Ranaviral disease in wild Australian amphibians

Fact Sheet

December 2016

Introductory statement

Ranaviruses have been associated with disease outbreaks causing significant mortality and morbidity in wild amphibians, reptiles, and both cultivated and wild fish [1]. Ranaviruses have been reported from most continents, including Australia [2, 3]. In 2008, ranaviral disease of amphibians was included as a notifiable list disease by the World Organisation for Animal Health OIE [4]. Amphibian ranaviral disease is considered an emerging infectious disease, as it is now detected over an increasing species and geographic range [5]. Bohle iridovirus (BIV), a ranavirus, has been reported in amphibians in Australia. A recent online book on ranaviruses [1] summarises current knowledge on taxonomy, ecology, immunity and diagnosis and readers are referred to this source for further information.

Aetiology

In amphibians, ranaviral disease is caused by several species of closely related double-stranded DNA viruses belonging to the genus *Ranavirus* in the family *Iridoviridae*. There are four other genera in the family: *Iridovirus* and *Chloridovirus* (insect viruses), and *Lymphocystivirus* and *Megalocytivirus* (marine and tropical fish viruses) [6].

Natural hosts

Ranaviruses have been identified in a range of ectothermic[1] vertebrates, including fish, amphibians (frogs, toads, salamanders) and reptiles (lizards, turtles, snakes). Some ranaviruses can infect a broad host range across all these taxa. Trans-taxon infection between fish and amphibians [7], and reptiles and amphibians is suggested by surveillance data from wild populations in the USA [8], and by experimental infection [9].

World distribution

Amphibian ranaviruses have been reported from many countries and regions including Asia, North America, Latin America and Europe (see Appendix 1).

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[1] An animal that relies on external sources of heat: a “cold blooded” animal e.g. frogs, fish and reptiles.

The geographical range of Bohle iridovirus (BIV) (previously only reported in Australia) has recently expanded to the USA, where it caused high mortality in captive, endangered boreal toads (*Anaxyrus boreas boreas*) [10]. A virus isolated from a diseased leaf tailed gecko (*Uroplatus fimbriatus*) in Germany [11] has been shown to be extremely closely related to BIV [12].

Occurrences in Australia

Data on the geographical origin and time of emergence or introduction of ranaviruses into Australia is not known. Only limited data are available on geographical and species range of ranaviruses in Australia.

Bohle iridovirus (BIV; a *Ranavirus*) was isolated from metamorphs[2] of captive ornate burrowing frogs (*Limnodynastes ornatus*), from Bohle in northern Queensland, where it caused mortality during and after metamorphosis [13]. Laboratory studies in Australia have also shown that both cane toads and native frogs are vulnerable to BIV [14, 15]. Tadpoles appear the most susceptible, while juvenile frogs were more susceptible than adults. In challenge experiments, juvenile *L. caerulea, Litoria alboguttata, Cyclorana brevipes* and *P. corieacea* were acutely susceptible whilst adult *L. rubella, L. inermis, L. caerulea, Cophixalus ornatus, Taudactylus acutirostris* and cane toads were less susceptible [15].

In Darwin, an irido-like virus was associated with disease and mortalities in captive magnificent tree frogs (*Litoria splendida*) and green tree frogs (*Litoria caerulea*) [2].

Wild, moribund adult *Litoria caerulea* from Townsville and captive juvenile red-backed toadlets (*Pseudophryne coriacea*) from Sydney have tested positive for BIV with PCR [14, 15].

While disease from ranavirus has not been frequently observed in wild amphibians in Australia, antibodies to ranaviruses have been detected widely in cane toads (*Rhinella marina*) in NSW, Qld and NT. The overall prevalence was 2.7% (range 0-18%). The identity of the ranavirus that induced the antibodies is unknown and no viruses were isolated from any individuals [16].

Epizootic haematopoietic necrosis virus (EHNV), a ranavirus, was associated with disease and mortality in Australian wild redfin perch (*Perca fluviatilis*) but does not appear to infect wild amphibians.

Some ranaviruses not found in Australia have been shown to cause disease in native Australian amphibians in experimental challenges for example, Venezuelan Guatopo virus was able to kill *L. caerulea* in experimental trials [17]. Green python Wamena virus (a *Ranavirus*) was isolated in Australia, but originated from a python illegally imported from Indonesia [3].

Epidemiology

Globally, ranaviruses can cause >90% mortality in the wild during mass mortality events [18]. However, ranaviruses have not been implicated in mass mortality events in Australia.

