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Successful treatment of Anchor Worm (Lernaea cyprinacea) using Lufenuron in the Mexican Axolotl (Ambystoma mexicanum)

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Successful Treatment of Anchor Worm (*Lernaea cyprinacea*) Using Lufenuron in the Mexican Axolotl (*Ambystoma mexicanum*)

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Abstract

A 3-year-old, female, captive Mexican axolotl (*Ambystoma mexicanum*) presented with a significant *Lernaea* infestation. The animal had a history of poor husbandry prior to being rescued by the current owner. The axolotl was anesthetised using a buffered 0.15% tricaine methanesulfonate (MS-222) immersion bath and then moved to a buffered 0.1% MS-222 bath for maintenance. The adult anchor worms were removed manually under anesthesia. Following recovery, the axolotl enclosure was treated with 0.1mg/L lufenuron, which was added to the water once a week for five treatments. Six months following treatment there has been no recurrence of the *Lernaea* infestation. This is the first documentation of successful treatment of *Lernaea* in the Mexican axolotl using lufenuron.

Key words: *Ambystoma mexicanum, Lernaea,* anchor worm, lufenuron

Introduction

Anchor worms (*Lernaea cyprinacea*) are parasitic copepod crustaceans that predominantly affect fish kept in freshwater environments (Hossain *et al*., 2018). Following mating, the adult female burrows into the flesh of the host, with site specificity for the gills, head, and fins (Hangan *et al*., 2013; Hossain *et al*., 2018). While fish can survive with low burdens of anchor worms, chronic infestations can result in poor growth and secondary infections that can lead to death. Due to the parasite's low host specificity, anchor worm has been reported to affect numerous amphibian species (Carnevia and Speranza, 2003; Huacuz, 2002; Kupferberg *et al*., 2009; Nagasawa *et al*., 2007; Takami and Une, 2017; Wellborn and Lindsey, 1970). Despite well documented treatment protocols for fish, guidelines for treatment in amphibians remain anecdotal.

This report describes the clinical findings, treatment method, and follow-up of successful treatment for anchor worm using lufenuron on a Mexican axolotl (*Ambystoma mexicanum*).

Case Report

A 3-year-old, female Mexican axolotl was presented to the Exotics Service at the Beaumont Sainsbury Animal Hospital, Royal Veterinary College, London, United Kingdom due to numerous anchor worms seen on the gills and skin. The axolotl had been rescued from a private owner five days prior, where husbandry conditions were reported to be poor, with numerous axolotls being kept in various tanks of unknown water quality and evidence of ammonia burns and missing limbs on some specimens. Since rehoming, the axolotl had been mixed with two other axolotls in a large plastic storage box in 20L (5 gallon) of water in a dark room. Water temperature was maintained at 18-19°C (64-66°F). The water was treated with a conditioner (Prime®, Seachem, Madison, GA, USA) and a complete water change performed every 24 hours. Water quality testing was not carried out in this case because of the history of such regular, complete water changes. The owner reported they had been fed feeder fish by the previous owner, but were now being fed a diet of earthworms and frozen bloodworms.

On initial examination, the affected axolotl was quiet but alert and responsive, in good body condition, and weighed 106g. There were greater than twenty, 3 mm, thread-like Y-shaped projections protruding from the gills and the skin by the gills, ventrum, and front limbs consistent with embedded, gravid female *Lernaea* sp. (Figure 1). The other axolotls were also examined but had no evidence of *Lernaea* sp. infestation.

The following day, the animal was anesthetised for manual removal of the adult anchor worms. Anesthesia was induced by immersion in a 0.15% tricaine methanesulfonate (Tricaine Pharmaq, PHARMAQ®, Hampshire, UK) solution until the loss of the righting reflex was observed (approximately 10 minutes following initial immersion) (Menger *et al*., 2010; Zullian *et al*., 2016). The patient was then transferred into a 0.1% MS-222 solution for maintenance of anesthesia during the procedure. The induction was smooth and the anesthetic depth was considered adequate for a short, non-surgical procedure of this nature. Water temperature was maintained at 18°C (64°F) and both MS-222 baths were buffered to a pH 7 using sodium bicarbonate prior to patient immersion. Heart rate was continuously monitored using Doppler ultrasound (Model 811-B, Parks Medical Electronics, Inc, Aloha, OR, USA) and was measured at 20-25 beats per minute during induction and maintenance . Analgesia was provided with an intramuscular injection of 0.4mg/kg meloxicam (Metacam®, Boehringer Ingelheim Ltd, Berkshire, UK) (Wright *et al*., 2014) into the forelimb (Figure 2). Anchor worms were identified and removed using mosquito artery forceps by firmly gripping the anchor worm as near to the epidermis or gill filaments of the axolotl as possible (Figure 3). Traction was then applied to remove the anchorworm, taking care to remove the entire parasite. Once all copepods were removed, the axolotl was placed in a recovery bath of fresh dechlorinated water. Recovery was assisted by simulating branchial and cutaneous irrigation by moving the axolotl through the water slowly and deliberately, mimicking normal movement. Recovery was rapid once moved into the freshwater bath, as heart rate increased to 80 beats per minute and voluntary movement returned within 10 minutes. The total anaesthetic time was 30 minutes, including induction and recovery.

