Safe laboratory management for arachnids of medical importance

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Summary

Safe animal management is key to any facility; this is especially true of those housing animals of significant medical importance. Procedures detailed here demonstrate how dangerous arachnids including scorpions (Family *Buthidae*), wandering spiders (Family *Ctenidae*), recluse spiders (Family *Sicariidae*) and the *Theridiidae* family containing the widow spiders can be safely managed in the laboratory environment. This dramatically reduces the risk of envenomation during husbandry and experimental procedures. Risks are reduced with modified enclosures that allow separation from the animals during most husbandry procedures and use of anaesthesia where contact is inevitable. The equipment and techniques presented here thus allow for greatly improved safety when working with dangerous invertebrates.

Introduction

Unlike most laboratory animal species, invertebrates, such as spiders, are not protected by UK law. However, there are still compelling reasons for refining the husbandry of such animals, particularly as these species are of increasing interest as a source of novel therapeutics. Good husbandry will increase the lifespan and breeding potential which may have financial implications and it is recognised that healthy animals, given the opportunity to exhibit "normal" behaviours, make more valid experimental subjects. Possibly even more importantly for professional animal technologists is the principle that we have a duty of care to any living creature held in captivity and good practice demands that we do our best to ensure each creature remains, at the very least, healthy and comfortable. In the case of venomous invertebrates, there is another important reason for cultivating good husbandry practice; understanding behaviours and methods of safe handling of venomous species could have serious health implications to the handler. The basic principles of good animal husbandry are the same for all species; maintenance of appropriate, hygienic environmental conditions including provision of appropriate quantities of good quality food, a good understanding of the species' "normal" behaviour and frequent, careful observation to identify any deviation from that normal behaviour. In addition, the husbandry of arachnids of medical importance poses more significant challenges in containment and safe handling (for both animal and technician) due to the size, speed, aggression and venomous characteristics of many species.

This paper describes a method of performing routine husbandry by which risk of envenomation is significantly reduced along with risk of escape or injury of the animals and was originally presented as a poster at the Laboratory Animal Science Association (LASA) Winter Meeting and an oral presentation at the Institute of Animal Technology (IAT) Congress 2011.

Medical importance

Several species of Araneomorpha spiders produce venom of significant medical importance, such as the armed spiders of South America (Family *Ctenidae,* species *Phoneutria fera* (Perty, 1833) and *P. nigriventer* (Keyserling 1897)), brown recluse spiders (Family *Sicariidae*, species *Loxoceles laeta* (Gertsch, 1961; non Nicolet, 1849)) and the *Theridiidae* family members – *Latrodectus mactans* (Fabricius, 1775) and *L. hasselti* (Thorell, 1870). Envenomation from arachnids of these genera have long been known to cause significant health effects in humans and are thus considered medically important. Of the species maintained within the Venomtech facilities the widows, such as the black widow (*Latrodectus mactans*) and the redback spider (*Latrodectus hasselti*), are the most likely to produce fatal envenomation, despite the fact that according to LD_{50} studies their venom is of lower potency than that of other arachnids discussed herein, LD_{50} 1.34mg/kg[1]. The widow's neurotoxic venom leads to immediate intense pain due to the stimulation of neurotransmittor release through increased exocytosis, from nociceptive (pain) synapses but this effect is not specific to the nociceptive neurones and leads to muscle cramps, convulsions, vomiting, perfuse sweating and occasionally death. Prior to the development of an effective antivenin approximately 6% of bites were fatal, this dropped to 0.2% with effective diagnosis and treatment[11]. The true role of effective diagnosis was emphasised recently when an otherwise healthy female died from an undiagnosed spider bite suspected to be *Latrodectus mactans*[2]. To reduce this potential it is vital that medical professionals know where dangerous spiders are being kept outside of their natural range such that diagnosis is swift. Brown recluse spiders (*Loxosceles* species) are a source of significant morbidity throughout the tropics and sub-tropical regions, due to the fact that they are common in human dwellings and their bite often goes unnoticed and therefore treatment is delayed due to lack of diagnosis. Although the mortality rate is low at 0.03%[3] the high morbidity is due to the high level of cytotoxic enzymes, such as phospholipase, hyaluronidase and sphingomyelinase, that breakdown the skin and muscle leaving large ulcers that are difficult to treat effectively. In Paraná state, Brazil, 50% of *Loxosceles* bites are classified as moderate (characteristic lesion, erythrema and petechiae) and 2% severe (with moderate symptoms but with added haemolysis)[3] so they have great potential to impact the quality of life of victims and therefore require specialist husbandry techniques to minimise this risk.

