

ATTACHMENT 4: Summary of formidable infectious diseases of amphibians

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This document contains a list of key points about the formidable infectious diseases of amphibians. The term "formidable" is used in the sense that the disease below are capable of causing epidemic disease with a high mortality rate.

This summary was delivered by Rick Speare at the conference, "Getting the Jump! On Amphibian Disease", held 26-30 August 2000 in Cairns, Australia. This was the initial presentation in the workshop session (28-29 Aug) to develop feasible recommendations based on best available evidence. During the presentation the points were commented on by the participants and modified as required. The points were again reviewed and slightly modified by the core working group.

Since some of the points made were based on poor quality evidence, additional research is required to improve the confidence of the statement. Where the point is important for improving the evidence base for management decisions, the statement is followed by a **✘ = more research needed**. Some statements not followed by a **✘** may benefit in the scientific sense from research, but if those results will not substantially influence management decisions, a **✘** has not been added.

A glossary of terms is added at the end of the points.

CHYTRIDIOMYCOSIS

1. Chytridiomycosis is a disease of amphibians caused by *Batrachochytrium dendrobatidis*.
2. It is an emerging infectious disease since it has been newly discovered and has been detected over an increasing geographic range.
3. In Australia *B. dendrobatidis* currently appears to occupy four zones with earliest known cases detected at different times: 1) an east coast zone with emergence in Southeast Queensland or northern New South Wales in the late 1970s (first case detected in December 1978) and subsequent spread north and south, 2) a southwest zone in Western Australia with first case detected in 1985 and spread south, north and west, 3) a South Australian zone in the Adelaide region with first case detected in 1996, and 4) a northwest zone in the Kimberley with first case detected in 1999. ✘

BIOLOGY

4. *B. dendrobatidis* has low host specificity, and can probably infect any species of amphibian, even highly terrestrial species.
5. *B. dendrobatidis* is transmitted via a zoospore that requires water as a medium.
6. *B. dendrobatidis* appears unlikely to have a resting phase that is resistant to dehydration. ✘
7. Zoospores and zoosporangia are killed by drying.
8. *B. dendrobatidis* appears to move naturally through the environment at a rate unaffected by human activity; the average rate of movement is 100 km/yr.
9. Once detected in an area, *B. dendrobatidis* can be detected subsequently in that area.
10. *B. dendrobatidis* can grow and multiply without an amphibian host in the laboratory.
11. In nature *B. dendrobatidis* may multiply in a reservoir other than amphibians, and this could be the environment alone independently of an animal host. ✘

PATHOGENICITY

12. *B. dendrobatidis* is a pathogen capable of causing a high incidence of morbidity and mortality in amphibians in captivity.
13. Tadpoles are readily infected but do not suffer obvious clinical effects.
14. Tadpole movement assists with dispersal in a local environment.
15. *B. dendrobatidis* is a pathogen capable of causing a high incidence of morbidity and mortality in some species of amphibians in the wild.
16. Why amphibians with chytridiomycosis die is not known. ✘
17. The species of the host has an effect on the severity of chytridiomycosis. ✘
18. Some strains of *B. dendrobatidis* may be better adapted to the host they are isolated from than to another host. ✘
19. Environmental factors determine the severity of chytridiomycosis.
20. Low temperature appears to increase the severity of chytridiomycosis, while other environmental factors that may do this have not been identified. ✘
21. The probability of an amphibian self-curing after infection with *B. dendrobatidis* is unknown. ✘

DIAGNOSIS / DETECTION

22. Clinical signs of chytridiomycosis in juvenile and adult amphibians include: neurological signs (abnormal posture - hind legs held out from flanks, depressed or absent righting reflex; abnormal behaviour - nocturnal frog sitting out in daylight, lack of flee response, fitting when handled), thickened epidermis (barely visible roughening of skin surface, sloughing of skin surface), sudden death.
23. The best sample to make a diagnosis of chytridiomycosis is skin, particularly the most superficial epidermal layer on the feet.
24. The current routine techniques for diagnosing chytridiomycosis in amphibians (direct mounts, histology, culture) are insensitive. ✘
25. The immunoperoxidase technique will improve sensitivity and specificity. ✘
26. An ELISA technique using skin may improve sensitivity when based on polyclonal antibodies, and specificity when based on monoclonal antibodies. ✘
27. Techniques to detect zoospores using monoclonal antibodies specific to zoospores will enable the force of infection to be calculated for water bodies under different environmental circumstances and will add immensely to the understanding of the epidemiology and natural history. ✘
28. Techniques to detect zoosporangia using monoclonal antibodies specific to zoosporangia will enable a better understanding of the ability of *B. dendrobatidis* to grow in the environment and may answer the question about whether the substrate itself is a reservoir. ✘
29. Analysis of sequences from the ITS (internal transcribed spacer) region of rDNA indicates that up to 6 % variation exists among isolates of *B. dendrobatidis* from Australia, however much baseline work needs to be done before making conclusions about species or strains. ✘

