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Blood Biochemical Reference Intervals for Free-Ranging Olive Baboons (*Papio anubis*) in Kenya

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Abstract

Biochemical reference intervals are important for assessing the health of target species and populations by identifying abnormalities in key blood parameters. Although reference intervals have been established for baboons in captivity, the lack of data from free-ranging individuals makes it difficult to interpret the results of their blood chemistry panels or to assess and monitor the health of wild baboon populations. The goal of this study was therefore to establish serum biochemical reference intervals for free-ranging olive baboons (*Papio anubis*) in Kenya. We evaluated 14 biochemical parameters from 28 baboons sampled at the Mpala Research Center, Nanyuki, Kenya. Reference intervals obtained from this wild population were comparable to those from captive baboon populations. Alkaline phosphatase (ALP) and phosphorus levels differed significantly among age classes; both were higher in subadult and juvenile baboons than in adults. However, none of the components of the blood biochemistry panel differed significantly between the sexes. The reference intervals we report provide a baseline for the evaluation and treatment of free-ranging olive baboons and provide context for interpreting the biochemical profiles of captive individuals.

Keywords Chemical immobilization · Health status · Primate · Serum chemistry

Introduction

Well-defined, normal blood biochemistry parameters provide an important tool for assessing primate health at both the individual and population level. Because many

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factors can cause variation in blood biochemistry, including age, sex, and large-scale geographical location, samples from many individuals are needed to define the normal range of variation within a species. As a consequence of this need for large sample sizes, the reference intervals that veterinarians and researchers use to interpret the results of blood biochemistry tests are often based on data from captive, rather than wild, primate populations. However, components of blood chemistry panels may differ between captive and wild populations owing to a range of factors including diet, husbandry practices, and levels of physical activity and exertion and dehydration (Brenner *et al.* 2002; Junge and Louis 2005; Percin and Konyalioglu 2008; Rangel-Mendoza *et al.* 2009). Such discrepancies, if they exist, could have a potentially important impact on our ability to assess and monitor the health of wild primates.

The olive baboon (*Papio anubis*) is one of two common, widespread baboon taxa in Kenya. Its close association with human settlements and agriculture make it a frequent patient of wildlife veterinarians. Further, the importance of olive baboons to public health in Kenya because of their role as potential carriers of zoonotic disease highlights a need for accurate reference values to assess baboon health. Although there are some compilations of normal blood biochemistry in captive baboons (Harewood *et al.* 1999; Teare 2002), few data exist from free-ranging individuals (Foy *et al.* 1965; Melton 1982), and no studies report the results of complete blood biochemistry panels for free-ranging olive baboons. The goal of this study was to establish sets of reference values for common blood biochemistry test results from free-ranging olive baboons, taking variation due to age and sex differences into consideration.

Methods

Study Location

We conducted this research at the Mpala Research Center (MRC), located in central Kenya near the town of Nanyuki in Laikipia County. MRC covers 48,000 acres of unfenced savannah and woodland habitat. MRC is home to a diverse wildlife community that includes elephants, wild dogs, Grevy's and plains zebras, leopards, lions, and two nonhuman primate species: vervet monkeys and olive baboons. Olive baboons are numerous in the conservancy and surrounding settlements. Despite extensive shooting, trapping, and poisoning by humans, olive baboon populations in Kenya are on the rise and the species is listed as being of least concern (Kingdon *et al.* 2008).