With venom nearly twice as potent as that of the black widows[4], the armed spiders, often called wandering spiders (*Phoneutria* species), are, unexpectedly, rarely fatal. These spiders are large, fast, aggressive and have the ability to produce frequent systemic envenomation but rarely do, in fact only in 0.5 % of cases.[5] This could be an example of venom dose metering by spiders as has been observed in *Parabuthus* scorpions.[6] This is possibly because venom is likely to be energetically expensive to produce, so large doses are only delivered in life threatening circumstances, the arachnids preferring to warn potential predators with threat displays, dry bites (those without envenomation) and low dose bites.

Although all scorpions produce feelings of fear and anxiety in many people, only a few are of significant medical importance and therefore listed on the Dangerous Wild Animals Act, 1976. Scorpions of the *Buthidae* family such as *Leiurus quinquestriatus* (Ehrenberg, 1828), *Parabuthus liosoma* (Ehrenberg, 1828) and *Androctonus australis* (Linnaeus, 1758) are the main species capable of fatal envenomation[8] and therefore listed. Despite the fact that venom from the death stalker *Leiurus quinquestriatus* is nearly five times more potent in subcutaneous LD_{50} studies with white mice[9] than that of the black widow *Latrodectus mactans*, it is responsible for very few fatalities. This is another potential example of dose metering[6] or could be due to a low venom yield. Even so, scorpion envenomation is a serious event and may often require medical attention as many other *Buthidae* scorpions do have a higher fatality rate.

The pharmacological potential of these venoms is of great interest as a source of biological tools and therefore present a need for Venomtech staff to maintain these animals in captivity. Captive husbandry of these species presents a serious risk to the health and safety of staff, visitors, nearby businesses and the public, risks which are greatly reduced by this husbandry protocol. The use of carbon dioxide (CO_2) for anaesthesia in arachnids has been documented several times but the integral use of anaesthesia as part of the husbandry program has not been described previously to the best of our knowledge.

Methods

All procedures were performed in accordance with the ethos of the Animals (Scientific Procedures) Act, 1986, even though the arachnids are not protected species. All animals were obtained from private breeders at the Terraristika expo, Hamm, Germany, March 2010.

Enclosures – The substrate is a 1-5 cm thick covering of vermiculite (Peregrine Livefoods Ltd) moistened according to the humidity needs for the species being housed, as suggested for laboratory husbandry of tarantulas[9]. Water is provided via a 5 cm diameter plastic water bowl (Vanishing World).

Accommodation for small scorpion species (average body length 2.97 cm +/- 0.48 cm) are standard 0.7 litre secure locking plastic boxes (Really Useful Products Ltd) with eight, 3 mm holes along each side for ventilation. Larger (4cm body length) *Parabuthus liosoma* (Ehrenberg, 1828) are housed as above but in 1.7 litre flat Really Useful boxes (Really Useful Products Ltd). Scorpion body lengths are measured from chelicerae to the terminal wide segment of the mesosoma. A shallow dish is provided for water and

Figure 1. Modified Really Useful box for housing spiders of medical importance, showing feeding aperture in the lid (with closure bolt) and CO_2 line for anaesthesia.

small sections of UPVC roundline guttering (Wickes Ltd) are used to replicate natural crevices and burrows.

Typical containment for true spiders, many of which are listed in the Dangerous Wild Animals Act 1976, as amended 2010 (e.g. *Loxosceles sp.*), is made from a 2.1 litre plastic box with secure fitting lid (Really Useful Products Ltd) (Figure 1). For larger spiders (greater than 5 cm conscious leg-span) the boxes are scaled up accordingly so most large *Phoneutria* species are housed in 3 litre enclosures. All *Phoneutria* species are kept at ~80 % relative humidity while the *Loxosceles* and *Latrodectids* are kept much drier (~30 % relative humidity). A 10 mm diameter aperture for feeding (crickets and flies) and topping up the water bowl is drilled into the lid and secured with an M10 16 mm nylon bolt (Nylons and Alloys Ltd).

