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Venous blood gases, plasma biochemistry, and hematology of wild-caught common chameleons *(Chamaeleo chamaeleon)*

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Abstract

The purpose of this study was to determine a wide range of selected hematologic, venous blood gases, and plasma biochemistry analytes in common chameleons *(Chamaeleo chamaeleon)*. Blood samples were collected from the ventral tail vein of 41 common chameleons to determine reference intervals for 30 different blood analytes. The calcium-to-phosphorus ratio, packed cell volume (PCV), refractometric total solids (TS), blood cell counts, and differentials were also determined. The microscopic evaluation of blood smears revealed inclusion bodies in monocytes in 7 of the samples. Females showed significantly higher values of plasma proteins and calcium and cholesterol concentrations and males showed significantly higher values of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) plasma concentrations. Significant differences were found between similar analytes determined by different testing methodologies in the PCV/hematocrit, electrolytes (sodium, potassium), and plasma proteins [TS, total protein (TP) and albumin]. Blood analytes determined in this study can provide baseline data that may be useful when evaluating the health status of common chameleons, taking into consideration the potential effects of gender and the type of analyzer used.

Résumé

Des prélèvements sanguins ont été obtenus de la veine caudale ventrale sur 41 caméléons communs (Chamaeleo chamaeleon) *dans l'optique d'établir des intervalles de référence pour 30 analytes sanguins. Le ratio calcium/phosphore, le PCV, les solides totaux par réfractométrie (TS), la numération-formule sanguine ont aussi été déterminés. L'évaluation microscopique des frottis sanguins ont révélé la présence de corps d'inclusions ressemblant aux corps d'inclusions a Chlamydia dans les monocytes sur sept échantillons. Les femelles avaient des valeurs significativement plus élevées pour l'AST et la GGT. Des différences significatives entre les mêmes analytes mais d'après différentes techniques analytiques ont été trouvées pour le PCV/hématocrite, des électrolytes (sodium, potassium) et les protéines plasmatiques (TS, TP et albumine). Les analytes sanguins rapportés dans cette étude pourront fournir des données de base utiles pour l'évaluation de l'état de sante des caméléons communs, tout en considérant les effets potentiels du sexe et de l'analyseur utilisé.*

(Traduit par les auteurs)

Introduction

The common chameleon *(Chamaeleo chamaeleon)*, also known as the Mediterranean chameleon, is a medium-sized, arboreal lizard species that inhabits natural forests and plantations in the Mediterranean zone and is widely distributed in southern Europe, northern Africa, and southwestern Asia (1). Chameleons are slow-moving, diurnal lizards, and in Israel, they are mostly active during the warm months (May to November) (2).

Reptile medicine and research relies on laboratory analyses to evaluate health status, but reference ranges for most hematology, biochemistry, and other physiological variables of common chameleons are limited (3,4). Only a few studies of a small number of common chameleons ($n < 11$) were found to report selected hematology and blood biochemistry parameters (3–5).

Physiologic parameters, such as electrolytes, enzymes, metabolites, and proteins, are important factors that can be used to assess metabolic and homeostatic disturbances, tissue perfusion, and the overall health status of the animal (6,7). The number of parameters that can be analyzed at any given time, however, is limited by the small body size, low total blood volume (5% to 8% of total body weight in reptiles), and challenging venous access that allows only a small blood sample to be collected (7). Point-of-care (POC) analyzers may allow rapid decisions to be made in critical patients and provide field researchers and zoological veterinarians with prompt results while using a relatively small volume of blood (7–11). After

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comparing various POCs and laboratory analyzers, it is recommended that analyzer-specific reference intervals be developed (8,12,13).

The purpose of this study was to determine a wide range of selected hematologic, venous blood gases, and plasma biochemistry analytes in common chameleons. The specific biological hypotheses tested in this study were that gender would affect results and therefore may require separate reference intervals and that using different testing methodologies may produce different values when testing the same analytes.

Materials and methods

Animals

Common chameleons sampled in this study were wild-caught from the central Mediterranean coastal region of Israel (roughly between 32°N–33°N and just west to 35°E). Blood sampling for this study was done during the summer months of June and July when the average day/night temperatures were 32°C/25°C and the mean relative humidity 70%. The chameleons were judged to be healthy following a complete physical examination that showed no overt signs of disease. For each chameleon, the weight was accurately obtained using a digital gram scale and the snout-to-vent length (SVL) was accurately measured to the nearest 1 mm using a ruler. Although the age could not be accurately determined in these wild chameleons, only animals with an SVL of $>$ 90 mm were sampled in this study and were estimated to be mature and at least 1 y old (14). The gender was determined by observing the bulges of the 2 hemipenes at the base of the tail in mature males and the smooth taper from the vent area towards the tail in females. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC#3407) of the College of Veterinary Medicine at the Kansas State University and under a permit from the Israeli Nature and Parks Authority (2014/40513).

