Refining laboratory husbandry of venomous snakes of the family Elapidae

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Summary

Unlike rodent management, captive maintenance of venomous snakes poses a considerably greater risk to operators and those around them: specific protocols are, therefore, required. Traditional techniques used by hobbyists and professionals involve frequent direct contact; agreed-upon safety procedures exist in the form of antivenom and the knowledge of its use although this is a reactive solution as opposed to proactive avoidance of a bite in the first place. This paper discusses novel maintenance procedures that greatly reduce the risk presented by traditional techniques. Venomtech Ltd work with over fifty species of venomous snake but this paper will focus on species of the family Elapidae. Many elapids are nervous, agile and possess fast-acting neurotoxic venom which, combined with a willingness to use it, means separation between operator and snake is critical. Standard husbandry techniques such as feeding and cleaning therefore need to maintain this separation in order to cultivate a safe working environment. Procedures presented here show contact-free management of elapids through the use of modified enclosures based on Really Useful Boxes (Really Useful Products Ltd) and utilising the snakes' natural behaviours. Cobras (genus *Naja*), mambas (genus *Dendroaspis*) and taipans (genus *Oxyuranus*) are used as example species, but these techniques are also applicable to a wide variety of other venomous snakes. Use of these novel techniques as part of the laboratory management procedure reduces stress from traditional handling techniques and minimises the risk of envenomation subsequently refining safe laboratory management of dangerous snakes.

Introduction

The results of a venomous snakebite can vary; if not fatal, an envenomation can result in a number of serious pathologies such as necrosis, paralysis and oedema[1]. Despite this the snake as a laboratory animal allows for many novel opportunities, as it is many of these pathologies that can be exploited for beneficial applications. Snakes are markedly different in behaviour and physiology from commonly used laboratory animals and the consequences of aggressive contact can be dire, therefore novel methods of husbandry must be utilised in order to maintain them to an appropriate laboratory standard.

Venomous snakes are worked with in captivity for a number of reasons, including education (such as zoological establishments), biological research and amongst private keepers out of interest, however at Venomtech and many other scientific facilities it is for their venom itself. Venom constitutes a diverse library for drug discovery and has already yielded a number of drugs, including Captopril (an Angiotensin Converting Enzyme [ACE]) inhibitor developed from the venom of the fer-de-lance [*Bothrops jararaca*][2] and Integrilin (a pro-clotting factor developed from the venom of Southwestern pygmy rattlesnake [*Sistrurus miliarius barbouri*])[3]. Venom is also critical for the production of antivenom to serve regions with a high incidence of venomous snakebite. With these innovations already in existence and the search for new drugs only getting more intense it is likely that venomous snake management in laboratories will only increase, it is all the more important to develop appropriately safe husbandry techniques.

Snakes are found on every continent (with the exception of Antarctica) comprising 456 genera and around 2,900 species, with venomous snakes making up around 600 of these[4]. Most venomous snakes are found in tropical regions with those from cooler climates undergoing brumation during winter periods. Venomtech works with three venomous snake families, *Viperidae*, *Colubridae* and *Elapidae*, described below:

- ***Viperidae*** (vipers) is divided into two subfamilies, the *Viperinae* (true vipers) and *Crotalinae* (pit vipers), which are physiologically similar; members of the family *Crotalinae* however possess a heat sensing 'pit' for locating prey. This family contains species such as the rattlesnakes (*Crotalus* spp) and Asian pit vipers (*Cryptelytrops* spp). The primary differences in venomous snakes can be highlighted through examination of their dentition – vipers have comparatively long fangs that are highly mobile, folding out to bite and back when the mouth is closed (known as solenoglyphous)[5]. Most vipers have cytotoxic venom that lyse cells, causing massive necrosis and severe pain. Vipers are mostly nocturnal, sedentary ambush predators, preferring to wait for prey to come to them rather than move to seek it out – therefore they were not included in this study due to our protocol requiring movement to food.