The individual enclosures are stacked in a glass fronted shelving unit that is back heated (according to species, typically 27 +/- 3 °C) with a 100 W heat cable (Eurorep Ltd) and thermostatically controlled with a Thermocontrol Prostat II (Lucky Reptile). This produces a thermal gradient that the animals use to naturally thermoregulate. All enclosures are labelled with species name and ID code and similar species are kept together. Velcro backed laminated labels on the front of the glass cabinet display details of species (scientific and common names) complete with antivenin codes (where appropriate); these are to be removed and taken to hospital with the casualty should envenomation occur (see emergency procedure below). Water and humidity levels are checked regularly and animals are fed feeder insects every 1-3 weeks as appropriate for the size and species. Spiders are fed prey insects just smaller than their opisthosoma, young spiders (up to 4 mm leg-span) are fed one fruitfly *Drosophila melanogaster* (flightless mutant) (Livefoods Direct Ltd) every week, all other arachnids are fed appropriate sized crickets or locusts (*Gryllus* species and *Schistocerca gregaria*) (Concrete Jungle Ltd) weekly. Removal (spot cleaning) of discarded food boluses and uneaten food is performed, after the spider is anaesthetised, as below. With the scorpion enclosures, discarded food boluses and uneaten food can be removed with 30 cm forceps.

A 5 mm diameter occluded tube, with pinprick holes for CO_2 anaesthesia and water spray (via syringe) is fitted just above the substrate level. At the top of the opposite side of the box are eight 3 mm ventilation holes such that a rising concentration of carbon dioxide (CO_2) can be achieved. CO_2 anaesthesia is achieved through connection of the occluded tube to a low range CO_2 flow meter (Harvard apparatus) opened to 0.5 litre/minute for 0.7 litre boxes and 1 litre/minute for all other enclosures. Spiders undergoing anaesthesia are closely monitored and are only considered anaesthetised when a response to firm tapping of the box is no longer detectable and when the spider can be inverted by tipping up the enclosure. This abolishment of the natural right-reflex is a firm measure of sufficient anaesthesia depth. Time to anaesthesia is recorded and at this point the CO_2 supply is stopped. The anaesthetised spider is then placed straight into a clean enclosure and allowed to recover in fresh air. Should a spare, clean enclosure not be available then the spider can be placed into a 670 ml plastic food box, (which is also used as an anaesthesia chamber), ventral side up, in fresh air as most spiders will recover before the enclosure can be fully cleaned. The time to recovery is recorded as the time taken for the right reflex to return. Only when the spider is secure can the home cage be cleaned. Lab CO_2 levels are monitored with a CO_2 Dräger Pac 7000 (Fisher Scientific) to ensure compliance with the UK occupational exposure limit of 5000 ppm over 8 hours or 15000 ppm in any 15 minute period. Another reason that Really Useful boxes are appropriate is that they are autoclavable, and resilient to chemical sterilisation such as 70% isopropanol wipes (Hygiene for Less Ltd) and 2% Virkon (Fisher Scientific). The time for the spider to right itself is recorded as this gives the most accurate 'end-point' measure for full recovery. Most spiders will recover before the enclosure has been cleaned so when the clean enclosure is ready, anaesthesia is repeated and the anaesthetised spider replaced into the clean enclosure and monitored to confirm recovery.

Due to the scorpions' lack of agility and inability to climb vertical plastic surfaces they can be easily manipulated by carefully, but firmly, grasping the last few segments of the metasoma, just anterior to the telson, using a pair of 30 cm rubber tipped forceps (Cornish Crispa Company Ltd). The animal can then be securely lifted swiftly into a clean enclosure. This system also attends to the animals' welfare needs as the majority of predatory arachnids are ambush hunters and therefore do not need large enclosures to exercise in, as long as there is provision of shelter and or material to attach silk to build a retreat with.

