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Reference Intervals for Plasma Biochemical Variables by Point-of-Care Testing in Captive Black-tailed Prairie Dogs (*Cynomys ludovicianus*)

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Black-tailed prairie dogs (*Cynomys ludovicianus*) are kept in zoological collections, maintained as companion pets, and are tested in field and laboratory settings. Biochemical analysis for routine health and research purposes can be performed by using point-of-care (POC) testing; however, analyzer- and species-specific reference intervals need to be determined. In this prospective study, 50 captive-raised sexually intact prairie dogs (16 females, 34 males) underwent plasma biochemical analysis by using a veterinary POC biochemical analyzer. We used a manufacturer-predetermined profile of 14 analytes: albumin, ALP, ALT, amylase, BUN, calcium, creatinine, glucose, potassium, sodium, phosphorus, total bilirubin, total protein and globulin. A subset of 17 samples was tested concurrently for the same 14 analytes by using a reference laboratory analyzer, and we determined RI for the POC analyzer for these 14 biochemical analytes. Sex had a significant effect on albumin and creatinine values, which were higher in females than males, and on ALT, which was lower in females. In addition, age had an effect on 9 plasma analytes: juvenile animals had higher plasma concentrations of albumin, ALP, ALT, BUN, and glucose than adult animals, whereas adults had higher concentrations of creatinine, sodium, total protein, and globulins. Only calcium and BUN had acceptable analytical agreement between the POC and reference analyzers. The reference intervals determined in this study can aid clinicians and researchers performing POC plasma biochemical analysis in prairie dogs, providing that they consider potential analyzer-, sex-, and age-related effects.

Abbreviations: ASVCP, American Society for Veterinary Clinical Pathology; POC, Point of care; RI, reference interval

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The black-tailed prairie dog (*Cynomys ludovicianus*) is a non-hibernating burrowing member of the order Rodentia and the family Sciuridae.[24] It is a keystone species in the grasslands of North America and is the most common prairie-dog species found in zoological collections, as privately owned pets, and in research facilities.[24] Due to its biliary similarities to humans and a propensity to develop various zoonotic diseases, this species is often used as a model for related research.[4,23,28]

Likewise, laboratory testing, including blood biochemical analysis, for general health monitoring, assessment of clinical disease, and research, is commonly performed in this species.[5,8,11,12,15,18,19,22,28,33,36,38,43,48] Erroneous interpretation of test results can occur because species-and method-specific reference intervals (RI) are not always available or have not been established according to current guidelines of the American Society of Veterinary Clinical Pathologists (ASVCP).[13] For example, the previously published biochemistry RI for prairie dogs were derived from multiple populations (either captive, wild, or pet animals) and comprised fewer than 20 animals. Differences in environment, diet, sex, weight, age, geographic distribution, sampling technique, and assay methodology may preclude the use of these published RI in various populations of prairie dogs.[10,13,28]

Point-of-care (POC) biochemical testing is commonly used in clinical and research settings and compared with traditional laboratory analysis, provides the advantages of smaller sample volume, immediate test results, and analyzer portability.[26,41] The veterinary bench-top biochemical analyzer we used in the current study (VS2, Abaxis, Union City, CA) requires only 0.1 mL of whole blood, plasma, or serum for the analysis of multiple physiologic analytes and has previously been used in prairie dogs in both laboratory and field settings.[11,12,22,50,51] One previous report regarding hematology and biochemistry RI in this species had used its human-equivalent biochemical analyzer (Piccolo, Abaxis) in a small group of wild-caught animals that were sampled repeatedly.[28]

Current ASVCP guidelines for the validation of an assay for use in a new species requires, among other steps, documentation of the analytical performance of the new method and comparison of the new assay with an existing reference ('gold-standard') method.[2,3,25,32,35] In exotic and wildlife species, challenges in creating species-specific standard materials complicates the assessment of the analytic performance of different assay methodologies. Comparison studies between this veterinary POC analyzer with laboratory biochemical analyzers in other species have demonstrated variable agreements and significant proportional errors or bias between the POC and reference values for several analytes.[2,3,17,32,35] After similar comparisons, the development of analyzer-specific RI was recommended.[25,35,45]

