

RANAVIRUSES IN AMPHIBIANS

Ranaviruses are a genus of the Iridoviridae family of viruses. Ranaviruses are capable of causing diseases with formidable mortality in amphibians (Speare et al 2001). Ranaviral disease of amphibians is listed as globally notifiable by the OIE.

The Australian Wildlife Health Network Fact Sheet on Ranavirus can be found at: <http://www.wildlifehealth.org.au/FactSheets.aspx>

A detailed review of ranaviruses can be found in:

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/>

Information on ranavirus in the UK can be found at: <http://www.froglife.org/what-we-do/disease-ranavirus-2/>

1. Ranaviral disease in amphibians is caused by multiple "species" of closely related viruses placed in the genus Ranavirus.
2. Ranaviruses are icosahedral, enveloped viruses containing double stranded DNA, and ranging in diameter (vertex to vertex) from 152 to 157 nm (Hyatt et al 2000).
3. Ranaviral disease is an emerging infectious disease of amphibians globally since it is being detected over an increasing geographic range and in more species (Table 1).
4. Ranaviruses have only recently been reported from Asia (Table 1) in association with amphibian mortality.
5. In Australia the evidence implicating ranaviruses in amphibian declines is inconclusive.

BIOLOGY AND PATHOGENICITY OF RANAVIRUSES

6. Some ranaviruses, e.g. Bohle iridovirus, can infect 3 classes of vertebrate (amphibia, reptilia and pisces).
7. Most ranaviruses produce systemic necrosis of haematopoietic tissues except RUK#13 from UK which may be associated with skin ulcers only (Hyatt et al 2000).
8. Ranaviruses have low host specificity in general (i.e. most can infect many species of host (Moody and Owens 1994; Hyatt et al 2000), but some species may have high host specificity (Jancovich et al 2001).
9. Ranaviruses are highly infectious since inoculating doses can be very low.
10. Ranaviruses are robust viruses capable of surviving for extended periods of time even in dried material (Landon 1989).

11. Acclinical carrier states with ranaviruses occur, and are probably the most common state in wild amphibians.
12. 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.
13. Once detected in an area, ranaviruses are not consistently detected thereafter.
14. Ranaviruses may be able to survive in the environment without a host, but will not multiply.
15. Ranaviruses are capable of causing a high incidence of morbidity and mortality in amphibians in captivity and experimentally.
16. Ranaviruses can cause a high incidence of morbidity and mortality in some species of amphibians in the wild.
17. In Australia there have been no epidemics of ranaviral disease detected in wild amphibians.
18. The pathological outcome of infection of amphibians with ranaviruses is variable and difficult to predict.
19. Some factors which determine this outcome are known (age of host, viral characteristics), but the environmental factors that determine the outcome are unknown (e.g. pollution, UV, climate).
20. Chronic ranaviral disease in amphibians can occur experimentally and in the wild.
21. The significance of chronic ranaviral disease on wild amphibian populations is unknown.
22. The potential for amphibian carriers of ranaviruses to release viral particles into the environment is unknown.

Table 1: Ranaviruses reported from amphibians.

Location	Virus	Species	Reference
Asia			
China	Tiger frog virus (TFV)	Tiger frog <i>Rana tigrina</i>	He et al 2002
	Rana grylio virus (RGV)	<i>Rana grylio</i>	Zhang QiYa et al 2001
Thailand	RTV	Tiger frog <i>Rana tigrina</i>	Ahne & Essbauer 2001
Oceania			
Australia	Bohle Iridovirus (BIV)	Ornate burrowing frog <i>Limnodynastes ornatus</i>	Speare & Smith 1992
Europe			
UK	RUK	Common frog <i>Rana temporaria</i>	Drury et al 1995; Hyatt et al 2000

	<i>Rana esculenta</i> iridovirus (REIR)	Edible frog <i>Rana esculenta</i>	Ahne et al 1998
	BUK	Common toad <i>Bufo bufo</i>	Essbauer & Ahne 2001
North America			
Canada	Regina ranavirus (RRV)	Tiger salamander <i>Ambystoma tigrinum diabolic</i>	Bollinger et al 1999
USA	Tadpole edema virus (TEV)	North American bullfrog <i>Rana catesbiana</i>	Wolf et al 1968
	Frog virus 3 (FV3), (FV1, 2, 9-23), LT1-LT4	Leopard frog <i>Rana pipiens</i>	Hyatt et al 2000
	<i>Ambystoma tigrinum</i> virus (ATV)	Tiger salamander <i>Ambystoma tigrinum stebbinsi</i>	Jancovich et al 2001
	T6-20	Red eft <i>Diemictylus viridescens</i>	Essbauer & Ahne 2001
	NVT	<i>Notophthalmus viridescens</i>	Essbauer & Ahne 2001
	TEV, Redwoodvirus	Red legged frog <i>Rana aurora</i>	Essbauer & Ahne 2001
	FV1-3, FV9-23	Leopard frog <i>Rana pipiens</i>	Essbauer & Ahne 2001
	XV	African clawed toad <i>Xenopus laevis</i>	Essbauer & Ahne 2001
South America			
Venezuela	Guatopo virus	Cane toad <i>Bufo marinus</i>	Hyatt et al 2000
	LSV	Tiger frog <i>Rana tigrina</i>	Essbauer & Ahne 2001

