Amphibian pet trade pathogen testing report

Participant ID: 9999
Samples received: 06/30/2023
Report generated: 07/03/2023

Summary of results

	Number of habitats (e.g., tanks)				
	Positive[1]	Tested	Reported in facility	Prevalence[1]	(95% CI)[2]
Bd	0	10	43	0%	(0% — 28%)
Bsal	0	10	43	0%	(0% — 28%)
Rv	1	10	43	10%	(2% — 42%)

[1] Assumes no false positives or false negatives
[2] Range of values of prevalence most consistent with these data and the number of habitats reported.

The estimates of prevalence of each pathogen among habitats within your facility are approximate. They assume that our testing was perfect, meaning that we would have detected an infection if it was present in a habitat. They also assume that you sampled tanks at random, so there was no bias. The range of prevalence values in the 95% CI is meant to give a sense of how precise this estimate of prevalence is, given these assumptions. If we sample more of the habitats within your facility we will have a more precise estimate than if we sampled relatively fewer.

Full sample details

Table 2: Full results from your facility. Each row of the table represents the results from one "habitat" or housing container, which may have contained multiple individual animals. Each column is a pathogen. A "1" represents a detection, a "0" a non-detection, and 'NA" indicates the sample was not successfully tested. Habitat IDs and associated information correspond to what was written on the sample labels.

Habitat ID	Species	Stage	Sample type	Rv	Bd	Bsal
5	Rana potteri	Adult	Filter	0	0	0
39	Dendrobates grangere	Adult	Swab	0	0	0
19	Dendrobates grangere	Adult	Swab	0	0	0
12	Rana potteri	Larvae	Filter	0	0	0
1	Rana potteri	Larvae	Filter	0	0	0
29	Rana potteri	Larvae	Filter	0	0	0
31	Rana potteri	Larvae	Filter	1	0	0
16	Rana potteri	Larvae	Filter	0	0	0
4	Agalychnis weaslei	Larva	Filter	0	0	0
27	Agalychnis weaslei	Adult	Swab	0	0	0

What "positive" and "negative" mean in the context of this project

Samples consist of either a batch of swabs (from up to 5 individual animals within in a single habitat and processed as a single batch), or water filters designed to sample every animal at once inside an aquatic habitat. These samples were tested by the Brunner Lab at Washington State University for the presence of DNA sequences specific to each pathogen using a test called a real-time quantitative Polymerase Chain Reaction (qPCR), akin to those used to test for COVID-19.

A "positive" sample indicates that the DNA sequence of the pathogen in question was detected in the group of swabs or in the filtered water sample that was provided. A positive sample does not necessarily indicate infection, which requires that the pathogen is living and reproducing in or on the amphibian; however, it is evidence that the microbe is present. A "negative" sample indicates a lack of detection. Lack of detection does not necessarily indicate the absence of (at least a low-level) infection with the pathogen, because sometimes low-grade infections are not detected by qPCR. An "NA" means that this test was not successfully run (e.g., the sample was lost, produced ambiguous results, etc.).

Information on pathogen for which we tested

- ***Batrachochytrium dendrobatidis* (Bd)**: is a species of "chytrid" fungal pathogens that affects the skin of amphibians. It is known to cause the declines and likely extinctions of myriad amphibian species around the world. It is also known to occur in captive populations and in amphibians sold in the live animal trade. It's impact on amphibians varies from lethal over several weeks from exposure to inapparent, subclinical infections to the infection being cleared depending on host species and Bd strain, host condition, and environmental conditions, especially temperature. Signs of Bd infection and disease include: excessive skin shedding, lethargy, paralysis, and lack of appetite. See Publication #2 on the Southeast PARC website for additional information on Bd.