- ***Elapidae*** (Elapids) contains venomous snakes with immobile fangs (proteroglyphs)[5] located at the front of the mouth, such as mambas (genus *Dendroaspis*), cobras (genus *Naja*) and taipans (genus *Oxyuranus*). Elapids are generally diurnal, highly active hunters. Their fast metabolism gives them a healthy appetite; Elapids in captivity can eat a lot and in the wild will actively seek out prey in their nests or burrows. They rely on their speed, agility and highly toxic venom to apprehend food items. Elapid venom is neurotoxic, bites exhibiting neurological symptoms such as ptosis and flaccid paralysis[1]. Elapid venom usually causes death through respiratory paralysis with rapid onset of symptoms[1]. Their tendency to move and seek out food makes them ideal for this contact-free protocol.
- ***Colubridae*** (colubrids) is a 'catch-all' group of snakes for those that cannot be classified into the other families, therefore not all colubrids are related and many are phenotypically different, for example some colubrids (and therefore those Venomtech work with) are venomous. Venomous members of the family are known as opistoglyphs[5], named for a set of fixed, grooved teeth located at the back of the mouth that function as 'rear fangs'. These lack enclosed venom injection apparatus and therefore most rear-fanged snakes deliver their venom by mastication. Certain colubrids such as twig snakes (*Thelotornis spp*) and boomslangs (*Dispholidus typus)* are considered medically significant[5], with haemotoxic venom and highly developed delivery systems that do not require mastication to envenomate. Many species of colubrid are physiologically similar to elapids in terms of the protocol described in this paper, therefore a single species, the False Water Cobra (*Hydrodynastes gigas*), was included in this study to test the protocol's reliability across other families.

There is little to no standardised protocol for venomous snake maintenance with the only utilised baseline for maintenance techniques arising from private keepers to whom safety and a scientific outlook is not necessarily a priority. David Warrell's work on management of exotic snakebite[6] paints a grim picture of the outcomes of carelessness and bad protocol amongst private keepers, as well the growing number of exotic snakebites from captive specimens occurring throughout the Western hemisphere. Many of these bite cases involve alcohol intoxication or inappropriate handling[6] and can be considered a result of the lack of true standardised, safety-conscious protocols for the care of these animals. Current husbandry techniques focus around manipulation of the snakes with lengthy handling tools and manual contact to arrest motion, however many, if not all, species have the ability to climb their own bodies or handling tools rapidly; others strike great distances faster than human response or can use their own tail as a leverage point for a strike over their own back[7]. Due to their remarkable agility and the consequences of contact with a venomous snake, any husbandry technique that encourages manual contact or exposure to a venomous snake is inherently unsafe.

Due to the risk of severe injury from a venomous snake, it is not possible to carry out any maintenance within the enclosure with the animal present; therefore removal of the animal to a holding enclosure is required. As this is typically a weekly event it is essential that protocols for movement of the animal do not carry unacceptable risk. Traditional feeding protocols also carry a substantial level of risk. Commonly, a prey item (such as a dead rodent) is grasped in tongs and placed into an open enclosure and the snake encouraged to strike at the food. In a feeding state, these animals are ready to deliver a lethal dose of venom and utilising the traditional method there is no operator protection. Limiting any contact between the animal and technician during this time is vital for safe practice.

This paper focuses on novel techniques for low-risk enclosure maintenance and feeding protocols. This is key in bringing traditional venomous snake protocols more in line with the Health and Safety hierarchy of risk control[8] which is essential for minimising risk in any establishment.

Establishing contact-free methods is critical in minimising exposure to venomous animals. Conditioning, particularly that involving animals moving to a particular place on cue, has been recorded by Kubie and Halpern in garter snakes (*Thamnophis radix)*[9], Weiss and Wilson in Aldabra tortoises (*Geochelone gigantea*)[10] and Kellog and Pomeroy in water snakes (*Nerodia fasciata)*[11] and our protocols utilised this approach to improve efficiency, reliability and repeatability.