Analyzers

The VetScan VS2 Analyzer (Abaxis Veterinary Diagnostics, Union City, California, USA) is a veterinary bench-top analyzer that is marketed for use in reptiles (12). The small analyzer (15 × 32 × 20 cm, 5.1 kg) uses plastic single-use rotors containing dry reagent beads configured for either specific health profiles (kidney, liver, comprehensive, other) or species (equine, canine, large animal, avian/reptile) and requires 100 μL of whole blood, serum, or plasma for the analysis. The rotor designed for birds and reptiles [VetScan Avian/Reptilian Profile Plus (VSARP); Abaxis Veterinary Diagnostics] measures 11 analytes and calculates 1 value [Globulins (Glob)] within 15 min. The VSARP provides concentrations of aspartate aminotransferase (AST, U/L), bile acids (BA, μmol/L), creatine kinase (CK, U/L), uric acid (UA, mg/dL), glucose (Glu, mg/dL), total calcium (Ca, mg/dL), phosphorus (P, mg/dL), total protein (TP, g/dL), albumin (Alb, g/dL), globulins (Glob, g/dL), potassium (K, mmol/L), and sodium (Na, mmol/L).

The VetScan Mammalian Liver Profile (VSMLP) reagent rotor (Abaxis Veterinary Diagnostics) was also used in this study as it measures several biochemistry analytes that are not included in the VSARP. The VSMLP measures 8 analytes, including alanine aminotransferase (ALT, U/L), albumin (Alb, g/dL), alkaline phosphatase (ALP, U/L), bile acids (BA, μmol/L), total bilirubin (Tbil, mg/dL), cholesterol (Chol, mg/dL), gamma-glutamyl transferase (GGT, U/L), and blood urea nitrogen (BUN, mg/dL).

The iSTAT system (Abaxis Veterinary Diagnostics) consists of 2 main components: a handheld, battery-operated analyzer and disposable cartridges with micro-fabricated biosensors (15). This assay requires only 95 μL of heparinized whole blood and the analysis is completed within approximately 2 min, which is an advantage when testing blood gases in small-body chameleons with limited blood volumes (7,15,16). This analyzer was chosen for this study as it was advocated for use in wildlife field studies in general and specifically in reptiles (7,11,16–21). The specific iSTAT cartridge used in this study [CG8+ cartridges (iSTCG8), Abbott Laboratories, Abbott Park, Illinois, USA] can provide multiple parameters, including the hematocrit (Hct, %), hemoglobin (Hb, g/dL), blood pH, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), base excess (BE), bicarbonate (HCO_3), total carbon dioxide content (TCO_2), saturation of oxygen (SO_2), potassium (K, mmol/L), sodium (Na, mmol/L), ionized calcium (iCa, mmol/L), and glucose (Glu, mg/dL).

Blood sample collection and analysis

The chameleons were manually restrained and blood samples were collected aseptically from the ventral tail vein. A venous blood sample was collected using a 25–27G 1/2 to 5/8″ hypodermic needle attached to a 1-mL syringe (BD SafetyGlide Syringe, Becton Dickinson, Franklin Lakes, New Jersey, USA) and placed in a 0.5-mL lithium-heparin collection tubes (BD Microtainer; Becton Dickinson).

All blood gases and biochemistry analyses were carried out immediately. The basic biochemistry profile was done on all of the blood samples using the VSARP and the calcium-to-phosphorus ratio (Ca:P) was later calculated for each chameleon. For each blood gas or biochemistry analysis, a 100-μL aliquot of blood was placed into a VetScan rotor or an iSTAT cartridge. Each rotor or cartridge was used within 10 min of removing it from refrigeration and immediately after opening its protective pouch. Tests were then analyzed immediately after the rotor or cartridge was filled. Before this study, the VetScan VS2 and the iSTAT analyzers were functioning without a problem on a routine basis and their software was updated regularly according to the manufacturer's instructions. All samples were run by the same operator.

The PCV was determined routinely by the microhematocrit method in heparinized capillary tubes and centrifuged at 12 000 × *g* for 5 min and the total solids (TS) were read from the spun and broken tube using a handheld, temperature-compensated refractometer. Blood smears were prepared using a squash preparation and were air dried until later staining using an automated stainer (Aerospray Stainer; Wescor, Logan, Utah, USA) with a modified Wrights stain (Wescor Cytology Reagents). The total white blood cell count (TWBC) was estimated from the smear by counting 10 fields at 400× magnification. The microscope-calibrated formula used was: TWBC (in cells × 10^9/L) = average WBC per 400× × 1.5. The blood smears were examined in order to conduct a 100-leukocyte differential, used to calculate absolute values for each cell type and to

assess the morphology of all cells. The hematology profile included WBC and rubricytes count and heterophils, lymphocytes, monocytes, azurophils, eosinophils, and basophils (counts and %).

Data analysis

Reference intervals were determined according to the ASVCP and Clinical and Laboratory Standards Institute (CLSI) guidelines (22,23). Because of the smaller sample size (< 120), the reference limits were calculated using a robust approach, both when normally distributed and after Box-Cox transformation when not normally distributed (23,24). Ninety percent confidence intervals (CI) of the reference limits were obtained using a bootstrap approach to assess precision of the reference interval limits as recommended. Outliers were detected using Tukey and Dixon methods and removed accordingly. Only the median and range were reported for $10 \leq n < 20$.

