Chytrid fungus in Australian frogs

Fact sheet

July 2023

Key points

- The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) infects the keratinised tissues (skin or tadpole mouthparts) of amphibians around the world, potentially resulting in the disease chytridiomycosis.
- The impact of *Bd* infection on a host population can vary.
- Outbreaks of *Bd* have caused repeated mass mortalities in Australian frogs, implicated in the extinction or decline of 43 of Australia's 238 frog species [1].
- Chytridiomycosis is listed on the WOAH Animal Diseases List [2].
- Infection with *Bd* is a nationally notifiable disease; you must notify animal health authorities if you suspect an animal has a *Bd* infection (see *Surveillance and management* below).

Aetiology

Batrachochytrium dendrobatidis (Bd) is the fungus, phylum Chytridiomycota, order Rhizophydiales, that causes the disease chytridiomycosis in amphibians.

One Health implications

Wildlife and the environment: Chytridiomycosis has contributed to the decline or extinction of 501 amphibian species globally; 43 in Australia [3]. These numbers may continue to rise, with the potential for climate change to alter the geographical overlap of *Bd* with susceptible frog species in Australia [4].

Humans and domestic animals: There is no evidence that *Bd* is zoonotic. It will not grow above 28°C and dies if held at 37°C for 4 hours. Homeotherms are considered unsuitable hosts.

Natural hosts

The fungus is found in all three amphibian orders: *Anura* (frogs and toads), *Caudata* (salamanders and newts) and *Gymnophiona* (caecilians).

The fungus has been found in over 600 amphibian species, and is the primary cause of decline in over 501 species [3][1]. Of these, anurans account for 93% of severe declines (proportional to

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[1] These figures have been disputed. See 5. Lambert MR, Womack MC, Byrne AQ, Hernández-Gómez O, Noss CF, Rothstein AP, . . . Koo MS (2020) Comment on "Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity". Science, **367**(6484): eaay1838.

taxonomic abundance, as they make up 89% of all amphibian species). Although chytridiomycosis is lethal to caecilians, species declines due to the disease have not been recorded.

The fungus has also been detected by PCR in a number of other non-amphibian hosts, with crayfish and reptiles in particular being identified as potential vectors for *Bd* transmission [6].

World distribution

Chytrid fungus has been detected in all continents where amphibians occur.

Occurrences in Australia

History

In Australia, the oldest record of *Bd* is from a museum frog specimen collected in south-east Qld near Brisbane in 1978 [7], which coincides with sudden frog declines in a number of species and two species extinctions in the region [8, 9]. Subsequent amphibian declines in central coastal Qld (1985-86) and the Wet Tropics (1990-95) suggest that *Bd* spread north to its current northern limit at Big Tableland near Cooktown [10-12]. In southern Australia, the spread of *Bd* has been poorly documented but its distribution extends down the entire east coast to Tas (first detected in 2004) [13, 14]. Two separate foci occur in other states, one in southwest WA, where the earliest record dates to 1985, and another around Adelaide in SA (earliest record 1995) [15].

Current situation

Batrachochytrium dendrobatidis is now endemic in Qld, NSW, ACT, Vic, Tas and WA [16]. Much of the continent is considered too hot and/or dry to sustain *Bd*. It has been found in wild amphibian populations on the east coast of Qld and NSW on or between the Great Dividing Range and the coast, in the ACT, Vic, Tas and in southwest WA. Little is known about *Bd* in SA. The NT is currently considered amphibian chytrid free [17].

