

PIJAC Pet Amphibian Trade Microbe Surveillance Pilot Study

Participant ID: 1234
 Samples received: 08/03/2021
 Results finished: 08/13/2021
 Report produced: 08/26/2021

Summary of results

	Samples		Prevalence	
	Positive	Screened	Estimate ¹	(95% CI) ²
Harmful				
Bd	2	9	22%	(3% <U+2014> 59%)
Bsal	0	8	0%	(0% <U+2014> 37%)
Rv	2	8	25%	(3% <U+2014> 64%)
Beneficial				
Jliv	8	8	100%	(63% <U+2014> 100%)

¹Estimates of prevalence assume our testing never produces false negatives or false positives. This is certainly not true, but we cannot know for certain how well they work in this context.

²The 95% CI represents the range of values of prevalence most consistent with these data and the number of tanks reported on the forms.

Information on microbes for which we tested

Harmful microbes

- ***Batrachochytrium dendrobatidis* (Bd)**: Bd is a species of 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 known to occur in captive populations and in animals sold in the life animal trade. It's impact on animals vary from lethal over several weeks from exposure to inapparent 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 known to cause the declines of European salamanders, after its likely introduction from Southeast Asia in trade. 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. North America is home to the greatest biodiversity of salamanders in the world, hence Bsal's introduction to North America could have significant ecosystem impacts. Bsal is known to occur in captive populations in Europe and native species in Vietnam and China. Its impact on animals vary from lethal within several weeks to subclinical infections or resistance, 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 fishes). They are commonly found in the wild in North America and around the world, often associated with mass-mortality events. They are known to occur in captive populations and in animals sold in the life animal trade. Their impacts on animals vary from rapidly lethal (days to weeks) to inapparent depending on host and virus species, host condition, and environmental conditions. Symptoms of ranavirus infections include edema (swelling); red blotches on the belly, hands, or near the cloaca; and lack of appetite. More information on ranaviruses is available in this online Open Access book published by Springer: [Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates](#).

Beneficial microbes

- ***Janthinobacterium lividum* (Jliv):** Jliv is a bacterial species with experimentally demonstrated antifungal and anti-Bd properties. It is known to occur on wild amphibians and fish, and is common in the environment. There are no known health risks to humans. To our knowledge, it has been yet to be detected in captive populations, hence detection is an important discovery and demonstrates that healthy microbes exist in amphibian trade. Jliv can protect amphibians from disease caused by Bd, and perhaps Bsal, infections. If Jliv was detected in your facility, we recommend keeping environmental conditions constant because its presence will help improve amphibian health. This paper (<https://rdcu.be/cswSs>) explains the benefits on Jliv in the skin microbiome of amphibians to protect against Bd infections.

Full sample details

Full results from your facility. Each row of the table represents the results from one “tank” or housing container, which may have contained multiple individual animals. Each column is a microbe. A “1” represents a detection, a “0” a non-detection, and 'NA' indicates the sample was not successfully tested. Tank numbers and associated information correspond to what was written on the sample labels.

Tank	Spp	Stage	Sample_Type	Rv	Bd	Bsal	Jliv
1	common frog	larval	filter	0	1	0	1
2	common frog	larval	filter	0	0	0	1
3	common frog	juvenile	filter	0	0	0	1
3	common frog	juvenile	swabs	0	0	NA	1
4	common frog	adult	swabs	1	0	0	NA
5	rare salamander	adult	swabs	NA	0	0	1
6	rare salamander	juvenile	swabs	1	1	0	1
7	rare salamander	adult	swabs	0	0	0	1
8	rare salamander	adult	swabs	0	0	0	1

What “positive” and “negative” mean in the context of this project

Samples were tested by the [Brunner Lab](#) at Washington State University for the presence of DNA sequences specific to each pathogenic or beneficial microbe using a test called a real-time quantitative Polymerase Chain Reaction (qPCR). Recall that samples correspond to a given tank, terrarium, enclosure, or other animal housing container: water filters. Water filters sample from all of the individuals in the tank While swab samples were collected from individual animals in a tanks, all of the swabs from a tank were processed as a batch.

A “positive” sample indicates that the DNA sequence of the organism in question was detected on at least one individual amphibian swabbed or in the filtered water sample that was provided. A positive sample does not necessarily indicate infection, which requires that the organism is living and reproducing in 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 microbe, 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.).

Treatment options

If beneficial microbes were detected in your facility or collection, terrific! This is great news! Keep doing whatever it is you are doing! We know very little about this microbe in captive settings, so we are excited to see this result. Thanks!

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 steps to follow if you have positive tests for harmful microbes are as follows:

1. **Isolate and disinfect:** Isolate and quarantine the affected animals (i.e., those in tanks 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 and/or hand-washing can minimize the spread of pathogens, but is especially effective when combine with tank-specific equipment that.
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 PIJAC) 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 30 C (86 F) constant 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 PIJAC (Josh Jones, josh@pijac.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.
 - Ranavirus (Rv): Unfortunately, there are currently no good treatment 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 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.