Values were compared between genders using 2-sided, 2-sample *t*-tests for normally distributed variables with homogeneous variances and 2-sample Wilcoxon tests for other variables. Values were compared between monocyte inclusion body positive and negative animals using 2-sample Wilcoxon tests for other variables.

The agreement of selected pairs of variables (TS and VetScan TP, PCV and iSTAT hematocrit, albumin VSARP *versus* VSMLP, VetScan *versus* iSTAT for K, Na, and glucose) was investigated using Passing-Bablok regression analysis (25). The constant bias is represented by the intercept of the regression line and should be different from 0 to be significant (0 not included in the 95% CI), whereas the proportional bias is represented by the slope of the regression line and should be different from 1 to be significant (1 not included in the 95% confidence interval). A cumulative sum of residuals (CUSUM) test of linearity was carried out. The 95% limits of agreement around the mean bias were obtained by the following formula:

$$\text{bias} \pm 1.96 \sqrt{\sigma^2}$$

with σ^2 the variance of the bias. Clinical allowable error limits were defined as 10% (26). Since globulins were a calculated value for the VetScan, agreement statistics were not done on this analyte.

Graphs were produced with one method as the x-axis, the second method as the y-axis, the biochemistry data points, the equality line, the clinical allowable error limits around the equality line, the Passing-Bablok regression line (mean bias), and the 95% limits of agreement around the mean bias. The more disagreement there was, the more divergent the Passing-Bablok regression line (bias line) was from the equality line. For clinically acceptable agreement, 95% of the data points (or the limits of agreement) should be contained within the clinical allowable error limits. Spearman correlation coefficients were also obtained.

When not specified otherwise, an alpha of 0.05 was used for statistical significance. The R software [R development core team (2012), R foundation for statistical computing, Vienna, Austria. http://www.r-project.org] was used for statistical analysis with the R-package "MethComp" [MethComp: functions for analysis of agreement in method comparison studies. R package version 1.22 (2013). http://cran.r-project.org/package=MethComp]. The R package was used for Passing-Bablok regression analysis. Reference values were determined using Reference Value Advisor (27).

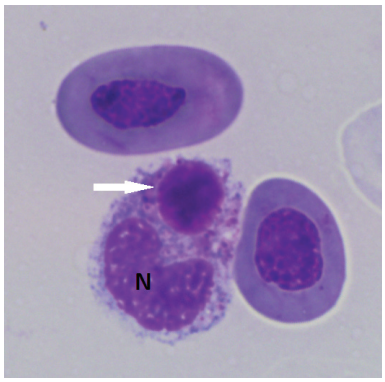

Figure 1. Photomicrograph of a circulating monocyte of a common chameleon in Israel. In addition to the host cell nucleus (N), there is a distinct inclusion (white arrow). Modified Wrights stain; ×400 magnification.

Results

The study included 41 chameleons (18 females and 23 males). There were no differences in weight ($P = 0.72$) or length ($P = 0.79$) between male and female chameleons in this study, showing (mean ± SD) 58.8 ± 22.5 g and SVL 12.2 ± 1.8 cm, respectively.

Light microscopy of blood films showed granular magenta-colored inclusions within monocytes in 7/41 of the chameleons in this study (Figure 1). The inclusions varied in size from approximately 3 to 12 μm and occasionally appeared to be composed of smaller round-to-pleiomorphic structures. The hematological data summary is presented in Table I.

As many of the blood samples obtained were low in volume due to the small size of the animals, the available blood was divided between the VSMLP (n = 27:12F/15M) and the iSTAT (n = 17:7F/10M) analyses. The VSARP failed to report measurements for several analytes, including CK (10/41) and UA (1/41). All BA (41/41) were below the built-in cut-off value of < 35 mg/dL and Ca (3/41) and P (1/41) were above the cut-off value of > 20 mg/dL (all females) of the VSARP. The VSMLP showed values below the reportable range for Alb (1/41, < 1 g/dL) and BUN (19/27, < 2 mg/dL). Individual outliers were identified for several analytes in this study and removed from the final analysis. The biochemistry and venous gases data summary is presented in Table II. Several gender-related significant differences were observed in some of the blood analytes and are summarized in Table III. Agreement statistics are presented in Table IV. The level of agreement between selected analytes measured by different tests in this study is given in Figure 2.

Discussion

When assessing the health of animals, clinicians and researchers look for species-specific baseline values for blood analytes that can be measured with relative ease using commercial blood gas and chemistry analyzers. Species-specific hematological data is especially important in reptiles due to the diverse environmental conditions in different habitats that can affect the blood profiles of each species (19). Our study provides a broad-view data set for blood gas, biochemistry, and hematology measures in common chameleons.

Table I. Hematology determined in common chameleons.

