RESEARCH NOTE

BLOOD COLLECTION TECHNIQUES IN THE BADAK KERBAU *Dicerorhinus sumatrensis* IN SUNGAI DUSUN RHINO CONSERVATION CENTRE

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Introduction

Blood collection in rhinoceros was carried out for scientific, diagnostic and management purposes. The size of the patient and the quantity of blood required determine the anatomical site of venipuncture (Fraser and Mays, 1989). The site chosen for bleeding an animal must provide sufficient space and security to the operator while carrying out the procedure. In addition, it should give the best result or blood collection with minimum stress on the animal. Blood collection sites vary with species and individuals. Previous collection of blood from the marginal ear vein of Badak Kerbau involved restraint by gently stroking the pereneum and medial thigh until the animal goes on lateral recumbency (Zainal-Zahari, 1989). In the Badak Kerbau, the three possible bleeding sites include the marginal ear vein of the ear, the cephalic and radial vein of the forelimb and the coccygeal vein of the tail. A comparative study was carried out on the different bleeding sites in the Badak Kerbau and 30 collections were made monthly for two months.

Materials and Methods

Bleeding were carried out twice a week in five Badak Kerbau in the Rhino Conservation Centre, Sungai Dusun for plasma progesterone profiles, serum chemistry and complete blood count. The animals were conditioned for 2-3 weeks prior to collection. They were baited to enter the chute and handled physically for the bleeding manipulations. The chute measures 2.5m long, 1m wide and 1.25m high consisting of circular vertical galvanised iron posts buried into concrete floor. Gaps between each posts ranges from 15-18cm. As the animal enters the chute from the back, vertical posts are slotted into holes in the floor and gradually the animal is squeezed from the back and laterally. Conditioning for collection from the marginal ear vein involves placement of a tourniquet at the base of the ear. This is followed by handling the ear firmly, as the animal moves its head vertically or horizontally. No conditionings were necessary for collection sites involving the tail and forelimbs. Each collection method and site was compared for ease of operation, success of collection and safety to the animal and handler. Each

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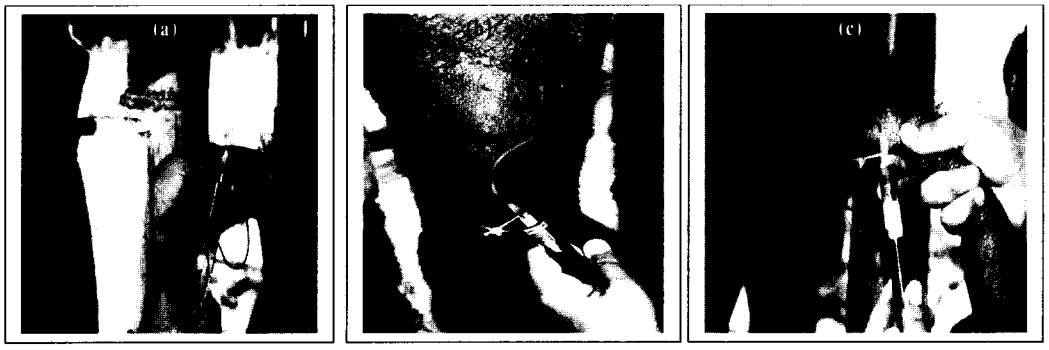
bleeding technique would require a winged-infusion set (SURFLOâ winged infusion set, Terumo Corporation, Japan), vacutainer tubes (Vacutainerâ, Becton Dickinson, United Kingdom), 5-10 ml syringes (Terumoâ, Terumo Corporation, Japan) and 18G vacutainer needles (Precision Glideâ, Becton Dickinson, United Kingdom) for the tail venipuncture.

Plate 1: Various blood collections sites in the Badak Kerbau (a) Ear, (b) Forelimb and (c) Tail.



Marginal Ear Vein

The operator would position himself, standing, next to the head of the rhino and secure a tourniquet at the base of the ear to expose the veins. The site of collection is swabbed with alcohol prior to bleeding. A 23G winged-infusion set is used for the initial puncture and as blood starts to flow down the catheter, a 5-10 ml syringe is used to further aspirate the blood. The blood is then transferred into a vacutainer tube.

Cephalic or Radial Vein

The operator would position himself squatting or kneeling immediately behind the left forelimb of the rhino, if bleeding were on the right and vice versa. The bleeding site, located on the medial surface is swabbed with alcohol prior to venipuncture. Venipuncture is done with the 23G needle and as soon as blood starts to flow down the catheter, a 5-10 ml syringe is attached and blood aspirated. Blood is then transferred to a vacutainer tube. Bleeding could be attempted from the fetlock to the carpus, medially, with the veins running caudoventrally. In some of the rhinos, these veins are vaguely visible. During the procedure, the operator needs to extend the arm under the abdomen of the rhino. Additional restraint is required during bleeding, to hold down the free forelimb, from kicking and removing the winged- infusion set.