Approximately 18% of frog species (43 of 238) in Australia have suffered declines or become extinct due to chytridiomycosis [1]. Species from three endemic families (*Hylidae, Myobatrachidae, Microhylidae*) and one introduced (*Bufonidae*) family are affected [15]. Six Australian frog species, the mountain mist frog (*Litoria nyakalensis*), northern gastric brooding frog (*Rheobatrachus vitellinus*), southern gastric brooding frog (*R. silus*), northern tinkerfrog (*Taudactylus rheophilus*), sharp-snouted day frog (*T. acutirostris*) and southern day frog (*T. diurnus*), all from Qld, have not been observed in the wild following *Bd's* spread throughout eastern Australia in the late 1970s-80s, with all but one (*T. rheophilus*) having since been declared extinct [18]. At least 10 other species, including the waterfall frog (*Litoria nannotis*), common mist frog (*L. rheocola*), spotted tree frog (*L. spenceri*) and lace-eyed tree frog (*Nyctimystes dayi*) have seen dramatic declines due to *Bd* infection [17].

Many persisting species remain at lower abundance and smaller distributions than the levels recorded before the species were affected by *Bd*, some are continuing to decline and significant mortality from the disease is ongoing even decades after its introduction [17].

Epidemiology

There is no known sex- or age-linked predisposition to infection, although there is age-linked mortality. Deaths in infected tadpoles have not been reported, while juveniles and adults of some species display high mortalities. Mortality in susceptible species is, in general, higher in metamorphs than adults.

Time from exposure to clinical signs and death usually ranges between 14-70 days. When frogs show clinical signs, death usually follows within 2-3 days. In captivity and in challenge and transmission experiments, some adult frogs are capable of surviving and clearing infections but mortality rates of up to 100% are common [16, 19]. Prevalence of infection in apparently subclinical frogs in infected populations in Australia can approach 100%.

Sources of agent and transmission

- Shedding of zoospores from infected skin. Zoospores leave the host via discharge papillae projecting through the surface of an epidermal cell (Figure 1). Zoospores require water to survive, (a film is adequate).
- Skin on frogs and mouthparts on tadpoles are the only tissues that become infected.
- Zoospore invades the outer epidermal layers to infect the new host.
- Frogs are subclinically infected for most of the duration of the infection. *Bd* is not an obligate parasite and can exist and grow in moisture in the laboratory. However, it is easily outcompeted by environmental microorganisms. Hence, frogs can be infected from water containing zoospores generated either from frogs or, potentially, from non-parasitic growth that might occur under certain conditions (this has not been demonstrated to date).

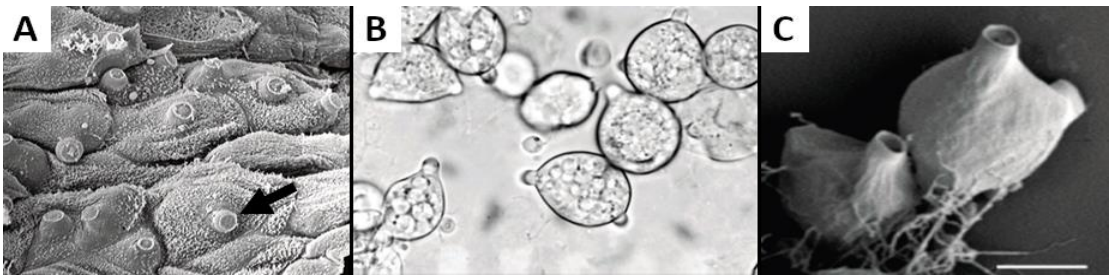

Figure 1. Micrographs of *Batrachochytrium dendrobatidis*. A) Image of infected skin from a scanning electron microscope, fungal discharge tubes are protruding through the surface (arrow), B) *Bd* in culture, C) Scanning electron micrograph of zoosporangia with open discharge tubes, and rhizoids at the base. Images courtesy Lee Berger.

Pathogenicity and virulence

The relationship between *Bd* and its amphibian hosts is complex and widely variable [20]. Variations in pathogenicity, and genetic and extrinsic variations in virulence all affect the impact of infection on the host. Pathogenicity varies with host species, fungal strain, exposure dose and period, temperature and body size.

