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BIOCHEMICAL VALUES IN FREE-RANGING WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*) IN KRUGER NATIONAL PARK, SOUTH AFRICA

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Abstract: Biochemical panels were analyzed on 181 individual free-ranging white rhinoceros (*Ceratotherium simum*) from Kruger National Park, South Africa. These animals were immobilized between July 2006 and May 2010 for management purposes. Serum and heparinized plasma samples were analyzed using an in-house chemistry analyser (ABAXIS VetScan2). The objectives of this study were to establish biochemical references ranges for Kruger National Park's population of white rhinoceros; to assess differences in values obtained using sera or plasma; and to assess differences in values between gender and different age categories. Significant differences between plasma and serum values were found in most measured parameters except minerals (calcium and magnesium). Because all animals appeared clinically healthy at the time of blood collection, it is hypothesized that choice of anticoagulant may affect certain parameters. Comparison between age categories and gender also resulted in significant differences in a few measured parameters. Identifying differences are important when establishing baseline reference ranges for wildlife populations to allow accurate monitoring of trends that may change over time. The paucity of data on normal biochemical ranges for free-ranging white rhinoceros demonstrates the value of this study and importance of evaluating potential confounding variables.

Key words: Biochemistry panel, *Ceratotherium simum*, heparinized plasma, serum, white rhinoceros.

INTRODUCTION

Blood biochemical values have been a foundation of veterinary medicine in assessing health status, along with physical examination, hematology, and other ancillary tests. Imbalances in physiologic values can be exacerbated by stress of capture and transport.[8,19] Therefore, screening of animals before transport may reduce risk of complications by identification of potential "at risk" individuals that should potentially be released rather than subjected to further stressors. Because of the logistics of performing laboratory tests in wildlife, especially in field situations, this has not been routinely done until the advent of portable, field-friendly technology. Wildlife veterinarians and biologists can now benefit from information gained during an immobilization in some cases before translocation of the animal by running samples in the field using portable equipment that can provide results within 5–15 min. However, baseline reference ranges need to be developed to interpret results for management and medical decisions.

Kruger National Park has the largest single free-ranging white rhinoceros (*Ceratotherium simum*) population of the African range countries, with an estimate of 7,000–9,000 animals (Buss, pers. comm.). As many as 100+ white rhinoceroses may be immobilized in a single year for translocation, snare removal, or other procedures, thereby providing an opportunity to collect biologic samples such as blood for analyses and biobanking. The objectives of this study were to establish biochemical references ranges for Kruger National Park's population of white rhinoceros, to assess differences in values obtained using sera or plasma, and to assess differences in values between gender and different age categories. By achieving these objectives, the information will provide a baseline for monitoring animals during adaptation to bomas, translocation, or captivity as well as a reference for other populations of free-ranging and captive white rhinoceros.

MATERIALS AND METHODS

Animals

Samples were available from 181 individual free-ranging white rhinoceros that were immobilized for management procedures in Kruger

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National Park (23°49′60′′S, 31°30′0′′E) between July 2006 and May 2010, according to Standard Operating Procedure for the Capture, Transportation, and Maintenance in Holding Facilities of Wildlife. This protocol was approved by South African National Parks Animal Use and Care Committee. Animals appeared healthy based on body condition and physical examination. There were 15 juveniles, 116 subadults, and 49 adults in the sample set; 96 males and 85 females were analyzed for sex effects. Animals were grouped into age categories based on horn size for drug administration: juvenile (calf with dam), young or subadult (2.5–7 yr), or adult (>7 y).[18] Immobilizing drugs used were a mixture of etorphine (M99, Novartis, Kempton Park, 1619 South Africa), azaperone (Stressnil, Janssen Pharmaceutical Ltd., Halfway House, 1685 South Africa), and hyaluronidase (Hyalase, Kyron Laboratories, Benrose, 2011 South Africa). Doses were as follows: juvenile, 1–1.5 mg of etorphine, 20 mg of azaperone, 5,000 IU. of hyaluronidase; subadult, –2.0 to 3.5 mg of etorphine, 20–40 mg of azaperone, 5,000 IU. of hyaluronidase; and adult, –3.5 to 4.2 mg of etorphine, 40 mg of azaperone, and 5,000 IU. of hyaluronidase. Butorphanol (Torbugesic, Fort Dodge Animal Health, Fort Dodge, Iowa 50501 USA) was either added directly to the dart or administered intravenously in an auricular vein. The butorphanol dose administered was calculated at 10–20 mg for each 1 mg of etorphine.

