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TECHNICAL BULLETIN

F10 treatment of chytridiomycosis

Background on chytridiomycosis

Globally 36% of amphibian species are recognized as threatened by the IUCN red list, a fact reflected most notably by threats including habitat destruction and more recently chytridiomycosis. Chytridiomycosis is a disease of amphibians caused by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). Although fungi in the Phylum Chytridiomycota ("chytrids") are widely distributed in water and moist environments, only *Bd* has been associated with amphibian disease. Bd invades the *stratum corneum* and *stratum granulosum*, the two top layers of the epidermis. Chytridiomycosis is the most formidable disease faced by amphibians with potential to cause high mortality rates in captive and wild populations. Given susceptible species in an environment that favours the amphibian chytrid fungus, *Bd* can eliminate complete populations and even drive species to extinction.

The wide occurrence and range of amphibian taxa from which *B. dendrobatidis* has been detected, regardless of the degree of habitat conservation, suggests that any amphibian species can become infected. A difference in the severity of the disease among species has been attributed to ecological niche and life history strategy of the species as well as pathogen lineage. The infective stage of the chytrid fungus is a motile zoospore that can either reinfect the same individual or transmit to other hosts when released into water. Transmission can occur either directly through contact between frogs or indirectly through water, animate vectors including humans, birds etc. and through inanimate vectors such as footwear and equipment. Transmission between countries is by movement of infected amphibians in the scientific, pet or food frog trade.

Chytridiomycosis manifests as clinical symptoms related to the central nervous system including abnormal behaviour and body posture i.e. nocturnal frogs squatting unprotected during the day with limbs in an unusual position away from the body, lethargy and loss of righting reflex, and causes sloughing of the skin. Tadpoles infected with *Bd* are usually asymptomatic. On close examination, areas of depigmentation may be observed in the keratinized mouthparts of some species. The primary diagnostic tool for chytridiomycosis screening is a molecular based real time PCR Taqman test. A skin swab taken from especially the ventral surfaces of the animal including feet, thighs and stomach region are submitted for molecular screening.

Investigation into the efficacy of F10 against *Bd*

The need exists to control infectious diseases in order to further amphibian conservation. Currently, not all fungicides are environmentally friendly or are safe to use across both tadpole and adult life-stages of amphibians. Additionally, disinfectants often consist of by-products such as alcohol, phenol and iodine, which may damage the mucus/wax layers of amphibians which prevents dehydration of amphibians as well as aiding in the prevention of microbial infections. This prompted a research project into the efficacy of F10SC Veterinary Disinfectant in treating chytridiomycosis, initiated by the North-West University, South Africa.

The study took a four tiered approach to ensure that F10SC Veterinary Disinfectant was not only effective at killing *Bd*, but that effective doses were safe to use on the amphibian host. Both tadpoles and frogs of the Guttural toad (*Amietophrynus gutturalis*) were included in the test sample. First, an infection protocol was established to ensure that infected individuals were available for study. The contact time necessary to kill live *Bd* cultures was then determined for various F10SC concentrations. Next, the F10SC toxicity range was established for the toads through a LOEC observation trial. Once these parameters were established the protocol was tested on infected subjects.

The study found that the larval and adult stages of amphibians react quite differently to F10SC exposure. This can be attributed to obvious morphological and physiological differences that exist between the life stages. For this reason different treatments have been suggested for tadpoles and frogs that are both effective against *Bd* and safe to use for the animals. The various benefits of F10SC such as efficacy at low concentrations with minimal tissue irritation and short contact times, as well as it being biodegradable, make F10SC a safer and highly effective treatment of chytridiomycosis. A limiting factor of the study is that only one species of frog was tested. Variation in sensitivity may occur for different species and further investigation is required into the relationship between animal subject age and size and sensitivity to F10SC.

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F10 treatment protocol

The following biosecurity measures should be applied:

- Frogs in quarantine should be housed individually in enclosures with minimal habitat enrichment. Conditions should resemble their natural life style (e.g. aquatic, semi-aquatic, terrestrial).
- Enclosures can be disinfected by spraying a 1:250 disinfection solution on hard surfaces and leave for 15 minutes before rinsing off with distilled water.
- A clean pair of non-powdered surgical gloves should be worn every time a frog is handled.
- Each enclosure should have its own dedicated equipment (e.g. nets, thongs, filters, air stones etc.)

Treatment of juvenile and adult frogs

Place frogs in a water bath containing 1:3000 dilution of F10SC for 10 minutes. The treatment should be repeated for 9 consecutive days. Use containers small enough to restrain the movement of the frogs, as some species are prone to climb onto the sides of enclosures and thus do not remain in contact with the treatment solution. During the treatment schedule frogs should be switched between the treatment containers and a disinfected quarantine enclosure.

Treatment of tadpoles

Place tadpoles in a water bath containing 1:10000 dilution of F10SC for 10 minutes. The treatment should be repeated for 9 consecutive days. Tadpoles can remain inside their treatment containers as long as the treatment solution is rinsed out with distilled water and replaced with usual aquarium water.

References

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This report is the advance notice of an international pending paper.

John Temperley

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