Follow up treatment was then prescribed with an in-water suspension of lufenuron, based on similar successful treatment regimes in fish. A 6.78mg/ml solution was created by crushing 67.8mg lufenuron tablets (Program®, Elanco™ Indianapolis, IN, USA) into 10ml of water. 9mg (1.3ml of 6.78mg/ml solution) was added to a 90-litre tank to create a 0.1mg/L immersion bath for all three axolotls. The treatment was repeated once a week for five weeks, with complete water changes every 7 days to coincide with fresh immersion baths. The 90-litre tank was the permanent enclosure for the axolotls at the time of discharge, and a canister filter system (Fluval® 107, Rolf C. Hagen Inc. Quebec, Canada) was added to improve water quality.

The owner reported that after the first dose of treatment there was no further evidence of adult anchor worms. Approximately six months following treatment, the owner reported that all three axolotls were thriving in their new enclosure with no evidence of anchor worm infestation.

Discussion

While *Lernaea* sp. has been reported in various amphibian species, including foothill yellow-legged frogs (*Rana boylii*), adult bullfrogs (*Rana catesbeianus*), and the Lake Patzcuaro salamander (*Ambystoma dimerilii*) (Kupferberg *et al.*, 2009; Huacuz, 2002; Wellborn and Lindsey, 1970), the first reported epizootic infestation in axolotls occurred in commercially bred animals contaminated by goldfish (*Carassius auratus*) in Uruguay (Carnevia and Speranza, 2003). When considering the history in this particular case, it is suspected that this axolotl was likely housed in an enclosure that had been exposed to contaminated feeder fish. As the other two axolotls that presented did not have visible adult *Lernaea* infestations, it was likely that these individuals were housed without exposure to the parasite prior to presentation. In a retrospective study (Takami and Une, 2017), a single case of *Lernaea* infestation was documented in an axolotl but no history or treatment plan was discussed.

To successfully treat infestations of *Lernaea*, the clinician needs to consider the life cycle of the parasite. *Lernaea cyprinacea* has a direct life cycle, with the female permanently attaching to the host after copulation and causing intense focal inflammation and hemorrhage (Hossain, 2018). The male anchorworm may be found on the gills, but they are not permanently attached and die after copulation (Avenant-Oldewage, 2012). The development of egg sacs on the female occurs approximately four days after attachment, and eggs hatch into larvae in one to three days (Hossain, 2018). The resulting larvae can cause disruption and necrosis of the gill tissue before developing into adults (Avenant-Oldewage, 2012). Adult female parasites can be very resistant to treatment and manual removal is recommended; whereas free-swimming larval stages require medical treatment, which can be aided by regular heavy water changes to dilute the population (Maclean, 2006).

There are numerous treatment options for *Lernaea* in fish, but not in axolotls (Avenant-Oldewage, 2012). Historically, one of the most effective and commonly used drugs for this condition is the benzylurea chemical diflubenzuron. Diflubenzuron was not available in this case, so another treatment was needed. Salt at 4.8 g/L for 30 days has been used to prevent the development of early life stages of *Lernaea* sp. in food fish species that are tolerant of salinity (Steckler and Yanong, 2012). However, adult female *Lernaea* are tolerant of salinity up to 22.4 g/L, so removal of potential host fish from the tank for seven days is required to break the life cycle and ensure the deaths of the copepodid larvae when using this method. Additional alternative treatments that have been suggested for treating *Lernaea* infestations include macrocytic lactones, such as doramectin, and another benzoylurea pesticide, lufenuron (Hemaprasanth *et al*., 2008; Wolfe *et al*., 2001). Due to known efficacy of benzylurea chemicals in axolotls and ease of access of lufenuron, the authors chose to use the latter in this case. The dose was selected based on recommended therapy for *Argulus* infestations in axolotls and widely published efficacious doses used in fish medicine (Maclean, 2006; Mayer *et al*., 2013; Mutschmann, 2015).

Anecdotally, the use of praziquantel, potassium permanganate, and formalin have been described, but doses and safety for use in axolotls is unclear. *Lernaea* are also known to be susceptible to organophosphates, but these drugs are considered toxic to larval stages of axolotls and therefore were not considered in this case (Robles-Mendoza *et al*., 2009).

This study describes a safe and effective protocol for treatment of *Lernaea* infestation in the Mexican axolotl using manual removal under anaestheta and in-water treatment with lufenuron.

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Figure legends:

Figure 1. Y-shaped female *Lernaea* sp. visible on the gills during anaesthetic induction.

