

# Ranaviruses in wild reptiles in Australia

## Fact sheet

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### Introductory statement

Ranaviruses have been associated with disease outbreaks causing significant mortality and morbidity in wild amphibians, reptiles, and cultivated and wild fish. Systemic infection caused by a ranavirus (Wamena virus) has been reported in confiscated green tree pythons (*Morelia viridis*), transported to Australia from Irian Jaya. This was the first report of the infection in any species of snake, and the first in a reptile in Australia. Studies indicate that ranaviruses have the potential to infect and kill a wide range of poikilotherms (fish, amphibians, reptiles) (Duffus et al. 2015). This information indicates there is a major risk associated with the national and international trade in wildlife and the co-translocation of pathogens (Hyatt et al. 2002). An online book on ranaviruses (Gray and Chinchar 2015b) summarises current knowledge on taxonomy, ecology, immunity and diagnosis and readers are referred to this source for further information.

### Aetiology

**Family:** Iridoviridae

**Genus:** *Ranavirus*

Iridoviruses (genera *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Lymphocystivirus*, *Megalocytivirus*) are large double stranded cytoplasmic DNA viruses that can occur as enveloped and non-enveloped forms (Jancovich JK et al. 2012).

A variety of species of ranavirus have been isolated from reptiles, including frog virus 3 (FV3)-like virus, Bohle iridovirus (BIV)-like virus and common midwife toad virus (CMTV)-like virus (Marschang et al. 2016).

### Natural hosts

Ranaviruses infect fish, amphibians and reptiles (Jancovich James K et al. 2015) (see also WHA Fact Sheet “Ranaviral disease in wild Australian amphibians”). At least fourteen families of reptiles have been reported to be susceptible to ranavirus infection, including Agamidae, Anguidae, Boidae, Chameleonidae, Chelydridae, Dactyloidae, Emydidae, Gekkonidae, Iguanidae, Lacertidae, Pythonidae, Testudinidae, Trionychidae and

Varanidae (Duffus et al. 2015; McKenzie et al. 2019; Peiffer et al. 2019). In Japan, ranavirus has been isolated as a potential co-infection from dermatophilosis skin lesions in captive reared inland bearded dragons (*Pogona vitticeps*), a native Australian species (Tamukai et al. 2016).

Trans-taxon transmission of ranaviruses between fish and amphibians (Mao et al. 1999) and reptiles and amphibians is suggested by surveillance data from wild populations in the USA (Currylow et al. 2014), and by experimental infection (Brenes et al. 2014a). Within the Iridoviridae family, the genera *Iridovirus* and *Chloriridovirus* infect invertebrates and *Lymphocystivirus* and *Megalocytivirus* are found in fish only (Jancovich James K et al. 2015).

## World distribution

Ranaviruses have been identified on all continents other than Antarctica (Duffus et al. 2015) with systemic ranaviral infection documented in reptiles in the North America (Duffus et al. 2015; Kimble et al. 2017; McKenzie et al. 2019; Peiffer et al. 2019), Europe and Asia (Duffus et al. 2015).

## Occurrences in Australia

The sole report of naturally infected reptiles within Australia is that of Wamena virus in confiscated green tree pythons (*Morelia viridis*), recently transported to Australia from Irian Jaya (Hyatt et al. 2002). Within Australia, ranaviruses have been isolated from fish (epizootic hematopoietic necrosis virus [EHNV]) (Langdon et al. 1986) and from the ornate burrowing frog (*Limnodynastes ornatus*) and magnificent and green tree frogs (*Litoria splendida* and *L. caerulea*) (Bohle iridovirus [BIV]) (Speare and Smith 1992; Hengstberger et al. 1993; Weir et al. 2012). An erythrocytic virus suspected to be a non-ranaviral iridovirus has been found infecting a diamond python (*Morelia spilota spilota*) in Australia (Hyatt and McCracken, unpublished observations).

Experimental infection trials have shown significant virulence of BIV in hatchling Krefft's river turtles (*Emydura macquarii krefftii*) and saw-shelled turtles (*Myuchelys latisternum*) and juvenile eastern water dragons (*Intelligama lesueurii lesueurii*) (Ariel et al. 2015; MacLaine et al. 2018; MacLaine et al. 2019; Wirth et al. 2019). Despite this seeming virulence, no outbreaks in free-ranging Australian reptiles have been described.

