

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.

Wright KM, Whitaker BR. 2001. Amphibian Medicine and Captive Husbandry. Krieger Publishing, Malabar, Florida.

CONTENTS

Viral Diseases of Amphibians	3
Frog Erythrocytic Virus	4
Lucké tumor herpesvirus	5
Other Viruses Pathogenic to Amphibians	5
Viruses that Can Use Amphibians as Reservoir Hosts	6
Bacterial Diseases of Amphibians	6
Bacterial septicaemia	6

Non-haemolytic group B <i>Streptococcus</i>	7
Chlamydia	8
Mycobacteria	8
Salmonella.....	8
Leptospira	10
Fungal Diseases of Amphibians.....	10
Fungi Discussed Elsewhere	10
<i>Basidiobolus ranarum</i>	10
Saprolegniasis	11
APHANOMYCES IN tadpoles	11
Chromomycosis.....	11
<i>Dermocystidium</i> and <i>Dermosporidium</i>	12
Parasitic Diseases of Amphibians.....	12
Pathogenic protozoa.....	12
Microsporidia	12
Myxidium	12
Trypanosomes.....	13
<i>Myxobolus hylae</i>	13
<i>Ichthyophonus</i> -like organisms	14
Pathogenic Helminths.....	14
Rhabdias.....	14
<i>Pseudocapillaroides xenopi</i>	15
Filaroids.....	15
Spargana	15
Trematode metacercaria	16
Acanthocephala	16
Pathogenic arthropods	16

Diptera	16
Arachnids	17
Nutritional Diseases	17
Metabolic Bone Disease.....	17
Other Diseases	17
Literature Cited	17
Web Sites	30

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 <i>Bufo marinus</i>	Venezuela	Necrosis of haematopoietic tissue	Hyatt et al 2000
Amphibian erythrocytic viruses	North American bullfrog <i>Rana catesbiana</i> Green frog <i>R. clamitans</i> <i>R. septentrionalis</i>	Canada	Anaemia; reduced survival	Gruia-Grey et al 1992
	Northern leopard frog <i>R. pipiens</i>	USA	Unknown	Bernard et al 1969
	<i>Leptodactylus ocellatus</i>	Brazil	Unknown	de Sousa & Weigl 1976
	<i>Phrynohyas venulosa</i>	Brazil	Unknown	de Matos et al 1995

	Cane toad <i>(Bufo marinus)</i>	Costa Rica	Unknown	Speare et al 1991
	<i>Ptychadena anchieta</i>	South Africa	Unknown	Alves de Matos & Paperna 1993
	<i>Bufo gargarizans</i>	China	Unknown	Werner 1993
	<i>R. boulengeri</i>	China	Unknown	Werner 1993
	<i>R. nigromaculata</i>	China	Unknown	Werner 1993
Amphibian leucocyte virus	<i>R. catesbiana</i>	Europe (captive source Mexico)	Lethargy, skin ulceration	Briggs & Burton 1973
Lucké tumor herpesvirus	<i>Rana pipiens</i>	USA	Lucké renal adenocarcinoma	Lucké 1938; McKinnell & Carlson 1979
Calicivirus	<i>Ceratophrys orata</i>	USA (captive)	Pneumonia, death	Smith et al 1986
Herpesvirus-like particles in skin	<i>Rana dalmatina</i>	Europe	Epidermal vesicles	Bennati et al 1994
Flaviviruses				
Sindbis virus	<i>Rana ridibunda</i>	Europe	Unknown	Kozuch et al 1978
West Nile virus	<i>Rana ridibunda</i>	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 Iridoviridae.
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 Minnesota (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 pyogranulomatous 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. chelonae subsp abscessus* was isolated from 4/66 *B. marinus* and 2/86 *B. granulosis* 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 Enterobacteriaceae, 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	<i>Rana perezei</i>	60%	Monzon Moreno et al 1995
Australia			
North Queensland	<i>Bufo marinus</i>	12.7% (19/150)	O'Shea et al 1990
	<i>Litoria caerulea</i>	One isolate	Taylor et al 2000
Asia			
India	<i>Bufo</i> spp.	7% (40/570)	Sahib Singh et al 1979
	<i>Bufo</i> spp.	36.7% (121/329)	Sharma et al 1974; Sharma 1979
Central America			
Trinidad, Grenada	<i>Bufo marinus</i>	41.6% (25/60)	Everard et al 1979
	<i>Hyla minuta</i>	50% (1/2)	
South America			

Surinam	<i>Bufo marinus</i>	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. melanostictus* (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 chlamydozoospores. 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 intracoelomic 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 proceroid 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. mansonii* (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.

