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A method for blood sampling the Galápagos tortoise, *Chelonoidis nigra* using operant conditioning for voluntary blood draws

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ABSTRACT - Here we outline the methodology of implementing a blood draw training protocol for use with Galápagos tortoises (*Chelonoidis nigra*) using operant conditioning in order to obtain blood samples for routine blood analysis. The procedure is minimally invasive and does not require manual restraint.

INTRODUCTION

The Galápagos tortoise *Chelonoidis nigra* (Quoy & Gaimard, 1824) is the largest living species of tortoise and one of the heaviest living reptiles, with some specimens reaching up to 5 feet in length and weighing up to 400kg (Caccone et al., 1999). Listed on the IUCN Red List as a vulnerable species (IUCN, 2015), *C. nigra* is protected by the Ecuadorian government. This large species grazes on grass and browses on leaves, cacti and native fruit and is an important seed disperser (Blake et al., 2012).

One of the biggest challenges in managing captive chelonians is to reduce the amount of stress for individual specimens surrounding diagnostic and treatment processes. Tortoises, like all animals, can suffer from stress (Fazio et al., 2014), be that psychological or physical, in a number of ways and this may make it difficult to interpret behaviour and can affect diagnostic results.

Tortoises do not often exhibit obvious clinical signs in the early stages of a disease (pers. com, Divers, 2014) and this, combined with their cryptic behaviour, often leads to clinical misinterpretation of health status and wellbeing. Thus routine blood sampling is often necessary for early diagnosis, prognosis, and treatment of disease. Blood analysis is an important mechanism in the diagnosis of health and disease status (Fazio et al., 2014). In addition, blood draws enable keepers to make a quantifiable assessment of an element of a tortoise's husbandry with parameters such as total 25-hydroxyvitamin D_3 (Selleri & Girolamo, 2012) which can inform and evolve husbandry practices, specifically the provision of UVB radiation. It is becoming much more accepted that blood sampling is an essential tool for optimal captive husbandry within modern zoological collections.

At ZSL London Zoo five Galápagos tortoises are maintained in the Land of the Giants exhibit. Historically, blood sampling has not been routine due to the need for manual restraint and the perceived stress that this would have on the animals. In order to obtain routine blood samples from our *C. nigra* we utilised their finch response and through a programme of desensitisation training and operant conditioning successfully obtained blood samples from the optimal site in a minimally invasive and minimally stressful way. This was achieved through a process of active desensitisation (Hellmuth et al., 2012), where a new stimulus is introduced; in this case the keepers used their fingers to tap and apply pressure to the necks of *C. nigra* in order to simulate the pecking of the finches; this elicits a response of the specimen stretching its neck and going into a trance like state which facilitates access to the veins in the neck for blood withdrawal.

Since the early 1990s there has been a dramatic increase in the use of operant conditioning techniques to train exotic animals for husbandry purposes (Fleming & Skurski, 2014). There has been some published success in using both classical and operant conditioning in training Aldabran tortoises (*Aldabrachelys gigantea*) (Weiss & Wilson, 2003) but this technique has not been documented for use in *C. nigra*. Operant conditioning is a form of learning. It relies on a simple premise, which is that actions have consequences and this can be taught by using reinforcement (positive or negative) and can mean that a desired behaviour can be strengthened and is more likely to occur again in the future. In this paper we describe how by using operant conditioning, blood draws can be obtained in Galapagos tortoises. This technique has facilitated the development of a training protocol that can be used for captive *C. nigra*.

MATERIALS AND METHODS

The first step in any training programme should be to develop and implement a plan and shaping document. This document should outline the methods, as well as the desired outcomes and should take into account factors such as enclosure layout or limitations, as well as staffing and species limitations. The most important element of any training programme is consistency (Swaisgood & Stephenson, 2004). The method was developed as follows for the operant conditioning of 2.3 *C. nigra* at ZSL London Zoo.

A focal individual was first conditioned to allow handling and finching to take place. Because the finch response is a natural behaviour, this did not pose too much of a challenge; the animal just needed to associate the finch response with the positive reinforcement of being touched. Early conditioning attempts were hindered by conspecific animals. Non-target specimens would often interact with the animal being trained. We therefore incorporated separation into our protocol. Once the finch response was evoked by the tactile stimulation from the keeper, non-target conspecific animals were separated behind a fence in order to prevent disturbance of the target animal during the training process.

The focal individual was touched and lightly scratched; first on the legs and then the neck to elicit the finch response. When the specimen achieved the finch response, with neck and legs fully extended and an even weight distribution on all four limbs, a second keeper, acting as a stand-in for a member of the veterinary team approached the animal and touched the neck in order to locate the vein; it lies in a groove easily felt when the neck skin is pulled taught. Once the vein had been located, a finger was then pushed onto the neck skin to try to desensitise the animal to the feeling of pressure being placed on this area, in anticipation of when the needle goes in on an actual blood draw event. Other stimuli were trialed to replicate the sensation of needle penetration but finger pressure was the method of choice. Once this process had been repeated four times, the animal was rewarded with a high value food item such as a small piece of carrot, and this concluded the conditioning session.