Virulent *Bd* infections are typically the result of high pathogen loads and resulting severe skin pathology [20]. However, high infection loads do not always correlate with high mortalities, and tolerant species and individuals can act as super spreaders [21]. The explanation behind the innate differences between species leading to absence of lesions and mortality in these tolerant animals is not yet fully understood. However, for all species, the environmental context (e.g., temperature, season, altitude, latitude) of infection is known to play a significant role in susceptibility [20].

In terms of host factors regulating pathogenicity, the large, range-restricted anurans of Australia and the Americas have suffered the greatest declines from chytridiomycosis [3]. Recently metamorphosed frogs appear to be the most sensitive to the disease in some species. Infections of tadpoles are limited to their keratinized mouthparts and often appear to have no negative effects (implicating them as potential disease reservoirs), although some evidence suggests some species may lose body condition and suffer reduced survival. Tadpole infections can be carried through metamorphosis and cause high mortality rates in young, newly metamorphosed frogs of some species, and in fully grown adults of others [22]. Factors favouring host resistance may include previously acquired immunity, coinfections, host genotype, body condition and size, life stage and age and behavioural traits (e.g. basking, use of retreat sites) [19, 20].

Clinical signs

Clinical signs resulting from *Bd* infection can range from fatal to none, depending on host species and life stage. Central nervous system signs predominate behavioural change, slow and uncoordinated movement, abnormal sitting posture, tetanic spasms, loss of righting reflex and paralysis. Skin changes in chytridiomycosis are typically microscopic although abnormal skin shedding occurs (skin shed more frequently and in smaller amounts) and redness may be seen (Figure 2). Following the onset of clinical disease, death may occur rapidly, with severe infections often causing cardiac arrest and mass mortalities.

Figure 2. Great barred frog (*Mixophyes fasciolatus*), a lethargic frog with shedding skin accumulating on the body. Image courtesy of Lee Burger.

Diagnosis

Chytridiomycosis is diagnosed by detecting *Bd* in the skin of amphibians using light microscopy or PCR.

Light microscopy: zoosporangia detected in skin samples. There are two routine tests: 1) examination of skin slough or smear with or without staining, or 2) examination of fixed histological sections. Magnification of x400 is used to confirm zoosporangia presence [23].

Molecular tests: the most common test is the real-time Taqman qPCR which can quantify the amount of DNA in the sample [23].

Laboratory diagnostic specimens and procedures

For PCR: swab of skin from feet webbing (5 strokes per hind foot), inner thighs (5 strokes per hind leg) and ventral body surface (5 strokes on each side of the abdominal midline) [24]. Swab mouthparts of tadpoles. Sterile gloves should be worn and changed between individual animals to prevent cross contamination. Store swabs at -20°C until testing.

For histopathology: skin (stratum corneum) of feet or toe tips is often adequate but whole frog for necropsy is best to aid diagnosis and rule out other diseases. Fix samples in 10% buffered neutral formalin. Samples may be stained using haematoxylin and eosin [23].

Clinical pathology

There are no consistent changes of haematology and biochemistry, except for impaired electrolyte balance and oxygen regulation occurring in the late stages of disease [25, 26]. This depletion of Na^+, K^+ and Cl^- noted in some frogs may cause the cardiac arrest associated with chytridiomycosis, by diminishing cardiac electrical function [27].

Pathology

Gross lesions: in most cases no gross changes are seen. Occasional cases have increased sloughing of skin and erythema.

Histology/ microbiology: there is local hyperkeratosis of infected and adjacent cells with presence of sporangia inside cells. Epithelial cells in the layer beneath the superficial layer undergo dissolution, often leading to sloughing of the most superficial layer. There is usually no associated inflammatory reaction in dermis. Sites of predilection are the feet and ventral surfaces, but in heavy infections other sites on the body are infected. Infected tadpoles may display loss of pigmented jaw sheaths and teeth rows [16].