Animals were located and darted using a helicopter. Drugs were delivered remotely using 3.0-ml plastic darts with a 60-mm uncollared needle propelled by a compressed air rifle (DAN-INJECT, International S.A., Skukuza, 1350 South Africa).

Once animals were immobilized, samples were collected and rhinoceroses were loaded into transport crates. All animals received intravenous diprenorphine (M5050, Novartis Animal Health, Bedson Africa Pty. Ltd., Lynwood Ridge, 0040 South Africa) calculated at 3 times the etorphine dose (in milligrams) and 50 mg of zuclopenthixol acetate i.m. (Clopixol-Acuphase, H. Lundbeck Pty. Ltd., North Riding, 2194 South Africa).

Sample collection and analyses

Blood was collected in lithium heparin and serum separator vacutainers (Fisher Scientific, Suwanee, Georgia 30024 USA) from the auricular vein of a rhinoceros within 15 min of immobilization. Samples were kept in a cooler on ice blocks until they could be processed at the laboratory. Vacutainers were centrifuged at 2,500 rpm for 10 min, and plasma or serum was decanted. Samples were frozen at –20°C until analyses were performed. Adequate volumes of both heparinized plasma and sera from all rhinoceroses were not available, resulting in unequal sample sizes for analyses. Heparinized plasma and sera were used for chemistry analyses with the ABAXIS VetScan2 chemistry analyser (ABAXIS, Inc., Union City, California 94587 USA) using a Large Animal chemistry rotor (Large Animal profile, ABAXIS Inc.). Measured analytes (preselected on rotor) included albumin (alb), alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium (Ca), γ-glutamyltransferase (GGT), total protein (TP), globulin (glob), blood urea nitrogen (BUN), creatinine phosphokinase (CK), phosphorus (P), and magnesium (Mg).

Statistical analyses

Data from each biochemical parameter were first assessed for normality visually using a histogram with a density curve and then more formally by using the Shapiro–Wilk test for normality. The distribution of each parameter (mean, SEM, and 95% confidence interval for the mean, as well median and the 95% confidence interval for the median) is summarized in tables by sample type (plasma vs. serum) as well for potential confounding factors (sex and age categories). As a first screening step, the Wilcoxon rank test was used to compare median values between plasma and serum for each parameter (univariable analysis). To account (adjust) for other factors in the analysis, a multivariable regression model using ranks was used to compare the median value for each parameter between plasma and serum while adjusting for the effect of age and sex of the rhinoceros. Statistically significant differences between sera and plasma for each biochemical parameter were considered at $P < 0.05$. Records were stored on an Excel sheet and STATA software (Stata Statistical Software, Release 11, StataCorp LP, College Station, Texas 77840 USA) was used for the statistical analysis.

RESULTS

The distribution of data for each parameter indicated that data were nonnormally distributed; the histogram showed skewed distribution and the Shapiro–Wilk test was significant at $P < 0.05$ for each parameter. The distribution (mean, SEM, 95% CI of the mean, median and 95% CI for the

Table 1. Distribution (mean, SD, SEM, and mean 95% CI, median and 95% CI for the median) for heparinized plasma and serum biochemical values for free-ranging white rhinoceros in Kruger National Park, 2006–2010.