This conditioning was carried out daily in the afternoon over three consecutive days prior to the first blood draw attempt. Attempting to take blood from a *C. nigra* in the afternoon was considered advantageous as it allowed the animal enough time to bask in the morning which resulted in increased body temperatures by the afternoon and improved engorgement of jugular veins due to peripheral vasodilation, allowing for a blood sample to be obtained more easily (Dessauer, 1970). We elicited the finch response with the target animals at other random times of the day to disassociate it from any possible negative response with the blood draw procedure. The technique used to elicit the finch response on these occasions was kept exactly the same as for the actual blood draw procedures.

On the day of an actual blood draw, a veterinarian or veterinary nurse took the place of the stand-in secondary keeper whilst the first keeper remained ‘finching’ the tortoise from behind. The veterinarian first inserted a butterfly needle into the vein, and left the needle inserted whilst the tortoise would inevitably retract its neck. When the animal was successfully re-finched, a syringe was then attached to the butterfly needle and the blood taken. The animal was then instantly rewarded with food, indicating the end of the procedure.

Figure 1: Veterinary team member palpates the neck of *C. nigra* in order to find the jugular vein, once the animal has been finched.

Figure 2: Veterinary team member inserts the butterfly needle into the jugular vein of *C. nigra* whilst the animal maintains the finch position.

RESULTS AND DISCUSSION

Since the implementation of the training protocol we have now been able to obtain routine blood samples from all of our five *C. nigra* under operant conditioning and we have achieved an 85% success rate of the behaviour learned. The options of blood sampling sites from *C. nigra* are often restricted to dorsal tail vein, the sub-carapacial venous sinus and the jugular veins. The lymphatic system in tortoises is extensive and any lymph contamination of blood samples renders results spurious and misleading. The jugular veins are considered the venipuncture site with the least risk of lymph contamination and therefore the optimal site for blood sampling (Wright, 2009). Examination of a blood sample should be a component of a routine medical work up of any chelonian medical case (MacArthur, Wilkinson & Meyer, 1988) but the procedure of obtaining the blood could cause stress (Fazio et al., 2014). Manual restraint of large chelonians is likely to compromise their welfare, as well as being impractical due to their large size and strength, and should be avoided where possible.

C. nigra exhibit a classic example of a symbiotic relationship with some Galápagos finches, (e.g. *Geospiza fuliginosa*) (Christian, 1980). The ectoparasite-eating finch stimulates a reaction in the tortoise that evokes a change of posture to one with neck fully extended and standing as high as possible and therefore exposing as much skin as possible. This improves feeding access for the finches and facilitates the removal of ectoparasites. This behaviour is maintained in captive populations despite the absence of the finches. During the finch response the tortoises go into a trance-like state, in which they do not react to stimuli such as the presence of food (pers. obs).

The training protocol is now established and working effectively, but during the process there were a number of obstacles to overcome. Many factors can affect the success rate of this process, such as the ability of the veterinary team member to successfully locate the vein. This process was made more difficult by the fact that the vein often moves, making locating it on a first attempt more difficult. Historically, multiple attempts to obtain a blood samples were made. There were concerns that target specimens could become shy of needles and that the skin and vein could be damaged. In order to minimise this, a three strike rule was implemented which meant that only three attempts of needle insertion could be made and if these were unsuccessful after the third try, the session would conclude regardless of the success in obtaining a blood sample. A seven day rest period between blood draws was also implemented, which meant that if a blood draw attempt was unsuccessful, another attempt could not be made until another seven days had elapsed.

Keeper shyness is a term we have given to represent the behaviour of an animal when the presence of a keeper is associated with any negative stimuli and this often leads to a specimen displaying unwillingness to exhibit the desired finching behaviour. On such occasions there was little or no finch response present. Anecdotally, in extreme circumstances this can manifest itself as a sudden physical percussing of the plastron on the floor. This behaviour can be rectified or avoided by making sure that the training is positively reinforced regularly without the negative stimuli being present and by interacting with the tortoises outside the blood draw sessions.

Initially when the de-sensitisation process was attempted, we used many methods to try to prepare the animal for the sensation of the needle being inserted into the vein. These included blunt needles pressed against the skin as well as rubber bands flicked on the neck to try and recreate the sensation for training without the needle actually being used. However, after these methods were trialed, we did not feel anything could replicate the sensation of the pain response once a needle was actually inserted. We changed the methods to applying pressure with a forefinger and focused on the ability of the keeper to move the neck and head of the tortoise to the correct angles which better exposed the neck vein and facilitated blood sampling events.

We now have a method that is effective and allows us to routinely monitor the health status of our *C. nigra* group. Carrying out routine blood screening allows us to gather data to create a detailed profile of tortoise health which can then be correlated to other data such as transcoelomic ultrasound scans to track many aspects of tortoise husbandry, reproduction and the treatment of illness.

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