Analyte	Unit	*n*	Mean	Median	SD	Range	RI	Lower limit 90% CI	Upper limit 90% CI
PCV (centrifuged)	%	40	28	27.5	6.5	18 to 60	14 to 41	12 to 17	37 to 44
TS (refractometry)	g/dL	41	5.6	5.4	1.3	3.2 to 8.8	2.7 to 8.2	2.2 to 3.3	7.5 to 8.9
Hct (iSTAT)	%	17	26	26	5	19 to 34	NA	NA	NA
Hb (iSTAT)	g/dL	17	8.7	8.8	1.7	6.5 to 11.6	NA	NA	NA
WBC*	$\times 10^9$/L	41	9.6	6.8	8.3	1.1 to 36.1	1.5 to 37.5	1.0 to 2.0	25.0 to 56.2
Heterophils*	%	39	46.2	42.0	21.4	12.0 to 94.0	12.8 to 97.0	0.0 to 16.2	81.4 to 100.0
Lymphocytes*	%	39	28.6	24.0	16.6	5.0 to 65.0	2.6 to 72.0	0.7 to 5.6	62.1 to 84.7
Monocytes (including azurophils)	%	39	14.8	11.0	12.5	0.0 to 50.0	0.0 to 37.4	0.0 to 0.0	28.9 to 46.2
Azurophils	%	39	5.8	4.0	5.5	0.0 to 22.0	0.0 to 17.0	0.0 to 0.0	13.1 to 19.9
Eosinophils*	%	39	1.9	0.0	3.6	0.0 to 12.0	0.0 to 9.2	0.0 to 0.0	5.9 to 11.7
Basophils*	%	39	1.8	1.0	2.7	0.0 to 10.0	0.0 to 7.4	0.0 to 0.0	5.0 to 9.2
Rubricytes	$\times 10^9$/L	35	51.5	25.0	44.6	0.0 to 175.0	0.0 to 126.9	0.0 to 0.0	90.2 to 158.4
Heterophils	$\times 10^9$/L	39	4.0	3.0	3.0	0.4 to 10.8	0.4 to 12.7	0.3 to 0.6	9.8 to 15.7
Band heterophils	$\times 10^9$/L	40	0.0	0.0	0.0	0.0 to 0.0	0.0 to 0.0	0.0 to 0.0	0.0 to 0.0
Lymphocytes*	$\times 10^9$/L	38	2.5	1.3	2.8	0.2 to 12.3	0.2 to 13.7	0.1 to 0.3	8.4 to 22.3
Monocytes (including azurophils)*	$\times 10^9$/L	39	1.9	0.6	2.6	0.0 to 10.8	0.0 to 10.2	0.0 to 0.0	5.2 to 16.0
Azurophils*	$\times 10^9$/L	38	0.4	0.3	0.5	0.0 to 1.7	0.0 to 2.1	0.0 to 0.0	1.5 to 3.0
Eosinophils*	$\times 10^9$/L	39	0.1	0.0	0.2	0.0 to 0.9	0.0 to 0.9	NA	NA
Basophils*	$\times 10^9$/L	38	0.1	0.01	0.1	0.0 to 0.4	0.0 to 0.3	NA	NA

* Box-Cox.

SD — standard deviation; RI — reference intervals for hematological analytes determined using a robust method with or without Box-Cox transformation; CI — confidence interval; PCV — packed cell volume; TS — total solids; Hct — hematocrit; Hb — hemoglobin; WBC — white cell count; NA — not applicable.

Although the relatively small sample size in this study ($n = 41$) is less than the ideal sample size ($n > 120$) and thus precludes the calculation of formal reference intervals (24), these results provide a useful starting point for clinicians and researchers (28) and are in line with what is often available when working with wild species (19).

Due to technical challenges in the field, primarily the low volume of the blood samples obtained in this species, total WBC counts were not done using a hemocytometer. Blood smears were used for estimating WBC counts and the differential, but were also important for evaluating cell morphology and the potential presence of blood parasites (6,7). Careful attention was placed on proper smear preparation to create a fine monolayer of cells with minimal cell lysis and disturbance of the relative cell distribution in the blood. Because cell counts are based on the relative number of cells, the estimated WBC can be influenced by the PCV and, in cases of anemic or dehydrated animals, a correction formula can be used: Total WBC × Actual PCV ÷ Normal PCV = Corrected WBC (6,7). Blood smears should ideally be prepared immediately from fresh uncoagulated samples. Heparin was used in this study as it is considered the anticoagulant of choice in reptiles in general (6,7), but its effect on the blood properties of common chameleons is yet to be determined.

The chameleons in this study were sampled from the ventral (coccygeal) tail vein, which is a venipuncture site commonly used in lizard species (4,16,29–31). As concerns about this venipuncture site include traumatic tissue injuries and transient darkening of the tail after sample collection, jugular sampling was advocated as a replacement venipuncture site (4,31). However, a comparative report did not demonstrate any major differences in measurements between samples obtained from either the tail vein or the jugular in this species (4). As more clinicians are familiar with using this venipuncture site, the tail vein was chosen for consistency to be used in this study.

