AMPHIBIAN HEALTH

Amphibian fungal disease in the live animal trade

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The infectious amphibian disease, chytridiomycosis caused by Batrachochytrium dendrobatidis (Bd) is causing amphibian declines on a global scale. The trade in live amphibians has been implicated in the introduction of Bd in novel areas with susceptible amphibian communities. With many species threatened or even extinct due to Bd, the threats of spreading the disease outside its indigenous range are evident. Nevertheless, measures to stop or minimize its spread and that of other amphibian diseases through the live animal trade have barely been implemented. In this article we provide information on Bd and the role of the live animal trade in its spread. We also discuss the preventive measures which can be taken to halt the spread of Bd.

The chytrid fungus, Batrachochytrium dendrobatidis (Bd), is the causative agent of the infectious disease, chytridiomycosis, in anurans (frogs and toads), urodelans (salamanders and newts) and caecilians (limbless, snake-like amphibians). Chytridiomycosis results from a sustained cutaneous infection by Bd, which may lead to hyperkeratosis (thickening of the outermost layer of the epidermis), extensive shedding, and impaired osmotic regulation, eventually causing cardiac arrest. Bd is closely related to B. salamandrivorans (Bsal), which specifically infects urodelans (OFI Journal 82).

Although anurans seem to be affected to a greater extent, Bd can cause disease in an extremely wide host range, which is exceptional among vertebrates. It is an emerging infectious disease of amphibians, causing mass mortality and population declines worldwide. In particular, amphibian communities in Central and South America and Australia have experienced dramatic declines due to Bd, and several species have gone extinct after they came into contact with the fungus.

Of the 6,260 amphibian species assessed by the IUCN, nearly one third (32.4%) are globally threatened or have gone extinct, which puts them among the most endangered group of vertebrates. Besides other factors, such as habitat loss, environmental pollution and climate change, diseases such as Bd are important drivers in the global decline of amphibians.

Emergence, persistence and human-mediated spread

The exact origin of Bd is still unknown. Africa, North America, Brazil and Asia have been suggested as its ‘cradle’, but it is not inconceivable that several lineages of Bd have an independent history. So far, at least six major Bd lineages are being recognized, including a hypervirulent global panzootic (i.e. widespread) lineage (BdGPL). This invasive lineage is associated with massive declines and extinctions that spread in a wave-like manner once introduced into a new area and was involved in major epizootics (infectious animal disease outbreaks) in the Americas, Australia and Europe (Spain, French Pyrenees).

The exact outcome after introduction in nature is unpredictable owing to many biotic and abiotic variable factors, such as climate, virulence, host susceptibility, competition with other microorganisms, etc. However, once established in wild amphibian communities, Bd is virtually impossible to eliminate. Complicating factors are the wide host range and variable susceptibility of the different hosts. In particular, species which carry Bd without obvious adverse health effects can act as reservoirs and vectors.

Atelopus hoogmooldi. Central and South American harlequin toads (Atelopus species) are greatly affected by Bd, with many species endangered or already extinct. PHOTO: MAARTEN GILBERT
from which the chytrid fungus can infect other, potentially susceptible species. In this way Bd can persist and spread without going extinct itself.

Although the origin of Bd is still not entirely clear, the nature of Bd-associated amphibian mortalities and molecular evidence suggest that its emergence and epidemic spread are recent, human-mediated events. Especially the worldwide trade in African clawed frogs (Xenopus laevis), which were used for pregnancy testing and are currently still used for research, has been implicated in the extensive spread of pathogenic Bd variants across the globe. Nevertheless, other host species have also been implicated in the spread of pathogenic Bd variants, such as the American bullfrog (Lithobates catesbeianus), which is extensively farmed and traded as a food source.

Bd is among the world’s 100 worst invasive alien species. It has been recognized by the Aquatic Animal Health Standards Commission (OIE) ad hoc Group on Amphibian Diseases as one of the two pathogens of particular importance in amphibians (the other one being Ranavirus, while the listing of Bsal is currently being evaluated). In 2008, chytridiomycosis caused by Bd was added to the OIE’s list of notifiable diseases owing to increasing evidence of the spread of the pathogen through the live amphibian trade.