THERAPY / DISINFECTION

30. Antifungal agents can kill *B. dendrobatidis* in culture, but the effect in the infected tadpole, juvenile and adult is variable in terms of cure.
31. Fluconazole and itraconazole are most promising, but effective regimes need to be established. ✘
32. Saline solutions and heat at levels that do not harm tadpoles need to be evaluated either as sole therapeutic regimes or for use in conjunction with antifungal agents. ✘
33. Ethyl alcohol, glutaraldehyde and sodium hypochlorite (bleach) are effective disinfectants. Artificially generated ultraviolet light is probably also effective as a disinfectant. ✘

EPIDEMIOLOGY

34. To accurately understand the epidemiology of any infectious disease of amphibians, a key concept is distinguishing between infection and disease/death.
35. *B. dendrobatidis* appears not to be present in some countries and in some regions within countries. However, detection may be dependent on the extent of searching. ✘
36. In Australia *B. dendrobatidis* is present in 6 of the 7 states, except it may not be present in the Northern Territory. ✘
37. More testing is needed to evaluate the current and historic range of *B. dendrobatidis* in Australia. ✘

38. From a disease control perspective *B. dendrobatidis* appears to 1) have emerged in Australia in the 1970s in Southeast Queensland or northern New South Wales and spread north and south, 2) emerged in southwest Western Australia in 1985 and spread south, north and west, 3) emerged in the Adelaide region sometime before 1996, and 4) emerged in the Kimberley region in 1999. ✘
39. New foci apparently not contiguous with established zones of *B. dendrobatidis* will most probably be by movement of infected amphibians or of contaminated water recently in contact with an infected amphibian. ✘
40. Amphibians being 1) accidentally moved with produce, nursery plants, and building materials, 2) moved deliberately in the commercial animal trade, and 3) species expanding their range constitute an ongoing risk. ✘
41. Estimated rate of spread from 3 poor quality data sets is 100 km/yr; theoretical calculations indicate this figure is possible. ✘
42. Epidemics of chytridiomycosis in wild amphibians have occurred in Australia, Ecuador, New Zealand, Central America, Spain, and USA.
43. These epidemics appear to be continuing in Australia.
44. Epidemics appear to have a strong seasonal cycle in some parts of Australia showing an increased prevalence and mortality in colder months, with additional marked variation between years. ✘

SAFETY

45. Individuals working with live *B. dendrobatidis* must realise that they are dealing with a pathogen that is highly virulent to amphibians and must adopt suitable standards of biocontainment to prevent release of laboratory cultures to the wild.
46. *B. dendrobatidis* is unlikely to infect human skin because it does not multiply at temperatures above 31° C.
47. The risk of various activities (handling, etc) conveying *B. dendrobatidis* among amphibians needs to be quantified to enable best practices to be chosen.
48. Strategies need to be developed to decrease the risk of commercial culture of amphibians on a mass scale polluting the natural environment with *B. dendrobatidis*. ✘

RANAVIRAL DISEASE

1. Ranaviral disease in amphibians is caused by multiple "species" of closely related viruses placed in the genus Ranavirus.
2. Ranaviral disease is an emerging infectious disease of amphibians globally since it is being detected over an increasing geographic range and in more species.
3. In Australia the evidence implicating ranaviruses in amphibian declines is inconclusive.
4. Since some ranaviruses can infect 3 classes of vertebrate (amphibia, reptilia and pisces), the epidemiology / control of ranaviral disease must have a broader perspective beyond amphibians alone.

BIOLOGY

5. Ranaviruses have low host specificity in general, but some species may have high host specificity.

6. Ranaviruses are highly infectious since inoculating doses can be very low.
7. Ranaviruses are robust viruses capable of surviving for extended periods of time even in dried material.
8. Aclinical carrier states with ranaviruses occur, and are probably the most common state in wild amphibians. ✘
9. 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. ✘
10. Once detected in an area, ranaviruses are not consistently detected thereafter. ✘
11. Ranaviruses may be able to survive in the environment without a host, but will not multiply. ✘