Coccygeal Vein

The operator squats or stands posterior to the hindlimb of the rhino. Bleeding sites ranges from the area 10cm from the tail base to area 25cm from the tail tip, at the annular rings or folds of the tail. The lowest or middle section of the tail is chosen for the venipuncture. Prior to bleeding, the ventral site of a tail ring is swabbed with alcohol. Blood collection is made at the ventral midline section of tail using a 23G winged-infusion set which is attached to an 18G-vacutainer needle improvised by the author at the Rhino Conservation Centre. During the procedure, venipuncture is made using the 23G winged-infusion needle and as blood flows down the catheter, the 18G-vacutainer needle is attached to the vacutainer tube.

Results and Discussions

The main problems are associated with obtaining sufficient blood samples in the quickest possible time without over stressing the animal or injuring the operator. In addition, the smaller size of the Badak Kerbau as compared to its relatives, the White, Black, Javan and Indian rhinoceros makes it more difficult as its blood vessels are relatively very much smaller. Collection using the marginal ear vein is time consuming and does not provide sufficient working space, while endangering the operator whom is working between the posts, next to the head, mouth and horns. The possibility of the animal moving its head dorso-ventrally further endangers the operator. Blood collected is limited in volume as it is dependent on the amount of time between puncturing the vein, connecting the 5 or 10ml syringe and aspirating the blood. However, this method might be possible in very few animals after prolong conditioning as observed in a female at Cincinnati Zoo, USA (Romo, S, pers comm). In Zoo Melaka, blood was successfully collected from laterally recumbent Badak Kerbau that was physically restraint by stroking the thighs and pereneum. However, only 3mls of blood were obtained in 83% of the rhino. During the collection, the rhino was restraint in the paddock and could easily endanger the handler (Zainal-Zahari, 1987).

Similarly, working with the forelimb medially endangers the operator who approaches the animal laterally, posterior to the elbow. Subsequently, the operator has to work in-between the vertical posts in order to reach the cephalic or radial vein. The chances of being stepped or kicked by the hind or fore limbs are high. It is almost impossible to approach the medial surface of the forelimb from the front as its possible for the animal to use its head to injure the operator's arm. The hooves of the rhinos are hard and sharp enough to traumatise the arm or hand. The amount of blood collected is limited as it depends on the amount of time post venipuncture and the ease of connecting the winged infusion set to the 5 or 10ml syringe and aspirating the blood. In the black rhinoceros *Diceros bicornis*, a small amount of blood could be obtained from the marginal ear vein but in a sedated,

possibly restrained animal, a large amount can be obtained from the medial radial vein of the forelimb (Miller, 1993). In an anaesthetised Black rhinoceros, 1-8 litres of blood could be collected from the medial carpal vein. However, the ear vein is most commonly used in a non-sedated rhinoceros although some venipunctures were done using the medial carpal vein (Miller, 1999). Although the use of the cephalic or radial veins in the Badak Kerbau was initially easy, it became more difficult with the subsequent collections.

The approach from the tail provides the safest and easiest method of bleeding and allows ample time for the operator to locate the vessel. Bleeding success is 80-100% using the coccygeal vein or artery. With the operator squatting or kneeling behind the posts and the animal, the risk of being step or kicked or horned by the rhino is negligible. However, it must be bore in mind that the Badak Kerbau has the capacity to kick backwards with its hind leg, one leg at a time. Therefore, it is always safer to position one self not too directly behind the animal during venipuncture. Conditioning is only necessary to get the animal into the chute but not for bleeding. Provided it is being fed, venipuncture using the coccygeal is possible in an unconditioned animal. Needle of 23G was better than 25G or 18G winged infusion. Venipuncture should be done cranially at an angle of 45°-75° and once blood starts to flow down the winged infusion set, it is connected via the 18G-venoject needle to a collecting vial, which could be renewed rapidly. The amount of blood collected averaged 10mls.

During the initial study, a total of 80 venipunctures were done from five rhinoceros using the three sites. The venipuncture was more effective using the coccygeal vein or artery as compared to the cephalic or the marginal ear vein (Table 1). The blood volume collected and success rate is significantly higher with the former method. In addition, there is minimum danger to the animal and handler.

Table 1: Comparative Bleeding Sites and Blood Collection in the Badak Kerbau (ratings of 1-4+)

Bleeding Sites	Vein Visibility	Ease of Bleeding	Danger to Operator	Danger to Animal	Average Volume of Blood (mls)	Success Rate
Ear	++++	+	++	++	5	<60%
Forelimb	++	+	++	++	5	<60%
Tail	-	++++	-	+	10	>80%

During venipuncture, a minimum of three operators is necessary for the procedure; one to feed the rhino, one to carry out the venipuncture and one to assist during the collection. Conditioning of the rhinos to enter the chute should be carried out routinely in order to shorten the procedure. All bleeding procedures

should be executed in the shortest possible time as the animal would get more restless and stressed if confined for long periods. Individual variations between rhinos include tolerance to handling and vein visibility. In addition, vein visibility is markedly reduced in animals that were allowed prior to bleeding. Similarly, the rhinos are more relaxed in the early hours of the morning as the ambient temperature is lower as compared to the afternoon. Whatever method of blood collection used, the welfare of the animal should always be of a priority.

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