

# Bsal outbreak in captive urodeles : a treatment protocol

## Rationale

To minimize risk of exposure of wild urodele populations to the chytrid fungus *Batrachochytrium salamandrivorans* (Bsal), potential sources of spillover should be identified and eliminated. One important source are Bsal infected urodeles in captivity. Although prevalence of Bsal in captive urodeles appears to be low to very low (i.e. the majority of collections are assumed to be Bsal negative), large scale Bsal outbreaks have occurred in Germany, the Netherlands, the United Kingdom and Spain. Early diagnosis and treatment should curb the risk of Bsal being spilled over to wild amphibians. Through raising awareness, keepers should be encouraged to have any unusual mortality in their urodeles examined by a veterinarian, which includes necropsy and molecular tests for Bsal. Keepers may be encouraged to do so, if costs for Bsal detection could be reimbursed. However, the occurrence of Bsal in captive urodeles does not necessarily result in disease outbreaks. The fungus may persist in captive urodeles unnoticed (many species kept in captivity should be considered potential Bsal reservoirs, for example belonging to the genera *Cynops*, *Paramesotriton*, *Tylotriton*). Ideally, Bsal should be eliminated from these asymptomatic reservoirs as well. This, however, requires extensive sampling. Reimbursement of sampling and analysis would provide a strong incentive for keepers to have their collection tested.

## Diagnosis

Bsal will be considered to be present in captive urodeles when the presence of its DNA is demonstrated in at least one sample from that respective collection, using the qPCR developed by Blooi et al. (2014). Although several factors, including postmortem decay, may result in false positive results (Thomas et al., 2017), the qPCR was demonstrated to be robust and when adhering to a precautionary principle, the limited proportion of false positive results should be considered positive unless proven otherwise (for example when sequencing or histopathology fail to confirm the result).

## Treatment of a Bsal infected urodele collection

Once the presence of Bsal in a captive collection has been confirmed, the following steps need to be taken :

### 1. Implementing biosecurity measures

Until the collection has been declared free of Bsal, the owner must strictly adhere to the following principles :

a) All materials, sick or dead animals and terrarium content that have come into contact with captive salamanders in the contaminated collection need to be disinfected before disposal, and this needs to be done until the collection has been declared free of Bsal. This can be done either by heating (5 minutes at min 70°C), disposal with household waste that will be heat treated or using disinfectants (for example hypochlorite (bleach) at 4%).

b) All animal movements should be avoided during the treatment period.

c) Visits by fellow keepers should be avoided during the treatment period.

d) Preferably, the keeper should wear disposable gloves and wear dedicated footwear (only used in the hobby chamber) during the entire treatment.

e) Every enclosure should be considered a separate entity and no materials, animals, water etc must be exchanged between enclosures. Preferably, the keeper wears a separate set of disposable gloves for each container.

## 2. Identification of infected groups of animals

a) Preferably, all animals in the collection are sampled using cotton tipped swabs. Individual samples are optimal; for cost reduction, one swab can be used to sample up to 5 animals from the same enclosure. Swabbing should preferably be done by an experienced person. It is very important that the tip of the swab is rubbed across the animal firmly.

b) During sampling, it is mandatory to wear disposable gloves, that need to be changed at least between each enclosure to limit the risk of further spread of the infection.

c) swabs should be sent immediately to the diagnostic laboratory. In case swabs need to be stored, this is done best in a freezer.

## 3. Treatment of groups of infected animals

a) It is mandatory that in a group of co-housed urodeles that contains one positive animal, all animals in the same enclosure are treated.

b) Treatment is initiated immediately in groups of animals that show signs of Bsal associated disease. For other urodele groups that are asymptomatic, treatment of a contained group must be initiated immediately if the analysis of the swabs returns at least one positive result.

c) Treatment of the animals consists of transferring them to an enclosure, lined with moist tissue for terrestrial animals or containing water for aquatic animals. Hiding places (eg pcv tubes) should be present and the temperature should be kept at 25°C (minimum) during 10 days. During this period, the keeper should monitor the temperature and should ascertain that a minimum temperature of 25°C is maintained at all times. Failure to do so may result in the persistence of Bsal. Many urodeles do not tolerate such high temperatures well, especially in an advanced stage of the disease. However, this is the most efficient treatment and this treatment has produced satisfactory results in many species thus far. Daily exchange (and proper disposal) of the tissue will reduce the occurrence of further health problems. In case heat treatment is likely to result in severe decrease of the animal's health (for example in species with thermal maxima lower than 20°C), consulting a knowledgeable veterinarian for medicinal treatment (combined voriconazole / colistin) is advised.

d) After transfer of the animals to the treatment enclosure, their terrarium / aquarium and its contents need to be thoroughly disinfected.

e) After the 10 days of the heat treatment, the animals are kept in the same enclosure for at least two weeks. Optimally, at 6 weeks after ceasing treatment, all animals (including those who have not been treated) should be re-tested for the presence of Bsal. If all swabs return negative results and all animals are clinically healthy, the status of the collection can be changed to negative. However, it is strongly advised that any further mortality event in such a collection is thoroughly examined.