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EFFECT OF VENIPUNCTURE SITE AND ANTICOAGULANT ON SELECTED HEMATOLOGIC VALUES IN BLACK RHINOCEROS (*DICEROS BICORNIS*)

Michele Miller, D.V.M., Ph.D.

Abstract: Paired blood samples were collected from the ear and radial vein of four captive healthy adult black rhinoceroses (*Diceros bicornis*). Samples were collected using heparin or ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Packed cell volume (PCV) and total protein (TP) values were compared between samples drawn from the two venipuncture sites and treated with the two anticoagulants to determine whether statistically significant variation occurred. No significant difference in the grouped values was observed when venipuncture sites (ear and radial vein) were compared using the same anticoagulant (heparin). However, when comparing different anticoagulants (EDTA and heparin) used to collect blood from the radial vein, the grouped-heparinized samples had higher mean PCV and TP values than did the EDTA-treated samples. These differences may be important when performing serial sampling in a sick rhinoceros and suggest that the choice of anticoagulant should be consistent, although selection of venipuncture site may be less important when monitoring selected hematologic values in black rhinoceroses.

Key words: Black rhinoceros, *Diceros bicornis*, packed cell volume, total protein, venipuncture, anticoagulant.

INTRODUCTION

Captive black rhinoceroses (*Diceros bicornis*) suffer from a number of hematologic and other systemic diseases.[9,13,14,16,18,21] Therefore, medical management of this captive species requires the collection of blood samples for hematologic and biochemical analyses. Serial samples are crucial for monitoring the progression of disease and the response to therapy in black rhinoceroses with hemolytic anemia, intravascular hemorrhagic syndrome, and hemosiderosis.[13,18,19] Different anticoagulants may be required for specific types of analyses. Black rhinoceros serum has a tendency to form fibrin clots that reduce yield, so it has been recommended that biochemical analyses be performed on heparinized plasma (R. E. Miller, pers. comm.).

With increased use of behavioral conditioning techniques and restraint devices, more institutions are able to obtain samples from nonanesthetized rhinoceroses. Recommended venipuncture sites include the ear and radial veins.[13,15] Facility design and individual animal temperament may dictate the accessibility of the venipuncture site. Frequent sampling or disease conditions may complicate blood collection from only one site.

Logistic factors may affect the selection of the venipuncture site, the collection method (including the type of anticoagulant required for specific tests), and the volume obtained. The unknown effects, if any, of venipuncuture site and anticoagulant type on hematologic values confound the interpretation of results. This report compares selected hematologic values in nonanesthetized healthy adult black rhinoceroses, using paired samples collected from the ear and radial veins to determine recommendations for serial sample analyses.

From the Department of Veterinary Services, Disney's Animal Kingdom, P.O. Box 10,000, Lake Buena Vista, Florida 32830-1000, USA. Correspondence should be directed to Dr. Miller.

MATERIALS AND METHODS

Sample collection

Between July 1999 and February 2001, blood samples were collected from four adult black rhinoceroses (two males, two females). All animals were housed at Disney's Animal Kingdom in Lake Buena Vista, Florida. None of the animals exhibited significant clinical problems during the collection period. Blood was collected using behavioral conditioning methods to allow voluntary venipuncture from the ear and radial veins of standing animals. Weekly samples were collected for a total of 73 paired samples.

Skin over the venipuncture site was cleaned with chlorhexidine scrub (Nolvasan scrub, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA) and 70% ethanol. A 23-gauge needle was inserted into the ear vein and the blood collected in heparinized capillary tubes from the needle hub. A 19-gauge, 0.75-in. butterfly needle and extension set (ref. #4919, Becton Dickinson, Franklin Lake, New Jersey 07417, USA) was used to collect blood from the radial vein into vacutainer tubes containing potassium EDTA (ref. #366485, Becton Dickinson) and lithium heparin (ref. #366454, Becton Dickinson). Because of differences in training level, not all samples were collected from each animal in

each session. A total of 89 paired samples were collected from the radial vein of the four rhinoceroses, using EDTA and heparin vacutainers. Only paired samples collected during the same training session were used in the comparisons.

Hematologic and biochemical analyses

Samples were analyzed within 2 hr of collection. Packed cell volume (PCV) was measured by centrifuging capillary tubes of whole blood and by reading replicate tubes manually. Plasma from these capillary tubes was used to measure total protein (TP) by refractometry (veterinary refractometer, Westover model RHC-200, China). Additional hematologic and biochemical analyses were performed every month as part of the routine health-monitoring program; however, values from outside laboratories were not included in the results presented in this article.

Statistical analyses

Statistical analyses were conducted with the SAS System software version 8.2 by an outside consulting firm (BioSTAT Consultants, Inc., Portage, Michigan 49024, USA). Comparisons were deemed statistically significant at $P < 0.05$ for two-sided tests. The paired *t*-test was used to compare the results from the use of different anticoagulants in the radial vein of the four rhinoceroses (leg-hep vs. leg-EDTA) and from the heparinized blood taken from different venipuncture sites (ear-hep vs. leg-hep) in each animal. Analyses within sex were not conducted because of the small sample size. Analyses comparing venipuncture sites (ear-hep vs. leg-hep) and anticoagulants (leg-hep vs. leg-EDTA) were performed on the differences in paired observations for all animals using analysis of variance (ANOVA).