Shaw SD, Bishop PJ., Harvey C, Berger L, Skerratt LF, Callon K, Watson M, Potter J, Jakob-Hoff R, Goold M, Kunzmann N, West P and Speare R. 2012. Fluorosis as a probable factor in metabolic bone disease in captive New Zealand native frogs (*Leiopelma species*). *Journal of Zoo and Wildlife Medicine*, 43 (3): 549-565.

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.

LITERATURE CITED

Ahne W, Bearzotti M, Bremont M, Essbauer S. 1998. Comparison of European systemic piscine and amphibian iridoviruses with epizootic haematopoietic necrosis virus and frog virus 3. *Zentralbl Veterinarmed [B]* 45(6):373-383.

- Alves de Matos AP, Paperna I. 1993. Ultrastructure of erythrocytic virus of the South African anuran *Ptychadena anchietae*. *Diseases of Aquatic Organisms* 16:105-109.
- Amborski RL, Snider TG, Culley DD. 1983. A non-hemolytic, group B *Streptococcus* infection of cultured bullfrogs, *Rana catesbiana*, in Brazil. *Journal of Wildlife Diseases* 19:180-184.
- Anderson IG, Prior HC, Rodwell BJ, Harris GO. 1993. Iridovirus-like virions in imported dwarf gourami (*Colisa lalia*) with systemic amoebiasis. *Aust Vet J.* 70(2):66-67.
- Anver MR, Pond CL. Biology and diseases of amphibians. 1984. In: *Laboratory Animal Medicine*. Eds J. G. Fox, B. J. Cohen, F. M. Loew. Academic Press, New York. Pp 427-447.
- Aplin K, Kirkpatrick P. 2000. Chytridiomycosis in southwest Australia: historic sampling documents the date of introduction, rates of spread and seasonal epidemiology, and sheds new light on chytrid ecology. *Proceedings Getting the Jump! On Amphibian Disease*, 26-30 August, Cairns. Pp24.
- Ariel E. 1997. Pathology and serological aspects of Bohle iridoviral infections in 6 selected water-associated reptiles in North Queensland. PhD thesis: James Cook University, Townsville.
- Babudieri B, Carlos ER, Carlos ET. 1973. Pathogenic leptospira isolated from toad kidneys. *Tropical and Geographical Medicine* 25:297-299.
- 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.
- Bardsley JE, Harmsen R. 1973. The Trypanosomes of Anura. *Advances in Parasitology* 11:1-73.
- Barton DP. 1994. A checklist of helminth parasites of Australian amphibia. *Records of the South Australian Museum* 27:13-30.
- Barton DP, Richards SP. 1996. Helminth infracommunities in *Litoria genimaculata* from Birthday Creek, an upland rainforest stream in northern Queensland, Australia. *International Journal for Parasitology* 26:1381-1385.
- Beneke ES. 1978. Dermatiaceous fungi in laboratory-housed frogs. *Pan American Health Organisation Scientific Publication*, Washington DC 356:101-108.
- Bennati R, Bonetti M, Lavazza A, Gelmetti, D. 1994. Skin lesions associated with herpesvirus-like particles in frogs (*Rana dalmatina*). *Veterinary Record* 135:625-626.

- Bennett LJ. 1978. The immunological response of amphibia to Australian spargana. *Journal of Parasitology* 64:756-759.
- Berger L. 2001. Diseases in Australian frogs. PhD Thesis: James Cook University, Townsville.
- Berger L, Speare R, Daszak P, Green ED, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proceedings of National Academy of Science* 95:9031-9036.
- Berger L, Speare R, Thomas A, Hyatt AD. 2001. Mucocutaneous fungal disease in tadpoles of *Bufo marinus* in Australia. *Journal of Herpetology* 35:330-335.
- Berger L, Skerratt LF, Zhu XQ, Young S, Speare R. Severe sparganosis in Australian tree frogs. *J Wildl Dis.* 2009 45:921-9.
- Berger L, Volp K, Mathews S, Speare R, Timms P. 1999. *Chlamydia pneumoniae* in a free-ranging giant barred frog (*Mixophyes iteratus*) from Australia. *Journal of Clinical Microbiology* 37:2378-2380.
- Bernard GW, Cooper EL, Mandell ML. 1969. Lamellar membrane encircled viruses in the erythrocytes of *Rana pipiens*. *Journal of Ultrastructural Research* 26(1):8-16.
- Bernard J, Bremont M, Winton J. 1992. Nucleocapsid gene sequence of a North American isolate of viral haemorrhagic septicaemia virus, a fish rhabdovirus. *Journal of General Virology* 73(4):1011-1014.
- Blaustein AR, Hokit DG, O'Hara RK, Holt RA. 1994. Pathogenic fungus contributes to amphibian losses in the Pacific northwest. *Biological Conservation* 67:251-254.
- Bollinger TK, Mao J, Schock D, Brigham RM, Chinchar VG. 1999. Pathology, isolation, and preliminary molecular characterization of a novel iridovirus from tiger salamanders in Saskatchewan. *Journal of Wildlife Diseases* 35(3):413-429.
- Bool PH, Kampelmacher EH. 1958. Some data on the occurrence of *Salmonella* in animals in Surinam. *Antonie van Leeuwenhoek* 24:76-80.
- Bosch J, Martinez-Solano I, Garcia-Paris M. 2000. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad in protected areas of Central Spain. *Proceedings Getting the Jump! On Amphibian Disease*, 26-30 August, Cairns. Pp36.
- Bradley GA, Rosen PC, Sredl MJ, Jones TR, Longcore JE. 2002. Chytridiomycosis in native Arizona frogs. *Journal of Wildlife Diseases* 38(1):206-212.