Figure 2. An intramuscular injection of meloxicam was administered following anaesthetic induction.

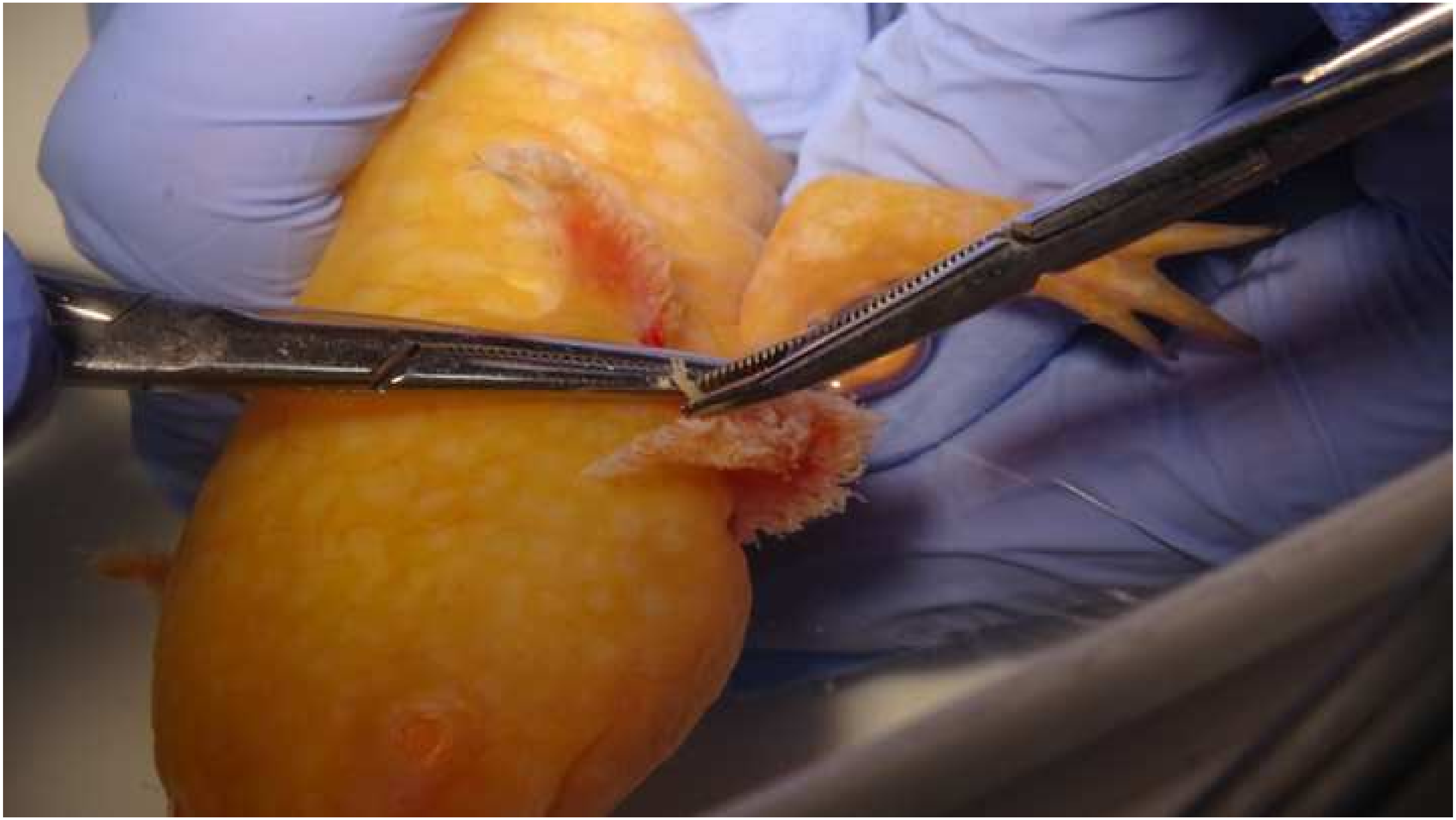
Figure 3. Adult *Lernaea* sp. being removed using mosquito artery forceps.

Figure 2

Click here to access/download;Figure;Figure 2.png

Figure 3

Click here to access/download;Figure;Figure 3.png



Rebuttal letter for: **Successful treatment of Anchor Worm (*Lernaea cyprinacea*) using Lufenuron in the Mexican Axolotl (*Ambystoma mexicanum*)**

Dear Associate Editor and Reviewers,

Thank you for you time, expertise and constructive comments.

I have copied each point made by the reviewer(s) in bold with my response alongside.

1. **Line 8, p. 3. add the word anchor in front of worm and then modify the remainder of the sentence to read "to remove it in its entirety".** – Corrected and now reads as directed.
2. **Line 12, p.4. change the word house to housed** – Corrected.

I look forward to your feedback and any further suggestions you may have.

Thank you all again for your time.

Yours sincerely,

Jack MacHale