- ***Batrachochytrium salamandrivorans* (Bsal)**: Similar to Bd, Bsal is a fungal pathogen that attacks the skin of amphibians. It is native to Southeast Asia (found in native species in Vietnam and China) and was apparently introduced via trade into Europe. It is known in captive collections in Europe and the UK and has caused the declines of European salamanders. It is not yet known to occur in North America, but if it were introduced it has the potential to cause massive impacts, including likely extinctions of our unique and extensive biodiversity of salamanders. Bsal's impact on animals vary from lethal within several weeks to subclinical infections to recovery, depending on host species, host health, and environmental conditions, especially temperature. Bsal signs are similar to Bd: abnormal skin shedding, lethargy, paralysis, and lack of appetite. Some species may also develop circular, necrotic lesions through the epidermis that appear like "cigarette burns" or holes through the skin. Some lesions may bleed and heavily infected toes can fall off. See Publication #23 on the SE PARC website for additional information on Bsal. Also, Bsal Task Forces in Europe (http://bsaleurope.com/) and North America (http://bsaleurope.com/) have been established to help prevent the spread of Bsal.

- **Ranaviruses (Rv)**: Ranaviruses are viral pathogens of cold-blooded vertebrates (amphibians, reptiles, and bony fishes). They are commonly found in the wild in North America and around the world, often associated with mass-mortality events. They are also known to occur in captive populations and in animals sold in the live animal trade. Their impacts on animals vary from highly lethal within days to weeks to mild or even inapparent depending on the host and virus species, host condition, and environmental conditions. Symptoms of ranavirus infections include edema or swelling, especially around the legs, red blotches on the belly, hands, or near the cloaca; and lack of appetite. Occasionally, clear or whiteish papules might be seen on the skin. However, most viral damage is internal and some animals die with no obvious signs of infection. More information on ranaviruses is available in the free book Ranavirues: Lethal Pathogens of Ectothermic Vertebrates.

Treatment options

If pathogenic microbes were detected in your facility, we would strongly encourage you to treat the affected animals and your facility or collection in order to prevent their 1) potential impacts on other animals you keep (these pathogens are capable of rapid spread between tanks or containers), 2) further spread to other facilities or collections, and 3) accidental escape into wild populations.

The basic steps to follow are:

1. **Isolate and disinfect**: Isolate and quarantine the affected animals (i.e., those in habitats where the detection occurred) and any others that have likely been exposed to them directly (e.g., in contact) or indirectly (e.g., shared equipment, recirculating water). The quarantine should continue until you can be sure the animals are uninfected. During and after the quarantine we recommend special attention to disinfecting water, substrates, housing tanks, and all equipment that comes into contact with animals or their habitats. (See disinfectant options, below.) Disposable gloves that are changed between habitats and/or hand-washing between habitats should be used to minimize the spread of pathogens.
2. **Confirmatory and further testing**: False positives do occur occasionally with qPCR and other forms of testing. We thus recommend confirming positive results with a second, independent lab before proceeding to treatment options. Testing tanks not included in this pilot study is also warranted to ensure you know the scope of infection. A list of fee-for-service laboratories that can help with testing are below. Note that if Bsal was found in your facility or collection, we (the researchers and the Pet Advocacy Network) will help facilitate this testing.
3. **Treatment options**: We recommend consulting with a veterinarian prior to treatment and follow-up testing of animals after treatment to ensure the infections have been cleared. Below are some pathogen-specific options.

- *Batrachochytrium dendrobatidis* (Bd): One of the most effective treatments for eliminating chytrid fungus infections is heat. Warming your amphibian to a constant 30 C (86 F) for 10 days will clear Bd infections. If your amphibian species cannot tolerate high temperatures, soaking them in a water bath of 0.0025% Itraconazole for 5 minutes per day for 5 days will generally clear infections. Another option is Terbinafine hydrochloride, which is the active ingredient in Lamisil. Soaking an amphibian in a water bath with 0.02% Terbinafine for 30 minutes per day and 5 days will generally clear Bd infections.
- *Batrachochytrium salamandrivorans* (Bsal): Heat and antifungal treatment options exist for Bsal. However, if Bsal was detected, we ask that you contact the Pet Advocacy Network (Ashley at ashley@petadvocacy.org, 202-452-1525 x1040) for additional free testing. Bsal originates from Asia, is causing salamander declines in Europe, and has not been detected yet in the U.S. Thus, if you had a positive detection, it is important to verify that the test result is not a false positive, which can occur occasionally with qPCR. If Bsal is confirmed with a second test, our team will work with you to eradicate it from your facility. We can work with you while maintaining your anonymity. For more details, see https://www.healthyamphibiantrade.org/pathogen-response
- Ranavirus (Rv): Unfortunately, there are currently no good options for treating ranaviral infection in amphibians. We recommend you humanely euthanize the infected and exposed animals, and disinfect your tanks following the procedures below.