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The aim of this prospective study was to determine RI according to the current ASVCP guidelines for selected biochemical analytes in heparinized plasma from captive-raised black-tailed prairie dogs (*Cynomys ludovicianus*) by using POC testing. In addition, we sought to determine whether sex (male compared with female) or age (juvenile compared with adult) had an effect on these biochemical variables, because such bias occurs in other rodent species. In addition, because both the veterinary POC and laboratory analyzers are used when working with prairie dogs, we performed a comparative analysis of the same 14 selected plasma analytes in a subset of 17 animals. We hypothesized that agreement between the POC and laboratory analyzer would be unacceptable and that measurements of various analytes would differ according to the sex or age of the prairie dogs tested in this study.

Materials and Methods

Animals. Clinically healthy captive-raised, black-tailed prairie dogs (*Cynomys ludovicianus*) were sampled in this study as part of an overall health evaluation. The animals were group-housed in a concrete-lined room bedded with hay and kept at a constant temperature range (21 to 23 °C). Water and grass hay were offered free choice, and the rest of the provided diet consisted of a mix of vegetables and commercial rodent blocks. Several plastic dog kennels (40 in. × 27 in. × 30 in.) and PVC tubing were provided for enrichment and hiding. All animal care procedures conformed to guidelines established by the IACUC at Kansas State University (approval nos. 3311, 3548, and 3729).

Isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL)-anesthetized prairie dogs underwent a health evaluation, which included a complete physical examination, accurate body weight, identification by microchipping, CBC, plasma biochemical panel, and fecal examination. All prairie dogs were determined to be healthy.

Experimental design and sample collection. For anesthesia, each prairie dog was chamber-induced by using 5% isoflurane gas in 2 L/min oxygen. After induction, each prairie dog was maintained under general anesthesia by using a tight-fitting small face mask and nonrebreathing circuit with 2.0% isoflurane delivered in 1.5 L/min of oxygen. The animals were allowed to breathe spontaneously. Body temperature (approximately 37 °C) was monitored rectally by using a handheld digital thermometer and maintained by using a warm-water blanket. Vital signs were monitored by using a stethoscope, a Doppler flow detector (model 811B, Parks Doppler System, Parks Medical Electronics, Aloha, OR) and a pulse oximeter (model N20PA, Nellcor Handheld Pulse Oximeter, Covidien, Dublin, Ireland). Once the animal was stable under anesthesia (less than 5 min), a venous blood sample (0.5 mL) was collected from either the jugular vein or cranial vena cava by using a 1.0-mL syringe (Kendall Monoject, Tyco Healthcare Group, Mansfield, MA) with a 25-gauge, 0.5 × 16 mm needle (Hypodermic needle, Exelint, Los Angeles, CA) and placed into a 0.5-ml lithium–heparin-coated blood collection tube (BD Microtainer, Becton Dickinson, Franklin Lakes, NJ). Lactated Ringers solution (30 mL) was administered subcutaneously before anesthesia was discontinued.

Plasma biochemical analysis. Immediately after collection, each blood sample was centrifuged (10 min at 3000 × *g*); the separated plasma was transferred to an individual microcentrifuge tube. For analysis by using the veterinary POC analyzer, a 100-µL aliquot of plasma was removed from each collection tube by using the standard pipette provided by the manufacturer and placed into a Comprehensive Diagnostic Profile reagent rotor (Abaxis, Union City, CA). This predetermined biochemical profile comprises albumin, ALP, ALT, amylase, BUN, Ca^{2+}, creatinine, glucose, K^+, Na^+, phosphorus, total bilirubin, and total protein, all of which are analyzed directly, as well as globulins, which is calculated from the measured albumin and total protein. In this study, each rotor was used within 15 min of removal from refrigeration and immediately after opening the protective pouch. Tests were then analyzed immediately after rotor filling and according to the manufacturer's directions. Prior to this study, the veterinary POC analyzer was functioning without a problem on a routine basis, and its software was updated regularly as provided by the manufacturer. The operator's manual states that the analyzer includes an internal self-calibration quality control program.[1] All samples were run by the same operator (DE). None of the samples showed evidence of lipemia, icterus, or hemolysis after visual plasma inspection and according to the analyzer's internal quality-control program.