Study Design and Sample Collection

We collected blood samples from 29 free-ranging olive baboons in July 2012 at the MRC as part of a larger study on baboon behaviour (Farine *et al.* 2017; Strandburg-Peshkin *et al.* 2015, 2017). Only healthy individuals were selected for blood draws based on a brief visual exam and after being assessed as having body conditions scores of good (4/5; thin layer of subcutaneous fat, hip bones and spine are visible and readily palpable but not prominent) or excellent (5/5; well-developed musculature and subcutaneous fat layer, pelvis, ribs and spine were palpable with gentle pressure but not visible). We determined individual age and sex via physical examination, performed

after the individual had been chemically immobilized. We estimated the age of each individual based on the pattern of dental eruption and evidence of sexual maturation. Individuals with deciduous dentition were classified as juveniles. Subadult and adult males were distinguished based on their body size and the development of secondary sexual characteristics, including their mantle, musculature, canine size, and morphology. We considered females to be adult if they had full, permanent dentition and were parous (based on the elongation and darkening of nipples) or showed evidence of cycling (based on the morphology of their sexual skin). Nulliparous females that were cycling but still had one or more deciduous teeth were classified as subadult.

For 1 mo before capture and sampling, we prebaited two open areas ca. 500 m apart with maize to habituate members of an olive baboon group to our traps. From July 21 to 29, 2012, we trapped 33 of the 46 members of this focal baboon group in 1 × 1 × 1 m cage traps. Four individuals deemed too small were immediately released. We immobilized the other 29 baboons using a 10–15 mg/kg dose of ketamine HCl (Ketamine 100®, Pantex, Hapert, The Netherlands). We then weighed and measured these individuals, drew blood samples (3–4 ml, depending on body weight) and, except for two individuals, fit GPS collars (e-Obs Digital Telemetry, Gruenwald, Germany). The GPS collars were equipped with SureDrop automated breakaway units (Advanced Telemetry Systems, Isanti, MI, USA) that were preprogrammed to trigger on September 7, 2012, causing the collars to fall off. We successfully recovered all collars. We drew blood from the greater saphenous vein and placed it immediately into serum-separator tubes. We placed sample tubes in a darkened cooler with ice packs and centrifuged them to obtain serum as soon as we returned to the MRC labs (within a maximum of 9 h post-collection). It took ca. 15 min to complete the procedures for each baboon, after which we returned individuals to a cage trap to recover in the shade. All individuals were released without complications after 30–45 min and rejoined the rest of their group.

Sample Analysis

Serum chemistry analysis was performed with a VetScan VS2® Analyzer (Abaxis Inc., Union City, CA, USA) using VetScan VS2® Comprehensive Diagnostic Profile cassettes. The VetScan VS2® is commonly used by veterinarians to process wildlife samples in the field, and despite a lack of published manufacturer's precision guidelines, it has shown acceptable reproducibility compared to central lab testing when analyzing nonhuman primate samples (Snider *et al.* 2009). Before field data collection, we also processed a serum-based control cassette to verify accuracy and ensure quality control while processing free-ranging baboon samples in the field. In total, 14 serum chemistry parameters were measured (albumin, alkaline phosphatase [ALP], alanine transaminase [ALT], amylase, total bilirubin, blood urea nitrogen [BUN], calcium, phosphorus, creatinine, glucose, sodium, potassium, total protein, globulin). The VetScan® analyzer recorded 26 of 28 sampled individuals as having high (2+ or 3+) levels of hemolysis. Hemolysis was severe enough in the samples from five individuals that the machine was unable to read potassium levels and, in one case, total bilirubin. Thus, samples from some baboons could not be used for all tests; the exact number of baboons contributing data to each chemistry parameter is listed in Table I.

Table I Descriptive statistics, intervals on means and medians, and 90% intervals on reference limits for 14 sets of blood biochemical results from olive baboons caught in Laikipia, Kenya in July, 2012