Note: If you do choose to keep infected animals (e.g., perhaps they are rare or threatened and apparently healthy), we ask that they are quarantined and equipment and housing materials is kept separate between infected and uninfected groups of animals to minimize the risk of transmission to others and accidental escape into the wild. Of course we also ask that infected animals not be sold or sent to other facilities or owners.

Disinfecting Procedures

Several common disinfectants can inactivate Bd, Bsal and ranavirus. The minimum concentrations are shown in the table, below. It is recommended that the disinfectant is in contact with the contaminated surface or water for at least 10 minutes. More information can be found in Appendix 1 (pp. 350-351) of this paper by Gray et al. (2017).

Pathogen	Commercial Bleach	NaOCL (active ingredient in bleach)	Ethanol	Virkon	Novalsan (chlorhexidine acetate)	UV Light
Bd	4%	0.2%	70%	1%	1%	Ineffective
Bsal	20%	4%	70%	1%	Not Tested	Not Tested
Rv	4%	0.2%	70%	1%	1%	Yes

Fee-for-service diagnostic laboratories

- **Amphibian Disease Laboratory** at the University of Tennessee Institute of Agriculture https://amphibiandisease.tennessee.edu/diagnostic-services/ (Full disclosure: project director Dr. Matthew Gray is associated with this laboratory.)
- **Pisces Molecular** https://www.pisces-molecular.com/
- **Research Associates Laboratory** (RAL) https://www.vetdna.com/test-type/reptiles

Methods of euthanasia

With all of these methods, please be sure that the individuals have lost their righting reflex by turning them upside down and ensuring there is no movement. Keep the animals exposed to the chemical agents for at least 10 minutes after the loss of this reflex to ensure the individuals are dead. Following euthanasia, animals can be frozen and double-bagged before disposal to ensure pathogens are not accidentally released.

- **Orajel (benzocaine-HCL gel)** : Amphibians can be humanely euthanized with benzocaine, which is the active ingredient in Orajel®. A pea-size amount of Orajel® (maximum strength, with 20% benzocaine-HCl) spread evenly on the back of the amphibian. Orajel is somewhat soluble in water, but can be added to a shallow water bath holding the amphibian.
  Powdered benzocaine is also effective, but not soluble in water and must first be dissolved in >95% ethanol before dissolving this alcohol solution in water. One can find benzocaine-HCl, which is water soluble, from some suppliers online, but it is not always clear that these white powders are as pure as advertised. In either case, aim for 1/2 ounce benzocaine or benzocaine-HCL per 8 gallons of water (0.5 gram per liter) and add an equal amount of baking soda (to buffer this otherwise acidic solution). This solution can then be used for bath euthanasia.
- **Tricaine-S or Finquel (MS 222)**: MS 222 is often used for fish and amphibian anesthesia and euthanasia, though it is being phased out of research settings because of concerns over its toxicity and carcinogenicity. MS 222 is an eye and lung irritant, so be sure to wear gloves, proper eye protection, and make the solution in an area with good ventilation. As a likely carcinogen, disposal of the solution can also be problematic, so we recommend placing the solution in a sealed container before disposal or letting it dry and disposing of the crystals. For euthanasia, use 1 ounce per ~2 gallons of water (5 gram per liter) and an equal amount of baking soda (to buffer this otherwise acidic solution) to create a solution in which aquatic stages can be euthanized.
- **Eugenol or clove oil and freezing**: Clove oil can be purchased for fish anesthesia. While it is effective as an anesthetic with amphibians, it may not lead to death, even at higher concentrations.

Thus, you may anesthetize amphibians and then euthanize them by freezing or a physical method such as decapitation.