Parameter	*n*	Mean	SD	SEM	95% CI	(mean)	Median	95% CI	(median)	*P*[a]
Plasma										
Albumin (g/dl)	73	1.7	0.9	0.1	1.5	1.9	1.2	1.1	1.3	
ALP (U/L)	73	80.5	29.8	3.5	73.6	87.5	75.0	69.0	84.6	
AST (U/L)	73	63.0	21.3	2.5	58.0	68.0	64.0	55.6	70.0	
Ca (mg/dl)	73	11.9	1.5	0.2	11.5	12.2	12.0	11.8	12.2	
GGT (U/L)	73	16.1	5.5	0.6	14.9	17.4	15.0	14.0	16.0	
TP (g/dl)	73	10.4	1.4	0.2	10.1	10.7	10.5	10.3	10.6	
Globulin (g/dl)	57	8.6	1.4	0.2	8.3	9.0	9.1	8.5	9.4	
BUN (mg/dl)	73	11.4	4.0	0.5	10.5	12.3	11.0	10.0	13.0	
CK (U/L)	73	195.3	116.6	13.6	168.1	222.5	175.0	159.6	189.0	
P (mg/dl)	73	4.5	1.0	0.1	4.3	4.7	4.6	4.2	4.8	
Mg (mg/dl)	73	3.2	0.6	0.1	3.1	3.3	3.2	3.1	3.3	
Serum										
Albumin (g/dl)	107	2.5	0.8	0.1	2.4	2.7	2.8	2.7	2.8	<0.001
ALP (U/L)	108	56.1	16.8	1.6	52.9	59.3	57.0	53.3	59.7	<0.001
AST (U/L)	108	38.6	15.7	1.5	35.6	41.6	36.0	34.0	39.0	<0.001
Ca (mg/dl)	108	11.6	1.6	0.2	11.3	11.9	11.9	11.8	12.0	0.22
GGT (U/L)	108	11.9	3.5	0.3	11.2	12.6	12.0	11.0	12.0	<0.001
TP (g/dl)	108	9.5	1.0	0.1	9.3	9.6	9.4	9.2	9.6	<0.001
Globulin (g/dl)	93	6.6	1.0	0.1	6.4	6.8	6.6	6.5	6.8	<0.001
BUN (mg/dl)	108	9.7	2.9	0.3	9.1	10.2	9.0	9.0	10.0	0.001
CK (U/L)	108	127.6	52.7	5.1	117.5	137.6	117.0	109.3	124.7	<0.001
P (mg/dl)	108	5.0	7.5	0.7	3.6	6.5	4.4	4.1	4.5	0.042
Mg (mg/dl)	108	3.1	0.4	0.0	3.0	3.2	3.1	3.0	3.2	0.23

[a] *P* value obtained for the difference between plasma and serum median values for each parameter after controlling for age category and sex in a multivariable model using ranks.

median) of heparinized plasma and serum biochemical values is shown in Table 1. Value distribution by sex is shown in Table 2 and by age category in Table 3 for both values obtained using plasma and serum, respectively. Observed differences in Table 1 between plasma and serum median values were statistically significant using univariable analysis (Wilcoxon rank sum test, $P < 0.001$) for all measured analytes except the minerals (Ca, $P = 0.22$; Mg, $P = 0.23$). Heparinized plasma samples had higher median values compared with serum samples for ALP (75 vs. 57 U/L), AST (64 vs. 36 U/L), GGT (15 vs. 12 U/L), TP (10.5 vs. 9.4 g/dl), glob (9.1 vs. 6.6 g/dl), BUN (11 vs. 9 mg/dl), and CK (175 vs. 117 U/L). Only the median alb was significantly higher in serum compared with plasma (2.8 vs. 1.2 g/dl, respectively; $P < 0.001$). After controlling for the potential confounding effect of sex and age (multivariable analysis), all the overall observed differences remained statistically significant. Differences between plasma and serum P median values become statistically different ($P = 0.042$) after adjusting for differences observed among different age categories, with an overall lower median value for phosphorus in serum samples.

The results of the multivariable model indicated that when controlling for the combined effect of sample type and age category in the analysis (i.e., keeping sample type and age category constant), there were no statistically significant differences ($P > 0.05$) between female and male rhinoceroses for most of the parameters studied (Table 2). The only differences observed were higher median ALP values in male rhinoceroses compared with female rhinoceroses on serum samples (ALP 61 vs. 55 U/L, respectively; $P = 0.024$) and higher median Mg values in male rhinoceroses compared with female rhinoceroses on plasma samples (Mg, 3.4 vs. 3.0 mg/dl, respectively; $P = 0.019$).

When controlling for the combined effect of sample type and gender in the analysis (i.e., keeping sample type and sex constant), there were no statistically significant differences ($P > 0.05$) between rhinoceroses in different age categories for most of the studied parameters (Table 3). Adult white rhinoceroses had a significantly lower

Table 2. Distribution (mean, SD, SEM, and 95% CI for the mean, median and 95% CI for the median) for plasma and serum biochemical values for females and males free-ranging white rhinoceros in Kruger National Park, 2006–2010.