Analytes	SI units	Age group	*N*	Mean	SD	Median	Minimum	Maximum	95% CI mean[a]	95% CI median[b]	Reference interval	Lower reference limit 90% CI	Upper reference limit 90% CI	Distribution	Method
Albumin	g/L	All	27	34	6	36	16	39	31.4–36.4	33–38	22–50	18–29	43–54	NG	R
ALP	U/L	J, SA	15	705	129	666	535	950	634.3–776.7	606–791	486–1080	436–548	902–1333	G	PT
		Adult	13	299	206	254	84	650	174.6–424.0	143–413	0–702	NE	NE	NG	R
ALT	U/L	All	28	43	14	41	20	79	38.1–48.7	37–47	11–69	4–21	59–78	NG	R
Amylase	U/L	All	28	136	39	129	79	257	120.7–151.3	115–151	47–214	22–73	184–241	NG	R
Total bilirubin	μmol/L	All	27	6	1	6	4	8	5.3–6.1	5.0–6.0	4–8	4–5	7–9	G	
BUN	mmol/L	All	28	3.8	0.9	3.6	2.3	5.6	3.5–4.2	3.2–4.4	2.1–6.0	1.8–2.5	5.3–6.7	G	PT
Calcium	mmol/L	All	28	2.3	0.1	2.4	2.0	2.6	2.26–2.34	2.26–2.41	2.0–2.6	1.9–2.1	2.5–2.6	G	PT
Phosphorus	mmol/L	J, SA	15	1.5	0.3	1.5	1.0	1.9	1.3–1.7	1.29–1.66	0.8–2.1	0.6–1.0	1.8–2.3	G	P
		Adult	13	1.1	0.3	1.0	0.6	1.6	0.94–1.26	0.87–1.42	0.5–1.9	0.4–0.7	1.5–2.3	G	PT
Creatinine	μmol/L	All	28	40	20	35	18	123	31.8–47.8	30.0–44.0	17–90	15–21	69–120	NG	RT
Glucose	mmol/L	All	28	6.4	2.0	6.0	2.7	10.4	5.6–7.2	5.3–6.7	3.1–11.5	2.6–3.7	9.7–13.4	G	PT
Sodium	mmol/L	All	28	142	3	143	136	149	141.1–143.8	141–144	134–149	131–137	147–150	G	PT
Potassium	mmol/L	All	23	5.9	1.0	5.9	4.7	8.9	5.5–6.3	5.2–6.3	4.3–9.0	4.1–4.7	7.7–11.3	G	PT
Total protein	g/L	All	28	80	6	80	72	98	77.8–82.4	77.0–80.0	67–91	63–70	86–96	NG	R
Globulin	g/L	All	27	46	9	43	35	67	42.1–49.3	40.0–46.0	34–79	33–36	60–185	G	PT

J = juvenile; SA = subadult; *N* = number of individuals; CI = confidence interval; G = Gaussian; NG = non-Gaussian; P = parametric method; PT = parametric method after Box–Cox transformation; R = robust method; RT = robust method after Box–Cox transformation; NE = variable could not be estimated

[a] The 95% confidence intervals about the mean are based on normal distribution results

[b] Confidence intervals about the median are computed (in each case more stringently than 95%) by the exact binomial calculations, which were computed to obtain the probability $P(Y_k \leq P_{100k} \leq Y_{k+1})$ for the $100p$th = 50th percentile with limits Y_k and Y_{k+1}

Statistical Analysis

We calculated descriptive statistics for each of the blood chemistry variables and determined the corresponding reference intervals using Reference Value Advisor (Geffré *et al.* 2011). All analyses conformed to CSLI guidelines; as the sample size was small ($N \leq 28$) and the population variances were unknown, we averaged values and confidence intervals for each biochemical marker based on both the median and the mean because, in general, the median is less likely than the mean to be influenced by outliers and effects of skewed distributions in small sample sizes. The mean for each variable was estimated using the Student's *t*-distribution. Distribution-free confidence intervals were developed about each of the median values. We used the *k*th order statistic, Y_k, which is a random variable, to develop these intervals for the median. The probability that our sample blood chemistry variables fall into the probability interval about the median is $P \geq 0.95$ with $N = 29$, or less, depending on missing values and subsets of the data. We can assume each observation falls independently into or outside of this interval so that the desired probability is distributed as binomial (N, p). These exact probabilities were calculated in Mathcad20 rather than using the normal approximation, despite some sample sizes being >20. General linear models were used to test for the effect of sex, age class, and their interaction on mean differences in biochemical markers. Levene's test for variances tested the assumption of homogeneity of variances. For the two variables, ALP and phosphorous, for which age category showed significant differences, separate reference confidence intervals were developed (Table I). One sample was excluded from analysis because of multiple obvious outliers and/or analyzer reading errors in the biochemistry results.

