



## **Chytridiomycosis in the Pet Industry**

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## **Worldwide Amphibian Decline**

- One of the biggest threats facing amphibian species worldwide is the fungal disease chytridiomycosis, caused by the chytrid fungus, *Batrachochytrium dendrobatidis*.
- The sudden appearance of chytridiomycosis indicates that *B. dendrobatidis* was recently introduced into new regions and has subsequently infected novel host species.
- Dispersal of *B. dendrobatidis* between countries is most likely caused by the global transportation of amphibians.

## ***Batrachochytrium dendrobatidis***

- The amphibian chytrid fungus has been placed in a new genus, *Batrachochytrium* (Phylum Chytridiomycota, Class Chytridiomycetes, Order Chytridiales). *B. dendrobatidis* was named after an isolate from a blue poison dart frog (*Dendrobates azureus*) that died at the National Zoological Park in Washington, DC.
- The earliest isolate of chytridiomycosis found was in a *Xenopus laevis* frog preserved in 1938.
- Chytridiomycosis was a stable endemic infection in southern Africa for 23 years before any positive specimen was found outside Africa.

## **Environmental Conditions**

- Most chytrids occur in aquatic habitats. They have motile flagellated zoospores that develop within a sporangium.
- The fungal infection prevalence is greater in environments with high moisture or humidity.
- *Batrachochytrium dendrobatidis* grows within a wide range of temperatures (4–25°C) and grows optimally at 17–25°C. This wide range of temperatures lets this pathogen persist in many environments. The ability to persist at 4°C allows *B. dendrobatidis* to survive winters.

## Clinical Pathology

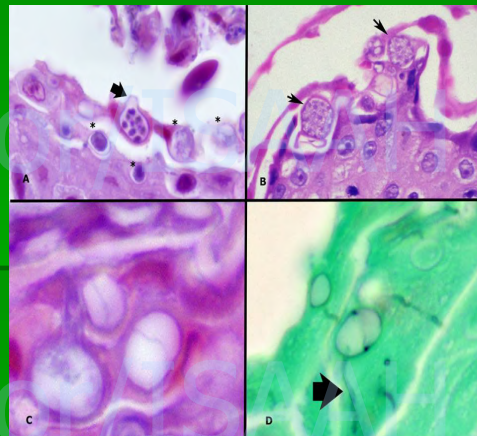
- Lesions associated with chytridiomycosis include hyperplasia of the epidermis and hyperkeratosis of the stratum corneum.
- Typical clinical signs in frogs with chytrid are lethargy, inappetence, skin discoloration, presence of excessive sloughed skin, and sitting unprotected during the day with hind legs held loosely to the body.
- Frogs become moribund in terminal stages with loss of righting reflex, and death usually occurs a few days after the onset of clinical signs.

## Histopathology

Detailed morphologic features of *Batrachochytrium dendrobatidis* thalli:

- A) Mature sporangium with discrete basophilic zoospores and a discharge tube (arrow) and there also are several uninucleate developing thalli;
- B) Developing multinucleated thalli;
- C) Three "empty" thalli that have previously discharged their zoospores;
- D) A Gomori's methenamine silver (GMS) stained section demonstrating the rhizoids (arrow).

(from *A Manual For Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs*, Proceedings from a Workshop: 16–18 February 2009 San Diego Zoo; p. 116.)



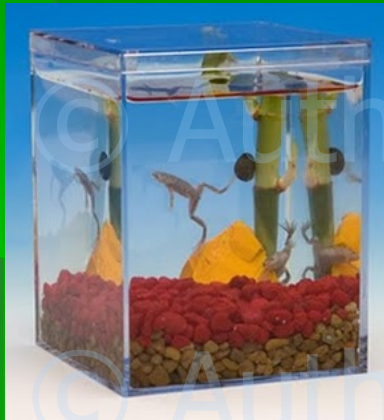
## African Dwarf Frog

- Kingdom: Animalia
- Phylum: Chordata
- Class: Amphibian      Subclass: Lissamphibia
- Superorder: Batrachia      Order: Anura
- Family: Pipidae
- Genus: Hymenochirus Boulenger, 1896
- **Species:** *Hymenochirus boettgeri*  
*Hymenochirus boulengeri*  
*Hymenochirus curtipes*  
*Hymenochirus feae*



African dwarf frogs are small totally aquatic frogs native to tropical and subtropical regions of Africa, primarily near the Congo.

## African Dwarf Frogs can be kept separately or with fish



## **Pet Frog Sales**

- African Dwarf Frogs are the most popular amphibian pet. They can be kept in aquariums with community tropical fish.
- A breeder in California produces over one million AD Frogs annually for the pet trade.
- We sell over 50,000 AD Frogs per month.
- Started testing frogs in 2007 and found positive Chytrid. We instigated a preventive treatment protocol and subsequent periodic testing to ensure treatment is successful.