For comparison, residual plasma samples from a subset of 17 prairie dogs (11 males, 6 females) were concurrently evaluated by using an automated wet-biochemistry analyzer (Cobas c501, Roche Diagnostics, Indianapolis, IN) at the Kansas State Veterinary Diagnostic Laboratory. For the purpose of this study, the same 14 biochemical analytes were evaluated. Before the laboratory analysis, the plasma samples were kept away from light and refrigerated (4 °C) and were analyzed within 4 h of sample collection. The laboratory analyzer was maintained and calibrated according to the manufacturer's instructions and internal laboratory standard operating protocols. Instrument performance was monitored daily by internal quality control and using commercial quality-control materials (PreciNorm and Precipath, Roche Products) with 1_{2s} or 1_{3s} rules, depending on the analyte measured. This laboratory analyzer was subject to a quarterly external quality-assurance program (Veterinary Laboratory Association Quality Assurance Program, Atlantic Veterinary College, University of Prince Edward Island, Canada).[9,20] The laboratory analyzer was operated by trained laboratory medical technologists.

Statistical analysis. Reference intervals were determined according to ASVCP guidelines, with modifications according to recommendations for small sample sizes.[13,31] Because of the low sample size (40 to 60), the reference limits were calculated by using a standard approach (mean ± 2 SD) when normally distributed and a nonparametric approach when not normally distributed. For normality testing, Anderson–Darling tests were used with a significance level of 0.2.[31] To assess the precision of the RI limits, 90% CI of the RI were obtained by using a bootstrap approach as recommended.[13] Outliers were detected by using the Dixon method and removed accordingly.

Values were compared according to the animals' sex and age by using linear regression for parametric variables with homogenous variances and a Kruskal–Wallis rank sum test for nonparametric variables. Assumptions of linear regression were checked on residual plots.

The agreement between the laboratory and POC analyzers evaluated by using Passing–Bablok regression. A CUSUM test for linearity was performed; in addition, 95% limits of agreement were reported as $\pm 1.96 \times 1\ SD_{bias}$. Results were then compared with the highest reported allowable total errors for companion mammals for interpretation of clinical agreement.[20] The 2 methods were considered in clinical agreement when more than 95% of measurements were within the clinically allowable error limits (95% limits of agreement within these error limits).

R (http://www.R-project.org/) was used for statistical analysis, and the R package MethComp was used for Passing–Bablok

Table 1. Reference intervals for plasma biochemical analytes of captive black-tailed prairie dogs (*Cynomys ludovicianus*) determined by using a veterinary POC analyzer with parametric or nonparametric methods

							90% CI	
Analyte	*n*	Mean	Median	1 SD	Range	RI	Lower limit	Upper limit
Albumin (g/dL)	49	3.3	3.3	0.4	2.6–4.1	2.6–4.1	2.4–2.7	3.9–4.2
ALP (IU/L)[a]	50	85	78	36	23–187	24–180	23–37	152–187
ALT (IU/L)[a]	50	50	40	28	26–148	26–145	26–27	114–148
Amylase (IU/L)	50	154	148	33	76–220	87–220	74–101	207–234
Total bilirubin (mg/dL)[a]	50	0.4	0.4	0.1	0.3–0.5	0.3–0.5	0.3–0.3	0.5–0.5
BUN (mg/dL)	50	31	31	7	16–43	17–44	14–20	41–47
Calcium (mg/dL)	49	9.7	9.8	0.5	8.7–10.8	8.7–10.7	8.6–8.9	10.5–10.9
Phosphorus (mg/dL)	50	5.7	6.0	1.9	1.9–9.3	1.9–9.5	1.2–2.8	8.7–10.2
Creatinine (mg/dL)[a]	49	0.7	0.7	0.2	0.3–1.2	0.3–1.2	0.3–0.4	1.1–1.2
Glucose (mg/dL)[a]	50	156	152	26	108–220	109–217	108–120	203–220
Na (mmol/L)[a]	50	141	141	3	134–148	134–148	134–137	146–148
K (mmol/L)	50	4.8	4.8	0.7	3.3–6.6	3.3–6.4	3.0–3.6	6.0–6.6
Total protein (g/dL)	48	6	6	0.5	5–7	5–7	5–5	7–7
Globulin (g/dL)[a]	49	2.8	2.8	0.6	1.7–4.3	1.7–4.3	1.7–1.8	4.1–4.3