A serological survey of various free-ranging northern Australian reptiles has revealed antibodies to BIV antigen in freshwater turtles (*E. m. krefftii* and *M. latisternum*), freshwater crocodiles (*Crocodyls johnstoni*) and in five species of snakes (*Boiga irregularis*, *Dendrelaphis punctulatus*, *Morelia spilota*, *Liasis childreni* and *Tropidonophis mairii*) (Ariel et al. 2017).

## Epidemiology

Information on the epidemiology of ranaviruses in reptiles is scant. In chelonians in North America, outbreaks occur for the duration of the animals' active (non-hibernating period) as opposed to summer outbreaks seen in amphibians (Brunner et al. 2015). Disease appears to be acute with seemingly high mortality given low seropositivity of populations, even following known outbreaks (Johnson et al. 2008; Johnson et al. 2010; (Belzer and Seibert 2011; Allender et al. 2013a). However, this pattern does not appear to hold for aquatic turtles in the USA and aquatic turtles and freshwater crocodiles in Australia in which seroprevalences range from 15-59% (Goodman et al. 2013; Ariel et al. 2017), indicating much greater resistance to the infection.

Transmission between reptiles and from amphibians and fish to reptiles has been demonstrated experimentally with frog virus 3 (FV3)-like virus (Brenes et al. 2014a; Brenes et al. 2014b). Similarly, BIV isolated from amphibians has been used to successfully infect and cause disease in Australian turtle species (Ariel et al. 2015). The high fatality rate and low seroprevalence of ranavirus in chelonians suggests these are spillover events, with transmission suspected to occur from amphibians (Brunner et al. 2015). It is not clear whether aquatic turtles and freshwater crocodiles may be maintaining the infection within their own populations based on the higher seroprevalences found.

Clinical carrier states with ranaviruses occurs in amphibians and fish. Experimental infection in turtles has created subclinical infections, indicating the same may be true for reptiles (Brenes et al. 2014b; Brunner et al. 2015). Movement of ranaviruses into an area will most probably be by movement of infected amphibians, fish or reptiles and infected equipment and other inanimate objects that have been contaminated by ranaviruses (Brunner et al. 2015). It has also been proposed that a natural source of infection could be from invertebrates. (Just et al. 2001; Marschang et al. 2005).

Generally, ranaviruses have low host specificity (Langdon 1989; Jancovich James K et al. 1997). The virus is highly infectious and capable of surviving for extended periods of time, even in dried material (Langdon 1989).

## Clinical signs

Clinical signs vary between different taxonomic groups of reptiles and possibly with viral aetiology. Respiratory distress, anorexia, palpebral oedema, swelling of the head, neck and legs, ocular and nasal discharges, stomatitis and skin ulcers have been reported in chelonians infected with FV3-like virus (Johnson et al. 2008; Ruder et al. 2010; Belzer and Seibert 2011; Miller et al. 2015; McKenzie et al. 2019). Hatchling freshwater turtles experimentally challenged with BIV were judged to be moribund one day prior to death, having been active and alert until then (Ariel et al. 2015). Various species of lizards demonstrate ulcerative cutaneous lesions associated with ranaviral infections (Stöhr et al. 2013; Tamukai et al. 2016) with the addition of lethargy, anorexia and ocular discharge seen in some species (MacLaine et al. 2019; Peiffer et al. 2019). Granulomatous lesions in the skin and tongue have also been noted in infected lizards (Marschang et al. 2005; Behncke et al. 2013). Lethargy, anorexia and ulceration of the buccal and nasal mucosa is reported in pythons (Hyatt et al. 2002).

## Diagnosis

Diagnosis is by a combination of history, clinical signs, and diagnostic laboratory techniques including PCR, virus isolation, histopathology, immunohistochemistry, electron microscopy and serological techniques such as ELISA. Samples on which one or more of these techniques can be used include swabs, toe/tail clips, whole animals, fixed tissues and blood (Miller et al. 2015). Detection using PCR on bone marrow in decomposed samples has been described by Butkus et al. (2017). The sample and technique most appropriate for the investigation should be discussed with the diagnostic lab prior to sample collection, if possible.

A list of laboratories that routinely conduct ranavirus testing is maintained on the Global Ranavirus Consortium website ([www.ranavirus.org](http://www.ranavirus.org)). OIE reference laboratories for ranavirus in Australia include the CSIRO Australian Animal Health Laboratory in Geelong and the University of Sydney Faculty of Veterinary Science, Camden campus (Miller et al. 2015)

## Clinical pathology

Culture of lesions to exclude the diagnosis of primary bacterial or fungal infection is recommended. However, mixed infections of ranavirus and bacteria have also been described, so identification of a bacterial or fungal agent does not necessarily rule out ranavirus as a diagnosis (Tamukai et al. 2016).