Methods

This protocol is a modification of feeding technique and therefore no part of this work required a licence under the Animals (Scientific Procedures) Act 1986. All animals were housed appropriately according to the guidelines in force at the time.

Snakes were housed in 50 litre polypropylene boxes (Really Useful Products) with locking lids appropriate for a venomous snake enclosure. 5mm holes are drilled for ventilation, along with one 8mm hole for insertion of a pipe to refill water bowls without entry into the enclosure. Enclosures are furnished with a 3 litre polypropylene box (Really Useful Products) or naturalistic water bowl (Exo-Terra) in the case of *Naja, Dendroaspis and Oxyuranus* species or a 5 litre plastic box (Really Useful Products) in the case of *Hydrodynastes* species, for hydration, with water ad libitum. A smaller dish is adequate for most animals, however *Hydrodynastes* are a species that lives amongst marsh and bodies of water in the wild[12] and tend to immerse themselves completely in their water dish. A large upturned flowerpot (Meadow Grange Nurseries) is provided as a shelter with a 10cm diameter hole bored in the side for entry and exit. Loose substrate is not used, with several layers of clean newspaper serving the same purpose and providing an easily disposable cage lining.

Snakes are ectotherms and rely on the temperature of their surroundings to regulate their body temperature and therefore their enclosure must be heated. Venomtech's snakes are heated with external under-enclosure heat mats (Peregrine Live Foods Ltd) with daytime temperatures of 29˚C +/– 2.1˚C, dropping to 25˚C +/– 1.5˚C at night and kept on a 10 hours on, 14 hours off day-night cycle. These temperatures are maintained using in-enclosure thermostats (Habistat). None of the species included in the study require a consistently elevated humidity level, however *Hydrodynastes* enclosures are occasionally misted with water from a 5l Hoselock Killaspray garden sprayer (Meadow Grange Nurseries) as their wild environment is very wet.

Figure 1. The Feeding Airlock (patent pending GB1205301) accessible safely to the operator (left image) and with the outer cap closed and ready for snake feeding (right image)

Fitted to the front of the enclosure is the contact-free feeding airlock (patent applied for GB1205301). Figure 1 shows the two-gate system with an unscrewable outer cap and an inner locking gate that is opened and closed by an external handle. This creates a chamber that is accessible to the technician but not the snake if the outer cap is open and the inner closed and conversely creates a chamber that the snake can gain entry to without an opening to the outside, where the technician is. The housing is bolted securely to the enclosure, and the inner gate can be locked closed via a loop on the handle, leaving no possibility of the snake gaining escape through the gate.

Food items utilised for this protocol consisted of pre-killed mice (*Mus musculus)* average weight *36.6 g* (range 28-54 g) and pre-killed rats (*Rattus norvegicus*) average weight 82.6 g (range 54g-141g), dependent on the head size and species of the snake.

This first protocol minimises contact with a venomous snake during feeding. The outer cap was unscrewed and appropriate food item (a pre-killed rat or mouse) was placed in the space between the two gates. The outer cap was then replaced and then the inner gate was opened, allowing the snake access to the food item. The time taken from the gate's opening to the snake biting the food was recorded and observations of behaviour were noted. This procedure was repeated with the snakes every week over eight weeks. The snakes were maintained in all other aspects between these feedings, however this is an appropriate feeding rate due to their slower metabolism.

The second protocol utilised a second 50l polypropylene box (Really Useful Products) as a holding enclosure for the snake being fed. A feeding airlock (patent pending GB1205301 see Figure 1) was attached to the side of this enclosure, at the same level as the gate on the home enclosure, with a detachable PVC tube (Midwest Tongs) in place of the screw cap. When the cap is removed from the gate on the home enclosure, this tube can be securely attached, creating a linked system. Our safe husbandry protocol entailed placing a defrosted prey item in the holding enclosure whilst not attached to the home enclosure. The holding enclosure is then closed up, attached to the home enclosure and then the gates are opened and the snake's reactions observed. The time taken from the opening of both gates to the snake moving entirely into the holding enclosure was recorded.