	Parameter	*n*	Mean	SD	SEM	95% CI of mean		Median	95% CI of median	
Plasma										
Females	Albumin (g/dl)	33	1.7	1.0	0.2	1.4	2.0	1.2	1.1	1.6
	ALP (U/L)	33	80.8	22.0	3.8	73.0	88.6	81.0	72.3	88.0
	AST (U/L)	33	61.2	19.8	3.4	54.2	68.3	63.0	54.3	69.0
	Ca (mg/dl)	33	11.9	0.8	0.1	11.6	12.2	12.0	11.6	12.4
	GGT (U/L)	33	15.7	4.0	0.7	14.3	17.1	15.0	13.3	17.0
	TP (g/dl)	33	10.3	0.6	0.1	10.1	10.5	10.4	10.0	10.6
	Globulin (g/dl)	26	8.5	1.2	0.2	8.0	9.0	9.0	7.7	9.5
	BUN (mg/dl)	33	11.2	4.2	0.7	9.7	12.7	11.0	9.3	13.0
	CK (U/L)	33	185.5	91.2	15.9	153.2	217.9	172.0	154.7	193.3
	P (mg/dl)	33	4.4	0.8	0.1	4.2	4.7	4.4	4.1	4.9
	Mg (mg/dl)	33	3.1	0.3	0.1	3.0	3.2	3.0	2.9	3.2
Males	Albumin (g/dl)	40	1.7	0.9	0.1	1.4	2.0	1.3	1.1	1.5
	ALP (U/L)	40	80.3	35.2	5.6	69.0	91.6	71.0	63.8	89.0
	AST (U/L)	40	64.5	22.7	3.6	57.2	71.7	66.5	48.0	75.5
	Ca (mg/dl)	40	11.9	2.0	0.3	11.2	12.5	12.0	11.8	12.2
	GGT (U/L)	40	16.5	6.5	1.0	14.4	18.6	15.0	14.0	16.0
	TP (g/dl)	40	10.4	1.8	0.3	9.9	11.0	10.6	10.3	10.9
	Globulin (g/dl)	31	8.7	1.5	0.3	8.2	9.3	9.1	8.1	9.6
	BUN (mg/dl)	40	11.6	3.9	0.6	10.3	12.8	12.0	10.0	13.7
	CK (U/L)	40	203.4	134.6	21.3	160.3	246.4	178.0	152.6	197.7
	P (mg/dl)	40	4.5	1.1	0.2	4.2	4.9	4.6	4.1	5.0
	Mg (mg/dl)	40	3.3	0.7	0.1	3.1	3.5	3.4	3.1	3.5
Serum										
Females	Albumin (g/dl)	51	2.5	0.8	0.1	2.3	2.7	2.8	2.7	2.8
	ALP (U/L)	52	51.3	17.1	2.4	46.6	56.1	55.0	48.4	57.6
	AST (U/L)	52	40.1	14.9	2.1	36.0	44.3	39.0	34.4	41.6
	Ca (mg/dl)	52	11.6	1.6	0.2	11.2	12.0	11.9	11.7	12.0
	GGT (U/L)	52	11.3	2.8	0.4	10.5	12.1	11.0	10.4	12.0
	TP (g/dl)	52	9.3	0.7	0.1	9.1	9.5	9.4	9.1	9.6
	Globulin (g/dl)	44	6.6	0.7	0.1	6.4	6.8	6.6	6.3	6.7
	BUN (mg/dl)	52	9.4	2.7	0.4	8.7	10.2	9.0	8.4	10.0
	CK (U/L)	52	124.8	49.4	6.8	111.1	138.5	117.0	108.8	125.6
	P (mg/dl)	52	5.9	10.8	1.5	2.9	8.9	4.4	4.1	4.6
	Mg (mg/dl)	52	3.1	0.4	0.1	3.0	3.2	3.1	3.0	3.2
Males	Albumin (g/dl)	56	2.5	0.8	0.1	2.3	2.7	2.7	2.6	2.8
	ALP (U/L)	56	60.5	15.4	2.1	56.4	64.6	61.0	53.3	64.8
	AST (U/L)	56	37.2	16.3	2.2	32.8	41.5	34.5	33.0	36.8
	Ca (mg/dl)	56	11.7	1.6	0.2	11.2	12.1	11.9	11.5	12.2
	GGT (U/L)	56	12.4	3.9	0.5	11.4	13.5	12.0	11.0	13.0
	TP (g/dl)	56	9.6	1.2	0.2	9.3	9.9	9.5	9.3	9.7
	Globulin (g/dl)	49	6.6	1.3	0.2	6.2	6.9	6.6	6.4	6.9
	BUN (mg/dl)	56	9.9	3.1	0.4	9.1	10.8	9.5	8.0	10.0
	CK (U/L)	56	130.1	56.0	7.5	115.1	145.1	122.5	104.2	136.2
	P (mg/dl)	56	4.2	0.8	0.1	4.0	4.5	4.3	3.9	4.5
	Mg (mg/dl)	56	3.1	0.4	0.1	3.0	3.2	3.2	3.0	3.3