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[2] Amphibian larvae (e.g. tadpoles) in the process of turning into adults (e.g. frogs)

The epidemiology of ranavirus in amphibians in Australia is poorly understood but has been described in amphibians in North America and the United Kingdom, where epidemics associated with high mortality have been regularly reported [19, 20]. In the UK, recurrent annual mass mortalities have occurred since the 1980s. High amphibian mortality due to ranavirus in South America has also been described [21]. Risk is believed to increase with urbanisation and anthropogenic change to amphibian environments [22].

Tadpoles, metamorphs and juvenile frogs appear more susceptible to ranaviral disease than adult amphibians, but disease can occur in all life stages [15, 18]. Detailed information on mortality rate, incubation period and transmission is available for *Ambystoma tigrinum* [tiger salamander] virus (ATV):

Mortality rate: In the wild, most animals infected with ATV die, usually within 8–14 days. However, larvae leaving the ponds to overwinter as juveniles can be positive for ATV without any signs of infection for more than 5 months [23]. Brunner et al. 2005 [24] report that ATV had a mortality rate of 88.1% in a laboratory trial. In another study, all frogs that were experimentally infected with UK *Ranavirus* died with systemic haemorrhage 6-8 days after inoculation [25]. Mortality in captive boreal toads with BIV infection was 91% [10].

Incubation period: Experimentally, the inoculation period appears to depend on host characteristics and the dose of inoculation. In one experiment involving ATV, the number of days before clinical signs appeared ranged from 10-30 days, while time to death ranged from 15-35 days. The time to death after the onset of signs was typically short, between 3-10 days [24].

Transmission: ATV is efficiently transmitted via direct contact between animals (bumping, biting and cannibalism), as well as by necrophagy, and indirectly via water and fomites [24, 26, 27]. Salamander larvae become infectious soon after ATV exposure, while their ability to infect increases with time. In the wild, ATV appears to be reintroduced to ponds each year by adults returning from terrestrial refugia to breed, and from sub-lethally infected dispersing juveniles [23]. Indirect transmission of ATV (e.g. from infected water to susceptible larvae) can occur, presumably via blood, exudates, or other tissues shed into the environment [19]. In Canada, an FV3 (frog virus 3)-like *Ranavirus* infects multiple species in amphibian communities. The high prevalence in salamanders implicates them as hosts and also reservoirs for this virus [28]. Horizontal transmission through exposure to infected pond water is the most likely route of infection to sympatric anurans.

Some ranaviruses, including BIV, are environmentally resistant and can remain infectious for long periods in certain environments [29]. Studies with EHNV showed that it remained infective in water and after drying for over 100 days at 15 °C [30]. Heating to 60 °C for 15 minutes or 40 °C for 24 hours inactivated the virus.

Clinical signs

Clinical signs of acute ranaviral disease are seen in tadpoles, metamorphs, juveniles and adults. In general, tadpoles and metamorphs exhibit decreased activity, ascites, focal haemorrhages and death. Adults exhibit decreased activity, skin ulceration, focal and systemic haemorrhages and death [17, 31].

For BIV, clinical signs in the original epidemic included oedema of subcutaneous tissue, especially around the jaw and head, with the tongue protruding. Subcutaneous haemorrhages occurred on the ventral abdomen, inguinal areas and lower jaw (R. Speare, unpub. data).

Overseas, the UK *Ranavirus* in common frogs (*Rana temporaria*) causes two disease syndromes. The first (Ulcerative Syndrome; US) is characterised by skin ulceration, while the second (Haemorrhagic Syndrome; HS) is characterised by systemic haemorrhage. HS usually presents with large numbers of morbid or moribund frogs being found at a site. US is a chronic disease, with frogs usually being found in poor body condition but alive, while deaths occur more slowly at one site over a period of weeks or months [32].

In North America, tadpoles and salamanders with ranaviral disease are lethargic and slow moving, swimming in circles with buoyancy problems and an inability to remain upright. They have haemorrhages or swollen areas on the ventral surface, near the gills and hind limbs. Older tadpoles may show signs of oedema. Haemorrhagic and necrotic foci are seen in many internal organs. Clinical signs of disease caused by ATV in captive tiger salamanders include a combination of loose or bloody faeces, anorexia, vomiting (occasionally bloody), small pale raised foci in the skin and cutaneous erosions and ulcers. Death occurred within 48 hours of developing bloody faeces. The skin of infected salamanders became dark and speckled and shed skin was fragmented. Some individuals produced thick, sticky mucus from the back and tail causing several to become stuck to their cages [33].

