Ranaviruses in wild reptiles in Australia

Fact sheet

February 2020

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) [1]. This information indicates there is a major risk associated with the national and international trade in wildlife and the co-translocation of pathogens [2]. An online book on ranaviruses [3] 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 [4].

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 [5].

Natural hosts

Ranaviruses infect fish, amphibians and reptiles [6] (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* [1, 7, 8]. 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 [9].

Trans-taxon transmission of ranaviruses between fish and amphibians [10] and reptiles and amphibians is suggested by surveillance data from wild populations in the USA [11], and by

experimental infection [12]. Within the *Iridoviridae* family, the genera *Iridovirus* and *Chloriridovirus* infect invertebrates and *Lymphocystivirus* and *Megalocytivirus* are found in fish only [6].

World distribution

Ranaviruses have been identified on all continents other than Antarctica [1] with systemic ranaviral infection documented in reptiles in the North America [1, 7, 8, 13], Europe and Asia [1].

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 [2]. Within Australia, ranaviruses have been isolated from fish (epizootic hematopoietic necrosis virus [EHNV]) [14] and from the ornate burrowing frog (*Limnodynastes ornatus*) and magnificent and green tree frogs (*Litoria splendida* and *L. caerulea*) (Bohle iridovirus [BIV]) [15-17]. 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 (*Intellegama lesueurii)* [18-21]. 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*) [22].

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 [23]. Disease appears to be acute with seemingly high mortality given low seropositivity of populations, even following known outbreaks [24, 25]; [26, 27]. 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% [22, 28], 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 [12, 29]. Similarly, BIV isolated from amphibians has been used to successfully infect and cause disease in Australian turtle species [18]. The high fatality rate and low seroprevalence of *Ranavirus* in chelonians suggests these are spillover events, with transmission suspected to occur from amphibians [23]. 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 [23, 29]. 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 [23]. It has also been proposed that a natural source of infection could be from invertebrates. [30, 31].

Generally, ranaviruses have low host specificity [32, 33]. The virus is highly infectious and capable of surviving for extended periods of time, even in dried material [33].

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 [7, 25, 26, 34, 35]. Hatchling freshwater turtles experimentally challenged with BIV were judged to be moribund one day prior to death, having been active and alert until then [18]. Various species of lizards demonstrate ulcerative cutaneous lesions associated with ranaviral infections [9, 36] with the addition of lethargy, anorexia and ocular discharge seen in some species [8, 20]. Granulomatous lesions in the skin and tongue have also been noted in infected lizards [30, 37]. Lethargy, anorexia and ulceration of the buccal and nasal mucosa is reported in pythons [2].

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 [34]. Detection using PCR on bone marrow in decomposed samples has been described by Butkus et al. 2017 [38]. 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). 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 [34]

Laboratory diagnostic specimens and procedures

- Confirmation of *Ranavirus* presence within tissues can be achieved with PCR, electron microscopy, virus isolation, immunohistochemistry, in-situ hybridization and antigen-capture ELISA [34].
- Exposure to *Ranavirus* in reptiles can be tested serologically using ELISA [22].

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

Sample	Test	Storage	Limitations
Swab (oral, cloacal and skin lesions)	PCR, virus isolation	Frozen, preferably -80°C	False positives (environmental contamination), total DNA may be minimal, no histology
Toe or tail clip	PCR, virus isolation	Frozen, preferably -80°C	False positives (environmental contamination), no histology
Whole animal or internal organs	PCR, virus isolation, histology, IHC	Chilled; frozen if extended time period	Deceased animals; frozen specimens will have compromised histology
Fixed tissue	PCR, histology, IHC	10% neutral buffered formalin	DNA for PCR is compromised by fixation, no virus isolation
Blood	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
Marrow-containing bones	PCR	Frozen	Relatively easy in turtle shells, yet untested in snakes and lizards

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 [9].

Biochemical changes in chelonians include elevated urea, aspartate aminotransferase, creatinine kinase and lactate dehydrogenase. Toxic change in heterophils is reported in turtles [39].

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 [21, 25, 39]. Microscopically, lesions include fibrinoid vasculitis of multiple organs, including, the skin, mucous membranes, liver, and lungs [25, 39].Heterophilic inflammation with necrosis and haemorrhage is seen in the liver, spleen, pancreas and gastrointestinal tract of experimentally challenged hatchling turtles [18, 21]. 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 [25].

Lizards: Purulent to necro-ulcerative dermatitis and hyperkeratosis appears common to multiple species [20, 36] 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 [20].

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 [2].

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.

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 [40]. Allender et al. 2013 [41] 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 [42]. 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 [34].

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 [43].

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 [44]. 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 [45]. A dilute bleach solution is effective and inexpensive, but must be used with care for aquatic organisms [46] and preferably at a known final working concentration [2]. Ethanol (70%) is effective against EHNV [33] and can inactivate ranaviruses if given sufficient time or if used to flame equipment [45]. Quaternary compounds are also effective and are less corrosive than bleach;

however, careful rinsing is required to remove soapy residues [44]. Any disinfectant must be applied for the specified amount of time to be effective [46]. Glutaraldehyde and artificially generated ultraviolet light are also effective disinfectants [47]. 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 [48]. 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 [49].

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 [50]. *Ranavirus* infections in reptiles, particularly lizards, have often been identified in animals transported internationally for the pet trade [36]. 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.

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 [5]. The Global Ranavirus Consortium (www.ranavirus.org) has been created to facilitate this research.

More research is needed into the effectiveness of heat treatment and antiviral pharmaceuticals [34].

Surveillance and management

There are currently no formal surveillance programs for ranaviruses in reptiles in Australia. Infection with *Ranavirus* in reptiles is included in the WOAH list of wildlife diseases that are officially "not WOAH-listed diseases" but still of interest for surveillance and reporting (https://www.woah.org/en/disease/ranavirosis-wild-animals/). Confirmed cases of *Ranavirus* disease in reptiles should be captured in eWHIS.

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.

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

Statistics

There are a very small number of *Iridovirus* reports in reptiles in the National Wildlife Health Surveillance Database (www.wildlifehealthaustralia.com.au).

Human health implications

Ranaviruses will not infect humans and other endothermic vertebrates since they will not multiply above 32°C [51].

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.

Acknowledgements

We are grateful to the people who contributed to this fact sheet and would specifically like to thank Cheryl Sangster.

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.

Updated: February 2020

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