



## **Chytridiomycosis (Amphibian Chytrid Fungus Disease)**

Chytrid is an infectious disease impacting amphibians worldwide. This potentially lethal skin disease is caused by the chytrid fungus *Batrachochytrium dendrobatidis*, which has been detected on hundreds of species of amphibians in 35+ countries. The chytrid fungus is capable of causing sporadic deaths in some amphibian populations and 100% mortality in others. This disease is believed to have caused amphibian population declines in Australia, South America, North America, Central America, New Zealand, Europe, and Africa, and has been implicated in the extinction of some species of frogs.

Chytrid fungus typically lives in water or soil, although some are parasites living directly on plants and insects. The fungus reproduces asexually and has spores that move through the water. Only the amphibian chytrid fungus is known to infect vertebrates. Individual frogs are believed to contract the disease when their skin comes in contact with water that contains spores from infected animals. It is still unknown why the disease kills its host, how the fungus survives in the absence of amphibian populations, and the various ways the disease is spread.

How this disease interacts with other stressors (i.e. pollutants, invasive species, climate change) to exacerbate population declines is not clearly understood. Pesticides and other pollution can also decrease amphibian immune response, making them more susceptible to infectious disease. Climate change may alter the dynamics of parasite communities by making conditions more hospitable to growth of the pathogen, and it may also decrease the immune defenses of stressed amphibians that are not coping with sub-optimal climatic conditions.

### **How is the disease being spread?**

There are several ways the disease might be spread. People may unknowingly transport spores on their boots or field equipment from one water body to another, and infected captive frogs or salamanders may escape or be intentionally released into uninfected wild locations. Another possible way the disease is spread is when contaminated water from holding tanks of captive amphibians is poured into the environment.

### **What are the effects of chytridiomycosis on amphibians?**

The chytrid fungus invades the surface layer of the frog's skin, causing damage to the keratin layer. Infected frogs begin to die roughly 21 days post-infection, and though larvae stages (eggs or tadpoles) are susceptible to infection, deaths are generally restricted to the adult life stage. Though chytridiomycosis doesn't appear to cause larval mortality, it can significantly decrease body mass at metamorphosis and increase the duration of the larval stage, both of which are likely to negatively affect the amphibian's long-term survival.

While it is not completely understood how chytrid kills the amphibian, the fungus may release toxins that are absorbed through the skin, or it may directly affect water uptake and respiration (frogs drink and breathe through their skin).

### **Is there a cure or treatment for chytridiomycosis?**

While methods exist for effectively treating captive adult life stage animals infected with *Batrachochytrium dendrobatidis* with repeated soakings in a bath of itraconazole solution, it is not currently possible to eradicate the fungus from wild amphibian populations. Nor is it possible to protect an uninfected natural environment prior to the arrival of the chytrid fungus.

### **What can be done to stop or control this disease?**

Minimize or eliminate the risk of the spreading the disease by not releasing captive amphibians into the wild, do not move wild caught amphibians from one location to another, and disinfect your footwear and clothing and collecting equipment if you are working in or exploring aquatic environments.

### **How is chytridiomycosis diagnosed?**

A correct diagnosis generally requires laboratory testing by a pathologist because many infected frogs show no clinical symptoms of infection, and conversely sick or sick-looking frogs may have illnesses other than chytridiomycosis. There are no symptoms that necessarily implicate chytridiomycosis.

### **Which amphibians are susceptible to chytrid?**

While chytrid fungus has been confirmed on 14 amphibian species in Ohio including American toad, leopard frog, green frog, cricket frog, and spotted salamander, die-offs directly attributed to this disease have not been documented.

Not all chytridiomycosis-infected amphibian species at a site decline; nor do all species at an infected site become infected. Species vary in their inherent susceptibility to the fungus. This may be due to (1) differences in their ability to mount a sufficient immune response via anti-microbial peptides, or (2) differing microbiota on their skin -- bacteria that live on amphibian skin that can compete with or kill *B. dendrobatidis*. The naturally occurring bacterial communities on amphibian skin vary between populations and species.

Furthermore, certain species may have life history traits that predispose them to infection, such as a tendency to breed in permanent flowing water, or an avoidance of microhabitats that could render their skin less hospitable to the chytrid fungus (which grows best in cool temperatures). Species also vary in fecundity (some females lay 20 eggs while others lay 20,000) which impacts their ability to recover after a disturbance, such as the introduction of a new pathogen. Finally, species vary in the rate at which they can evolve defenses against newly introduced pathogens.