This study describes several hematological analytes in common chameleons. The PCV values in this study are similar and the WBC estimate is lower than values previously reported for this species (3). The differences in the WBC between the reports can be either due to the use of a different cytological estimation technique or because some of the animals in the other study were reported to be "rescued" and potentially sick (3). In addition, reference intervals may differ in the same species depending on the season, geographical location, and captivity status (6).

Some chameleons in this study (17%) were observed to have intracytoplasmic inclusion bodies in their monocytes. This novel observation resembles Chlamydia-like inclusion bodies in the monocytes of a flap-necked chameleon *(Chamaeleo dilepis)* from Tanzania that also had a concurrent pox virus infection with generalized disease (32,33). These intracytoplasmic inclusion bodies were not previously reported in common chameleons and further research is required to fully identify the potential pathogen of infection. If real, this infection showed no apparent disease and no measured hematological effects when infected chameleons were compared to those that were not infected.

Table II. Blood gases and biochemistry determined in common chameleons.

Analyte	Unit	*n*	Mean	Median	SD	Range	RI	Lower limit 90% CI	Upper limit 90% CI
VetScan Avian-Reptile Biochemistry Profile									
Aspartate aminotransferase*	U/L	40	456	425	189	156 to 899	167 to 928	135 to 210	798 to 1076
Creatine kinase	U/L	31	4398	4320	1813	1369 to 7955	563 to 8128	0 to 1407	7012 to 9051
Uric acid*	mg/dL	39	4.6	3.7	3.1	1.1 to 13.4	1.0 to 13.8	0.8 to 1.3	10.5 to 17.6
Glucose*	mg/dL	41	275	272	31	194 to 375	217 to 344	206 to 229	326 to 362
Calcium	mg/dL	38	13.4	12.8	3.0	9.3 to 19.0	7.0 to 19.5	5.5 to 8.1	17.8 to 20.8
Phosphorus*	mg/dL	40	8.8	9.0	2.6	3.6 to 15.7	3.5 to 14.1	2.4 to 4.7	12.9 to 15.3
Ca:P*		37	1.7	1.5	0.6	0.6 to 3.3	0.7 to 3.2	0.6 to 0.9	2.7 to 3.8
Total protein	g/dL	40	5.1	5.2	1.0	2.9 to 7.2	3.2 to 7.2	2.8 to 3.7	6.8 to 7.7
Albumin*	g/dL	41	2.2	2.1	0.5	1.1 to 3.7	1.2 to 3.2	1.1 to 1.4	3.0 to 3.5
Globulin	g/dL	41	3.0	3.1	0.7	1.4 to 4.4	1.5 to 4.5	1.2 to 1.9	4.2 to 4.9
Potassium	mmom/L	41	7.3	7.1	1.2	4.7 to 10.0	4.7 to 9.8	4.1 to 5.2	9.3 to 10.3
Sodium	mmol/L	41	143	142	6.5	129 to 159	129 to 155	126 to 132	153 to 158
Bile acids	μmol/L	41	< 35	< 35	NA	NA	NA	NA	NA
Vetscan Mammalian Liver Profile									
Alkaline phosphatase	U/L	27	11	11	4.7	2 to 23	1 to 21	0 to 4	17 to 23
Alanine aminotransferase*	U/L	26	8	7	5	5 to 26	4 to 44	NA	NA
Gamma-glutamyl transferase*	U/L	26	5	5	6	0 to 24	0 to 17	NA	NA
Bile acids	μmol/L	26	3	3	2	0 to 6	0 to 7	0 to 0	6 to 8
Total bilirubin*		27	1.4	1.7	08	0.4 to 2.7	0.1 to 4.6	0.1 to 0.4	3.2 to 5.9
Albumin*	g/dL	25	1.8	1.8	0.4	1.2 to 2.8	1.0 to 2.7	0.0 to 1.2	2.4 to 2.9
Blood urea nitrogen	mg/dL	27	NA	< 2	NA	< 2 to 2	NA	NA	NA
Cholesterol	mg/dL	26	259	272	77	111 to 379	95 to 426	56 to 139	390 to 465
iSTAT CG8+ Profile									
pH		17	7.17	7.13	0.19	6.9 to 7.5	NA	NA	NA
Partial pressure of carbon dioxide ($PaCO_2$)	mmHg	17	39.7	39.0	11.6	20.5 to 69.6	NA	NA	NA
Partial pressure of oxygen (PaO_2)	mmHg	17	101	98	32	61 to 180	NA	NA	NA
Base excess		17	16	16	6	8 to 25	NA	NA	NA
Bicarbonate (HCO_3)	mmol/L	17	23.9	18.1	13.8	5.6 to 44.7	NA	NA	NA
Total carbon dioxide (TCO_2)	mmol/L	17	17.3	16.0	9.7	6.0 to 38.0	NA	NA	NA
Saturation of oxygen (SO_2)	%	17	94	96	6	76 to 100	NA	NA	NA
Sodium	mmol/L	17	135	133	5	126 to 151	NA	NA	NA
Potassium	mmol/L	17	5.9	5.5	0.9	4.3 to 7.8	NA	NA	NA
Ionized calcium	mmol/L	17	1.4	1.3	0.3	1.0 to 2.1	NA	NA	NA
Glucose	mg/dL	16	281	277	31	246 to 369	NA	NA	NA

Reference intervals (RI) for biochemical analytes determined using a robust method with or without Box-Cox transformation.

