

Chytrid Fungus and Ranavirus in Chinese Giant Salamanders: Routine Checks, Symptoms and Treatments

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ABSTRACT: This paper outlines our experience in cases of chytrid fungus and ranavirus infection in the largest of amphibian species, the Chinese giant salamander (*Andrias davidianus*) (IUCN Red List Critically Endangered; CITES I). A fatal case of Chytridiomycosis occurred in 2014 with the chytrid fungus was discovered during necropsy of a salamander. The affected salamander was found dead and with thick viscous mucous on its skin. Chytrid fungus test results were positive. Sally, who had shared the same tank and life support system with the infected one, looked normal until she was tested positive for chytrid fungus. Treatment (Sporanox (itraconazole) bath) was provided and the test result afterward was negative. In 2017, we encountered a case of ranavirus infection in 10 confiscated salamanders that were temporarily held in our quarantined facility. At that time, initial symptoms included swelling, redness and lesions. All the salamanders were confirmed with ranavirus infection and then euthanized to avoid any possibility of disease transmission. After this case, we have enhanced our preventive measures. Besides routine screening tests for chytrid fungus, we have also included screening for Ranavirus in before they can be transferred to our facilities.

INTRODUCTION

In recent years, Chytrid fungus and Ranavirus have been health concerns to our Chinese giant salamander (*Andrias davidianus*) (IUCN Red List Critically Endangered; CITES I) collection in Ocean Park, Hong Kong. The amphibian collection in Ocean Park had been free from Chytrid fungus and Ranavirus until 2014 and 2017 respectively. This paper aims to share our experience in the handling of both pathogens in our Chinese giant salamander collection.

In 2014, a skin swab sample from the necropsy of a Chinese giant salamander had tested positive for *Batrachochytrium dendrobatidis* (Bd) using PCR screening. Subsequent screening of the rest of the collection had detected another two carriers, including Sally, an adult sized giant salamander, even though they were clinically healthy. Treatment with Itraconazole bath was used to clear the fungal infection from our collection.

In 2017, a batch of 10 juvenile Chinese giant salamanders was found infected with Ranavirus during the quarantine period. They were confiscated from the pet trade by the local authority, the Agriculture, Fisheries and Conservation Department (AFCD) and were held at the Ocean Park quarantine facility. Shortly after arrival, the salamanders started developing progressive necrotizing lesions on their limbs and oral cavity resulting in high mortality in the group. Necropsy findings confirmed that they had Ranavirus infection. Given concerns on the transmission of the virus, the remaining animals from the batch were humanely euthanized.

CHINESE GIANT SALAMANDERS AT OCEAN PARK HONG KONG

Four Chinese giant salamanders, including an adult female named Sally, are currently held in Ocean Park. These salamanders are either on exhibit at the Giant Panda Adventure facility or being held in a back of house facility. Ocean Park occasionally receives requests from AFCD to provide temporary holding for Chinese giant salamanders that are confiscated from either the local pet trade or illegally imported as food. Quarantine procedures for any collected animals are carried out in an isolated facility to prevent disease transmission.

CHYTRID FUNGUS

An annual veterinary check on our Chinese giant salamanders includes screening for Chytrid fungus (both *Batrachochytrium dendrobatidis* and *B. salamandrivorans*) to monitor and prevent any transmission of the disease to other animals in the collection. The samples are tested by our Environmental Lab using PCR assay. If the results are positive, the animal would be treated by our veterinarian using the itraconazole bath protocol outlined as below.

In 2014, a salamander was found dead with thick mucous on its skin and Chytrid fungus was first detected in our collection from skin swab test during the necropsy. Interestingly the histopathology report had found no evidence of Chytrid fungus lesions and the cause of death was attributed to unrelated disorder. Sally, an adult female (~1.4m and ~30kg) that had been in our collection since 2003, and another young

salamander had shared the same tank and life support system with the infected individual. Though both salamanders were without symptoms, their skin swabs had tested positive for Chytrid fungus (Bd).

Treatment using itraconazole bath (Une et al. 2012) was provided to both of them and it was found to be effective. Chytrid fungus was detected from Day 1 to 5. From day 6 to 10, the test results were negative. In this case, a single course of treatment over ten-consecutive-days was effective to clear the Chytrid fungus infection.

Operational procedures for treating Chytrid fungus-infected salamanders

1. Preparation of medication:

- 1.1. Distilled water was chilled with freeze packs to 18-20°C, which was similar to the temperature in the animal holding tanks.
- 1.2. Amphibian ringer powder was prescribed and added to the chilled water.
- 1.3. The solution was then poured into a pre-marked bucket for 15 liters. To prepare the medicated solution, Sporanox (Itraconazole, 10mg/ml) was used. 150ml of Sporanox was added to 15 liters of water, which is 0.01% concentration, to make up the treatment bath solution.