RESULTS

Comparison of values from different venipuncture sites

Paired samples were separately analyzed for each animal initially. Packed cell volume was not significantly affected by venipuncture site (ear vs. radial vein) in any of the individual animals ($P > 0.05$, paired *t*-test). The differences in the paired observations were then grouped and analyzed by ANOVA. Overall, there was no significant difference between the PCVs of blood collected from the ear (0.382 ± 0.024 [38.2 ± 2.4%]; mean ± SD) and the radial veins (0.372 ± 0.030 [37.2 ± 3.0%]; mean ± SD) using heparinized samples ($P = 0.147$).

Similarly, values for TP were compared between the two venipuncture sites using heparinized blood. In two individuals, no significant difference was observed in the measured values. One individual showed a statistically significant increase in the TP measured from the ear (76.0 ± 5.0 g/L [7.6 ± 0.5 g/dl]; mean ± SD) when compared with the radial vein (74.0 ± 5.0 g/L [7.4 ± 0.5 g/dl]; mean ± SD) ($P < 0.001$, paired *t*-test). However, because the increments of measurement in the refractometer are 2.0 g/L (0.2 g/dl), it is conceivable that these differences are not clinically significant and may be within the measurement error of the instrument. When the differences of the paired samples were grouped and compared using ANOVA, no statistical difference was observed in TP values for blood collected from the ear (73.0 ± 6.0 g/L [7.3 ± 0.6 g/dl]; mean ± SD) and the radial veins (71.0 ± 6.0 g/L [7.1 ± 0.6 g/dl]; mean ± SD) ($P = 0.21$).

Table 1 shows the statistical summary data from paired heparinized samples collected from the ear and radial veins of three adult black rhinoceroses (one animal had not yet been trained for sample collection from the ear during the study period). Overall, the group results appeared to be consistent with the individual animal comparisons of venipuncture sites. No statistically significant differences were observed in PCV or TP values measured in heparinized blood collected from the ear and the radial veins ($P > 0.05$; ANOVA).

Comparison of values using different anticoagulants

Unlike the results obtained from different venipuncture sites, individual animals showed statistically significant different PCV and TP values when different anticoagulants (heparin and EDTA) were used to collect blood from the radial vein. Packed cell volume was statistically higher in heparinized samples than in EDTA samples in three of the four animals tested (Table 2). Although TP was higher in heparinized samples than in EDTA samples in three of the four rhinoceroses, it was statistically significant in only one animal. A possible explanation for the lack of statistically significant differences in PCV in one of the rhinoceroses (rhinoceros 3) is the small sample size (three paired samples) available for comparison. Table 2 shows the statistical summary data of the differences among the paired samples collected from the radial vein using either EDTA or heparinized vacutainers. Again, the group results appear to follow the trends observed with the individual animal comparisons of PCV and TP, with both grouped means slightly higher in the heparinized samples.

Table 1. Comparison of PCV and TP values from heparinized samples collected from the ear and radial veins of three black rhinoceroses.[a]

	PCV		TP	
	Ear-hep (SI, %)	Leg-hep (SI, %)	Ear-hep (g/L, g/dl)	Leg-hep (g/L, g/dl)
Animal No. 1				
Mean ± SD	0.384 ± 0.029, 38.4 ± 2.9	0.382 ± 0.032, 38.2 ± 3.2	74.0 ± 6.0, 7.4 ± 0.6	72.0 ± 3.0, 7.2 ± 0.3
P (paired *t*-test)		0.07		0.11
Animal No. 2				
Mean ± SD	0.380 ± 0.020, 38.0 ± 2.0	0.370 ± 0.023, 37.0 ± 2.3	69.0 ± 4.0, 6.9 ± 0.4	69.0 ± 6.0, 6.9 ± 0.6
P (paired *t*-test)		0.14		0.92
Animal No. 4				
Mean ± SD	0.384 ± 0.026, 38.4 ± 2.6	0.373 ± 0.034, 37.3 ± 3.4	76.0 ± 5.0, 7.6 ± 0.5	74.0 ± 5.0, 7.4 ± 0.5
P (paired *t*-test)		0.17		<0.001*
Group				
Mean ± SD	0.382 ± 0.024, 38.2 ± 2.4	0.372 ± 0.030, 37.2 ± 3.0	73.0 ± 6.0, 7.3 ± 0.6	71.0 ± 6.0, 7.1 ± 0.6
ANOVA		0.15		0.21

[a] hep, heparinized; PCV, packed cell volume; TP, total protein; ANOVA, analysis of variance; SI, International system of units.

* Statistically significant at $P < 0.05$.