Brayton C. 1992. Wasting disease associated with cutaneous and renal nematodes, in commercially obtained *Xenopus laevis*. *Annals of the New York Academy of Science* 653:197-201.

Briggs RT, Burton PR. 1973. Fine structure of an amphibian leukocyte virus. *Journal of Submicroscopic Cytology and Pathology* 5:71-78.

Broz O, Privora M. 1951. Two skin parasites of *Rana temporaria*: *Dermocystidium ranae* Guyenot & Naville and *Dermosporidium granulosum* n. sp. *Parasitology* 42:65-69.

Brumpt E. 1924. Un cas de rupture de la rate avec hémopéritoine au cours d'une infection expérimentale a *Trypanosoma inopinatum* chez la grenouille verte (*Rana esculenta*). *Annales de Parasitologie* 4:325-326.

Bryan FL, Fanelli MJ, Riemann. 1979. *Salmonella* infections. Chapter 11. In: Food-borne infections and intoxications. 2nd ed. Ed: Riemann H, Bryan FL. Pp 74-130.

Calef GW. 1973. Natural mortality of tadpoles in a population of *Rana aurora*. *Ecology* 54:741-758.

Canning EU, Elkan E, Trigg PI. 1964. *Plistophora myotrophica* spec. nov., causing high mortality in the common toad *Bufo bufo* L., with notes on the maintenance of *Bufo* and *Xenopus* in the laboratory. *Journal of Protozoology* 11:157-166.

Carey C. 1993. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. *Conservation Biology* 7:355-362.

Carey C, Cohen N, Rollins-Smith LA. 1999. Amphibian declines: An immunological perspective. *Developmental Comparative Immunology* 23:459-472.

Carlson DL, Sauerbier W, Rollins-Smith LA, McKinnell RG. 1994. The presence of DNA sequences of the Lucke herpesvirus in normal and neoplastic kidney tissue of *Rana pipiens*. *Journal of Comparative Pathology* 110(4):349-55.

Carr AH, Amborski RL, Culley DD, Amborski GF. 1976. Aerobic bacteria in the intestinal tracts of bullfrogs (*Rana catesbiana*) maintained at low temperatures. *Herpetologica*. 32(3):239-244.

Centers for Disease Control and Prevention. 1994. Addressing emerging infectious disease threats: a prevention strategy for the United States. Atlanta, Georgia: U.S. Department of Health and Human Services, Public Health Service.

Cicmanec JL, Ringler DH, Beneke ES. 1973. Spontaneous occurrence and experimental transmission of the fungus, *Fonsecaea pedrosoi*, in the marine toad, *Bufo marinus*. *Laboratory Animal Science* 23(1):43-47.