Common Name	Scientific Name	Sex	Age (years)	Weight (g)	Inventory Code
Black Mamba	*Dendroaspis polylepis*	Male	6	1420	D.pol02
Spectacled Cobra	*Naja naja*	Male	4	500	N.naj03
Spectacled Cobra	*Naja naja*	Female	4	550	N.naj04
Egyptian Cobra	*Naja haje*	Male	Unknown	750	N.haj01
Egyptian Cobra	*Naja haje*	Female	Unknown	500	N.haj02
Papuan Taipan	*Oxyuranus scutellatus*	Male	3	not recorded	O.scu01
Mozambique Spitting Cobra	*Naja mossambica*	Male	Unknown	515	N.mos02
Mozambique Spitting Cobra	*Naja mossambica*	Female	Unknown	390	N.mos03
False Water Cobra	*Hydrodynastes gigas*	Male	Unknown	1025	H.gig01
False Water Cobra	*Hydrodynastes gigas*	Female	Unknown	1000	H.gig02

Figure 2. The animals used in this study

The gates were then closed behind the snake, preventing it from re-entering the home enclosure and allowing cage maintenance to be performed. The home enclosure was then secured, the gates reopened and the time taken for the snake to return fully into its home enclosure. Snakes that seemed to adjust well to this technique were also tempted in the same way without the addition of food, in order to test the strength of any conditioned response.

All animals were sourced from either private reptile breeders or Emerald Exotics Ltd.

Results

Venomous snakes are generally quite diverse in behaviour and actions, however there were a number of key behaviours we were looking for during this test. It was important to differentiate when the snakes were interested in food (particularly when food was not present, showing a conditioned response) or whether they were simply exhibiting an aggressive or investigative response to foreign contact. Most snakes respond to a food stimulus initially with a tentative head-raise, followed by rapid tongue-flicking as the animal tastes the air to determine the direction and distance to prey. Most elapids react rapidly and confidently past this reaction – usually lunging for the food item with an open mouth followed by biting and envenomation. Snakes will often tongue-flick when approached with a number of different stimuli or when simply exploring their enclosure, however when food is present this is notably rapid in frequency, with each flick short in duration. A combination of rapid tongue-flicks, confident movement to the location of food and biting and consuming indicates a feeding response as opposed to mere investigation.

Contact-free Feeding

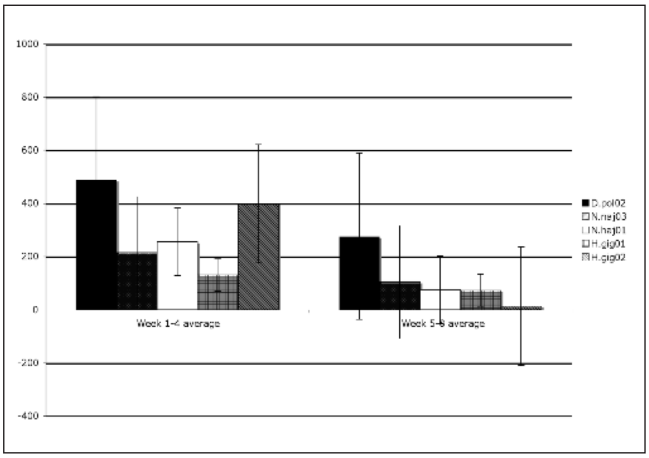

Figure 3. Time from gate opening to feeding

Figure 3 displays the average time from the inner gate being opened to the snake biting the food item. To give a definite indicator that the snake was aware of food in the enclosure, the behaviour of the snakes was observed, paying particular attention for food-related behaviours as mentioned earlier in this paper.