* Box-Cox.

SD — standard deviation; CI — confidence interval; Ca:P — Calcium-to-phosphorus ratio; NA — not applicable.

A pre-determined mammalian liver profile was also used in this study as it offers several parameters not found in the VSARP. Some of these parameters are either not useful, however, or their efficacy to evaluate the reptilian liver is yet to be determined (6,7). The alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities may increase in liver disease, but may not be specific (6,7). Although usually found in very low plasma concentrations in reptiles, gamma-glutamyl transferase (GGT) can be an indicator for hepatic or renal disease (7). Cholesterol is a good marker for liver disease, such as hepatic lipidosis, but can also vary depending on the diet and can increase in the reproductive female due to vitellogenesis (6,7). As observed in this study, the blood urea nitrogen (BUN) concentrations are usually low in the uricotelic terrestrial reptiles, but can increase due to dehydration in some chelonian species (6,7).

Baseline total bilirubin concentration was measured in this study. Reptiles do not usually produce high plasma bilirubin concentrations as they lack the biliverdin reductase enzyme and biliverdin is the end product of hemoglobin metabolism (6,7). For example, total bilirubin concentration measured in Mediterranean tortoises showed near-zero results (34), but both total and direct bilirubin

Table III. Gender-related significant differences observed in chameleons.

Analytes	Unit	Females (n = 18) Mean/median ± SD/(IQ)	Males (n = 23) Mean/median ± SD/(IQ)	P-value (Holm adjustment)
TS	g/dL	6.4 ± 1.3	5.0 ± 1.0	0.001
TP	g/dL	5.8 ± 1.2	4.8 ± 0.8	0.015
AST*	U/L	378 (164)	508 (208)	0.013
GGT*	U/L	0 (0)	8 (5)	< 0.001
Ca*	mg/dL	16.8 (3.3)	11.6 (3.0)	< 0.001
Cholesterol	mg/dL	335 ± 63	214 ± 64	< 0.001

SD — standard deviation; IQ — interquartile; TS — total solids; TP — total proteins; AST — aspartate aminotransferase; GGT — gamma-glutamyl transferase; Ca — calcium.

* Non-parametric statistics: median and Wilcoxon tests.

Table IV. Agreement statistics between selected variables using a Passing-Bablok linear regression analysis in common chameleons.

Pair of analytes	N	Constant bias (intercept)	95% CI	Proportional bias (slope)	95% CI	95% LOA	ρ	WCAEL
TS *versus* TP (VSARP)	41	0.84*	−0.03 to 1.67	0.79	0.64 to 0.96	−1.19 to 1.87	0.83	No
PCV *versus* Hct (iSTAT)	17	3.86*	−18; 13.38	0.71	0.38 to 1.5	−7.89; 18.01	0.73	No
Albumin (VSARP *versus* VSMLP)	25	−0.3*	−0.7; −0.3	1	1.0 to 1.2	0.1 to 0.6	0.95	No
Sodium (VSARP *versus* iSTAT)	17	61*	25 to 120	0.5*	0.1 to 0.8	−3 to 19	0.33	No
Potassium (VSARP *versus* iSTAT)	17	−1.6	−7.1 to 3.4	1.1	0.3 to 1.8	−0.6 to 3.8	0.40	No
Glucose (VSARP *versus* iSTAT	16	17	−27 to 84	0.9	0.7 to 1.1	−25 to 31	0.78	Yes

* Significant bias was encountered.

CI — confidence interval; LOA — limits of agreement; ρ — Spearman correlation coefficient; WCAEL — within clinical allowable error limits; TS — total solids; TP — total proteins; PCV — packed cell volume; VSARP — VetScan avian/reptile profile; VSMLP — VetScan mammalian liver profile; Hct — hematocrit.

All ρ had a P-value of < 0.001.

were measured in desert tortoises *(Gopherus agassizi)* with gender and seasonal-dependent variations (35). The clinical relevance of the measured bilirubin in this study is yet to be determined as is the validity of these results, although similar findings were also reported in Negev desert tortoises *(Testudo werneri)* tested using the same methodology (9).

Absolute values for bile acids (BA) were not recorded in any of the chameleons in this study when tested using the VSARP, which is similar to other studies in reptiles using this test (8–10,12,13,16). The VSARP measures bile acids, but has a calibrated cut-off value of 35 μmol/L. It has been suggested that most healthy reptiles would have concentrations lower than this concentration (6) and that same theory might also apply to the common chameleons sampled in this study. The low plasma concentrations of BA measured by the VSMLP may support this theory and are also in agreement with BA reported in Negev desert tortoises tested using the same methodology (9). It is also possible, however, that these assays do not measure the bile acid type(s) produced by this species. Future studies should compare different testing methodologies or test animals with known liver disease and increased plasma bile acid concentrations.

