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TECHNICAL REPORT

Technique for Venipuncture of the Transverse Facial Vein in the Black Rhinoceros (*Diceros bicornis*)

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Through the use of operant conditioning, the authors developed a technique to facilitate obtaining blood samples from a black rhinoceros diagnosed with idiopathic epilepsy. The technique involved operant conditioning to facilitate venipuncture of the transverse facial vein, at an anatomic landmark on the lateral side of the face ventral to the medial canthus of the eye, and dorsal to the lateral commissure of the mouth. The investigators used standard operant conditioning protocols to train the animal for desensitization to a needle puncture in the facial vein. Blood samples obtained from the facial location were free of excessive hemolysis and allowed for large volumes to be collected. The procedure was well-tolerated by the rhinoceros and could be performed regularly without complication. Zoo Biol. 35:570–573, 2016.

Keywords: *Diceros bicornis*; transverse facial vein; black rhinoceros; venipuncture

STATEMENT OF THE PROBLEM

Operant conditioning (OC) has been used in zoological collections for decades to facilitate veterinary medical procedures and animal husbandry. A wide range of species respond to training, and through diligent work and patience on the part of caretakers and veterinary personnel, it allows for the performance of minimally painful procedures in many species.

Operant conditioning and target training to facilitate venipuncture remains a regular component of the health care of captive black rhinoceros (*Diceros bicornis*). Venipuncture is routinely performed on this species to obtain blood and serum samples useful in the monitoring of reproductive status and serial monitoring for frequently described diseases such as iron storage disease [Miller et al., 2012; Capiro et al., 2014]. Present methods of venipuncture in this species use the radial vein on the medial surface of the forelimb for large volumes, or the smaller auricular veins of the ear for small volumes [Miller, 2003]. Typically this requires protected contact through a chute or restraint device along with operant conditioning for either blood collection method. Venipuncture of the radial vein often demands the phlebotomist to reach through chute bars and around to the medial aspect of the forelimb, creating a safety hazard. The auricular veins, while prominent, often provide the best site for catheterization, making them less desirable for serial blood collections as repeated venipuncture of these veins may increase the risk of thrombosis and venous collapse, as described in other species [Perry-Clark and Meunier, 1991].

The transverse facial vein is a large vessel on the lateral aspect of the cheek, which runs caudo-rostrally from the lateral commissure of the mouth following the anastomoses of the superior branch and inferior branch of the facial vein, which drain the upper and lower lip (Fig. 1). In equine patients, the same vein acts as an ancillary site for obtaining

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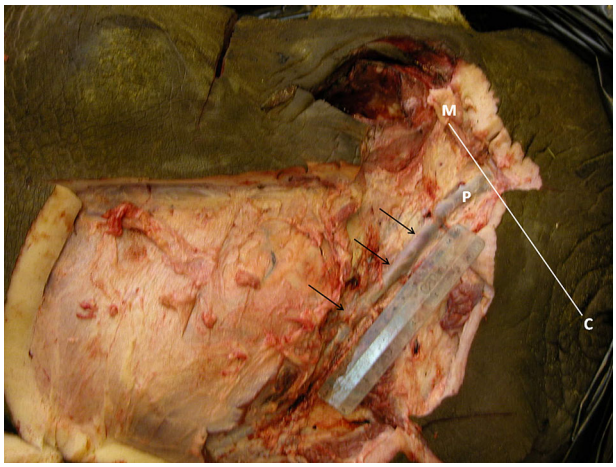

Fig. 1. Lateral view of a cadaver Black Rhinoceros head showing the anatomic relationship between the lateral commissure of the mouth (C), medial canthus of the eye (M), the transverse facial vein (arrows), and the proposed site of venipuncture (P).

small venous blood samples, although typically in horses attempts at venipuncture are limited to anesthetized animals [Hunter et al., 2013]. The authors successfully punctured the facial vein in an anesthetized rhinoceros with the advantage of obtaining large blood volumes (10–12 ml) from the site. Due to the ease of collection in an anesthetized animal, we developed a technique to perform facial vein collection from a standing, non-sedated rhinoceros.