Differential diagnoses

Other fungal infections of skin. Artefacts of skin are capable of being confused with sporangia by inexperienced diagnosticians. Amphibian mass mortality events may also be caused by ranaviral disease, the result of infection with *Ranavirus* spp. which can also occur as a co-infection with *Bd* [24]. *Batrachochytrium salamandrivorans* has not been detected in Australia but is a similar pathogen that typically causes deeper infection with ulceration. To diagnose by qPCR requires different primers to *Bd. Batrachochytrium salamandrivorans* is the subject of another WHA fact sheet ("Exotic - *Batrachochytrium salamandrivorans").*

Treatment

The success of current *Bd* infection treatment options is variable and unlikely to be 100% effective for all Australian amphibian species. However, some hosts can be cured by treatment, particularly if their infection is subclinical.

Itraconazole and voriconazole are the most widely used *Bd* treatments [23, 28]. Itraconazole baths and formalin and malachite green baths have been used to successfully treat metamorphosed frogs [29]. Daily itraconazole baths have also been effective in treating tadpoles [30]. The suitability of these methods has not been rigorously established for most amphibian species, toxicity issues exist and treatment regimens are logistically challenging [31, 32]. While itraconazole reduced *Bd* load for *in situ* mountain yellow-legged frogs (*Rana muscosa*), the inability of early life stages to maintain an effective long-term immune response meant that recruitment failure persisted [33].

Other treatment options are being investigated. A low dose of 1-butyl-1-methylpyrrolidinium bisimide (BMP-NTf2) was found to reduce *Bd* growth *in vivo* for the relatively chytridiomycosis-tolerant Pacific tree frog (*Pseudacris regilla*) but not for the highly susceptible dyeing poison frog (*Dendrobates tinctorius*) [31]. Ongoing research may reveal a dose and schedule that justifies its use as a future conventional *Bd* treatment agent.

Heating (>30°C) may be effective in treating heat tolerant amphibian species, such as the Chiricahua leopard frog (*Rana chiricahuensis*) [34].

Prevention and control

No vaccine against *Bd* is currently available. However, increased survivorship and lowered pathogen burden following low-virulence *Bd* inoculation has been observed in two frog species, potentially indicating a direction of future vaccine development [35].

Control and eradication of *Bd* in wild populations remains a challenge. Where the fungus cannot be entirely removed from the environment, increasing the salinity of ponds can create a refuge for wild salt-tolerant amphibians by compromising *Bd* transmission and growth [36]. Doses of salt up to 6 ppt[2] are thought to be safe for green and golden bell frogs (*Litoria aurea*) [37]. Further research is required to determine the context-dependent appropriateness of elevating water salinity for different Australian species and ecosystems.

Reducing fungal virulence without the need to eradicate it from a population and releasing captive bred animals with artificially increased resistance are current areas of exploration [38].

The use of conservation translocations to aid amphibians threatened by *Bd* is a well-established management action in Australia, with varying levels of success. A review of these studies was used to create a conceptual framework to inform future conservation translocations [39]. In Australia, control at a national level requires continued surveillance to ensure that maps of chytrid-free areas

[2] Ppt=parts per thousand

remain up to date, identification of possible candidate sites for translocations and reintroductions and development of the best methods to prevent spread of chytridiomycosis to these areas.

Appropriate biosecurity and disinfection is important when working with *Bd*. Table 1 is adapted from the WOAH Manual of Diagnostic Tests for Aquatic Animals [23] and outlines the physical disinfection techniques effective against *Bd* zoospores and zoosporangia.