As can be seen from the results, the time from the gate being opened to the snake biting the food item decreased over the course of the study. Time to first bite of prey was chosen to give a definite end point for accurate measurement – snakes frequently fast and will skip feeds for certain time periods, however many elapids in particular are curious and/or aggressive and will investigate a new addition to their enclosure regardless of desire to feed. Using the criteria for behaviour outlined above, we were able to use the action of a bite to determine a response particular to feeding and therefore a conditioned response between the gate and food. As the time decreased, it is reasonable to say conditioning was developing between the entrance to the gate and food, a reaction supported by observations made.

The male black mamba (*Dendroaspis polylepis*) showed a steady drop in time to find food from the feeding airlock (patent pending GB1205301). Upon first feeding the mamba seemed to be wary, repeatedly nose-bumping and then pulling back from the unfamiliar feeding airlock; from frequency of tongue-flicks however, the snake seemed aware that food was being presented through the gate. Through subsequent feedings a quick association developed between feeding and the gate. Mambas tend to be measured and deliberate in their reactions, startling easily and moving slowly but directly towards food as opposed to lunging and attacking like other elapids. The black mamba displayed excited, quickened breathing and rapid tongue-flicking whenever the gate was opened, even if the snake was not in full view of the open airlock chamber. It was notable that the mamba would raise its head and look in the direction of the airlock at the noise and vibration of the gate handle being manipulated, even if food was not present.

The spectacled cobras (*Naja naja*) both displayed similar reactions and a conditioned response to the gate. Cobras tend to be more aggressive and active feeders than other snakes and their responses to food reflect this – fast, forceful strikes are a common response to a presented food item and it is not uncommon for a cobra to miss a strike on a prey item several times in its excitement to catch it. Both animals reacted quickly to their first feed as is normal for any cobra with a food item in their enclosure but were initially wary around the unfamiliar environment of the feeding airlock (patent pending GB1205301). Despite this, they consistently found the food promptly with times decreasing as food was offered over concurrent weeks. Most notable with the spectacled cobras was the increasingly aggressive reaction to any movement around the feeding airlock (patent pending

GB1205301), such as fumbling with the outer cap or slight rocking of the gate handle. Attention to any part of the feeding airlock would usually attract the cobra out of its shelter and towards the gate, whether the airlock was within the animal's visual range or not, the animal reacting to the only vibration and sound of the gate apparatus. Rapid tongue-flicking and occasionally an opened mouth was observed as the animal progressed to the gate valve, showing an expectation of the presence of food.

The Egyptian cobras (*Naja haje*) took food from the gate in a decreasing amount of time as the weeks went on and displayed similar reactions to food. The cobras' reactions were less immediate than several of the other animals, such as the spectacled cobras (*Naja naja*). Upon the first feed the cobras nosed the prey item and pulled back into their shelters several times before biting the prey and immediately pulling it into concealment to devour. Egyptian cobras are highly reactive towards threats, often spreading their hoods and refusing to take their attention off any potential threat in the area[5] – this may explain the reluctance to take the food from such an exposed position. In addition, the female Egyptian cobra refused to eat one week as it was unwilling to remove its attention from the operator, only sitting and hooding. Despite this, the animals performed within expectations when given minimal distractions.

The false water cobras (*Hydrodynastes gigas*) showed substantial decrease in time over the course of the feedings and one of the most overt reactions to the feeding airlock. Animals were taking food in as little as 12 seconds by the end of the trial and both individuals demonstrated conditioning by waking up from sleep in their shelters and moving to the gate opening at a full on 'sprint' upon movement of the gate or airlock housing. The male was frequently found fully immersed in its water dish with head beneath the surface and would still exit the dish and move to the gate in short order following any activity around it.