Significant gender-related differences were observed in several blood analytes in this study. For reasons unknown, measurable plasma GGT concentrations were observed only in male chameleons in this study, which was also reported in male Negev desert tortoises tested using the same methodology (9). The plasma concentrations of the AST and CK in this study were higher than those previously reported in this species (3) and are suggestive of tissue damage that released these enzymes into the blood sample (6). However, male chameleons in this study showed higher plasma CK concentrations. Reports in several chelonian species showed higher concentrations of AST and CK in males during the warm months, which is attributed to hyperactivity during the mating season. This might also be true for male common chameleons in this study, which were tested during the Mediterranean summer (35–38). However, captive female Panther chameleons *(Furcifer pardalis)* showed higher values of AST and CK, as well as glucose and ALT, during the summer than males (31).

In this study, female chameleons showed higher concentrations in TS, TP, total Ca, and cholesterol plasma than male chameleons. Female reptiles often show higher total calcium concentrations due to reproductive activity and an increase in plasma proteins and cholesterol blood due to increased estrogen and calcium mobilization from bone and vitellogenesis (6,7,31,35,37,38). The chameleons in this study were sampled during the months of June and July,

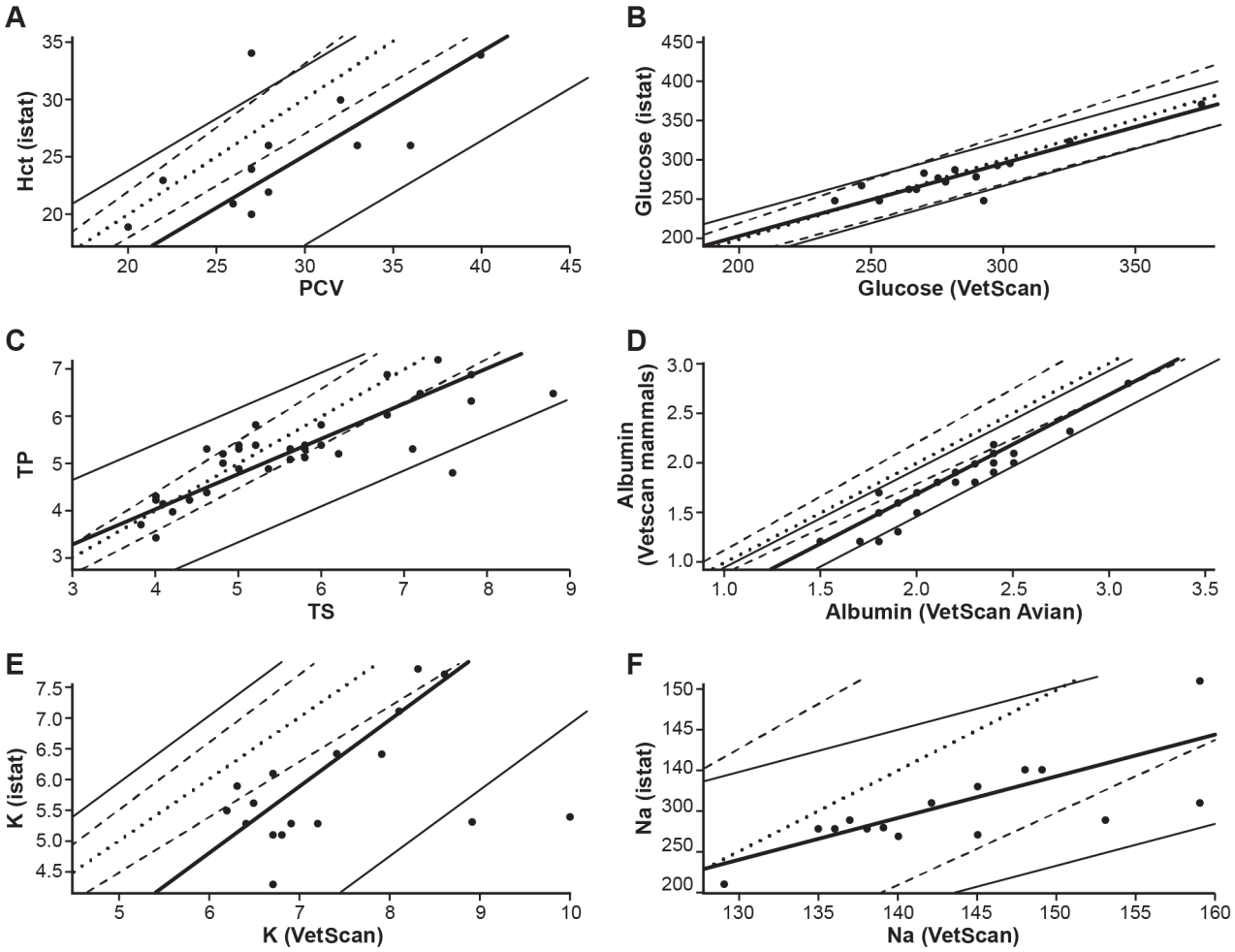

Figure 2. Comparison plots for different pairs of variables measured in common chameleons: A — packed cell volume (PCV)/hematocrit (Hct) [%]; B — glucose (mg/dL); C — total solids/total protein (TS/TP) (VetScan x2) [g/dL]; D — albumin (g/dL); E — potassium (K) VetScan vs iSTAT (mmol/L); and F — sodium (Na) VetScan vs iSTAT (mmol/L). The thick dashed line represents the line of perfect agreement (y = x), the thick plain line represents the regression line (mean bias), the thin lines represent the clinically allowable error limits, and the thin dashed lines represent the 95% limits of agreement.