Table 1. Disinfection techniques suitable for killing *Batrachochytrium dendrobatidis* (WOAH 2019)

Disinfection application	Disinfectant	Concentration	Minimum time of exposure
Surgical instruments and equipment	Benzalkonium chloride	2 mg / ml	1 minute
	Ethanol	70%	1 minute
Collection equipment and containers	Sodium hypochlorite (bleach)	1%	1 minute
	Path X or Quaternary ammonium compound 128	1:500	30 seconds
	Trigene	1:5000	1 minute
	F10	1:5000	1 minute
	Virkon	2 mg / ml	1 minute
	Potassium permanganate	1%	10 minutes
	Complete drying	-	>3 hours
	Heat	60 °C	30 minutes
		37 °C	8 hours
Footwear	Sodium hypochlorite (bleach)	1%	1 minute
	Path X or Quaternary ammonium compound 128	1:500	30 seconds
	Trigene	1:5000	1 minute
	F10	1:5000	1 minute
	Complete drying	-	>3 hours
Cloth	Hot wash	≥60 °C	30 minutes

Research

Understanding the medium- to long-term consequences of endemic chytridiomycosis for amphibians is critical for future management in the medium to long-term. Research is needed to enable better mitigation of the effects of chytridiomycosis in affected populations. Future avenues of research should include:

- What can be done to mitigate the impact of *Bd* where it is endemic and prevent its further spread?
- What areas of Australia are chytrid free and are they remaining so?
- Can resistance to infection or clinical disease caused by *Bd* be selected for?
- Can acquired immunity protect amphibians?
- Can *Bd* be eradicated from ponds or small standing water bodies?

- Can amphibian populations be treated or vaccinated?
- How do environmental characteristics of natural water bodies affect the biology of *Bd*?

Known research activities

- Testing of protocols for mapping regions with unknown chytrid status.
- Investigation of pathogenicity and epidemiology.
- Assessing effectiveness of management options such as conservation translocations.
- Determining whether innate immunity can be used to improve reintroduction success.
- Assessing the potential of selection for innate immunity in protecting amphibian populations.
- Assessing the effectiveness of treatment regimes.
- Predictive climatic and environmental modelling for risk of impact and spread.

Surveillance and management

Infection of amphibians with the amphibian chytrid fungus has been listed as a Key Threatening Process in Australia by the Commonwealth Department of Environment and Heritage and a Threat Abatement Plan (TAP), updated in 2016 [17], developed in consultation with key stakeholders and the National Chytrid Working Group. The main objectives of the updated TAP include identifying and prioritising key threatened species and populations and improving understanding of the impact of *Bd* upon them. This involves population monitoring, surveys, and mapping infected and chytrid-free areas, building on the tools developed for the initial 2006 TAP.

Risk analysis performed by Biosecurity Australia in "Quarantine requirements for the importation of amphibians or their eggs into zoological facilities" [40] did not list chytridiomycosis as a risk since it is endemic in Australia. However, this disregards the risk of importation into chytrid-free areas and the risks of introducing new lineages that may have higher virulence or that can hybridize with the current strains. Although chytridiomycosis is not specifically mentioned, the general hygiene strategies recommended will prevent the release of imported *Bd* strains during the initial two years. After two years, the amphibians can be released without testing for *Bd*. However, if being released into a chytrid-free area, the same requirements imposed on Australian bred amphibians under the Threat Abatement Plan would apply.

Chytridiomycosis is listed on the WOAH Animal Diseases List [2]. Infection with *Bd* is a nationally notifiable disease (see www.agriculture.gov.au/biosecurity-trade/pests-diseases-weeds/animal/notifiable). By law you must notify animal health authorities in your jurisdiction if you know or suspect that an animal has a notifiable pest or disease. Refer to advice in your jurisdiction (www.agriculture.gov.au/biosecurity-trade/pests-diseases-weeds/animal/notifiable) and on outbreak.gov.au on how to report [41].

The most complete dataset currently available on chytrid in Australia is on the Atlas of Living Australia (https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/fungi/60102877#records). Reports of chytrid are also captured in the National Wildlife Health Surveillance Database (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 captured from 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/ProgramsProjects/eWHIS-WildlifeHealthInformationSystem.aspx.

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