The Papuan taipan (*Oxyuranus scutellatus*) also showed a decrease in time, however it was not possible to gather enough data to plot properly on a graph. This was due to its reluctance to fully move into the gate. Taipans are extremely easy to startle when cornered[10] and this was encountered a number of times during testing with the animal often retreating to shelter upon catching sight of anyone outside the enclosure whether moving or not. Despite this, the animal showed willingness to feed from the airlock when undisturbed and if allowed time to feed without further activity in the room it could be maintained using this protocol.

Contact-free Maintenance

Our contact-free maintenance procedures yielded similarly promising results. The black mamba (*Dendroaspis polylepis*), female spectacled cobra (*Naja naja*) and both Mozambique spitting cobras (*Naja mossambica*) were trialled using this protocol, as these snakes responded well to the airlock for feeding and were showing evidence of conditioning. While evidence of conditioning appeared to some extent in all animals tested, only the animals most confident with the gate were utilised to test the full extent of the protocol.

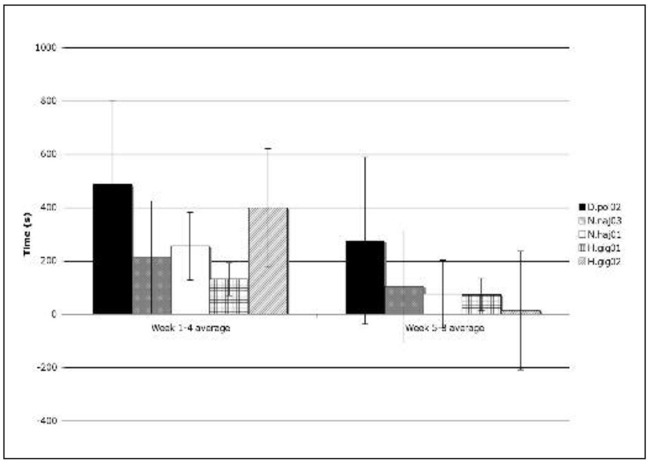

Figure 4. Time from opening of gate to relocation in holding enclosure

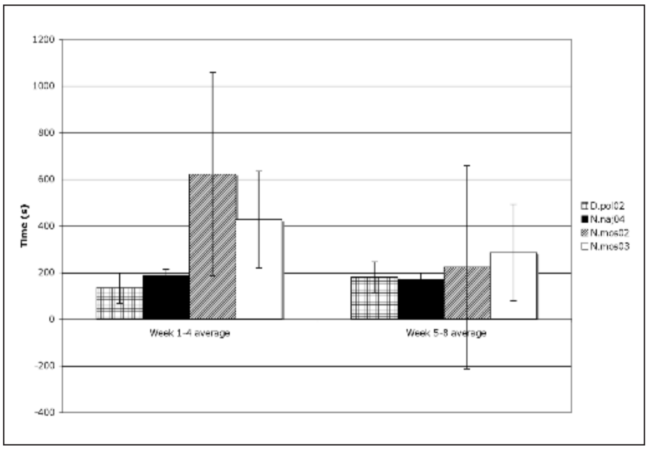

Figure 5. Time to return from holding enclosure without reward.

Figure 4 shows the change in average time the snake took to move fully into the holding enclosure from its home enclosure. Time to relocation was recorded over eight feedings with the contact-free maintenance system, from the opening of both gates to the time when the snake's tail passed into the holding enclosure. As can be seen from the graph, the black mamba (*Dendroaspis polylepis*) and female spectacled cobra (*Naja naja*) showed decreasing time to move to the holding enclosure over the course of the trial. The Mozambique spitting cobras (*Naja mossambica*) both showed decreases in time to move to the holding tub over the course of the trial. Figure 5 is the average time difference over the trial period for the snakes to return to their home enclosure from the holding enclosure, without any food stimulus. All snakes showed an average drop in time here except for the black mamba (*Dendroaspis polylepis*) which did not immediately return on the first repeat, preferring to investigate the holding enclosure thoroughly for any weaknesses. Once the mamba began to return to its home enclosure however, it did so with no hesitation.