### **What are the signs of the disease one might see on an infected amphibian?**

The typical symptoms of an animal infected with *Batrachochytrium dendrobatidis* include a red inguinal area ("red leg") and general lethargy. Observing multiple dead frogs in the same body of water may also be an indicator of the fungus but their demise could also be a result of other factors (e.g. pollutants).

### **What protocols should be employed to minimize the risk of transmitting disease on footwear, field equipment, or while handling amphibians in the wild?**

The following information is from: "Hygiene Protocol For Handling Amphibians In Field Studies. 2004. Speare R., Berger L., Skerratt L.F., Alford R., Mendez D., Cashins S., Kenyon N., Hauselberger K., Rowley J."

1. Amphibians can be handled using bare hands as long as the handler washes their hands between amphibians in water to which the animals would normally be exposed; this will ensure that the risks to frogs of exposure are not increased above environmental levels.
2. If no water is available for washing hands between amphibians, the handler should wear unused disposable gloves, or wear an unused plastic bag, or wipe their hands with a sterilizing alcohol-based hand disinfectant between amphibians.
3. If amphibians are held in a container prior to return to the wild, the container should not have previously have been used for holding other amphibians, or if previously used, the container should be disinfected prior to use using methods given in Table 1.
4. Surgical instruments, such as scissors used for toe tip clipping, should be sterilized between amphibians by chemical disinfection using 70% ethanol or other chemicals listed in Table 1.
5. When toe tip clipping is used, no more than 50% of the free length of the digit should be removed.
6. Amphibians should be handled and released as quickly as possible.
7. Amphibians should be released at the site from which they were captured.
8. No more than one terrestrial individual should ever be held in the same container simultaneously.
9. Tadpoles normally share water and placing them in a common container does not increase their rates of physical contact. They can therefore be held in groups in containers, as long as all members of the group are from the same site.
10. Tadpoles for release should not be held with batches of tadpoles collected from other sites in the same or different water bodies.
11. Non-surgical equipment used in a stream or water body should be disinfected using one of the methods listed in Table 1 prior to use in any other water bodies.
12. Footwear should be washed to remove any mud and disinfected using one of the methods listed in Table 1 prior to being used in a separate water catchment or water body isolated from the initial water body.
13. As there is no evidence that vehicles play a role in dissemination of the amphibian chytrid fungus, no action is required at this time.
14. Dead amphibians or amphibians that are obviously ill should be regarded as a higher infection risk than clinically normal amphibians and should be handled with gloves or plastic bags. If a sick or freshly dead wild amphibian is found, it should be collected, preserved and submitted for disease diagnosis.

Table 1: Disinfection strategies suitable for killing *Batrachochytrium dendrobatidis* and ranaviruses in field studies. Where concentrations and time are given, these are minimum shown to be effective. For *B. dendrobatidis* based on Berger (2001) and Johnson et al (2003) and for ranaviruses on Langdon (1989) and Miocevic et al (1993).

Purpose	Disinfectant	Concentration	Time	Pathogen killed
Disinfecting surgical equipment and other instruments (eg, scales)	Ethanol	70%	1 min	<i>B. dendrobatidis</i> Ranaviruses
	Vircon	1 mg/ml	1 min	<i>B. dendrobatidis</i> Ranaviruses
	Benzalkonium chloride	1 mg/ml	1 min	<i>B. dendrobatidis</i>
Disinfecting collection equipment and containers	Sodium hypochlorite (bleach)	1%	1 min	<i>B. dendrobatidis</i>
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	0.5 min	<i>B. dendrobatidis</i>
	Complete drying		3 hrs or greater	<i>B. dendrobatidis</i>
	Heat	60°C	5 min 15 min	<i>B. dendrobatidis</i> Ranaviruses
	Heat	37°C	4 hrs	<i>B. dendrobatidis</i>
	Sterilising UV light		1 min	Ranaviruses only
Disinfecting footwear	Sodium hypochlorite (bleach)	1%	1 min	<i>B. dendrobatidis</i>
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	1 min	<i>B. dendrobatidis</i>
	Complete drying		3 hrs or greater	<i>B. dendrobatidis</i>
Disinfecting cloth (eg, bags, clothes)	Hot wash	60°C or greater	5 min	<i>B. dendrobatidis</i>
			15 min	Ranaviruses