PATHOGENICITY

12. Ranaviruses are capable of causing a high incidence of morbidity and mortality in amphibians in captivity and experimentally.
13. Ranaviruses can cause a high incidence of morbidity and mortality in some species of amphibians in the wild.
14. In Australia there have been no epidemics of ranaviral disease detected in wild amphibians, but the level of searching has not been as high as for chytridiomycosis. ✘
15. The pathological outcome of infection of amphibians with ranaviruses is variable and difficult to predict.
16. Some factors which determine this outcome are known (age of host, viral characteristics), but the environmental factors (e.g., pollution, UV, climate) that determine the outcome are unknown. ✘
17. Chronic ranaviral disease in amphibians can occur experimentally and in the wild. ✘
18. The significance of chronic ranaviral disease on wild amphibian populations is unknown. ✘
19. The potential for amphibian carries of ranaviruses to release viral particles into the environment is unknown. ✘

DIAGNOSIS / DETECTION

20. 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.
21. The best samples to submit for laboratory diagnosis of ranaviral disease in live animals are not known. ✘
22. 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.
23. 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. ✘

24. The significance of serological tests for ranaviral antibodies in terms of indicating potential for viral shedding is unknown. ✘
25. Laboratory diagnosis of ranaviral disease in live animals cannot be done with a high level of sensitivity and confidence. ✘

THERAPY / DISINFECTION

26. Although no antiviral agents have been tested against ranaviruses, the chances of obtaining cure of chronically affected or carrier amphibians is very small.
27. Glutaraldehyde, bleach and artificially generated ultraviolet light are effective disinfectants.
28. Ethyl alcohol is not an effective disinfectant for ranaviruses.

EPIDEMIOLOGY

29. To accurately understand the epidemiology of any infectious disease of amphibians, a key concept is distinguishing between infection and disease/death.
30. The epidemiology of ranaviruses is best understood in North America and UK. ✘
31. 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. ✘
32. Serological studies on *Bufo marinus* show that ranaviruses are present in New South Wales, Queensland and Northern Territory. Fresh water tortoises in North Queensland also have antibodies against ranaviruses.
33. 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*. ✘
34. Of the two endemic ranaviruses in Australia, Bohle Iridovirus (BIV) and Epizootic Haematopoeitic Necrosis Virus (EHNV), only BIV appears capable of infecting amphibians.
35. BIV can also experimentally infected a number of native and introduced freshwater fish, freshwater turtles, and snakes.
36. Some other ranaviruses found outside Australia can cause experimental disease in Australian amphibians.
37. The potential of foreign ranaviruses and those intercepted in imported fish and reptiles to cause disease in Australian amphibians is unknown. ✘
38. From experimental trials and the epidemiology of ranaviruses overseas, the most likely outcome of a new ranavirus in Australia would be unpredictable local epidemics. ✘
39. This scenario means that ranaviruses may be highly significant to amphibians that have small populations confined to small geographic areas.

SAFETY

40. 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.
41. The standard of biocontainment needed for ranaviruses is higher than that required for *B. dendrobatidis*.

42. 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. ✘
43. Ranaviruses will not infect humans since they will not multiply above 33°C.
44. Strategies need to be developed to decrease the risk of commercial culture of amphibians on a mass scale polluting the natural environment with ranaviruses. ✘

OTHER DISEASES

1. Ongoing surveillance of amphibian diseases will give the best chance to detect any new disease capable of posing a threat to wild amphibians.
2. 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.

Glossary for Summary

Biocontainment	The procedures needed to ensure that infectious agents do not escape from laboratories or infected husbandry facilities.
ELISA	Enzyme Linked Immunosorbent Assay; a laboratory test developed to detect antigens of <i>B. dendrobatidis</i> by using an antibody detection system that binds to the specific antigen.
Emerging infectious disease	an infectious disease that has newly appeared or is increasing in incidence and geographic range.
Host specificity	the degree to which an infectious agent remains confined to one species of host or taxonomically related hosts. Low host specificity means that the infectious agent can infect many species of host, or species of host that are not closely related taxonomically.
Incidence	the number of new cases of a disease occurring at a location in a defined period of time.
Morbidity	clinical disease
Mortality	death
PCR	Polymerase Chain Reaction: a molecular biological technique performed in the laboratory to manufacture additional DNA strands from small numbers of DNA strands in the original specimen.

Resting phase	a stage in the life cycle of some chytrids which is resistant to dehydration. This stage does not appear to occur in <i>Batrachochytrium dendrobatidis</i> .
Self-cure	the process in which a host cures itself of an infecting agent.
Surveillance	the ongoing collection, collation, analysis and interpretation of disease specific data and dissemination to those who need to know to take steps to decrease the impact of the disease.
Zoosporangium	the spherical structure of <i>B. dendrobatidis</i> found in epidermis and from which zoospores are released.
Zoospore	the infectious stage of <i>B. dendrobatidis</i> that is motile in water.