Diagnosis

Iridovirus infections (including *Ranavirus*) cannot be diagnosed from clinical signs alone and infected amphibians may not show signs of disease. However, high mortality rates associated with haemorrhage, oedema, ascites or ulcers are suggestive of ranaviral disease. Histopathology showing severe necrosis of haematopoietic or other tissues is indicative and should be followed by more specific tests, including immunostaining, viral culture on cell monolayers, serology and molecular techniques, to identify the aetiological agent.

PCR analyses are required to distinguish among viral species and strains [34]. Transmission electron microscopy can classify the agent as a member of the *Iridoviridae*. ELISA and other serological tests can identify the genus *Ranavirus.*

Viral culture or PCR is needed to detect low level carrier infections. Marsh et al. 2002 [35] developed tests based on variation of the major capsid protein (MCP) gene sequence to distinguish among important ranaviruses from the regions of Australia, Europe and America.

Real-time PCR is useful as a research and screening tool, with the potential, given further development, to become a sensitive and specific method for detection and differentiation of ranaviruses [6]. A high-throughput real-time PCR procedure validated for EHNV is suitable for sensitive detection of amphibian ranaviruses including surveillance for subclinical infection [36].

Laboratory diagnostic specimens and procedures

For laboratory diagnosis of ranaviral disease in dead animals, fresh or frozen carcases, fresh or frozen tissues (e.g. spleen or kidney) and tissues fixed in 10% formalin should be submitted. If it is not possible to transport fresh/frozen tissues, samples should be fixed in 70% ethyl alcohol and submitted in addition to formalin fixed tissues [17].

For screening healthy animals, molecular tests of liver tissue can detect the presence of *Ranavirus*; however, this method is lethal [37]. In salamanders, non-lethal tail clips can be tested for *Ranavirus* [23]. Although toe-clips have been shown to yield sufficient DNA for PCR tests they do not reflect the location of subclinical ranaviral infection and are not validated for this purpose [38]. Non-lethal tests are less sensitive than testing of internal organs [39].

Testing can be done through the CSIRO Australian Animal Health Laboratory. Submission is generally through the state or territory diagnostic laboratory. See section 'Diagnosis'. Tests are available at the University of Sydney, co-host for the OIE reference laboratory for *Ranavirus* infection of amphibians. Details of the laboratory diagnostic procedures that are used are provided in the OIE Manual of Diagnostic tests [40].

Clinical pathology

Not reported.

Pathology

With BIV, typical pathology in natural and experimental infections included severe renal, pulmonary, hepatic, splenic and haemopoietic necroses and haemorrhages. *Ranavirus* immunoperoxidase stained many cell types in liver, lung, spleen, and in particular fibrocytes in extensive areas of swollen, necrotic dermis and glomeruli [17, 41]. Jerrett et al. 2015 [31] provide excellent illustrations of the histopathological changes and immunohistochemistry (IHC) staining in *Litoria splendida* and *L. caerulea*, with disease from a natural infection with Mahaffey Road virus (a BIV-like isolate). There was widespread lymphoid and fibroblastic necrosis with vasculitis in a wide range of tissues and intracytoplasmic basophilic inclusion bodies were present in several cell types including hepatocytes.

Pathology in infected hatching fresh-water turtles *Elseya (Myuchelys) latisternum* and *Emydura krefftii* that were experimentally infected with BIV included focal necrosis and infiltration of granulocytes in the pancreas, liver and sub-mucosa of the intestine [42]. Similar pathology was described for adult boreal toads with disease caused by BIV [10]

With UK ranavirus infections, histologic lesions include epidermal thickening and necrosis, with granulocytic inflammation, congestion and haemorrhage of internal tissues and intracytoplasmic inclusion bodies.

With North American ranaviruses, histological changes are similar to BIV, with widespread necrosis and haemorrhages in haemopoietic and other tissues. Basophilic intracytoplasmic inclusion bodies may be found in the liver or epidermis. Necrosis in the spleen, renal myeloid cells and glomeruli is typical [43].

Differential diagnoses

The only other disease with similar clinical signs that causes mass die-offs in wild adult amphibian populations is chytridiomycosis, caused by infection with *Batrachochytrium dendrobatidis*. Clinical signs include ventral reddening and occasionally skin ulcers. Frogs with severe chytridiomycosis are easily diagnosed by histology to detect spherical sporangia of *B. dendrobatidis* in the epidermis. Bacteria septicaemia ("red-leg") occurs in captivity, with similar clinical signs and gross pathology.