median ALP value compared with subadult rhinoceroses in plasma samples (57.0 vs. 81.0 U/L, respectively; $P = 0.004$) and in serum samples (46.0 vs. 60.0 U/L, respectively; $P = 0.004$). Juvenile rhinoceros had a lower median GGT value compared with subadults in plasma samples (GGT, 11.0 vs. 15.0 U/L, $P = 0.033$) but higher median CK values for juveniles compared with subadults in serum samples (CK, 142.0 vs. 111.0 U/L, respectively; $P = 0.001$). P varied between all

Table 3. Distribution (mean, SD, SEM, and 95% CI for the mean, median and 95% CI for the median) for plasma and serum biochemical values for free-ranging white rhinoceros of different ages in Kruger National Park, 2006–2010.

	Parameter	*n*	Mean	SD	SEM	95% CI (mean)		Median	95% CI (median)	
Plasma										
Subadults	Albumin (g/dl)	55	1.6	0.9	0.1	1.3	1.8	1.2	1.1	1.2
	ALP (U/L)	55	85.3	28.6	3.9	77.6	93.0	81.0	71.7	89.0
	AST (U/L)	55	65.1	20.0	2.7	59.7	70.6	68.0	58.4	71.0
	Ca (mg/dl)	55	12.1	1.2	0.2	11.7	12.4	12.1	11.8	12.4
	GGT (U/L)	55	16.8	5.4	0.7	15.3	18.2	15.0	14.7	17.0
	TP (g/dl)	55	10.4	0.9	0.1	10.1	10.6	10.4	10.1	10.6
	Globulin (g/dl)	42	8.6	1.4	0.2	8.2	9.0	9.1	8.8	9.5
	BUN (mg/dl)	55	11.8	3.9	0.5	10.7	12.8	12.0	10.0	13.3
	CK (U/L)	55	201.4	121.6	16.4	168.6	234.3	180.0	162.1	197.9
	P (mg/dl)	55	4.7	0.9	0.1	4.4	4.9	4.8	4.4	4.9
	Mg (mg/dl)	55	3.2	0.5	0.1	3.1	3.3	3.1	3.0	3.3
Adults	Albumin (g/dl)	15	1.9	1.1	0.3	1.3	2.5	1.5	1.1	3.0
	ALP (U/L)	15	66.4	30.3	7.8	49.6	83.2	57.0	42.4	86.3
	AST (U/L)	15	57.8	26.4	6.8	43.2	72.4	45.0	37.5	77.3
	Ca (mg/dl)	15	11.4	2.4	0.6	10.1	12.7	12.0	11.8	12.2
	GGT (U/L)	15	14.7	5.8	1.5	11.5	17.9	13.0	12.0	15.8
	TP (g/dl)	15	10.4	2.5	0.6	9.0	11.8	10.9	10.1	11.8
	Globulin (g/dl)	12	8.8	1.6	0.5	7.8	9.8	8.6	7.6	10.6
	BUN (mg/dl)	15	10.3	4.5	1.2	7.8	12.8	11.0	8.2	13.0
	CK (U/L)	15	178.1	108.5	28.0	118.0	238.2	165.0	128.5	178.6
	P (mg/dl)	15	3.9	1.1	0.3	3.3	4.4	4.0	3.3	4.4
	Mg (mg/dl)	15	3.1	0.8	0.2	2.7	3.6	3.2	3.1	3.6
Juveniles[a]	Albumin (g/dl)	2						2.2	1.3	3.0
	ALP (U/L)	2						66.0	37.0	95.0
	AST (U/L)	2						55.0	48.0	62.0
	Ca (mg/dl)	2						12.1	11.5	12.7