Alongside the safe husbandry methods detailed above specific emergency procedures are in place to reduce the risk of severe pathology developing should an envenomation incident occur. This includes a no lone-working with dangerous arachnids policy. All staff in the building are trained in essential life support first aid as well as the procedure detailed within. Further notification is required for the emergency services such that the local hospital and ambulance service are aware of the species kept and that includes the nearest specialist poison unit who manage the available antivenins. Unlike envenomation from a wild arachnid where the priority is to move away from the animal, which is often killed to be taken for identification in the laboratory environment, identification is known and the first action must be to contain the animal as arachnids can re-envenomate multiple times. It is therefore essential that they be secured for the sake of the casualty and the emergency responders. Scorpions can easily be returned to their home enclosure, using the technique detailed above. Spiders have greater agility and speed, thus the best emergency capture device is the domed top of a 2 litre plastic bottle, as this has a top for a handle and can be occluded with a small rigid plastic sheet. The entire device can then be placed into a suitable anaesthesia chamber. All nearby staff must be verbally alerted to the incident during attempts to secure the animal. If several people are available then emergency services should be called as soon as possible giving details of the number of casualties and approximate ages. The caller must explain that the casualty has been bitten/stung by an exotic venomous spider or scorpion (give species), indicate the location of the bite/sting, clarify that the casualty may require antivenin (give antivenin code from the cage label), and provide address details. Once the animal is securely contained then the procedure is divided into spiders and scorpions due to different treatment needs. The priority with spider envenomation is to prevent worsening of local symptoms and monitor for systemic exposure, whereas scorpion post-envenomation treatment should focus on prevention of systemic exposure as the only local effect expected is pain, and systemic envenomation is likely. If only two people are present, the casualty should be stabilised first by applying a cold compress and elevating the bitten limb. The emergency services can then be called. It is essential that the casualty is immobilised as quickly as possible as any delay can increase the potential onset of systemic symptoms.

For scorpions the casualty should be laid down in a semi-recumbent position and the affected limb bandaged from just below the site of envenomation up to the trunk in such a way as you would treat a sprain. This compression bandage is to limit the spread of the venom into the blood stream via the lymphatic system and is much the same as for a venomous snake bite[10]. It is critical that the casualty remains calm although it seems antagonistic to the natural response it is important and is best achieved through training and practice of emergency drills. The University of Adelaide has an excellent resource for envenomation and states many victims will be terrified, fearing sudden death and, in this mood, they may behave irrationally or even hysterically. The basis for reassurance is the fact that most venomous bites do not result in envenoming and the effectiveness of modern medical treatment greatly reduces the long-term effects[11]. At this stage the casualty's condition should be frequently monitored and any change in responsiveness, signs or symptoms should be noted. If the casualty loses consciousness and/or stops breathing then standard first aid procedures should be put in place, although due to the neurotoxic mode of action of the venoms apnea is likely without cardiac arrest and therefore artificial ventilation is required without cardiac massage if a palpable pulse is present.

Results

No adverse events (envenomations or escapees) have been identified within Venomtech Laboratories since its incorporation in March 2010. This can be accredited to these procedures and the associated risk assessment. Also we have observed many natural behaviours, such as normal ecdysis (shedding skin), feeding, mating and breeding in these animals, suggestive of the fact that they are not severely affected by the CO_2 anaesthesia and husbandry procedures in place. Detailing *Loxosceles laeta* breeding as an example of such breeding behaviour; the parents were introduced by placing their, 2.1 litre, enclosures side-by-side and opening the lids whilst contained in a 30 litre box with CO_2 line attached. On the 18th December 2010 mating proceeded as expected with the male signaling to the female by tapping the enclosure sides. After approaching cautiously, copulation was observed, followed by naturally rapid retreat of the male to avoid cannibalism. Both adults were anaesthetised and returned to their home enclosures. The egg sac was laid on the 23rd January 2011 and the female was anaesthetised in her enclosure such that the egg sac could be safely removed. The eggs were incubated separately in a 125 ml clear polypropylene containers (Fisher Scientific) at 24˚C and six spiderlings hatched on the 6th March. After a few days they were anaesthetised and separated out into individual pots with a plastic leaf for a hide. As young spiderlings they were fed flightless fruitflies (*Drosophila melanogaster*) and occasional small crickets (*Gryllodes sigillatus*) essentially following Donald Lowries' suggested method[12].