- Cooper EL, Wright RK, Klempau AE, Smith CT. 1992. Hibernation alters the frog's immune system. *Cryobiology* 29(5):616-631.
- Cort WW, Brackett S. 1938. A new strigeid cercaria which produces a bloat disease of tadpoles. *Journal of Parasitology* 24:263-271.
- Crump ML, Pounds JA. 1985. Lethal parasitism of an aposematic anuran (*Atelopus varius*) by *Notochaeta bufonivora* (Diptera: Sarcophagidae). *Journal of Parasitology* 7:588-591.
- Cunningham AA, Langton TES, Bennett PM, Lewin JF, Drury SEN, Gough RE, MacGregor SK. 1996a. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). *Philosophical Transactions of the Royal Society of London B*. 351:1539-1557.
- Cunningham AA, Sainsbury AW, Cooper JE. 1996b. Diagnosis and treatment of a parasitic dermatitis in a laboratory colony of African clawed frogs (*Xenopus laevis*). *Veterinary Record* 138:640-642.
- Cunningham AA, Daszak P, Hyatt AD. Amphibia. In: Woodford MH. (ed). Quarantine and health screening protocols for wildlife prior to translocation and release into the wild. IUCN Species Survival Commission's Veterinary Specialist Group, Switzerland and Office International des Epizooties (OIE), Paris. 67-71.
- Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R. 1999. Emerging infectious diseases of wildlife: role in amphibian population declines and global implications. *Emerging Infectious Diseases* 5:735-748.
- Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife – Threats to biodiversity and human health. *Science* 287:443-449.
- Davison AJ, Sauerbier W, Dolan A, Addison C, McKinnell RG. 1999. Genomic studies of the Lucke tumor herpesvirus (RaHV-1). *Journal of Cancer Research and Clinical Oncology* 125(3-4):232-238.
- de Matos AP, Paperna I, Lainson R. 1995. An erythrocytic virus of the Brazilian tree-frog, *Phrynohyas venulosa*. *Memorias Instituto Oswaldo Cruz* 90(5):653-655.
- de Sousa MA, Weigl DR. 1976. The viral nature of *Toddia* Franca, 1912. *Memorias do Instituto Oswaldo Cruz* 74: 213-230.
- Delvignier BLJ. 1986. *Myxidium immersum* (Protozoa: Myxosporidia) of the cane toad, *Bufo marinus*, in the Australian Anura, with a synopsis of the genus in amphibians. *Australian Journal of Zoology* 34:843-853.
- Delvignier BLJ, Freeland WJ. 1989. On some trypanosomes of the Australian anura. *Proceedings of the Royal Society of Queensland* 100:79-87.

- Dhaliwal SS, Griffiths DA. 1963. Fungal disease in Malayan toads: an acute lethal inflammatory reaction. *Nature* 197:467-569.
- Doi R, Oya A, Shirasaka A, Yabe S, Sasa M. 1983. Studies on Japanese encephalitis virus infection of reptiles. II. Role of lizards on hibernation of Japanese encephalitis virus. *Japanese Journal of Experimental Medicine* 53(2):125-34.
- Drury SEN, Gough RE, Cunningham AA. 1995. Isolation of an iridovirus like - agent from common frogs (*Rana temporaria*). *Veterinary Record* 137:72-73.
- Dusi JL. 1949. The natural occurrence of "red leg", *Pseudomonas hydrophila*, in a population of American toads, *Bufo americanus*. *Ohio Journal of Science* 49:70-71.
- Elkan E. 1965. Myiasis in Australian frogs. *Annals of Tropical Medicine and Parasitology* 59:51-54.
- Elkan E, Murray RW. 1951. A larval trematode infection of the lateral line system of the toad *Xenopus laevis* (Daudin). *Proceedings of the Zoological Society* 122:121-126.
- Elkan E, Philpot CM. 1973. Mycotic infections in frogs due to a *Phialophora*-like fungus. *Sabouraudia* 11:99-105.
- Emerson H, Norris C. 1905. "Red-leg" - an infectious disease of frogs. *Journal of Experimental Medicine* 7:32-58.
- Evans EE, Kent SP, Bryant RE, Moyer M. 1966. Antibody formation and immunological memory in the marine toad. In: *Phylogeny of Immunity*. (Ed. R.T. Smith, P.A. Mieschen and R.A. Good) University of Florida Press: Gainesville. Pp 218-226.
- Everard CO, Carrington DG, Korver H, Burke R, Everard JD, Gravekamp C. 1990. Leptospire in the whistling frog (*Eleutherodactylus johnstonei*) on Barbados. *Journal of Tropical Medicine and Hygiene* 93(2):140-5.
- Everard COR, Tota B, Bassett D, Ali C. 1979. *Salmonella* in wildlife from Trinidad and Grenada, W.I. *Journal of Wildlife Diseases* 15:213-219.
- Everard CO, Carrington D, Korver H, Everard JD. 1988. Leptospire in the marine toad (*Bufo marinus*) on Barbados. *Journal of Wildlife Diseases* 24(2):334-8.
- Faine S, Adler B, Bolin C, Perolat P. 1999. *Leptospira* and leptospirosis. *MediSci: Melbourne*.
- Flynn RJ. 1973. Parasites of Laboratory Reptiles and Amphibians. In: *Parasites of Laboratory Animals*. Iowa State University Press. Ames, USA. Pp 507-642.

Frank W. 1974. Mycotic infections in amphibians and reptiles. In: Proceedings of the Third International Wildlife Disease Conference. Ed L. A. Page. Plenum Press, New York. Pp 73-88.

Frank W, Roester U, Scholer HJ. 1976. Sphaerule formation by a *Mucor* species in the internal organs of amphibia. Zentrablatt für Bakteriologie und Parasitkunde 226:405-417.