Treatment

Currently, there is no effective treatment for iridoviral diseases, including ranaviral disease.

Prevention and control

In the absence of effective antiviral drugs or vaccines, the best control strategy for ranaviruses is prevention. Infected animals should be quarantined to prevent transmission to healthy animals via food, faeces and water [34].

When working with wild or captive amphibians, measures must be taken to minimise the risk of transmission or the exposure of amphibians to new strains of *Ranavirus*. This is best achieved through careful hygiene control and disinfection of potential fomites between sites and animals [5]. At the site level, equipment such as vehicles (boats, cars), traps, nets, boots and waders should be mechanically scrubbed and disinfected.

Common disinfectants (70% ethanol, 70% isopropyl alcohol, 10% household bleach) inactivate ranaviruses if applied liberally, for sufficient time, and in conjunction with mechanical scrubbing [44]. A dilute bleach solution is effective and inexpensive, but must be used with care for aquatic organisms [45] and preferably at a known final working concentration[3]. Ethanol (70%) is effective against EHNV (Langdon, 1989) and can inactivate ranaviruses if given sufficient time or if used to flame equipment [44]. Quaternary compounds are also effective and are less corrosive than bleach; however, careful rinsing is required to remove soapy residues [5]. Any disinfectant must be applied for the specified amount of time to be effective [45]. Glutaraldehyde and artificially generated ultraviolet light are also effective disinfectants [17]. Bleach (sodium hypochlorite) used at 3% final concentration and 1% Virkon S® were effective at inactivating an amphibian *Ranavirus* after 1 min exposure time and a 2% chlorhexidine product (Nolvasan®) provided a 10^3-fold reduction in ranavirus infectivity [46]. For disinfection using heat, appropriate temperature, application time and moist environment are essential. Residual infectivity in BIV was retained after exposure to 60°C for 5 min and 56°C for 1 h [29].

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[3] A final working concentration for sodium hypochlorite (household bleach) may have variable concentration. The available chlorine for a calculated final concentration may be less than expected for some sample matrices if biological load is not considered; see info from 46. Bryan LK, Baldwin CA et al. (2009) Efficacy of select disinfectants at inactivating *Ranavirus. Diseases of Aquatic Organisms*, **84**(2): 89-94.

A report for the Australian Government Department of Climate Change, Energy, the Environment and Water, *Hygiene protocols for the control of diseases in Australian frogs*, is available from https://www.dcceew.gov.au/sites/default/files/documents/frogs-hygiene-protocols.pdf

Research

The CSIRO Australian Animal Health Laboratory maintains an *Iridovirus* reference collection and can undertake a range of diagnostic tests. The main research group in Australia is led by Prof. Richard Whittington at the OIE Reference Laboratory for Infection with *Ranavirus* within the Faculty of Veterinary Science, Camden, NSW (+61 2 9351 1619).

Research focus should include investigating the risk and consequences of ranaviral disease emergence as well as the introduction of new ranaviruses into Australia. Basic risk assessment for Australian amphibians with respect to ranaviral infection is recommended, given the role that ranaviruses have played in causing mass mortality events and population declines in other parts of the world. This might include experimental transmission of UK and US ranaviruses to Australian species to determine their susceptibility and studies to predict, in Australia, the potential geographic range of ranaviruses known to cause mass mortality events overseas. The epidemiology of ranaviruses in the Australian context is poorly understood and warrants further focus.

Surveys of the number of potential host species, ranaviruses and their distribution are recommended. Testing of archived specimens may help to determine for how long ranaviruses have been present in Australia.

Surveillance and management

Infection with *Ranavirus* species is an WOAH listed disease [4]. Information on the disease, including epidemiology and surveillance is available in the OIE Aquatic Animal Health Manual: www.woah.org/fileadmin/Home/eng/Health_standards/aahm/current/2.1.03_RANAVIRUS.pdf. It has been recommended that the risk of importation could be more adequately addressed via the application of validated real-time PCR procedures and risk based surveillance methodology to regulate animals moving within trade systems and for mixing in captive conservation collections [35]. Gray and Chinchar 2015 [1] provide recommendations for surveillance and risk assessment for ranaviruses. Management strategies to control amphibian diseases can be found on the Amphibian Diseases Homepage, which is hosted by the ARWH: https://arwh.org/wp-content/uploads/2021/03/Ranaviruses-in-Amphibians.pdf

Wildlife Health Australia administers Australia's general wildlife health surveillance system, in partnership with government and non-government agencies. Wildlife health data is collected into a national database, the electronic Wildlife Health Information System (eWHIS). Information is reported by a variety of sources including government agencies, zoo based wildlife hospitals, sentinel veterinary clinics, universities, wildlife rehabilitators, and a range of other organisations and individuals. Targeted surveillance data is also collected by WHA. See the WHA website for more information https://wildlifehealthaustralia.com.au/Our-Work/Surveillance and https://wildlifehealthaustralia.com.au/Our-Work/Surveillance/eWHIS-Wildlife-Health-Information-System

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. Negative data are also valuable. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Statistics

There is one report of *Ranavirus* in a group of captive tree frogs in eWHIS [2].