	GGT (U/L)	2						11.0	8.0	14.0
	TP (g/dl)	2						10.6	10.4	10.8
	Globulin (g/dl)	2						8.6	7.8	9.4
	BUN (mg/dl)	2						9.5	8.0	11.0
	CK (U/L)	2						190.0	147.0	233.0
	P (mg/dl)	2						5.0	4.6	5.4
	Mg (mg/dl)	2						3.9	3.7	4.0
Serum										
Subadults	Albumin (g/dl)	61	2.4	0.9	0.1	2.1	2.6	2.8	2.6	2.8
	ALP (U/L)	61	60.6	14.1	1.8	57.0	64.3	60.0	57.0	63.0
	AST (U/L)	61	36.8	8.8	1.1	34.5	39.0	37.0	34.0	39.0
	Ca (mg/dl)	61	11.9	1.1	0.1	11.6	12.2	11.8	11.7	12.3
	GGT (U/L)	61	12.1	3.0	0.4	11.3	12.9	12.0	11.0	12.0
	TP (g/dl)	61	9.5	1.0	0.1	9.2	9.7	9.4	9.2	9.7
	Globulin (g/dl)	49	6.6	1.2	0.2	6.2	6.9	6.6	6.4	6.9
	BUN (mg/dl)	61	9.6	2.7	0.3	8.9	10.3	9.0	9.0	10.0
	CK (U/L)	61	114.1	31.8	4.1	106.0	122.3	111.0	105.3	119.0
	P (mg/dl)	61	5.5	10.0	1.3	3.0	8.1	4.4	4.0	4.5
	Mg (mg/dl)	61	3.2	0.3	0.0	3.1	3.2	3.1	3.0	3.2
Adults	Albumin (g/dl)	34	2.6	0.6	0.1	2.4	2.9	2.7	2.6	2.9
	ALP (U/L)	34	46.3	17.8	3.1	40.1	52.5	46.0	35.8	55.2
	AST (U/L)	34	38.7	18.9	3.2	32.1	45.3	35.0	32.0	42.0
	Ca (mg/dl)	34	11.4	1.7	0.3	10.9	12.0	11.9	11.5	12.0
	GGT (U/L)	34	12.1	4.0	0.7	10.7	13.5	12.0	10.8	13.0
	TP (g/dl)	34	9.6	1.1	0.2	9.2	9.9	9.6	9.2	10.2
	Globulin (g/dl)	32	6.8	0.9	0.2	6.5	7.1	6.7	6.4	7.1
	BUN (mg/dl)	34	10.1	3.4	0.6	9.0	11.3	10.0	8.0	11.0
	CK (U/L)	34	140.2	72.8	12.5	114.8	165.6	127.0	105.0	139.5

Table 3. Continued.

	Parameter	*n*	Mean	SD	SEM	95% CI (mean)		Median	95% CI (median)	
	Phosphorus (mg/dl)	34	4.0	0.8	0.1	3.7	4.3	3.9	3.7	4.4
	Mg (mg/dl)	34	3.0	0.6	0.1	2.8	3.2	3.2	3.0	3.4
Juveniles[a]	Albumin (g/dl)	12	2.8	0.3	0.1	2.6	3.0	2.8	2.6	3.1
	ALP (U/L)	13	60.3	15.6	4.3	50.9	69.7	57.0	51.0	73.6
	AST (U/L)	13	46.8	27.0	7.5	30.5	63.2	39.0	32.8	45.6
	Ca (mg/dl)	13	11.0	3.0	0.8	9.2	12.8	11.9	11.3	12.3
	GGT (U/L)	13	10.5	3.9	1.1	8.2	12.9	10.0	9.0	11.0
	TP (g/dl)	13	9.1	0.6	0.2	8.7	9.4	9.3	8.4	9.5
	Globulin (g/dl)	12	6.2	0.6	0.2	5.8	6.6	6.5	5.5	6.7
	BUN (mg/dl)	13	8.8	2.8	0.8	7.2	10.5	7.0	7.0	11.8
	CK (U/L)	13	157.8	52.8	14.6	125.9	189.7	142.0	119.4	208.3
	Phosphorus (mg/dl)	13	5.5	0.8	0.2	5.0	6.0	5.5	4.9	6.0
	Mg (mg/dl)	13	3.1	0.3	0.1	2.9	3.3	3.0	2.8	3.4