DESCRIPTION OF THE PROCESS

An 8-year-old male black rhinoceros (*Diceros bicornis*) housed in Lansing, Michigan, developed seizures at regular intervals and was presumptively diagnosed with idiopathic epilepsy. The diagnosis of epilepsy was made after significant diagnostic testing to exclude other potential causes of seizures and remains the first report of this condition in this species to the authors' knowledge.

After the control of initial seizure episodes, the rhinoceros required a daily anticonvulsant regimen of oral phenobarbital and later levitiracetam. In order to establish a baseline therapeutic range of the medications, assess animal compliance in ingesting the medication, and to monitor for extrapolated toxic serum levels, frequent blood collection needed to occur. Preservation of the auricular vasculature for long-term IV catheterization was paramount due to the persistent threat of a breakthrough seizure. Repeated venipuncture from weekly to biweekly sampling for serum drug concentrations could increase the risk auricular vein thrombosis and collapse, rendering them inaccessible for use in a future emergency. Although the rhinoceros was comfortable with radial vein venipuncture on the medial aspect of the forelimb, success in obtaining a sufficient sample was unreliable, and the process of the blood draw increased the safety risk for the phlebotomist and zookeepers involved.

The authors elected to use the transverse facial vein in standing venipuncture due to its accessibility, the docile behavior of the rhinoceros, increased safety for the personnel involved, and the ability to obtain a large enough volume to suffice for testing. The anatomic landmarks for the venipuncture site include the medial canthus of the eye, the lateral commissure of the mouth, and the bony facial crest. The site of needle insertion lay 1 cm caudal to the midpoint of a straight line drawn from the medial canthus of the eye, to the lateral aperture of the mouth at the rostral termination of the facial crest (Figs. 1 and 2).

Fig. 2. Standing venipuncture in the Black Rhinoceros from the facial vein showing site of venipuncture and blood collection apparatus.

The authors created a method using a stepwise approach, incorporating operant conditioning through target training with systematic desensitization procedures to facilitate this venipuncture method. The rhinoceros had previously been acclimated to a squeeze chute (Tamer, Fauna Research, http://www.faunaresearch.com/index.htm), which allowed extrusion of the animal's head to the level of the shoulder. With prior conditioning by the zookeeper staff, the rhino regularly responded to target training using a buoy-stick target and a food reward. Further training through shaping and positive reinforcement seemed to be a logical and appropriate method to build on previous conditioned behaviors and arrive at the point of toleration of a facial venipuncture [Crowell-Davis, 2008]. Tri-weekly training sessions of 20 min duration were planned consisting of a 3 week phased approach. Week 1 consisted of training sessions in which the veterinarian gently massaged the side of the face in the vicinity of the venipuncture site. During these sessions, the phlebotomist cleansed the puncture site using Chlorhexidine scrub. A mild lotion (Lotion Soft, Steris Corporation, 7501 Page Avenue, St. Louis, MO) applied to the venipuncture site simulated the application of lidocaine gel. Positive reinforcement, given in the form of a food reward of various produce or alfalfa cubes, allowed increasing tolerance of manipulation of the venipuncture site and lotion application.

Week 2 training sessions mirrored week 1 sessions, with the addition of applied pressure to the venipuncture site using a blunt needle cap to create a point pressure sensation without a needle stick. Preceding each blunt pressure stick, the phlebotomist spoke the key word "poke," and then the blunt stick applied. The rhinoceros tolerated all blunt stick procedures without change in demeanor or any apparent anxiety. Week 3 training sessions instituted the actual puncture of the skin using a 25 gauge needle, following the blunt pressure stick, and using the same "poke" cue word. At the outset of the training sessions, application of a lidocaine and prilocaine cream (Emla Cream, Akorn Pharmaceuaticals, Lake Forest, IL) to the site provided mild desensitization. Ten minutes of regular blunt stick training elapsed prior to puncture of the skin with the 25 gauge needle.

By week 4, full venipuncture of the site was possible with an 18–20 gauge needle. The venipuncture procedure followed in the same manner as the training sessions to ensure consistency. The syringe and needle apparatus consisted of an 1.5 inch, 18–20 gauge needle, a standard intravenous extension set, and 12 ml syringe (Fig. 3). The use of the extension set allowed for flexibility of motion with the rhinoceros' head during sample collection. Volumes of 6–10 ml of whole blood were routinely obtained from the venipuncture site, with repeated withdrawals each week as needed for drug monitoring. Following 1 month of continued weekly sampling, sampling frequency was reduced to every 2 weeks for the next 6 months, and then again reduced to monthly sampling for an additional 3 months with no regression or development of intolerance on the part of the rhinoceros.