Data Availability

The datasets generated and analyzed in this study are available from the corresponding author.

Ethical Note

The Smithsonian Tropical Research Institute IACUC (assurance #: 2012.0601.2015) approved all animal capture and treatment protocols. Protocols followed the guidelines prescribed by the Kenya Wildlife Service (KWS) Committee of the Department of Veterinary and Capture Services' guidelines on Wildlife Veterinary Practice (2006) and the Kenyan Veterinary Surgeons and Veterinary Para-Professionals Act (2011). The authors have no conflicts of interest to declare.

Results

Biochemical analyte interval values based on the mean vs. median were comparable in most cases (Table I). Three analytes—ALP, amylase, and creatinine—displayed more skewed distributions (average skew = 1.9), with the median differing from the mean. Age had a significant influence on both ALP (adults $\bar{x}$ = 299, SD = 206; nonadults $\bar{x}$ = 705, SD = 129; $t(20) = -6.13$, $P < 0.0001$) and phosphorus (adults $\bar{x}$ = 1.08, SD = 0.29;

nonadults $\bar{x}$ = 1.45, SD = 0.20; $t(26) = -3.35$, $P = 0.002$); juveniles and subadults exhibited higher levels of each than adults (see Table I). There was not a significant difference between juveniles and subadults for ALP (subadult $\bar{x}$ = 688, SD = 119; juvenile $\bar{x}$ = 753, SD = 162; $t(13) = 0.85$, $P = 0.41$) or phosphorus levels (subadult $\bar{x}$ = 1.52, SD = 0.29; juvenile $\bar{x}$ = 1.28, SD = 0.22; $t(13) = -1.49$, $P = 0.16$). Age was not a significant factor for the remaining analytes and none of the analytes was influenced significantly by sex (see Table II). Moderate to severe hemolysis was present in 27 out of 28 samples.

Reference intervals and 90% confidence intervals on the reference limits were calculated for 14 serum biochemical analytes using Reference Value Advisor (Geffré *et al.* 2011; Table I). In most cases, our analyte interval values based on mean and median were comparable.

Discussion

In general, our reference intervals appeared similar to existing blood biochemistry parameters from the commonly used reference database Zoological Information Management System (https://zims.Species360.org; Table III), as well as from studies of other baboon species in captivity (*Papio hamadryas*: Harewood *et al.* 1999). However, these published reference intervals for captive individuals appeared to be based on multiple samplings from the same individuals and thus were not suitable for statistical comparison to either our mean or median based reference intervals, which were calculated from one sample per individual.

Only two biochemical analytes—ALP and phosphorus—differed significantly across age groups. Elevated levels of these analytes are frequently seen in juveniles and subadults, likely owing to increased bone growth and circulating growth hormone in growing individuals (Gilbert and Barresi 2016). Similar findings have been reported in other primate species (Fox *et al.* 2008; Havill *et al.* 2003) and in other mammal species more generally (García *et al.* 2010; Maas *et al.* 2013). Although current guidelines do not recommend partitioning reference intervals by age or sex class when sample sizes are less than 40 (Friedrichs *et al.* 2012), we provide separate reference intervals for adults and for subadults and juveniles. However, owing to the statistical challenges in accurately defining reference intervals with small sample sizes, the age group specific reference intervals calculated for ALP and phosphorus should be viewed with caution.

