

Abstract

Many diseases of *Xenopus laevis* frogs have been described in the literature however, it is often difficult to find images which clearly illustrate associated clinical signs. This poster has been designed as a refinement to bridge this gap using photographs taken of frogs with clinical signs.

My aims are:

- To share my experiences, via images, to help improve the welfare and husbandry of this species.
- To stimulate a dialogue which results in further sharing of ideas and information.

Introduction

Between January 2016 and October 2018, cases of ill health and disease in a colony of *Xenopus laevis* frogs housed by the University Biological Services (UBS) at Cambridge University, have been documented via paper records and photographs. Over this 2 year 10 month period, there were over 2000 adult frogs housed in this facility, of which 1940 (97%) have shown no clinical signs of disease or other abnormality.

However, a number of incidents such as: Gas Bubble Disease, an infestation with nematodes, and a suspected adverse reaction to a batch of hCG hormone have all contributed to our usual low number of clinical cases.

Background to the Home Office designated biofacility

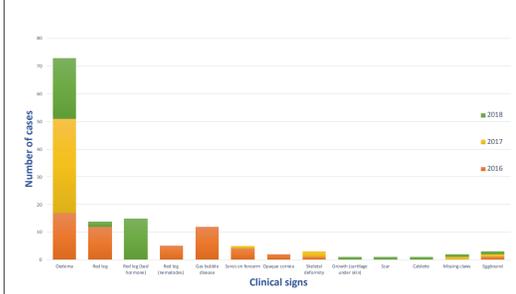
- Opened in 2005, the biofacility can hold up to 900 female and 240 male frogs in a Marine Biotech (now serviced by MBK Installations Ltd.) recirculating aquatic system.
- The system monitors parameters such as: temperature, pH, conductivity, and Total Gas Pressure.
- Frogs are maintained on mains fed water with a 20% water exchange.
- There are several filtration steps, including: biological, mechanical, activated carbon, and UV sterilization.
- Frogs are held under a 12 hour light/dark cycle.
- Each tank has a PVC enrichment tube (30cm x 10cm), with smoothed cut edges.
- Frogs are fed three times a week with a commercial trout pellet.
- The focus of the research undertaken in the biofacility is embryonic and developmental biology. All procedures are conducted under Home Office licence authority.
- Frogs are euthanized humanely using a Schedule 1 method.

Reported clinical signs associated with ill health and disease

- Swimming in circles, tilting or loss of righting reflex.
- Buoyancy problems
- Changes in activity e.g. lethargy or easily startled.
- Unusual amount of skin shedding.
- Weight loss, or failure to feed correctly.
- Coelomic (body cavity) distention.
- Whole body bloat.
- Fungal strands/tufts.
- Sores or tremors at extremities (forearms and toes).
- Redness or red spots.
- Excessive slime/mucous production or not enough (dry dull skin).
- Bite wounds, open cuts, lesions, abrasions of the skin or ulcerations.
- Cloudy corneas.
- Prolapsed cloaca.

(Reed 2005, Green 2010)

Clinical signs found between 2016 to 2018



Attributes of a healthy *Xenopus laevis* frog

- Alert and calm
- Clear eyes
- Muscular hind legs
- All claws intact
- Pear shaped with smooth, clear, slimy skin
- The dorsal pattern should continue over the frog's fore legs and tops of the toes. The abdomen should be a pearly solid paler colour, although freckles and spots are common



Opaque corneas

Description: One or both eyes appear cloudy.
Cause: Unknown, but could be a sign of ill health (Green 2010), stress, nematodes, or a developmental abnormality.
Treatment: None. The eye may decrease in size leaving a scar. This frog was still able to forage and behaved normally.



Loss of hind claws

Description: One or more claws are absent from the hind feet. Once a frog loses a claw the toe turns red or white over subsequent days. Claws do not regrow.
Cause: Unknown, histology is often inconclusive. Perhaps viral or autoimmune.
Treatment: Rehoused affected frogs and monitor. Drain tanks that occupied affected frogs and allow to air dry before filling back up.



Fluid filled cyst

Description: Bulge on the underside of the foot, filled with a clear fluid.
Cause: Unknown.
Treatment: Unknown, although the cyst appeared benign. The frog was euthanized as the cyst appeared to increase in size during the monitoring period.