[a]Nonparametric method

regressions. Reference values were determined by using Reference Value Advisor.[16]

Results

A total of 50 sexually intact prairie dogs (16 females, 34 males; age: 6 mo, $n = 28$; 24 mo, $n = 7$; 36 mo, $n = 12$; 54 mo, $n = 3$). For age-based comparisons, the animals were divided into groups of juveniles (age, 6 mo; $n = 28$ [female, 2; male, 26]) and adults (24 mo and older; $n = 22$ [female, 14; male, 8). Venous blood samples were collected from the cranial vena cava mainly ($n = 46$), with the remainder ($n = 4$) from the jugular vein.

The RI for 14 biochemical analytes as determined by using the veterinary POC analyzer are presented in Table 1. A few values were identified as outliers for albumin ($n = 1$), calcium ($n = 1$), creatinine ($n = 1$), total proteins ($n = 2$), and globulins ($n = 1$) and were removed from the final RI calculation.

Sex and age had significant effects on several plasma analytes measured in this study. For albumin, female prairie dogs had significantly higher plasma concentrations than males (mean difference of 0.3, $P = 0.027$). In addition, albumin was approximately 0.11 g/dL/y lower ($P = 0.02$) in older animals. Regarding total protein, plasma concentrations were significantly ($P = 0.008$) higher in 54-mo-old animals than 6-mo-olds (Figure 1 A), and globulin concentrations were higher in older animals than younger animals (mean difference of 0.2 g/dL/year, $P = 0.048$; Figure 1 B). In particular, 24-mo-old prairie dogs had higher ($P = 0.01$) globulin levels than juveniles. Although globulin levels seemed higher in 54-mo-olds than in 6-mo-old animals, the difference was not significant, partly due to the increased variability among samples from the older animals. For ALP, adults had lower plasma concentrations than juveniles (mean difference of 12.4 IU/L/y, $P = 0.002$; Figure 1 C), and for ALT, older prairie dogs and females had lower plasma concentrations than younger animals ($P < 0.005$, Kruskal–Wallis) and males ($P = 0.007$, Kruskal–Wallis), respectively.

Older animals had significantly lower BUN concentrations than younger animals (mean difference of 2 mg/dL/y, $P = 0.014$), and female prairie dogs had s higher creatinine concentrations than males (median difference of 0.1/mg/dL, $P = 0.003$). Creatinine concentrations were higher ($P = 0.004$) in 36-mo-olds than 6-mo-olds (Figure 1 D). Older animals had lower ($P = 0.003$) plasma glucose concentrations than younger animals (mean difference of 8.6mg/dL/year). Sodium concentrations were higher ($P = 0.007$) in older than younger animals (mean difference of 1 mmol/L/y). Descriptive statistics for the effects of age and sex on the measured plasma analytes are presented in Tables 2 and 3, respectively.

The comparison between the veterinary POC and laboratory reference biochemical analyzers for the same 14 analytes showed an acceptable clinical agreement for calcium and BUN only. Differences between methods for all of the remaining 12 analytes exceeded clinical allowable error margins. Passing–Bablok regression plots are presented in Figure 2. Agreement statistics for the comparison between the veterinary POC and laboratory reference analyzers are presented in Table 4.