Creatinine has been positively correlated with muscle mass and incidence of glomerulonephropathy in older animals, as well as in animals that eat meat-rich diets (García *et al.* 2010). Our sample population was slightly skewed toward younger individuals, with 15 juveniles and subadults to 13 adults. Adult males, which generally have the greatest muscle mass and consequently higher creatinine levels, comprised only 3 of 28 individuals in our sample population. It is possible the younger age of our sample population and smaller percentage of adult males resulted in a lower range for creatinine. In addition, free-ranging baboons have a different diet from captive baboons. Although our sampled individuals were assessed as being clinically healthy, it is likely that their diet is less calorie- or protein-rich than captive populations, which may result in comparatively lower creatinine levels.

The frequency and severity of hemolysis in our samples could have influenced results across multiple analytes, particularly potassium levels. Poor venipuncture

Table II Sex and age class comparisons of blood biochemical analytes from olive baboons captured in Laikipia, Kenya in July 2012

		Sex					Age				
Analyte	SI units	♀ $\bar{x} \pm$ SD	♂ $\bar{x} \pm$ SD	df	t	P	Adults $\bar{x} \pm$ SD	Nonadults $\bar{x} \pm$ SD	df	t	P
Albumin	g/L	32 ± 8	36 ± 3	17	−1.38	0.19	33 ± 8	35 ± 4	18	−0.63	0.53
ALP	U/L	449 ± 229	596 ± 289	26	−1.5	0.15	299 ± 206	705 ± 129	20	−6.13	<0.001*
ALT	U/L	45 ± 18	42 ± 7	18	0.61	0.55	43 ± 14	44 ± 14	26	−0.33	0.74
Amylase	U/L	142 ± 35	129 ± 45	26	0.82	0.42	137 ± 31	135 ± 47	26	0.14	0.89
Total bilirubin	μmol/L	5.5 ± 0.9	6 ± 0.9	25	−1.47	0.15	5.8 ± 0.9	5.7 ± 0.8	25	0.16	0.88
BUN	mmol/L	3.8 ± 1.0	3.7 ± 0.8	26	0.14	0.89	3.8 ± 1.0	3.7 ± 0.9	26	0.39	0.7
Calcium	mmol/L	2.3 ± 0.1	2.4 ± 0.1	26	−3.08	0.01	2.28 ± 0.14	32.38 ± 0.10	26	−2.23	0.03
Phosphorus	mmol/L	1.2 ± 0.4	1.3 ± 0.3	26	−0.67	0.51	1.1 ± 0.3	1.5 ± 0.3	26	−3.35	0.002*
Creatinine	μmol/L	34 ± 11	46 ± 27	15	−1.48	0.15	47 ± 27	33 ± 10	15	1.78	0.1
Glucose	mmol/L	6.6 ± 2.3	6.2 ± 1.7	26	0.49	0.63	6.0 ± 1.6	6.7 ± 2.3	26	−0.87	0.39
Sodium	mmol/L	143 ± 4	142 ± 3	26	0.28	0.78	143 ± 4	142 ± 4	26	0.36	0.72
Potassium	mmol/L	6.2 ± 1.2	5.5 ± 0.7	21	1.76	0.09	6.0 ± 0.9	5.9 ± 1.2	21	0.35	0.72
Total protein	g/L	83 ± 7	77 ± 3	18	2.79	0.01	82 ± 4	79 ± 7	26	1.25	0.22
Globulin	g/L	49 ± 11	42 ± 4	16	2.24	0.04	49 ± 10	43 ± 8	25	1.59	0.12

*Statistically significant difference at an $\alpha = 0.05$ level, after a Bonferroni correction for multiple tests (corrected $P < 0.0036$)