Human health implications

Ranaviruses will not infect humans since they will not multiply above 33°C.

Conclusions

Ranaviral disease in Australian amphibians has been detected sporadically but might become increasingly important with environmental change and the keeping of amphibians in captivity. Ranaviruses appear to be widespread (as suggested by the survey for antibodies in cane toads) and molecular epidemiology suggests that these viruses can spread worldwide. There is concern around the potential introduction into Australia of highly pathogenic *Ranavirus* species that have caused recurrent mortality events in other countries. Strict quarantine, testing and biosecurity are the management options of choice to reduce the risk to Australia.

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Wildlife Health Australia recognises the Traditional Custodians of Country throughout Australia. We respectfully acknowledge Aboriginal and Torres Strait Islander peoples' continuing connection to land, sea, wildlife and community. We pay our respects to them and their cultures, and to their Elders past and present.

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Appendix 1: Ranaviruses reported from amphibians

The table is adapted from that in the ARWH document https://arwh.org/wp-content/uploads/2021/03/Ranaviruses-in-Amphibians.pdf

Note, Table 2 in Duffus et al. 2015 [47] provides an up-to-date and comprehensive summary of this information.

Location	Virus	Species	Reference
China	Tiger frog virus (TFV)	Tiger frog (*Rana tigrina*)	He et al. 2002 [48]
	Rana grylio virus (RGV)	Rana grylio	Zhang et al. 2001 [49]
Thailand	*Rana tigrina* virus (RTV)	Tiger frog (*Rana tigrina*)	Essbauer and Ahne 2001 [50]

Australia	Bohle iridovirus (BIV)	Ornate burrowing frog (Limnodynastes ornatus)	Speare and Smith 1992 [13]
		Green tree frog (*Litoria caerulea)*	Speare 2000 [14]
		Cane toad (Rhinella marina)	Speare 2000 [14]
	Mahaffey Road ranavirus (MHRV)	Green tree frog Magnificent tree frog (*Litoria splendid)*	Weir et al. 2012 [2]
UK	*Rana* United Kingdom virus (RUK)	Common frog (Rana temporaria)	Cunningham et al. 1996 [51]; [52]
	Rana esculenta iridovirus (REIR)	Edible frog (*Rana esculenta*)	Ahne et al. 1998 [53]
	Bufo United Kingdom virus (BUK)	Common toad (*Bufo bufo)*	Essbauer and Ahne 2001 [50]
Canada	Regina ranavirus (RRV)	Tiger salamander (*Ambystoma* tigrinum diaboli)	Bollinger et al. 1999 [33]
USA	Tadpole edema virus (TEV)	North American bullfrog (*Lithobates catesbeianus*)	Wolf et al. 1968 [20]
	Frog virus 3 (FV3), (FV1, 2, 9-23), LT1-LT4	Leopard frog (*Rana pipiens*)	Hyatt et al. 2000 [52]
	Ambystoma tigrinum virus (ATV)	Tiger salamander	Jancovich et al. 2001 [19]
	T6-20	Eastern newt (Diemictylus viridescens)	Essbauer and Ahne 2001 [50]
	NVT	Notophthalmus viridescens	Essbauer and Ahne 2001 [50]
	TEV, Redwood virus	Red legged frog (*Rana aurora*)	Essbauer and Ahne 2001 [50]
	FV1-3, FV9-23	Leopard frog (*Rana pipiens*)	Essbauer and Ahne 2001 [50]
	Xenopus virus (XV)	African clawed toad (*Xenopus laevis*)	Essbauer and Ahne 2001 [50]
	Rana catesbeiana virus Z (RCV-Z)	North American bullfrog	Majji et al. 2006 [54]
	Zoo rana virus (Bohle-like iridovirus)	Boreal toads (Anaxyrus boreas boreas)	Cheng et al. (2014)
Venezuela	Guatopo virus (GV)	Cane toad	Hyatt et al. 2000 [52]

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