Discussion

To our knowledge, this study is the first to report RI for plasma biochemical analytes in captive-raised black-tailed prairie dogs (*Cynomys ludovicianus*) that were determined by using a veterinary POC analyzer according to the ASVCP guidelines and by taking into consideration the sex and age of the sampled animals. This analyzer is commonly used in clinical and research settings, and the data generated in this study can aid those working with this species.

The results of this study show variable disagreement with other studies in black-tailed prairie dogs that used similar POC biochemical analyzers, including one study that used the human-equivalent analyzer on wild-caught, 6-mo-old prairie dogs ($n = 18$) that were sampled every 3 to 4 d over a 30-d serum biochemistry trial.[28] Another study used the same veterinary POC analyzer as in the current study to evaluate a contraception vaccine and tested its effect on plasma biochemical analytes in wild-caught juvenile prairie dogs.[50,51] Some of the differences in RI between our current study and these previous studies likely reflect preanalytical factors; however, given the absence of published data regarding the analytical performance of the veterinary POC analyzer in prairie dogs, differences between analytical methods may contribute. These findings are recapitulated in the literature, given that results for this species that were obtained by using different commercial biochemical analyzers show great variability.[5,8,11,12,15,18,19,22,28,33,36,38,43,48] In addition, the

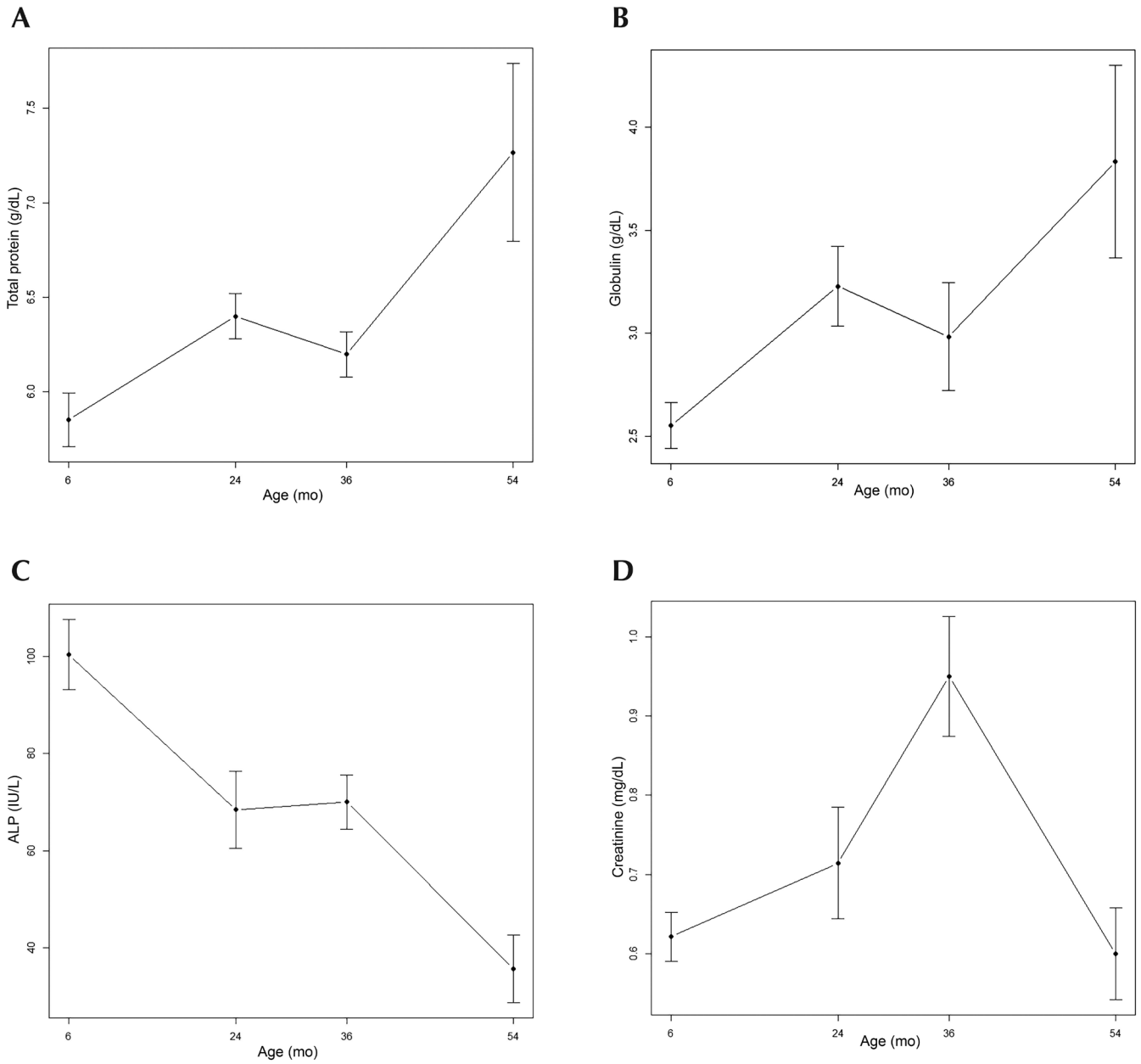

Figure 1. The effect of age on selected plasma biochemical analyte concentrations determined by using a veterinary POC analyzer for captive black-tailed prairie dogs (*Cynomys ludovicianus*) in this study. (A) Total proteins. (B) Globulin. (C) ALP. (D) Creatinine. The tested population included 6-mo-old (n = 28), 24-mo-old (n = 7), 36-mo-old (n = 12), and 54-mo-old (n = 3) prairie dogs.