Fraser, C. M. 1991. Merck Veterinary Manual, 7th edition. Merck and Co., New Jersey, USA.

Glorioso JC, Amborski RL, Amborski GF, Culley DC. 1974. Microbiological studies on septicemic bullfrogs (*Rana catesbiana*). American Journal of Veterinary Research 35:1241-1245.

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. Journal of Zoo and Wildlife Medicine 27:522-527.

Gravekamp C, Korver H, Montgomery J, Everard CO, Carrington D, Ellis WA, Terpstra WJ. 1991. Leptospire isolated from toads and frogs on the Island of Barbados. Zentralblatt für Bakteriologie 275(3):403-11.

Groff JM, Mughannam A, McDowell TS, Wong A, Dykstra MJ, Frye FL, Hedrick RP. 1991. An epizootic of cutaneous zygomycosis in cultured dwarf African clawed frogs (*Hymenochirus curtipes*) due to *Basidiobolus ranarum*. Journal of Medical and Veterinary Mycology 29:215-223.

Gruia-Gray J, Desser SS. 1992. Cytopathological observations and epizootiology of frog erythrocytic virus in bullfrogs (*Rana catesbiana*). Journal of Wildlife Diseases 28:34-41.

Gruia-Gray J, Petric M, Desser S. 1989. Ultrastructural, biochemical and biophysical properties of an erythrocytic virus of frogs from Ontario, Canada. Journal of Wildlife Diseases 25(4):497-506.

Gruia-Gray J, Ringuette M, Desser SS. 1992. Cytoplasmic localization of the DNA virus frog erythrocytic virus. Intervirology 33(3):159-64.

Hardouin J. 1991. A bull-frog rearing enterprise in the Philippines. (Un élevage de grenouilles-tareaux aux Philippines.) Tropicultura 9(1):34-36.

He JG, Lu L, Deng M, He HH, Weng SP, Wang XH, Zhou SY, Long QX, Wang XZ, Chan SM. 2002. Sequence analysis of the complete genome of an iridovirus isolated from the tiger frog. Virology 292(2):185-197.

Hedrick RP, McDowell TS. 1995. Properties of iridoviruses from ornamental fish. *Vet Res.* 26(5-6):423-7.

Honeyman, V. L., Mehren, K. G., Barker, I. K., Crawshaw, G. J. 1992. *Bordetella* septicaemia and chlamydiosis in eyelash leaf frogs (*Ceratobatrachus guentheri*). In: Proceedings of the Joint Meeting of the American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians. Pp 168.

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

Hubbard GB. 1981. *Aeromonas hydrophila* infection in *Xenopus laevis*. *Laboratory Animal Science* 31:297-300.

Hyatt AD, Gould AR, Zupanovic Z, Cunningham AA, Hengstberger S, Whittington RJ, Kattenbelt J, Coupar BEH. 2000. Comparative studies of piscine and amphibian iridoviruses. *Archives of Virology* 145(2):301-331.

Jancovich JK, Davids EW, Seiler A, Jacobs BL, Collins JP. 2001. Transmission of the *Ambystoma tigrinum* virus to alternative hosts. *Diseases of Aquatic Organisms* 46(3):159-163.

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

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

Kmety and Dikken. Classification of the species *Leptospira Interrogans* and history of its serovars.

Kostiukov MA, Alekseev AN, Bulychev VP, Gordeeva ZE. 1986. Experimental evidence for infection of *Culex pipiens* L. mosquitoes by West Nile fever virus from *Rana ridibunda* Pallas and its transmission by bites. *Med Parazitol (Mosk)* (6):76-8.

Kostiukov MA, Gordeeva ZE, Bulychev VP, Nemova NV, Daniyarov OA. 1985. The lake frog (*Rana ridibunda*)--one of the food hosts of blood-sucking mosquitoes in Tadzhikistan--a reservoir of the West Nile fever virus] *Med Parazitol (Mosk)* (3):49-50.

Kozuch O, Labuda M, Nosek J. 1978. Isolation of sindbis virus from the frog *Rana ridibunda*. *Acta Virol.* 22(1):78.

Langdon JS. 1989. Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, *Perca fluviatilis* L., and 11 other teleosts. *Journal of Fish Diseases* 12:295-310.