Table III Comparison of reference intervals for wild vs. captive baboons

Analytes	Wild *P. anubis* RIs (this study)	Captive *P. anubis* RIs (ZIMS[a])	Captive ♂ *P. hamadryas* Ris (Harewood *et al.* 1999)	Captive ♀ *P. hamadryas* RIs (Harewood *et al.* 1999)
Albumin (g/L)	22–50	29–45	33–50	32–50
ALP (U/L)	0–702	69–1219	0–1485	0–1400
ALT (U/L)	11–69	21–55	0–107	0–122
Amylase (U/L)	47–214	N/A	N/A	N/A
Total bilirubin (μmol/L)	4–8	0–6.84	0–5.54	0–4.08
BUN (mmol/L)	2.1–6.0	2.29–8.43	N/A	N/A
Calcium (mmol/L)	2.0–2.6	1.98–2.55	2.04–2.68	2.06–2.68
Phosphorus (mmol/L)	0.5–1.9	N/A	N/A	N/A
Creatinine (μmol/L)	17–90	44.2–123.6	0–173.3	34.1–93.8
Glucose (mmol/L)	3.1–11.5	2.89–10.70	2.58–7.97	2.20–9.28
Sodium (mmol/L)	134–149	135–158	139–153	138–153
Potassium (mmol/L)	4.3–9.0	3.0–4.7	2.65–4.75	2.52–4.63
Total protein (g/L)	67–91	53–81	58–80	59–84
Globulin (g/L)	34–79	19–46	N/A	N/A

[a] Zoological Information Management System (2018), http://zims.Species360.org/

technique, inadequate cold storage, and rough handling of samples can all cause damage to erythrocytes and lead to a hemolyzed sample in clinically healthy individuals (Ramer *et al.* 1995). In primates, serum potassium is elevated in the presence of even mild hemolysis due to high concentrations of intracellular potassium in erythrocytes (Ramer *et al.* 1995). Although proper sampling protocol was followed, the chaotic nature of collecting and transporting samples in the field likely led to a higher incidence of hemolysis in our samples than those in a more controlled captive setting and potentially also increased potassium levels. Given the likely increase in potassium due to hemolysis, the calculated reference intervals for potassium should be viewed with suspicion. There are no reference intervals of free-ranging olive baboons with which to compare creatinine or potassium. Therefore, it is also possible that these analytes are normally higher among free-ranging olive baboons.

In addition to the 90% CI obtained using Reference Value Advisor, we report 95% CI about both the sample means and medians. While the mean may be more useful in computing reference intervals for normally distributed data, the median is more appropriate for describing skewed distributions and less likely to be influenced by outliers. These effects are amplified by small sample sizes. Overall, however, the mean and median for each analyte were quite close and the distribution of values approximately symmetric. Thus, our computed reference intervals based about the mean were similar to those about the median for most analytes.

The analytes with more noticeable differences in these intervals included ALP, amylase, and creatinine. These analytes showed a more skewed distribution when compared to other tested analytes. Several analytes also had extreme outliers, most notably creatinine, for which one adult male sample had a value greater than four standard deviations higher

than the mean. Both of these factors may make the median-based interval a more appropriate measurement of "typical" values in ALP, amylase, and creatinine for this species of wild baboon. Median-based intervals for the individual age groups for ALP and phosphorus are the most useful because they are exact for these small sample-sized groups when compared to the mean, although again these reference intervals should be viewed with caution owing to the overall low sample size. Ultimately, the decision of whether to use a mean or median-based approach should be made on a case-by-case basis, taking into consideration the factors that we described in the foregoing.

We conclude that wildlife veterinarians and researchers can use these reference intervals as a preliminary tool to assess the health of free-ranging olive baboons. However, only olive baboons from the Mpala Research Center were used in this study, and individuals from other regions in Kenya or Central Africa may differ in blood chemistry parameters. We propose that these values can also be a first approximation for captive olive baboons owing to the limited literature available. The Zoological Information Management System (ZIMS; http://zims.Species360.org/), a commonly used reference database for blood biochemistry information, for example, relies on samples of only 16–22 individuals for its reference intervals for this species (ZIMS Expected Test Results for *Papio anubis* [2018, August 2009]. Species360 Zoological Information Management System. Retrieved from http://zims.Species360.org) while captive studies with larger sample sizes (e.g., Harewood *et al.* 1999) were conducted on other baboon species. However, a comparative study based on one sample per individual is needed to recommend our values as a true reference for captive specimens.

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