[a] Because of the small sample size ($n = 2$) for juvenile rhinoceros parameters measured in plasma, only the median is presented. The lower and upper confidence interval represents the minimum and maximum values, respectively.

three age categories. Adults had lower median P values compared with subadults in plasma samples (4.0 vs. 4.8 mg/dl, respectively; $P = 0.002$) and juvenile rhinoceroses had a higher median P values compared with subadults in serum samples (5.5 vs. 4.4 mg/dl, respectively; $P < 0.001$).

DISCUSSION

In this study, biochemical reference ranges for free-ranging white rhinoceros were established and examined for differences between sample type, age, and gender. Plasma and serum samples were collected from free-ranging white rhinoceroses that were immobilized for routine procedures. Biochemical parameters were selected based on commercial availability of tests for the chemistry analyzer and those that had been previously used in captive rhinoceroses (Miller, pers. comm.). Using this methodology, analytes were assessed for the Kruger National Park white rhinoceros population.

Comparison of serum values with the limited number of previously published values for free-ranging white rhinoceros showed that the Kruger population were very similar to those sampled in other studies. Mean values for serum albumin, AST, CK, GGT, TP, and ALP reported by van Heerden et al.[20] were similar to the median serum values in this study, despite using different laboratories and methodologies. In addition, serum biochemical parameter ranges overlapped between free-ranging black rhinoceros (*Diceros bicornis*) and white rhinoceros in some published reports.[14,20] Captive white rhinoceros biochemical values are also available; however, the type of sample (plasma vs. serum) and health status of the animals are unknown.[6] Despite these limitations, values are remarkably similar to those of free-ranging rhinoceros. These comparisons suggest that the methods used in this study provided reasonably reliable measurement of biochemical parameters in rhinoceros and permitted establishment of reference ranges for this population.

When serum and heparinized plasma were compared from white rhinoceros, overall, significant differences were found, with higher median plasma values for all measured parameters except Ca and Mg. Significant differences between serum and plasma were observed in most biochemical values in domestic horses (*Equus caballus*), although most were lower in citrated plasma except for P, Mg, and GGT.[11] Findings in domestic sheep (*Ovis aries*) resembled those of white rhinoceros with most biochemical parameters significantly higher in plasma, except for GGT, P, and bilirubin.[13]

The differences between plasma and serum may be species-dependent. For example, heparinized plasma resulted in significant increases in glucose, TP, alb, and P compared with serum in ostrich (*Struthio camelus*).[12] In contrast, the majority of biochemical values analyzed in Malaysian flying foxes (*Pteropus vampyrus*) did not differ significantly between heparinized plasma and serum.[4] It has been reported in domestic species that serum and heparinized plasma produce similar results in most biochemical tests, the exceptions being serum for bile acids and heparinized plasma for potassium (K).[9]

There may be several possible explanations for the observed differences in plasma and serum values in white rhinoceros in this study. One is the effect of sample handling and processing on fluid

analytes. Anticoagulants such as heparin prevent fibrin clot formation, which could bind some analytes, resulting in lower concentrations in serum samples as observed in Amazon parrots (*Amazona amazonica*).[3] Another potential effect of anticoagulants is the release of certain enzymes, electrolytes, or both into plasma from cells. This may be observed when an inappropriate ratio of anticoagulant to whole blood is used. In one study comparing human biochemical values using heparinized plasma and serum, when the concentration of heparin was increased (because of incomplete filling of the blood tube), ALT, AST, lipase, and K were affected, although no differences were found between plasma and serum when samples were properly collected.[2] Another possible but less likely scenario is the difference in reaction of serum and plasma samples in the chemical analyser. The ABAXIS VetScan2 and biochemical rotors have been developed for use with plasma or serum from an array of domestic veterinary species (ABAXIS, Inc.). Similarly, comparison of serum and heparinized plasma samples from human volunteers only showed significant differences in potassium between chemistry analyzers.[10]