- Laurance, W. F.; McDonald, K. R.; Speare, R. 1996. Epidemic disease and catastrophic decline of Australian rain forest frogs. *Conservation Biology* 10(2):1-9.
- Laurance, W. F.; McDonald, K. R.; Speare, R. 1997. In defence of the epidemic disease hypothesis. *Conservation Biology* 11:1030.
- Lips KR. 1999. Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology* 13:117-125.
- Longcore JE, Pessier AP, Nichols DK. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91:219-227.
- Lucké B. 1934. A neoplastic disease of the kidney of the frog, *Rana pipiens*. *American Journal of Cancer* 20:352-379.
- Lucké B. 1938. Carcinoma in the leopard frog: Its probable causation by a virus. *Journal of Experimental Medicine* 63:457-468.
- Lynch M. 2001. Amphibian quarantine protocols: Melbourne Zoo. Attachment 6. In: Speare, R. and Steering Committee of Getting the Jump on Amphibian Disease. Developing management strategies to control amphibian diseases: Decreasing the risks due to communicable diseases. School of Public Health and Tropical Medicine, James Cook University, Townsville. Pp 179-183.
- Maniero GD, Carey C. 1997. Changes in selected aspects of immune function in the leopard frog, *Rana pipiens*, associated with exposure to cold. *Journal of Comparative Physiology [B]* 167(4):256-263.
- Mao J, Green DE, Fellers G, Chinchar VG. 1999. Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. *Virus Res.* 63(1-2):45-52.
- Mao J, Wang J, Chinchar GD, Chinchar VG. 1999. Molecular characterization of a ranavirus isolated from largemouth bass *Micropterus salmoides*. *Dis Aquat Organ.* 37(2):107-14.
- Marquez R, Olmo JL, Bosch J. 1995. Recurrent mass mortality of larval midwife toads *Alytes obstetricans* in a lake in the Pyrenean mountains. *Herpetological Journal* 5:287-289.
- Mastura AB, Ambu S, Hasnah O, Rosli R. 1996. Spargana infection of frogs in Malaysia. *Southeast Asian Journal of Tropical Medical Public Health* 27:51-52.
- May R. 1998. Conservation and Disease. *Conservation Biology* 2:28-30.
- Mazonni R. 2000. Diseases in farmed American bull frog (*Rana catesbiana* Shaw, 1802) in Uruguay. *Proceedings Getting the Jump! On Amphibian Disease*, 26-30 August, Cairns. Pp37.

- McDonald K, Speare R. 2001. Banana frogs: What's in the box. Fourth National Banana Industry Conference, 7 June, Cairns.
- McKinnell RG. 1969. Lucké renal adenocarcinoma: Epidemiological aspects. In: Recent Results in Cancer Research (Special Supplement) Biology of Amphibian Tumours. Ed M. Mizell. Berlin: Springer Verlag. Pp 254-260.
- McKinnell RG. 1973. The Lucké frog kidney tumor and its herpesvirus. *American Zoologist* 13:97-114.
- McKinnell RG, Carlson DL. 1997. Lucke renal adenocarcinoma, an anuran neoplasm: studies at the interface of pathology, virology, and differentiation competence. *Journal of Cellular Physiology* 173(2):115-118.
- McKinnell RG, Gorham E, Martin FB, Schaad JW 4th. 1979. Reduced prevalence of the Lucke renal adenocarcinoma in populations of *Rana pipiens* in Minnesota. *J Natl Cancer Inst.* 63(3):821-4.
- Mikaelian I, Ouellet M, Pauli B, Rodrigue J, Harshbarger JC, Green DM. 2000. *Ichthyophonous*-like infection in wild amphibians from Quebec, Canada. *Diseases of Aquatic Organisms* 40:195-201.
- Miller EA, Montali RJ, Ramsay EC, Rideout BA. 1992. Disseminated chromoblastomycosis in a colony of ornate horned frogs (*Ceratophrys ornata*). *Journal of Zoo and Wildlife Medicine* 23(4):433-438.
- MokWY, Carvalho CM. 1984. Occurrence and experimental infection of toads (*Bufo marinus* and *B. granulosis*) with *Mycobacterium chelonae* subsp. *abscessus*. *Journal of Medical Microbiology* 18:327-333.
- Monzon Moreno C, Ojeda Vargas MM, Echeita A, Usera MA. 1995. Occurrence of Salmonella in cold-blooded animals in Gran Canaria, Canary Islands, Spain. *Antonie Van Leeuwenhoek* 68(3):191-194.
- Moody NJG, Owens L. 1994. Experimental demonstration of the pathogenicity of a frog virus, Bohle iridovirus, for a fish species, barramundi – *Lates calcarifer*. *Diseases of Aquatic Organisms* 18(2):95-102.
- Morehouse E, James TY, Ganley ARD, Vilgalys R, Berger L, Murphy P, Longcore JE. 2002. Genetic evidence suggests the fungal agent of chytridiomycosis in amphibians is a recently emerged, pandemic clone.
- Morgan J. 2000. Progress report on chytrid DNA project to Rick Speare and Lee Berger, from Jess Morgan, ex Parasitology, University of Queensland.
- Morse SS. 1995. Factors in the emergence of infectious diseases. *Emerging Infectious Diseases* 1:7-15.