The female spectacled cobra (*Naja naja*) showed initial reactions similar to the first feedings from the airlock – tentative exploration of the tube and a lot of tongue-flicking, before a characteristic 'charge' through the gate. The snake initially extended itself two-thirds of the way through the tube and pulled back a number of times before moving fully through – it is possible these behaviours were indicative of the animal dragging its tail end more comfortably forward in the low-traction tube or initial wariness over the transparent, exposed tube.

Upon initial introduction to the airlock and tube system, the black mamba (*Dendroaspis polylepis*) displayed an investigative nature typical of this individual as seen with the gate trials, quickly advancing past the inner gate and into the tube. After nosing the inside of the tube and moving back and forth within it, after approximately 6 minutes the mamba began to fully traverse into the tube, entering fully at 8 minutes 5 seconds after arresting motion for several minutes partway between both enclosures. The individual responded well to subsequent attempts utilising the system, displaying a much more confident response to the gate.

The Mozambique spitting cobras (*Naja mossambica*) showed an increase in time but this was for a number of reasons. Firstly, one week the female (*Naja mossambica*) took nearly sixteen minutes to move through into the holding enclosure, stopping in the tube in between and nosing the sides of the tube in a similar manner to when fossorial snakes dig. There are a number of possible explanations for this – firstly, we used a transparent tube for travel between the two enclosures and as such the snake may have been attempting to gain access to something outside the enclosure. Additionally, many cobras hunt burrowing rodents or rest in rodent burrows and therefore a tube may stimulate any burrowing behaviour. Kellogg and Pomeroy also noted use of the nose as a 'rough tactual organ'[11] and observations of nose bumping to investigate water mazes. Nosing of the tube and enclosure was also observed frequently in the Black Mamba (*Dendroaspis polylepis*) but to some degree in all animals involved.

One thing that was notable in all animals moving towards the gate was the frequency of tongue flicks and initial movement to the opening of the gate, which still appeared to be in relation to a food item being offered, despite the absence of one in the immediate airlock space. It was only when a snake gained sight of the different environment of the tube that their behaviour changed to somewhat investigative, however the tongue-flicks and heightened alertness persisted throughout, indicative of an awareness or expectation of food being offered. Movement back to the home enclosures was generally fairly direct as well and not a result of the animals finding the hole back by investigation – while initial trials with the tube system showed the snakes' first encounter with the system was generally an investigative one, returning to the home enclosure did not elicit these behaviours, with the animals generally moving through as confidently as with food present.

Discussion

Feeding and maintaining a venomous snake in the traditional manner carries substantial risk and our protocols have shown to minimise that risk and constitute a working protocol based on isolation and potential conditioning. In the time period throughout which this trial was carried out, every venomous snake was fed and had its enclosure cleaned using the feeding airlock (patent pending GB1205301) system to the same standard as if maintained using traditional methods. It is important to note that this study should be considered preliminary work that demonstrates a working protocol as opposed to a comprehensive study of conditioning. A number of factors such as snakes' feeding being disrupted by ecdysis (shedding of skin) and certain animals' attributes (such as *Naja haje*'s attitude to threats) meant the procedures did not always take place quickly but the improved safety greatly outweighs the minimal time delay. The time the snakes take to feed is somewhat inconsequential however, as the snakes do not have to be monitored while feeding and staff attention can be elsewhere.