In addition to type of sample, age and sex can influence biochemical values. Juvenile white rhinoceroses in this study had higher median P and CK than subadult rhinoceroses, and subadult rhinoceroses had higher median ALP and P compared with adults. Free-ranging subadult black rhinoceroses also had increased P and ALP compared with adults, similar to white rhinoceroses.[7] ALP and CK values were higher in both fillies and colts compared with adult horses, which mimicked findings in younger white rhinoceroses.[16] Age-related variations in ALP also were observed in free-ranging African elephants in Tanzania.[15] Age-specific changes in P and ALP values are usually attributed to bone activity associated with growth.[1]

It is unclear why male rhinoceroses had higher Mg values compared with females. However, blood values of Mg can be affected by dietary intake and lactation.[17] Other biochemical values were not significantly associated with age or sex in this study.

Blood values from free-ranging animals are often cited for comparison with captive counterparts, although in some species, there is a paucity of published literature. A variety of factors affect values, including season, age, geographic region, habitat quality, nutrition, health status, as well as extrinsic variables such as capture technique, sample collection and handling, anticoagulants, and the laboratory or analytical methods used. Samples from free-ranging wildlife are valuable resources for disease surveillance, establishing reference ranges and biobank sources for future genetic, serologic, and other studies. Therefore, determining the appropriate samples for different tests is an important part of any procedure. The advantages of serum sampling include its universal use in most immunological assays, biochemical profiles, and some disease detection tests.[5] The difference between serum and plasma is that serum does not contain clotting factors such as fibrinogen. The advantages of plasma collection are that they can be used for most biochemical tests, many immunologic assays, and often result in increased sample volume yield compared with serum. This has been anecdotally observed with rhinoceros samples that form large fibrin clots, reducing the yield of serum that can be harvested from a fixed amount of whole blood.

In this study, the mean value for each biochemical parameter and 95% confidence intervals (CI) of the mean were included for descriptive purposes only. Values for captive white rhinoceros have been reported as mean and SDs, but they are difficult to compare because of lack of information on health status, sample type, and laboratory methodology.[6] Because the distribution of data in this study were not normally distributed for any the biochemical parameters, median values represent the central tendency for values more accurately among different sample types (plasma and serum) and different categories (sex and age). Thus, we suggest using median values (and 95% CI of the median, rather than means) to interpret and make inferences about the "ranges" (distribution) of values from this study.

The study population included 181 animals, representing an important number considering these were all free-ranging white rhinoceroses. However, there were a few limitations to the study. One limitation was the incomplete set of biochemical parameters available for analyses. The selection of tests was based on a set profile that was commercially available for a benchtop analyzer and has been used for biochemical analysis in captive rhinoceroses to provide a frame of reference. A broader range of tests would create a larger database of values and could be achieved by combining several different rotor profiles. To obtain the large sample size, individuals from multiple years were tested. This may introduce some unknown confounding factors due to environmental changes, artifactual sample storage effects, or differences in capture conditions between years that could affect bio-

chemical values. Future studies to investigate the effect of these factors should be considered. Because not all individuals had paired serum and plasma samples available for analysis, a more robust determination of the differences between serum and plasma could be assessed through a prospective study of paired samples. Despite the relatively overall large sample size, it is recognized that only 15 juvenile white rhinoceroses were included in the analyses. This relatively small number could decrease the probabilities of declaring any observed differences statistically significant when comparing these younger animals to the animals in other age categories.

The significance of this report is the evidence that biochemical values can vary depending on the type of sample, a factor that is physiologically important when interpreting whether values are "normal" or "abnormal" based on a set of reference ranges. Values also may change based on characteristics such as age and sex. Understanding health in free-ranging white rhinoceros requires information on multiple aspects, including evaluation of physiologic parameters such as biochemical values. Therefore, careful collection and interpretation of samples are a crucial part of this process. Nonetheless, the results form a useful reference for biochemical parameters in free-ranging and captive populations of white rhinoceros.

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