The results presented below (Figure 2) show the anaesthesia parameters for induction and recovery of the species used when anaesthetised in their home enclosures. For the majority of individuals the time to induction is not significantly different to the recovery time (*Phoneutria* sp n=4 animals with 23 anaesthesias, p=0.94, and *Loxosceles* n=7 animals with 43 anaesthesias, p=0.89). In contrast to this the Latrodectids statistically differ from this pattern as the induction time is significantly greater than the recovery time (p=0.0005, Students T Test, n=7 animals, 11 anaesthesias). The outlier, *Phoneutria nigriventer*, displays the largest difference between induction and recovery (p=0.03, n=1 animal, 4 anaesthesias). Occasionally times outside these may be recorded. In our experience when there is a significant delay in time taken to induce narcosis, this is usually due to the animal going into pre-ecdysis. The potential reason for this is the development of the new exoskeleton and book lungs underneath the current cuticle as this is suspected to reduce the diffusion of CO_2 into the tissues. Unusually long recovery times have been observed on occasions with each species, often greater than 600 seconds. The cause of this phenomenon is unclear but further research is clearly needed.

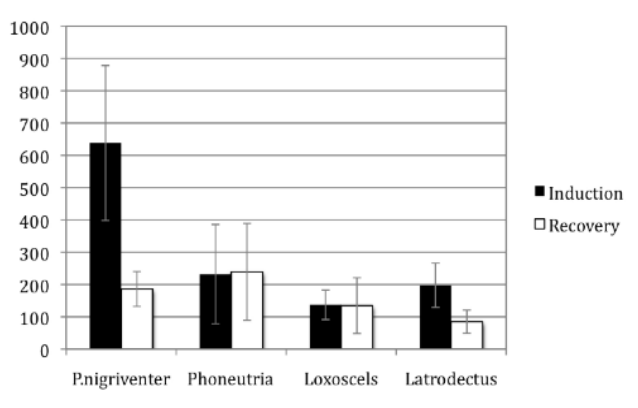

Figure 2. Summary of anaesthesia data for Araneomorph spiders, using their home enclosures, data averaged for genera except *Phoneutria nigriventer* due to this species being substantially larger, than the other members of the genus currently housed. Error bars show standard deviation of full data. The difference between induction and recovery times for *Phoneutria nigriventer* and *Latrodectius sp.* are significant ($p=0.03$ and $p=0.0005$ respectively).

Discussion

The authors believe these procedures allow dangerous arachnids to be maintained in a laboratory environment in a safe and effective manner, thus, attending to the welfare needs of the species at the same time as providing a physical barrier between the staff and the animals for the majority of routine procedures and utilising anaesthesia for those tasks, such as enclosure cleaning, where contact is unavoidable. Many aspects of natural behaviour have frequently been observed in these species being housed in this manner, including breeding of a few species. The CO_2 anaesthesia data presented here shows that the majority of species used in this study have similar induction and recovery times; this allows for prediction of recovery time in the majority of *Phoneutria* and *Loxoceles* species, thus predicting the safe working time. The *Latrodectus* species are an exception to this with a recovery time half that of the induction time. This could potentially be due to the enclosures although this phenomenon still exists when using a 670 ml anaesthesia chamber so it is more likely to be due to physiological differences. Further work is required in this along and with the single large *Phoneutria nigiriventer* as although the multiple anaesthesias show a difference between induction and recovery, studies have only been conducted on one animal due to the difficulty obtaining this species. In an ideal state a comparison would be performed between routine husbandry with and without the use of CO_2 anaesthesia, but the authors feel the risk of envenomation or escape are too great to warrant a trial. There have been anecdotal reports that the use of CO_2 anaesthesia may shorten the lifespan of spiders in captivity[13] but this has not been observed in this study, potentially due to the use of a rising concentration of CO_2 and careful observation to prevent over anaesthesia. Although CO_2 is a very cost effective anaethesthetic agent these procedures could potentially be used with volatile anaesthetics such as isoflurane with minor modifications such as placing the enclosures inside a scavenged anaesthesia chamber and monitoring of residual isoflurane.

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