneal wall and the rostral end of the maggot lay within the peritoneal cavity (Elkan 1965). A *L. caerulea* infected with a larva of *B. mertensis* was in poor body condition and did not eat well until the larva was surgically removed (Vogelnest 1994).

ARACHNIDS

Larval trombiculid mites infect the skin of frogs and toads and cause small vesicles in the skin (Flynn 1973). Ticks of the genus *Amblyomma* occur on *B. marinus* in Central and South America. They occur on all areas of the body and cause transient focal congestion and haemorrhage. Ticks have not been found on *B. marinus* in Australia or on native amphibians (Speare 1990).

NUTRITIONAL DISEASES

METABOLIC BONE DISEASE

Very common in captivity, can be caused by a combination of poor diet, inadequate calcium, inadequate UV and fluoride in water.

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OTHER DISEASES

Ongoing surveillance of amphibian diseases will give the best chance to detect any new disease capable of posing a threat to wild amphibians.

Although other known pathogens appear to have less potential for epidemic disease, the study of infectious diseases of amphibians is in infancy and this conclusion may be premature.

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