- Mutschmann F, Berger L, Zwart P, Gaedicke C. 2000. Chytridiomycosis in amphibians--first report in Europe. *Berl Munch Tierarztl Wochenschr.* 113(10):380-383.
- Newcomer CE, Anver MR, Simmons JL, Wilcke BW, Nace GW. 1982. Spontaneous and experimental infections of *Xenopus laevis* with *Chlamydia psittaci*. *Laboratory Animal Science* 32:680-686.
- Nyman S. 1986. Mass mortality in larval *Rana sylvatica* attributable to the bacterium, *Aeromonas hydrophila*. *Journal of Herpetology* 20:196-201.
- Obendorf DL, Peel BF, Munday BL. 1993. *Mucor amphibiorum* infection in platypus (*Ornithorhynchus anatinus*) from Tasmania. *Journal of Wildlife Diseases* 29:485-487.
- Olson ME, Gard S, Brown M, Hampton R, Morck DW. 1992. *Flavobacterium indologenes* infection in leopard frogs. *Journal of the American Veterinary Medical Association* 201:1766-1770.
- O'Shea P, Speare R, Thomas AD. 1990. Salmonellas from the cane toad, *Bufo marinus*. *Australian Veterinary Journal* 67(8):310.
- Petersen LR, Roehrig JT. 2001. West Nile Virus: A reemerging global pathogen. *Emerging Infectious Diseases* 7:611-615.
- Parker JM, Mikaelian I, Hahn N, Diggs HE. Clinical diagnosis and treatment of epidermal chytridiomycosis in African Clawed Frogs (*Xenopus tropicalis*). Manuscript in press.
- ProMED mail. Frog mortality, virus – UK. Fri 1 Feb 2002. Archive number: 20020201.3458
- Ragan MA, Goggin CL, Cawthorn RJ, Cerenius L, Jamieson AVC, Plourde SM, Rand TG, Soderhall K, Gutell RR. 1996. A novel clade of protistan parasites near the animal-fungal divergence. *Proceedings of the National Academy of Sciences, USA* 93:11907-11912.
- Ramakrishnan L, Valdivia RH, McKerrow JH, Falkow S. 1997. *Mycobacterium marinum* causes both long-term subclinical infection and acute disease in the leopard frog (*Rana pipiens*). *Infection and Immunity* 65:767-773.
- Reed KD, Ruth GR, Meyer JA, Shukla SK. 2000. *Chlamydia pneumoniae* infection in a breeding colony of African clawed frogs (*Xenopus tropicalis*). *Emerging Infectious Diseases* 6:196-199.
- Reichenbach-Klinke H, Elkan E. Amphibia. 1965. In: *The Principal Diseases of Lower Vertebrates*. Academic Press, New York. Pp 209-384.

- Rush HG, Anver MR, Beneke ES. 1974. Systemic chromomycosis in *Rana pipiens*. *Laboratory Animal Science* 24:646-655.
- Sandars DF. 1953. A study of Diphyllbothriidae (Cestoda) from Australian hosts. *Proceedings of the Royal Society of Queensland* 63:65-70.
- Sarvamangala Devi JN, Shivananda PG. 1983. Frogs as natural reservoirs of *Salmonella*. *Indian Journal of Medical Research* 78:465-470.
- Sastrawan P. 1978. Studies on the taxonomy and biology of the cestode *Spirometra erinacei*. Unpub Masters thesis, Dept of Parasitology, University of Queensland, Brisbane.
- Schmidt GD, Kuntz RE. 1969. Nematode parasites of Oceanica. VI. *Foleyella confusa* sp.nov., *Icosiella hoogstraali* sp.nov. (Filarioidea), and other species from Philippine amphibians. *Parasitology* 59(4):885-889.
- Scott ME. 1998. The impact of infection and disease on animal populations: Implications for conservation. *Conservation Biology* 2:40-56.
- Sharma VK, Kaura YK, Singh IP. 1974. Frogs as carriers of *Salmonella* and *Edwardsiella*. *Antonie von Leeuwenhoek* 40:171-175.
- Slocombe R, McCracken H, Booth R, Slocombe J, Birch C. 1995. Infectious skin diseases of captive frogs. In: *Proceedings of the Australian Society for Veterinary Pathology*, Melbourne. Pp 14.
- Smith AW, Anderson MP, Skilling DE, Barlough JE, Ensley PK. 1986. First isolation of calicivirus from reptiles and amphibians. *American Journal of Veterinary Research*. 47:1718-1721.
- Smythe L, Dohnt M, Norris M, Symonds M, Scott J. 1997. Review of leptospirosis notifications in Queensland 1985 to 1996. *Communicable Diseases Intelligence* 21(2):17-20.
- Somsiri T, Chinabut S, Soontornvit S. 1997. Challenge of cultured frogs (*Rana tigerina*) with *Aeromonas* species. In: *Diseases in Asian Aquaculture III*. Fish Health Section. Eds T. W. Flegel, I. H. MacRae. Asian Fisheries Society, Manila. Pp 15-18.
- Speare, R. 1990. Review of the diseases of the Cane Toad, *Bufo marinus*, with comments on biological control. *Australian Wildlife Research* 17:387-410.
- Speare R, Berger L, O'Shea P, Ladds PW, Thomas AD. 1996. The pathology of mucormycosis of free-ranging cane toads (*Bufo marinus*) in Australia. *Journal of Wildlife Diseases* 33:250-255.
- Speare R, Freeland WJ, Bolton SJ. 1991. A possible iridovirus in erythrocytes of *Bufo marinus* in Costa Rica. *Journal of Wildlife Diseases* 27:457-462.