small sample sizes used to generate RI in these other studies indicate that confidence intervals around the upper and lower reference limits are likely wide, thus perhaps also contributing to the observed differences between studies. These discrepancies in the data generated from prairie dogs using different blood biochemical analyzers indicate that these values should not be compared.

In this study, comparison between a reference laboratory analyzer and a veterinary POC analyzer yielded acceptable agreement for just 2 of the 14 tested analytes. The current ASVCP guidelines for method comparison recommend evaluating a minimum of 40 subjects and testing samples with analyte concentrations that span the working range of the assays. Despite the low number of patient samples for comparison and the inclusion of healthy animals only, the poor analytical agreement between methods confirms the need for method-specific RI. Because the POC analysis was near-immediate, whereas the laboratory testing occurred within 4 h after blood collection, some of the disagreement in results between the analyzers may also have been due to the timing of analyses. However, this time difference and the samples' refrigerated storage are accepted practices, and biochemical analytes are expected to remain stable within this time frame and method of storage.[7,44,49] In addition, similar disagreements between this veterinary POC analyzer and other laboratory analyzers have been reported for other species, thus leading to the recommendation that species- and analyzer-specific reference data should be generated and used.[2,3,7,17,35] This discrepancy further emphasizes the need to consider the tested animal population, sample handling, and analysis methods when interpreting biochemistry results.

To test the potential effect of age, we divided the prairie dogs in this study into groups of juvenile and adult animals. Comparison between these 2 groups showed significant age-related differences in 9 measured plasma biochemical analytes.

Table 2. Descriptive age-related statistics for plasma biochemical analytes of captive black-tailed prairie dogs (*Cynomys ludovicianus*) determined by using a veterinary POC analyzer