EPIDEMIOLOGY

23. The epidemiology of ranaviruses is best understood in North America and UK where local and general epidemics with high mortality have been reported (Wolf et al 1968; Drury et al 1995; Jancovich et al 2001).
24. In Australia the epidemiology of ranaviruses in wild amphibians is not understood since although ranaviruses occur there have been no outbreaks

- or disease detected in wild amphibians although field investigations have been limited.
25. Serological studies on *Bufo marinus* show that ranaviruses are present in New South Wales, Queensland and Northern Territory (Zupanovic et al 1998). Fresh water tortoises in North Queensland also have antibodies against ranaviruses (Ariel 1997).
 26. Serological studies have not been done on other amphibians in Australia since suitable techniques have not been developed for any species other than *B. marinus*.
 27. Of the two endemic ranaviruses in Australia, Bohle Iridovirus (BIV) and Epizootic Haematopoeitic Necrosis Virus (EHNV), only BIV appears capable of infecting amphibians.
 28. BIV can also experimentally infect a number of native and introduced freshwater fish, freshwater turtles, and snakes (Moody and Owens 1994; Ariel 1997).
 29. Some other ranaviruses found outside Australia can cause experimental disease in native Australian amphibians (Zupanovich et al 1998). Guatapo virus killed the green tree frog *Litoria caerulea*.
 30. The potential of foreign ranaviruses and those intercepted in imported fish and reptiles to cause disease in Australian amphibians is unknown.
 31. From experimental trials and the epidemiology of ranaviruses overseas, the most likely outcome of a new ranavirus in Australia would be epidemic disease of an unpredictable extent.
 32. This scenario means that ranaviruses may be highly significant to amphibians that have small populations confined to small geographic areas.

DIAGNOSIS / DETECTION

33. Clinical signs of acute ranaviral disease are seen in tadpoles, metamorphs, juveniles and adults: Tadpoles - decreased activity, ascites, focal haemorrhages, death. Metamorphs - decreased activity, anasarca, ascites, focal haemorrhages, death. Adults - decreased activity, skin ulceration, focal haemorrhages, death.
34. For laboratory diagnosis of ranaviral disease in dead animals submit fresh or frozen carcasses, fresh or frozen tissues (spleen or kidney is best), or tissues fixed in 10% formalin or 70% ethyl alcohol.
35. The current routine techniques for diagnosing ranaviruses in amphibians are histology, virus isolation from tissues, capture ELISA, and PCR. Low grade infections (carrier state) may only be detectable by PCR.
36. The significance of serological tests for ranaviral antibodies in terms of indicating potential for viral shedding is unknown.
37. Laboratory diagnosis of ranaviral disease in live animals is less sensitive
38. Marsh et al 2002 described a simple test using the major capsid protein gene to identify species of ranavirus once isolated in culture.

DISINFECTION

39. Glutaraldehyde, bleach and artificially generated ultraviolet light are effective disinfectants.
40. Ethyl alcohol is not an effective disinfectant for ranaviruses.

SAFETY

41. Individuals working with live ranaviruses must realise that they are dealing with pathogens that are highly virulent to amphibians and must adopt suitable standards of biocontainment to prevent release of laboratory cultures to the wild.
42. The standard of biocontainment needed for ranaviruses is higher than that required for *B. dendrobatidis*.
43. The risks in transmitting ranaviruses by various activities due to humans interacting with amphibians (handling, etc) need to be quantified to enable best practices to be chosen.
44. Ranaviruses will not infect humans since they will not multiply above 33°C.
45. Strategies need to be developed to decrease the risk of commercial culture of amphibians on a mass scale polluting the natural environment with ranaviruses.

REFERENCES

- 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.
- 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.
- 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.
- Drury SEN, Gough RE, Cunningham AA. 1995. Isolation of an iridovirus like - agent from common frogs (*Rana temporaria*). *Veterinary Record* 137:72-73.
- Essbauer, S., Ahne, W., 2001. Viruses of lower vertebrates. *J. Vet. Med., B* 48, 403–475.
- 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.
- 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.

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

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.

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. 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.

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.

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.