- Speare R, Smith JR. 1992. An iridovirus isolated from the ornate burrowing frog (*Limnodynastes ornatus*) in northern Australia. *Diseases of Aquatic Organisms* 14:51-57.
- Speare R. 2000. Steering Committee for Getting the Jump on Amphibian Disease. Nomination for listing of amphibian chytridiomycosis as a key threatening process under the Environment Protection and Biodiversity Conservation Act 1999.
- Speare R. 2001. Steering Committee for Getting the Jump on Amphibian Disease. Developing management strategies to control amphibian diseases: decreasing the risks due to communicable disease. School of Public Health and Tropical Medicine, James Cook University. Pp 1-209.
- Speare R, Thomas AD, O'Shea P, Shipton WA. 1994. *Mucor amphibiorum* in the cane toad, *Bufo marinus*, in Australia. *Journal of Wildlife Diseases* 30:399-407.
- Storey C, Lusher M, Yates P, Richmond S. 1993. Evidence for *Chlamydia pneumoniae* of non-human origin. *Journal of General Microbiology* 139:2621-2626.
- Taylor FR, Simmonds RC, Loeffler DG. 1993. Isolation of *Flavobacterium meningosepticum* in a colony of leopard frogs (*Rana pipiens*). *Laboratory Animal Science* 43:105.
- Taylor R, Sloan D, Cooper T, Morton B, Hunter I. 2000. A waterborne outbreak of *Salmonella Saintpaul*. *Communicable Diseases Intelligence* 24(11):336-340.
- Tinsley RC. 1995. Parasitic disease in amphibians: control by the regulation of worm burdens. *Parasitology* 111 Suppl:153-178.
- Waldman B, van de Woolfshaar KE, Klena JD, Andjic V, Bishop PJ, Norman RJ de B. 2001. Chytridiomycosis in New Zealand frogs. *Surveillance* 28(3): 9-11.
- Werner JK. 1993. Blood parasites of amphibians from Sichuan Province, People's Republic of China. *Journal of Parasitology* 79(3):356-363.
- Wilcke BW, Newcomer CE, Anver MR, Simmons JL, Nace GW. 1983. Isolation of *Chlamydia psittaci* from naturally infected African clawed toads (*Xenopus laevis*). *Infection and Immunity* 41:789-794.
- Williams RW. 1960. Observations on the life history of *Rhabdias sphaerocephala* Goodey, 1924 from *Bufo marinus* L., in the Bermuda Islands. *Journal of Helminthology* 34(1/2):93-98.
- Wolf K, Bullock GL, Dunbar CE, Quimby MC. 1968. Tadpole edema virus: a viscerotropic pathogen for anuran amphibians. *Journal of Infectious Diseases* 118:253-262.

World Health Organization/Food and Agriculture Organization Collaborating Centre for Reference and Research on Leptospirosis, Laboratory of Microbiology and Pathology, Brisbane, Queensland. Personal communication March 2002.

Zhang QiYa, Xiao Feng, Li ZhenQiu, Gui JianFang, Mao Jinghe, Chinchar, V. G. 2001. Characterization of an iridovirus from the cultured pig frog *Rana grylio* with lethal syndrome. *Diseases of Aquatic Organisms* 48(1): 27-36.

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". *Diseases of Aquatic Organisms* 32:1-8.

WEB SITES

Centers for Disease Control and Prevention. Japanese encephalitis.
<http://www.cdc.gov/japaneseencephalitis/>. Accessed 27 April 2002.