Skeletal deformity

Description: As frogs grow to adult size a small percentage (< 1%) often have skeletal deformities. The spine curves either upwards (as illustrated) or may bend to the left or right.
Cause: Unknown, but could be a genetic mutation or damage caused from the frog's environment when they was a small froglet or tadpole.
Treatment: None. If the deformity is impacting on the frog's welfare, causing difficulty in swimming, feeding, or any other behaviours, then the frog should be euthanized. Frogs with mild skeletal deformities still produce good oocytes.



Gas bubble disease

Description: Frogs float horizontally and develop redness in the webbing of their feet which may extend over the toes, hind legs and back.
Cause: Water that's been supersaturated with gasses.
Treatment: Shut down the system. Find and repair cracks) and allow time for the gasses to disperse. Frogs that show clinical signs should be removed and closely monitored until the system is repaired and the gasses dispersed. Frogs that have severe clinical signs (unresponsive and/or redness over more than 25% of their body) should be euthanized.



My experience

This happened at my facility when a small crack developed in the pipework attached to the pump allowing air to be drawn in and then pressurised. The first clinical sign noticed were frogs floating abnormally at the top of their tanks (approx. 10% of the colony). On closer inspection redness could be seen in the webbing of their feet. The system was immediately shut down. From this point onwards, 99% of the frogs made a rapid and excellent recovery. Those that didn't were euthanized using a Schedule 1 method.

Recovery

Frogs that displayed clinical signs such as redness on their hind legs, feet or abdomens were taken out of the re-circulating system and housed in a static tank and closely monitored. Water was changed daily. Most frogs made a full recovery within days and were returned to their home tanks once the crack in the pipe was repaired.

Recovery in photographs

The below photographs clearly illustrate the progress of a frog after exposure of Gas Bubble Disease. After two weeks this frog's clinical signs significantly improved.



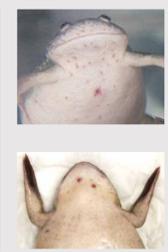
Red leg disease (General)

Description: Clinical signs include, redness of the hind legs, abdomen and/or red spotting that may extend to the frog's back, thickening of the skin, and/or swelling of limbs.
Cause: There are many types of Red leg, the way it presents can be attributed to different types of infectious organisms (*Aeromonas*, *Pseudomonas*, *Mycobacterium*, *Chryseobacterium*, etc). They may be carried concurrently (Hill et al 2010) and usually lie dormant in the frog, but can cause disease when frogs are exposed to a stressful event. If secondary fungal infection occurs this can confound the clinical signs.
Treatment: Red leg can be a complicated condition to treat especially where there is an underlying systemic disease (Green 2010). Salt baths have been found to help milder, early cases.



Red leg: *Aeromonas hydrophila*

Description: The photographs clearly demonstrate red lesions on the underside of affected frogs.
Diagnosis: Skin swabs taken from these lesions confirmed the causal agent was the bacterium *Aeromonas hydrophila*. In addition the bacterial cultures identified *Aeromonas hydrophila*, *Citrobacter freundii* and *Comamonas* spp.
Treatment: These frogs were euthanized. Their tanks were thoroughly cleaned and left to dry out.



Red leg: after new hormone batch

Description: Red patches developed on super ovulated frog's flanks and at the tops of their hind legs either during laying eggs or a few days later.
Diagnosis: These frogs were swabbed and bacterial cultures isolated both *Aeromonas* spp. and *Pseudomonas* spp.
Treatment: After ruling out husbandry practices, it was thought that the change to a new veterinary approved brand of hormone was the likely stressor. Upon returning to the old brand, no more cases were found. Frogs showing clinical signs were monitored where most made an excellent recovery.



Granular scarring

Description: Strange irregular granular scarring was observed on a frog's flank which did not heal.
Cause: Unknown, perhaps a chemical burn caused by Chlorhexidine which is found in many cosmetic products (Phillips et al 2015).
Treatment: None, the frog was euthanized as there was no improvement after monitoring. Histopathology was inconclusive.



Egg bound

Description: The frog may lay few or no eggs after superovulation. The next 1-2 days, the 'non-layer' has a bloated appearance and feels firm. The frog may be less responsive.
Cause: Eggs get blocked in the oviduct which can lead to a build up of bacteria inside the frog which, if left untreated, will result in the death of the frog.
Treatment: Close monitoring of 'non layers', then immediate euthanasia as soon as egg retention is recognised. Some frogs fail to lay due to other reasons such as technician error or faulty hormone.