Biochemical changes in chelonians include elevated urea, aspartate aminotransferase, creatinine kinase and lactate dehydrogenase. Toxic change in heterophils is reported in turtles (De Voe et al. 2004).

## Pathology

**Turtles:** Gross findings include subcutaneous and palpebral oedema, ulcerative and necrotising lesions in the skin, mouth and oesophagus, hyperaemic and oedematous lungs, splenomegaly, and enlarged, yellow livers (De Voe et al. 2004; Johnson et al. 2008; Wirth et al. 2019). Microscopically, lesions include fibrinoid vasculitis of multiple organs, including, the skin, mucous membranes, liver, and lungs (De Voe et al. 2004; Johnson et al. 2008). Heterophilic inflammation with necrosis and haemorrhage is seen in the liver, spleen, pancreas and gastrointestinal tract of experimentally challenged hatchling turtles (Ariel et al. 2015; Wirth et al. 2019). Basophilic intracytoplasmic inclusion bodies are sometimes reported in endothelial cells, macrophages, haematopoietic progenitor cells and epithelial cells of the oral mucosa, oesophagus, trachea and stomach (Johnson et al. 2008).

**Lizards:** Purulent to necro-ulcerative dermatitis and hyperkeratosis appears common to multiple species (Stöhr et al. 2013; Maclaine et al. 2019) with the addition of splenic congestion and miliary necrosis and petechial to ecchymotic haemorrhages in the alimentary tract in experimentally infected eastern water dragons. Microscopically, these water dragons exhibited splenic necrosis and necroheterophilic hepatitis with intracytoplasmic inclusions visible in skin, lung and liver (Maclaine et al. 2019).

**Snakes:** Gross lesions in green pythons consisted of oral mucosal ulceration. Microscopically, ulcerative rhinitis, thickening of the pulmonary alveolar walls, periacinar hepatic necrosis, focal peracute tubular necrosis in the kidney, myocardial infarction and diffuse acute necrosis of the spleen were noted (Hyatt et al. 2002).

## Differential diagnoses

- Snakes – including, but not limited to, ophidian paramyxovirus (OPMV) infection, bacterial or fungal stomatitis
- Chelonians – any infectious or non-infectious cause of oral ulceration, oedema, respiratory signs or sudden death.
- Lizards – other causes of bacterial and fungal dermatitis

## Laboratory diagnostic specimens

Table 1. Various potential samples, testing modalities and limitations (adapted from Miller 2015).

Sample	Test	Storage	Limitations
<b>Swab (oral, cloacal and skin lesions)</b>	PCR, virus isolation	Frozen, preferably -80°C	False positives (environmental contamination), total DNA may be minimal, no histology
<b>Toe or tail clip</b>	PCR, virus isolation	Frozen, preferably -80°C	False positives (environmental contamination), no histology
<b>Whole animal or internal organs</b>	PCR, virus isolation, histology, IHC	Chilled; frozen if extended time period	Deceased animals; frozen specimens will have compromised histology
<b>Fixed tissue</b>	PCR, histology, IHC	10% neutral buffered formalin	DNA for PCR is compromised by fixation, no virus isolation
<b>Blood</b>	PCR, virus isolation; ELISA if serum is separated; CBC if blood smear prepared	As per lab specifications	Can be difficult to collect, particularly from small animals
<b>Marrow-containing bones</b>	PCR	Frozen	Relatively easy in turtle shells, as yet untested in snakes and lizards

## Laboratory procedures

- Confirmation of ranavirus presence within tissues can be achieved with PCR, electron microscopy, virus isolation, immunohistochemistry, in-situ hybridization and antigen-capture ELISA (Miller et al. 2015).
- Exposure to ranavirus in reptiles can be tested serologically using ELISA (Ariel et al. 2017).

## Treatment

Most cases are diagnosed post-mortem. Treatment is unlikely a viable option for wild populations, but may have application in captive collections and for animals involved in translocation and recovery programs. Extrapolating from recent research of ranavirus infection in fish, antiviral agents may have effectiveness against ranaviruses (Li et al. 2014). Allender et al. (2013b) found markedly decreased mortality of ranavirus infected red-eared sliders (*Trachemys scripta elegans*) when held at 28°C as compared to 22°C. This finding is supported by reports of behavioural fever increasing resistance to ranavirus disease in frogs (Sauer et al. 2019). These studies indicate heat therapy may hold promise as a treatment, however this is contradicted by studies in some non-reptilian species that show increased virulence of ranaviruses at higher temperatures (Miller et al. 2015).