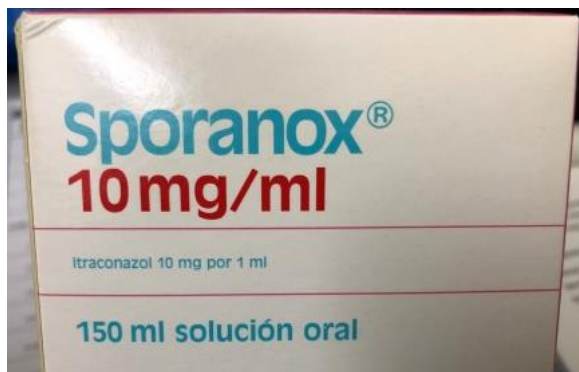


Fig. 1. Sporanox (Itraconazole, 10mg/ml)



Fig. 2. 3M Quick Swab

2. Treatment procedures:

- 2.1. Two oversized black plastic bags (prepared one inside another) were prepared for treating Sally due to her long body length. It is recommended that the most suitable treatment container be chosen based on animal size (e.g. length). For instance, a circular tarpaulin would be prepared

for smaller salamanders.

- 2.2. Swab samples were taken prior to treatment so as to screen for Bd. Each swab is rolled 5 times on each lateral body fold, plantar aspect of each hind foot, and on the ventral body (cranial to cloaca).
- 2.3. The medicated bath solution was added to the bag and the salamander remained in the bath for 10 minutes. In order to avoid having to prepare a large volume of the medicated bath and also for practical reasons, the animal was not totally submerged. Small buckets or cups were used to pour the medicated solution over the exposed areas. The duration of soaking in the medicated bath was extended from the recommended 5 minutes in the literature to 10 minutes and with no side effects noted. This was done to maximize the therapeutic effect of this medication with the smaller volume of medication used.
- 2.4. Should the same container be used for treating another animal in a similar manner, the container should first be rinsed with distilled water and also disinfected after each bath session.



Fig. 3. Skin swab sampling for Sally

3. Disinfection of salamander holding tanks

- 3.1. Disinfection was done when animals were moved out from their holding tank for treatment, on the 3rd, 6th and 9th day since treatment had begun. 1% sodium hypochlorite was applied to the tank system for at least 5 minutes. The level of the disinfectant solution was kept at only 1/3 the depth of the tank to maintain safety while using the bleach. The walls of tank were thoroughly with brushes that

were immersed in the disinfectant. The filter was then backwashed and rinsed. Once disinfected, the tank was emptied and filled with clean water. Subsequent test results for residual chlorine had to be negative before animals could be returned to the tank.

3.2. All reusable items were disinfected by soaking in 1:1000 Virkon solutions for at least 5 minutes. The surrounding area and floor was disinfected as well.



Fig. 4. Black plastic bag used for treatment and using a small bucket for pouring medicated solution over exposed areas



Fig. 5. Circular tarpaulin for treatment

RANAVIRUS

A confiscated batch of 9 juvenile Chinese giant salamanders was presented to Ocean Park. These salamanders weighed about 14 g and were about 17 cm in length, with one young individual at 28cm long. There were wounds found on the limbs of some of the juveniles and some had minor reddish swollen regions on sides of their lateral bodies. For the larger specimen, it had also found to have a serious injury on the lower jaw measuring about 2cm by 2cm, and the wound appearing with yellowish rotting epidermal tissues.

Before they were brought into our quarantine facility the skin swab samples of these salamanders tested negative for Chytrid fungus on 8th March 2017. After about 10 days in our quarantine facility, more individuals started developing necrotizing wounds on their limbs and lower jaws. Important events of this incident are shown in the following table.

This was our first encounter with Ranavirus infection. Despite the initial presentation of lesions; which were initially suspected to be wounds, this viral infection was not immediately diagnosed.

QUARANTINE

The quarantine procedure is the most important first-line of protection against the above pathogens. Keen observation on epidermal, appetite and behavioral changes are critical for early detection of the disease and in instituting of treatment.

Based on experience outlined above, apart from screening for Chytrid fungus before animals can be transferred to our facilities, we have since also required the screening for Ranavirus on all amphibians so as to enhance preventive measures.



Fig. 6,7. Picture showing necrotizing lesion on the oral cavity and limbs



Fig. 8. Picture showing necrotizing lesion on a hind limb

Table 1. Chronology of the disease outbreak happened in 2017.

Date	Event remark
11 th Mar	The animals were brought to an isolated facility in Ocean Park as per quarantine procedures.
14 th Mar	The larger specimen in the group was found dead. Tentative diagnosis was hepatitis with suspected infection.
21 st Mar	1 st juvenile died of trauma with degloving injuries on its limb.
22 nd Mar	2 nd juvenile died of trauma again.
25 th Mar	3 rd juvenile died and a Ranavirus infection was suspected.
27 th Mar	4 th death; suspected a Ranavirus infection; ruled out other bacterial/viral/parasitic skin disease possibilities.
29 th Mar	Severe necrosis of limbs. Prognosis poor. Humanely euthanized. Suspected a Ranavirus infection.
31 st Mar	Found dead with wounds, stomatitis and glositis. Suspected a Ranavirus infection.
1 st Apr	Rest of the salamanders (3 individuals) had swollen limbs and all were in a weak condition. Humanely euthanized. Suspected a Ranavirus infection.

REFERENCE FOR THE CHYTRID FUNGUS TREATMENT

UNE Y, MATSUI K, TAMUKAI K, GOKA K.
 2012. Eradication of the Chytrid fungus *Batrachochytrium dendrobatidis* in the Japanese giant salamander *Andrias japonicas*. *Disease of Aquatic Organisms*. **98**:243-247