Bloated hind legs

Description: Clear fluid accumulation under the skin of either one or both hind legs giving a bloated appearance. Frogs exhibit normal behaviours, continue to feed well and lay good eggs.
Cause: Unknown, but histopathology has revealed heart defects. This is a common clinical sign.
Treatment: Salt baths can reduce swelling, but swelling usually returns. If extreme, or the swelling spreads to the abdomen euthanasia should be performed.



Orange blotches

Description: Irregular orange/red marks appear on the abdomen.
Cause: Change in water quality. In this case the cause was due to a sudden switch from mains water to Reverse Osmosis (RO) water.
Treatment: Put source back to mains water supply. Note, when changing water quality/source, this should be done slowly until they are healthy). Separate and isolate affected animal with a different mate. Salt treatment if required, although this can add further stress to the frog.



Frogs will eat anything

Description: A frog was found with a sore on her back that would not heal.
Cause: The frog had eaten a cable tie (we don't know when this happened and we don't use this type of tie). The cable tie had passed through her digestive system down to her colon. The end of the tie had pierced through the colon and through the skin on her back.
Treatment: Be extremely careful and don't leave debris around tanks. Keep small inedible items away from tanks and procedure areas. Keep track of everything you use. Euthanize frog and record.



Wounds affecting males

Description: Red sores may occur on abdomen of male frogs.
Cause: Male gripping onto another male during amplexus (mating). This is common in male frogs housed together.
Treatment: Sores should heal in a couple of days (lesions should change from red to white, then decrease in size until they are healed). Separate and isolate affected animal with a different mate. Salt treatment if required, although this can add further stress to the frog.



Nematodes: *Capillaria xenopodis*

Description: The nematodes inhabit the epidermis (skin) typically on the frog's back that can then result in skin lesions (Green 2010, Reed 2000). This photo shows lesions on the underside of this frog.
Cause: Frogs show clinical signs when stressed. This frog was found positive for nematodes following laboratory tests.
Treatment: Anthelmintic (Levamisole or ivermectin). Thorough cleaning and drying of aquatic system.



Wound affecting males

Description: This male frog was found to have lines all over his body (Note: The redness on the right hand side was due to MS223).
Cause: This frog was probably trying to mate with another male who defensively kicked with his hind claws to get away or out of amplexus.
Treatment: Lines healed quickly. Isolation with another male and salt treatment if required, although this can add further stress to the frog.



Conclusion

- By sharing this photography I hope to expand and refine the material available to those working with *Xenopus* frogs. These cases were taken over a period of 2 years and 10 months and in some cases the cause was anecdotal, based on our experience at that time.
- If you work with *Xenopus* spp. and have experienced similar or different cases, or have any ideas or opinions on the cause and treatment, then please get in touch: tj222@cam.ac.uk it would be great to hear from you!

References

- Green, S.L. (2010) 'The Laboratory *Xenopus* sp.' CRS Press.
- Densmore, C.L., Green, D.E. (2007) Diseases of Amphibia. ILAR Journal, 48(3): 235-254.
- Hill, A.H., Newman, S.J., Craig, L., Carter, C., Czarra, J., Brown, J.P. (2010) Diagnosis of *Aeromonas hydrophila*, *Mycobacterium* species, and *Batrachochytrium dendrobatidis* in an African Clawed Frog (*Xenopus laevis*). J.Am.Assoc.Lab.Anim.Sci. 49(2): 215-220.

Acknowledgements

- Phillips, B.H., Crim, M.J., Hankenson, F.C., Steffan, E.K., Klein, P.S., Brice, A.K., Carty, A.J. (2015) Evaluation of Presurgical Skin Preparation Agent in African Clawed Frogs (*Xenopus laevis*). J.Am.Assoc.Lab.Anim.Sci. 54(6): 788-798.
- Reed, B.T. (2005). R.S.P.C.A Guidance on the housing and care of African clawed frog *Xenopus laevis*. Research Animals Department-RSPCA.

Acknowledgements

Cambridge University Biomedical Services, and The Gurdon Institute.