## 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 (Chinchar and Mao 2000).

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 (Hemingway et al. 2009). 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 (Brunner and Sesterhenn 2001). A dilute bleach solution is effective and inexpensive, but must be used with care for aquatic organisms (Speare et al. 2004) and preferably at a known final working concentration (Hyatt et al. 2002). Ethanol (70%) is effective against EHNIV (Langdon 1989) and can inactivate ranaviruses if given sufficient time or if used to flame equipment (Brunner and Sesterhenn 2001). Quaternary compounds are also effective and are less corrosive than bleach; however, careful rinsing is required to remove soapy residues (Hemingway et al. 2009). Any disinfectant must be applied for the specified amount of time to be effective (Speare et al. 2004). Glutaraldehyde and artificially generated ultraviolet light are also effective disinfectants (Australian Registry of Wildlife Health 2016). Bleach (sodium hypochlorite) used at 3% final concentration and 1% Virkon S<sup>®</sup> were effective at inactivating an amphibian ranavirus after 1 min exposure time and a 2% chlorhexidine product (Nolvasan<sup>®</sup>) provided a 10<sup>3</sup>-fold reduction in ranavirus infectivity (Bryan et al. 2009). 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 (La Fauce et al. 2012).

From experimental trials and the epidemiology of ranaviruses overseas, the most likely outcome of a new ranavirus in Australia would appear to be unpredictable local epidemics. Consequently, the presence of ranaviruses may be highly significant to species that have small populations confined to small geographic areas (Speare et al. 2005). Ranavirus infections in reptiles, particularly lizards, have often been identified in animals transported internationally for the pet trade (Stöhr et al. 2013). It is imperative that strict biosecurity regulations on imported fish, amphibians and reptiles, particularly for the pet trade, are upheld to protect against accidental introduction.

## Surveillance and management

Wildlife disease surveillance in Australia is coordinated by Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities and members of the public. Coordinators in each of Australia's States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. See the WHA website for more information:

[www.wildlifehealthaustralia.com.au/ProgramsProjects/eWHISWildlifeHealthInformationSystem.aspx#requests](http://www.wildlifehealthaustralia.com.au/ProgramsProjects/eWHISWildlifeHealthInformationSystem.aspx#requests)

There are currently no formal surveillance programs for ranaviruses in reptiles in Australia. Infection with ranavirus in reptiles is included in the OIE list of wildlife diseases that are officially “not OIE-listed diseases” but still of interest for surveillance and reporting

([www.oie.int/wahis\\_2/public/wahidwild.php/Diseaseinformation/popup/diseaselist](http://www.oie.int/wahis_2/public/wahidwild.php/Diseaseinformation/popup/diseaselist)). Confirmed cases of ranavirus disease in reptiles should be captured in eWHIS.

## Statistics

There are a very small number of iridovirus reports in reptiles in the National Wildlife Health Surveillance Database ([www.wildlifehealthaustralia.com.au](http://www.wildlifehealthaustralia.com.au)).

## Research

Wild transmission of reptilian ranaviruses needs further experimental investigation using a variety of reptile species, life stages and routes of transmission. The reptilian immune response to these viruses also needs further investigation (Wirth 2018). Further studies on viral epidemiology, biology and molecular structure are required to understand the relationship between ranaviruses of the three ectothermic vertebrate classes (Marschang et al. 2016). The Global Ranavirus Consortium ([www.ranavirus.org](http://www.ranavirus.org)) has been created to facilitate this research.

More research is needed into the effectiveness of heat treatment and antiviral pharmaceuticals (Miller et al. 2015).

## Human health implications

Ranaviruses will not infect humans and other endothermic vertebrates since they will not multiply above 32°C (Gray and Chinchar 2015a).

## Conclusions

Ranavirus disease in reptiles is an emerging threat overseas. The isolation of the virus from clinically affected pythons illegally brought into Australia highlights the risk of introduction of this pathogen into the country if appropriate biosecurity precautions are not followed. More study is required to better understand the epidemiology of the virus, and the potential risk to Australian reptile species.

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## To provide feedback on this fact sheet

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. If you can help, please contact us at [admin@wildlifehealthaustralia.com.au](mailto:admin@wildlifehealthaustralia.com.au).

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to [admin@wildlifehealthaustralia.com.au](mailto:admin@wildlifehealthaustralia.com.au). We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

## Disclaimer

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