We encountered no issues that would rule out utilising this system over a conventional feeding or husbandry method and substantial safety benefits. The clearest advantage provided by this system is that it removes all exposure to a venomous snake. Despite some issues with snakes taking longer periods of time to utilise the system described in the results section, all animals involved still completed the full cleaning and feeding procedures in less than 30 minutes and more often than not within 1-6 minutes. Waiting for any short period of time is still preferable in terms of safety to actually handling the snake and as the procedure is contact-free and requires no operator intervention, it is still highly efficient – several snakes can be left to feed or move to holding enclosures simultaneously, as opposed to the traditional methods of movement and feeding which involve all the operator's attention being on one snake at the point of maintenance. Efficiency of cleaning schedules can be improved as well – the holding enclosure utilised during the contact-free maintenance technique can be replaced with a fully-furnished new enclosure. This way, the full maintenance procedure can be completed with one movement of the animal, something that it is not possible at such a degree of safety using traditional methods.

In fact this procedure, once set up, requires minimal operator interaction; simple opening and closing of gates is the only action to ensure safety. From a laboratory management perspective this is positive and aids in efficiency, especially in larger animal units where the animals can be set up with food or a holding enclosure attached and other laboratory duties can be carried out simultaneously. It allows multiple staff to be inside the laboratory whilst venomous animals are being maintained and even allows staff to carry out duties that would normally be restricted to those with specialist skills. All of these actions are impossible or extremely dangerous if utilising the traditional methods of venomous snake management however ,with our system, the risk is reduced as far as practicably possible.

This system also greatly reduces the risk involved in feeding. Usual feeding practice entails grasping a defrosted prey item in a pair of long tongs, opening the lid or door of the enclosure and dangling the food in front of the snake. The snake will usually react in one of two ways; either strike and return to its resting position and await the food being placed on the floor, simulating 'death', or (often the case with elapids) the snake will move to the prey for an all out attack. This practice focuses around distance to provide safety from the snake and when carried out with usually agile and flighty elapids that distance can be closed rapidly. The contact-free feeding technique utilises a physical barrier and therefore is a step higher than the traditional method in the risk reduction hierarchy. It also discourages the traditionally normal striking during feeding, as the animals appear to forego the initial envenomating bite, simply biting down and eating the prey directly from the gate.

While the data collected is not strong enough for definite experimental proof of conditioning in the venomous snakes in our study, we found further evidence after the female spectacled cobra (*Naja naja*) and male Mozambique spitting cobra (*Naja mossambica*) were both allowed to enter the holding enclosure without food (not plotted on graph). Both snakes moved into the holding enclosure successfully. In terms of a working protocol this represents a great success as it shows a potential elimination of the need to feed during the husbandry process.

Our data also supports evidence from other research that indicates reptiles can be conditioned to find food from a particular place[10]. Based on this, it is reasonable to suggest that feeding the reptile from the same place that the enclosure is accessed, for example a lid or door, could result in the same association between food and the entrance point as was noticed with our feeding airlock (patent pending GB1205301) and elicit a feeding reaction towards a technician. Whilst up to 50% of defensive venomous snakebites are 'dry' and no venom is injected[22], during feeding elapids are highly aggressive and stimulated, possibly affecting this probability and the risk of a bite if conditioning between a lid or door and food develops. Feeding with the gate system prevents this by feeding the snake from an area the operator will never enter the enclosure from.

This system also eliminates the need to use potentially stressful restraint methods on a snake in order to remove it from its enclosure and allows the animal to choose where it wants to go as opposed to being manipulated. This protocol therefore has additional animal welfare benefits over the current techniques.

We believe these protocols constitute a significant risk reduction in laboratory management of venomous snakes. The ability to feed, clean and provide hydration to a venomous snake with a full barrier between operator and animal removes the main risk of contact with the animal, significantly lowering the danger normally associated with snake maintenance. While this study does not constitute experimental proof of conditioning in these animals, our data shows that the protocol is workable for safe venomous animal maintenance, as does the eight weeks of using the system to clean and feed snakes as part of normal husbandry without incident. As well as the safety improvements, there are significant advancements in efficiency, accessibility for less-trained technicians and reduction of animal stress during maintenance. We envisage the use of these protocols as a significant step towards standardised safe laboratory management protocols for the most dangerous of snakes.

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