Table 2. Comparison of PCV and TP values from EDTA and heparinized samples collected from the radial veins of four black rhinoceroses.[a]

	PCV		TP	
	Leg-hep (SI, %)	Leg-EDTA (SI, %)	Leg-hep (g/L, g/dl)	Leg-EDTA (g/L, g/dl)
Animal No. 1				
Mean ± SD	0.382 ± 0.032, 38.2 ± 3.2	0.354 ± 0.025, 35.4 ± 2.5	72.0 ± 3.0, 7.2 ± 0.3	68.0 ± 5.0, 6.8 ± 0.5
P (paired *t*-test)		0.001*		0.08
Animal No. 2				
Mean ± SD	0.370 ± 0.023, 37.0 ± 2.3	0.357 ± 0.019, 35.7 ± 1.9	69.0 ± 6.0, 6.9 ± 0.6	67.0 ± 4.0, 6.7 ± 0.4
P (paired *t*-test)		<0.001*		0.09
Animal No. 3				
Mean ± SD	0.325 ± 0.050, 32.5 ± 0.5	0.348 ± 0.048, 34.8 ± 4.8	81.0 ± 3.0, 8.1 ± 0.3	81.0 ± 8.0, 8.1 ± 0.8
P (paired *t*-test)		0.46		0.29
Animal No. 4				
Mean ± SD	0.374 ± 0.034, 37.3 ± 3.4	0.364 ± 0.028, 36.4 ± 2.8	74.0 ± 5.0, 7.4 ± 0.5	73.0 ± 5.0, 7.3 ± 0.5
P (paired *t*-test)		<0.001*		0.02*
Group				
mean ± SD	0.372 ± 0.030, 37.2 ± 3.0	0.357 ± 0.025, 35.7 ± 2.5	71.0 ± 6.0, 7.1 ± 0.6	69.0 ± 6.0, 6.9 ± 0.6
ANOVA		0.005*		0.034*

[a] hep, heparinized; EDTA, ethylenediaminetetraacetic acid; PCV, packed cell volume; TP, total protein; ANOVA, analysis of variance; SI, International system of units.

* Statistically significant at $P < 0.05$.

DISCUSSION

Results indicate that PCV and TP values do not vary significantly between the two most frequently used venipuncture sites in the black rhinoceros, i.e., the ear and the radial veins. Samples taken from the same site but placed in different anticoagulants did show statistically significant differences. Although reference blood values have been published for black rhinoceros, the effect of sample collection site and anticoagulant has not been examined in healthy animals.[8,10] Understanding what affects changes in PCV is especially important when monitoring a black rhinoceros suffering from one of the several anomalies that occur in this species.[9,13,16–18,21]

Studies have shown that a variety of factors can influence hematologic and biochemical analyses. Hemoglobin, serum albumin, neutrophil, and monocyte counts were significantly different between samples obtained from ear or finger sticks and venous blood from healthy human volunteers.[12] This could be partially explained by the hemoconcentration resulting from peripheral ultrafiltration and margination of cells. Sample site influences on blood values also have been reported in rats, tortoises, and birds.[2,3,7] Ear veins in the black rhinoceros may be so large that they are not subject to the changes associated with capillary blood, and therefore blood in these veins is similar to that in other peripheral veins. Preanalytic factors such as exercise, posture, and venous congestion can affect results in human patients.[20] All samples were collected from standing rhinoceroses early in the morning, before any significant activity. It is not known how activity, anesthesia, or recumbency would change these parameters. In one study comparing hematologic data from black rhinoceroses at the time of capture and after confinement for 3–4 wk, PCV dropped significantly only in the group that moved from high to low elevation during translocation.[11] However, TP increased in both groups of animals during the time of confinement. This may have been due to fluid loss or dehydration. These studies suggest that comparison of results should take into consideration the conditions of collection.

Although PCV and TP did not vary significantly between the two venipuncture sites used in this study, it is not known whether there were differences in white blood cell numbers or differential counts. Complete blood counts (CBCs) and plasma biochemical analyses were performed to monitor routine health parameters during this study, using only radial vein samples. Additional studies to evaluate potential differences in CBC and biochemical values should be undertaken.

The effect of anticoagulant may be due to the dilutional effect of using liquid EDTA, the result of underfilling blood tubes, or osmotic effects.[1,4] Excessive amounts of anticoagulant can alter PCV when measured by the standard centrifugation method, using blood from multiple species.[6] Artifactual decreases in PCV are caused by shrinkage of red blood cells exposed to excess EDTA.[4] Unlike the results seen with the rhinoceroses in this study, EDTA can cause overestimation of plasma proteins because of osmotic fluid shifts in canine and rabbit blood.[5] The use of vacutainers with a butterfly system minimizes the variables associated with incorrect volume in the collection tubes, as was seen in this study. Because postural or other anatomic variables were identical in these data (i.e., only samples collected from the same vein at the same time were compared), the observed differences are most likely due to other factors, such as those mentioned above.

CONCLUSIONS

Consistency in collection technique is important when comparing results from serial samples. The results from this study suggest that PCV and TP values are more likely to be artifactually altered by the choice of anticoagulant than by the choice of venipuncture site. Therefore, the same anticoagulant should be used for serial samples. The ability to distinguish artifactual from pathologic changes is crucial when evaluating the PCV and TP in a sick black rhinoceros. The factors that influence these values should be investigated to improve our knowledge and assessment of medical conditions of black rhinoceroses.

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