which is earlier than the described mating season (mid-August to mid-September) and egg-laying (mid-September to early November) (14,39). Copulation in wild common chameleons in Israel has been reported to take place from July to September (2) and the findings of this study may also support an earlier reproductive period or pre-ovulatory activity for female common chameleons in this geographical location.

Ionized calcium was measured in chameleons in this study using the iSTAT POC analyzer and this patient-side testing methodology was advocated for this purpose in the literature (7). The ionized calcium plasma concentrations measured in chameleons in this study (1.3 ± 0.3 mmol/L, mean ± SD) are generally in agreement with other limited data described in other reptile species (6,9,16,19).

Blood gases are rarely reported in reptiles but were measured in common chameleons in this study. Recently, several studies were published using the iSTAT for determining blood gases in reptiles (16–19,21). The normal blood pH of reptiles in general is suggested to be 7.5 to 7.7 at temperatures of 23°C to 25°C and some snakes and lizards can show pH values lower than 7.4 (6). The chameleons in this study showed a mean blood pH of 7.17 (± 0.17), which can either be normal for this species or lower due to higher ambient temperatures or excitement from the venipuncture restraint (6). Studies in Galapagos marine iguanas *(Amblyrhynchus cristatus)* and bearded dragons *(Pogona vitticeps)* reported similar pH ranges using the iSTAT testing methodology (16,19). While total CO_2 measurements in reptiles are expected to range from 20 to 30 mmol/L (6), many chameleons in this study showed lower values, as was also reported in bearded dragons tested with the iSTAT methodology (16). These total CO_2 values can either be normal for common chameleons or be caused by hyperventilation from the excitement observed during the manual restraint required for the venipuncture.

Measurements of several analytes were compared when different analyses were used for their determination in this study. There was a poor level of agreement between the TS and the TP when using the VSARP, with TS being higher, likely from measuring the total solutes in the plasma (glucose, lipids, etc.). The observed disagreement between the measurements of the albumin using the VSARP and the VSMLP had a constant bias and applying a correcting factor of −0.3 can provide a good agreement between the 2 different

analyses. For albumin determination, however, both the VetScan and the iSTAT analyzers use the bromocresol green (BCG) dye-binding method, which is currently considered unreliable for measuring protein in reptiles. Instead, protein electrophoresis is the recommended method for accurate measurements of plasma proteins (6,12,38,40,41). Significant disagreements were also observed between the 2 analyzers in measurements of Na and K concentrations. Good agreement was observed in blood glucose concentrations measured by the 2 analyzers, however, which was also previously described in a comparison study in black-tailed prairie dogs *(Cynomys ludovicianus)* (42). The disagreement observed in the higher PCV when compared to the calculated hematocrit (Hct, iSTAT) is expected and was previously described in humans and other species. It is probably due to the effect of the low plasma proteins in reptiles (< 6 g/dL), which lowers the Hct results when using the iSTAT methodology (9,43). Due to these potential interferences in measurement, a spun PCV is considered more accurate than a calculated Hct (9).

As blood testing in the current study was done during the summer months and as seasonality can have a significant effect on multiple blood analytes in chameleons (31), the results might reflect what is true for this species at this time of year. Future studies should therefore test common chameleons at different seasons for comparison. The iSTAT methodology used in this study was originally designed for human blood and this may not agree with reptiles, mainly in terms of blood protein concentrations, RBC form, and auto-correction for the human body temperature (37°C) (44). The importance of doing temperature correction for several parameters (pH, $PaCO_2$, and PaO_2) has been highlighted in reptiles and, although several differences showed when correcting for temperature in Galapagos green sea turtles *(Chelonia mydas)*, only minor and non-clinical differences were found (18). Temperature validation was not carried out in this study as the small body size of these chameleons did not allow large volumes of blood to be collected or rectal temperatures to be tested in most animals. Although these poikilothermic chameleons were sampled during the hot summer with ambient temperatures adequate for the iSTAT blood gases calculations (25°C to 32°C), it is recommended that future studies consider temperature validation of these blood gas results.

In conclusion, data reported in this study represent an important step toward determining the normal range of physiological values against which future blood gas, biochemistry, and hematology results can be compared in common chameleons. Such assessments are important for monitoring health and diagnosing disease. The results of this study add to a growing database of knowledge about health management in chameleon species. Differences in gender and testing methodologies exist and should be considered when interpreting diagnostic data.

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