**Prevention**
Because of the known potentially devastating effects of Bd and the inadequate means to eliminate it once established in nature, preventing its introduction into naïve, susceptible amphibian populations (i.e. populations that have not been previously exposed to the fungus) is of the utmost importance. Once sold to individual customers, escape or intentional release of captive amphibians, which potentially carry Bd or other infectious diseases into nature, cannot be completely prevented. Therefore, it is important that amphibians that ultimately reach a customer are free of Bd.

A first step would be testing the animals for Bd. Standardized pre-import screening of amphibians for Bd and, preferably, other high-impact amphibian pathogens (Ranavirus and Bsal) needs to be common practice in the live animal trade. Bd-positive animals should not be allowed to be traded.

To avoid the potential spread of Bd when transporting amphibians, high biosecurity measures need to be taken. Good practice would be to keep amphibians from the same population or collection well separated from those of other origins to prevent the spread of Bd. The containers used to transport the amphibians need to be discarded or thoroughly cleaned and disinfected when used for more than one shipment. Effective disinfection can be done with a 10% household bleach solution, or with a 1% Virkon-S solution, followed by extensive rinsing with clean water. These disinfectants can also be used to disinfect wastewater after transport. Heating is also effective: at least 30 minutes at 47°C, or at least five minutes at 60°C, will kill Bd. As Bd is intolerant to desiccation, thorough drying of materials can also help prevent its spread.

Furthermore, a quarantine period of at least two months is strongly recommended for newly acquired amphibians.

![The American bullfrog (Lithobates catesbeianus) is internationally traded in large numbers for consumption and as a pet animal, and is considered an invasive species in many parts of the world. This species is able to carry Bd without obvious adverse health effects, thereby spreading the pathogen unnoticed.](image)

**Treatment**
In contrast to the difficulties in eliminating Bd from amphibian communities in the wild, eliminating Bd from captive amphibian collections is feasible. However, it should be noted that no single overarching treatment is currently available and that effective treatment is species-dependent.

The safest and most effective way to treat captive amphibians and their enclosures may be thermal treatment. Bd can be killed by heating to 37°C for four hours. It is therefore recommended to hold heat-tolerant amphibian species at the highest temperature they can tolerate. Be careful, though, as many amphibian species will not tolerate these high temperatures.

Besides thermal treatment, the fungicide, itraconazole, can be used to treat Bd-infected amphibians by bathing the animals in a shallow bath and by oral administration. However, the potential negative effects of itraconazole on amphibians have not been well assessed.

**Future directions**
Despite the eminent risk posed by Bd and the prominent role of the amphibian trade in its spread across the globe, active measures to prevent further spread through the trade have barely been implemented.

From an ecological perspective, a complete ban on the commercial trade of wild-caught amphibians would be the best option. However, at this moment, the best solution would be a safe and sustainable trade of captive-bred amphibians, in which the spread of Bd and other pathogens outside their indigenous range is prevented.
Preventive measures, such as pre-import screening for Bd and strict biosecurity protocols are essential to reach this goal. A ban on the trade of particular high-risk invasive carrier species, such as the American bullfrog, should also be considered.

OFI has acknowledged the biosecurity risks that are associated with importing animals. A science-based pre-import screening for Bd and other relevant amphibian diseases should be compulsory for all imported amphibians, but the development and implementation of this could be long-term. In the meantime, awareness of this relevant issue needs to be created among suppliers, importers and consumers.

OFI and RAVON (Reptile, Amphibian and Fish Conservation the Netherlands) emphasize the importance of the screening of amphibians for Bd and other relevant amphibian diseases prior to shipment, the maintenance of high biosecurity standards, the testing of sick or dead amphibians, and the provision of information for the end-user not to release amphibians in the wild, nor to deposit wastewater outside.

Ultimately, trading healthy animals is beneficial for all parties and represents the best alternative to a complete ban on the amphibian trade. 

**FURTHER READING**
For further information regarding Bd, or RAVON, please contact: m.gilbert@ravon.nl and/or a.spitzen@ravon.nl

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