LETTER TO THE EDITOR

Challenges encountered while attempting anaesthesia of giant African snails (*Acathina fulica*)

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Conflict of interest statement

Authors declare no conflict of interest

The authors wish to report a frustrating experience whilst attempting to anaesthetize 12 giant African snails (*Acathina fulica*), presented to a referral practice (Ambulatorio Veterinario Montale, Naples, Italy) for diagnostic purposes. General anaesthesia was required to perform biopsies of the foot muscle of the snails to screen for parasites (*e.g. Angiostrongylus cantonensis*).

Little is known about anaesthesia of gastropods (Girdlestone at al. 1989; Woodall et al. 2003). One study (Gilberston & Wyatt 2016) described the use of 5% ethanol to anaesthetize five land snails. Although data about long-term complications were unavailable, the authors reported full recovery within 2 hours. Ethanol immersion was initially proposed to the owner and breeder of the study snails; however, she was concerned about the potential risks, knowing that higher concentrations of ethanol are used for euthanasia of snails, and opted for alternative anaesthetic techniques.

Anaesthesia of the first snail was attempted with an immersion technique previously reported in toads (Adami et al. 2015). The anaesthetic solution was prepared with 50 mL of dechlorinated mineral water obtained from the same source as that used in the terrarium water ponds (pH 7.0, 24 °C). Alfaxalone (0.1 mL; Alfaxan; Jurox, UK) was added to water to a concentration of 200 mg L^{-1}. The snail was partially immersed such that only the ventral part of its body was in contact with the anaesthetic solution. After 20 minutes of immersion, no signs of sedation (slower body movements with no attempt to climb) or anaesthesia (immobility and loss of tentacle withdrawal reflex) were detected. Therefore, the alfaxalone concentration was doubled for the second snail. This new concentration also failed to produce any degree of sedation. The third and fourth snails were immersed in 800 and 1600 mg L^{-1} alfaxalone solutions, respectively, without resulting in sedation. Consequently, alfaxalone (25 mg kg^{-1}; x mL) was injected intramuscularly (IM) into the foot muscle of the fifth snail, but with no sedation observed after 30 minutes. Alfaxalone, at the same dose was administered

via an intracoelomic injection in the sixth snail, with the assumption that this would be more effective than the IM route. The intracoelomic injection was performed with a 21 gauge hypodermic needle via an area of the foot skin hidden under the shell. None of these drug protocols resulted in sedation, therefore, in the seventh snail intracoelomic ketamine (50 mg kg^{-1}; product source) was administered, but with no appreciable effects. This dose was doubled for snails numbered eight and nine, same route, and resulted in slightly decreased movements, although the tentacle withdrawal reflex appeared to be well preserved in both snails. In the tenth snail, intracoelomic ketamine (200 mg kg^{-1}) resulted in more significant changes, and the snail withdrew into its shell, remaining for 24 hours that precluded completion of the clinical procedure because the foot muscle was inaccessible. The eleventh snail was administered a combination of intracoelomic ketamine (100 mg kg^{-1}), and dexmedetomidine (1 mg kg^{-1}; product information), resulting in slower movements but retention of tentacle withdrawal reflex. All 11 snails responded to the injections (both IM and intracoelomic) with foamy slime production and partial or complete retraction into their shells. Retraction was a major limitation since access to the biopsy site was obscured. For the twelfth snail, isoflurane was delivered into an induction chamber with the vaporizer at 5% and oxygen 5 L $minute^{-1}$ for 20 minutes. Pulmonated snails can breathe dry air (Barnes 1982). Although their respiratory anatomy differs from that of mammals, by lowering the respiratory cavity floor the pressure within the cavity is reduced, allowing inflow of air (Barnes 1982). No sedation or anaesthesia, or side effects, were observed in this snail.

Both land snails and slugs produce an external bodily secretion, the characteristics of which depend upon the nature of the stimulus initiating its synthesis and release. Under normal circumstances the slime is viscous and sticky; however, a snail that is overstimulated or undergoes excessive mechanical stimulation may release a more foamy, clear and abundant secretion. This suggests that foamy mucus production results from environmental

stress (Campion 1961). Moreover, excessive mucus production is the first symptom of poisoning in both slugs and snails. The inevitable manipulation of these snails and the exposure to anaesthetic drugs may have led to the copious foamy secretions observed here. Alternatively, the production of foamy mucus may have been an attempt to eliminate the anaesthetic agents, perceived by the body as toxic substances.

Anaesthesia of African giant snail seems to be extraordinarily challenging and none of the techniques attempted by the authors resulted in effective anaesthesia. The ability of land snails to excrete xenobiotic substances might be the reason for the lack of success reported in this letter; nevertheless, without measurements of drug concentrations in the excreted fluid, this remains a speculation not supported by evidence.

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