## **PCR Testing for Chytrid Fungus**

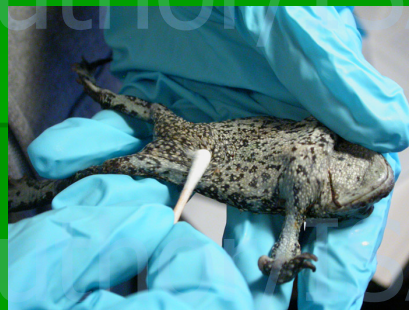
- PCR based testing of skin swabs for Chytrid fungus is available through both research and commercial laboratories and is strongly recommended as a part of routine quarantine procedures for amphibians.
- The PCR tests are very sensitive and detect small numbers of zoospores (the infective stages of chytrid fungus).

## Testing Protocols

- Frogs should be individually handled with fresh disposable gloves, and placed in individual disinfected containers prior to obtaining the samples, if testing for individual infection.
- Do not mix animals from different groups or populations to prevent spreading infection.
- Use clean, decontaminated equipment.
- Skin swabs are the preferred methods of collecting samples from live individuals.
- Use cotton swabs on 2mm-diameter wood stick, cut to lengths that fit into 2-ml tubes.
- Place sample swab into a 2-ml screw-cap tube containing 1 ml of 70 percent ethanol.

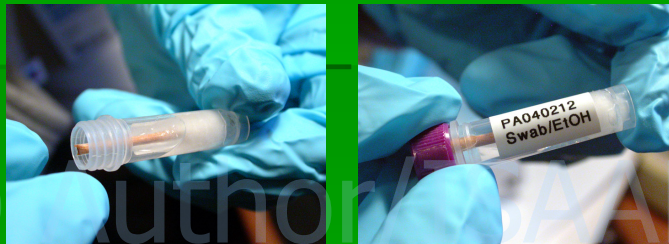
## Collecting the Sample

- To obtain the sample, hold the frog (using fresh gloves) in one hand, and gently but firmly swab with the cotton swab the ventral surface 25 times.



## Saving the Sample

- Place the swab into a sterile tube. Secure the lid and place in a rack or other container so that the tube remains upright.
- Label each tube with a unique sample number.



## Pooling of Test Samples

- Pooling of skin swabs from multiple frogs into a single PCR test has been used to reduce the costs associated with testing large numbers of animals for chytrid fungus.
- Batch testing is most useful for screening of populations from the same location or enclosure where results from individual animals are not as important.
- Statistically significant number sampled from population to evaluate whole population.

## Prevent the Spread

- Biosecurity practices that are helpful for controlling infection and transmission are:
- Avoid the transfer of animals, moist or wet substrates (e.g., soil, gravel, moss, plants), cage furniture or water between different enclosures.
- Disinfect nets, tools and equipment between uses in different enclosures.
- Quarantine and test incoming populations.

## Chytrid Preventive Treatments

### **Benzalkonium chloride (Net Dip):**

- Benzalkonium chloride used for 3 daily doses of 1 mg/L for a 60-minute bath, or 2 mg/L for a 20-minute bath.

### **Betadine:**

- Betadine is a commonly used topical antiseptic.
- The disinfectant contains 10% povidone-iodine, which is equivalent to 1% available iodine. Use Betadine at a dose of 100 ml/L of water for 10 minutes.

### **Heat:**

- *B. dendrobatidis* grows within a wide range of temperatures (4–28° C) and grows optimally at 17–25° C.
- Chytrid fungus dies within 96 h at 32°C, and within 4 h at 37°C. Housing frogs at 37°C for 16 h can clear them of the pathogen and prevent the development of infection.



## Holding Tanks Treatment

- AD Frogs are treated with Benzalkonium Chloride after arrival in their holding tanks.



## AD Frogs Floating in Tank



## Treating Active Infections

- For animals that are clinically ill with chytridiomycosis (showing signs of lethargy, anorexia, poor righting reflexes, excessive skin shedding, hunched posture) supportive treatment with supplemental electrolytes and antibiotics can be helpful.
- To correct electrolyte abnormalities, administer 12% Whitaker-Wright solution by stomach tube.
- Frogs that are clinically affected by chytridiomycosis are placed in an Ringers bath in order to encourage retention of electrolytes. The normal water source can be replaced with Ringers solution. The solution is changed daily.
- Empirical antibiotic treatment (e.g., enrofloxacin) is also administered to treat secondary bacterial infections.

## Chytridiomycosis Overview

- Chytridiomycosis (*Batrachochytrium dendrobatidis*) has been spreading across the globe and is linked to the decimation of wild frog populations. The global transportation of frogs and other amphibians for scientific research and the pet trade brings with it the risk of spreading this fungal infection.
- African Dwarf Frogs (*Hymenochirus curtipes*) are popular aquarium pets, but they can be carriers of this devastating fungal infection.
- The frogs can be treated for the fungus with a variety of topical antifungal medications (Betadine, Benzalkonium chloride), as well as through heating the water to a temperature that kills the fungus but is safe for the frogs.
- Preventive treatments using one of these methods will reduce the risk of spreading this serious fungal infection, and reduce the losses associated with the fungus in aquatic frogs kept as pets.

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**The End**