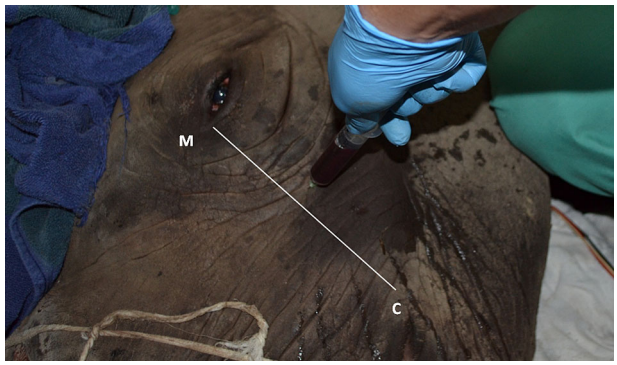

Fig. 3. Sampling from the facial vein in the immobilized Black Rhinoceros showing the withdrawal site 1 cm caudal to the midpoint of a virtual line drawn from the lateral commissure of the mouth (C) to the medial canthus of the eye (M).

DEMONSTRATION OF EFFICACY

Facial venipuncture in the black rhinoceros provides an additional site for sample collection, minimizing phlebotomist risk and allowing preservation of auricular veins for the long-term management of cases requiring repeated venipuncture. While no evidence of complication arose in this case, maintenance of sound aseptic practices decreases the likelihood of phlebitis, thrombosis, or other complication related to repeated puncture. Further investigation into the clinical agreement between serum blood values obtained from this site compared to other venipuncture sites should be done, although previous studies in horses suggest that blood measures obtained from transverse facial vein samples did not significantly differ from other sampling sites in the same animal [Hunter et al., 2013; Dahan et al., 2015]. Further, comparison samples from the radial and auricular vein in the black rhinoceros displayed similar agreement, suggesting that blood parameters from the facial vein may concur with other sites as well [Miller, 2003].

The administration of intravenous medications or fluids using this site has not been attempted despite the relatively easy access for venipuncture. Nearby anatomic structures, specifically the facial nerve, have the potential to be affected should an IV infusion extravasate in the area, causing unknown sequel. The auricular or radial sites still remain the preferred sites for catheterization or IV injection until further investigation into the suitability of the facial site for infusion can be accomplished. This site does provide an anesthetist with an easily accessible site for the collection of peri-operative or other needed samples in the course of immobilization. Immobilization constitutes a significant physiologic stressor in white rhinoceros as measured by standard arteriovenous blood gas parameters [Buss et al., 2015]. Samples such as venous blood gases, serum lactate, and others may be drawn easily from this site, without significant interruption to monitoring or ventilation in the recumbent, intubated patient.

Success of repeated sampling from the facial vein site requires OC. The management of most rhinoceros species in captivity involves the use of this technique for the facilitation of medical and basic husbandry procedures. Previous studies note that the use of OC has not resulted in significant stress to the rhinoceroses involved, as indicated by fecal corticoid output [Capiro et al., 2014]. Throughout the process of obtaining blood from the transverse facial site, the investigators appreciated no overt stress, as compared to other husbandry procedures for which the rhinoceros had been previously acclimated. Specific measurements of fecal glucocorticoids were not measured, nor would they be likely to valid in this case given the concurrent seizure episodes present in this patient.

CONCLUSION

Ultimately, transverse facial venipuncture was well-tolerated by the black rhinoceros and allowed for the collection of large volumes of blood for routine diagnostics. Training for this procedure, accomplished using standard OC methods, facilitates preservation of other sites when the need for repeated sampling is present. This method may prove safer for the phlebotomist, allowing the collection of large samples without having to reach circumferentially around the animal's limb to obtain it. Further investigation is needed into whether this site can easily accessed in other rhinoceros species as opposed to the black rhinoceros and whether blood parameters measured from this site differ significantly from those at other anatomic locations.

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