	Juvenile (n = 28)				Adult (n = 22)				Age-related
Analyte	Mean	Median	1 SD	Min-Max	Mean	Median	1 SD	Min-Max	effect
Albumin (g/dL)[a]	3.3	3.4	0.4	2.6–4.1	3.2	3.3	0.4	1.9–3.8	↓
ALP (IU/L)[a]	100.4	101	38.3	28–187	64.8	60.5	21.8	23–107	↓
ALT (IU/L)[a]	55.8	42	28.5	27–139	41.8	35	26.1	26–148	↓
Amylase (IU/L)	153	145	37.5	76–220	154.3	151.5	27.1	94–204	
Total bilirubin (mg/dL)	0.4	0.3	0.1	0.3–0.5	0.4	0.4	0.1	0.3–0.5	
BUN (mg/dL)[a]	33.5	33.5	4.8	24–41	26.7	25	6.9	16–43	↓
Calcium (mg/dL)	9.9	9.8	1.0	8.1–14.2	9.7	9.7	0.5	8.8–10.6	
Phosphorus (mg/dL)	6.6	6.7	1.5	3.2–9.3	4.6	4.3	1.7	1.9–8.6	
Creatinine (mg/dL)[a]	0.6	0.7	0.2	0.3–0.8	0.8	0.8	0.3	0.4–1.5	↑
Glucose (mg/dL)[a]	168.6	164.5	25.1	112–220	139.1	135.5	15.2	108–167	↓
Na (mmol/L)[a]	140.2	141	2.9	134–146	142.4	142	2.6	138–148	↑
K (mmol/L)	5.1	5.0	0.5	4.5–6.6	4.6	4.2	0.9	3.3–6.3	
Total protein (g/dL)[a]	5.9	5.9	0.7	3.4–6.9	6.4	6.3	0.6	5.5–8.2	↑
Globulin (g/dL)[a]	2.6	2.5	0.6	1.7–4.3	3.2	3.1	0.8	2.1–5.5	↑
Albumin: globulin ratio	1.4	1.3	0.4	0.8–2.4	1.1	1.1	0.4	0.4–1.7	

Arrows indicate the trend with increasing age.
[a]Value differed significantly ($P < 0.05$) between juvenile and adult animals.

Table 3. Descriptive sex-related statistics for plasma biochemical analytes of captive black-tailed prairie dogs (*Cynomys ludovicianus*) determined by using a veterinary POC analyzer

	Female (n = 16)				Male (n = 34)				Sex-related
Analyte	Mean	Median	1 SD	Min–Max	Mean	Median	1 SD	Min–Max	effect
Albumin (g/dL)[a]	3.4	3.4	0.2	3–3.8	3.2	3.25	0.5	1.9–4.1	↑
ALP (IU/L)	66.1	60.5	22.2	28–107	93.5	98.5	38.8	23–187	
ALT (IU/L)[a]	36.8	34	11.8	26–68	55.7	42	31.5	27–148	↓
Amylase (IU/L)	157.3	151.5	25.1	117–204	151.8	145	36.2	76–220	
Total bilirubin (mg/dL)	0.4	0.4	0.01	0.3–0.5	0.4	0.3	0.1	0.3–0.5	
BUN (mg/dL)	28.6	26	8.5	16–43	31.5	31	5.5	20–41	
Calcium (mg/dL)	9.6	9.7	0.5	8.8–10.6	9.9	9.8	0.9	8.7–14.2	
Phosphorus (mg/dL)	4.8	4.3	1.9	1.9–8.6	6.2	6.3	1.7	3.2–9.3	
Creatinine (mg/dL)[a]	0.8	0.8	0.2	0.6–1.2	0.65	0.7	0.2	0.3–1.5	↑
Glucose (mg/dL)	142.9	142	19.6	108–181	161.7	158	26.4	112–220	
Na (mmol/L)	141.9	141.5	2.6	137–148	140.8	141	3.1	134–148	
K (mmol/L)	4.5	4.25	0.8	3.3–6.3	5.0	5	0.6	3.8–6.6	
Total protein (g/dL)	6.2	6.25	0.4	5.5–6.8	6.0	6.05	0.8	3.4–8.2	
Globulin (g/dL)	2.9	3	0.6	2.1–4.3	2.8	2.7	0.8	1.7–5.5	
Albumin: globulin ratio	1.2	1.1	0.3	0.8–1.7	1.3	1.3	0.5	0.4–2.43	

Max, maximum; Min, minimum
Arrows indicate the trend in female prairie dogs.
[a]Value differed significantly ($P < 0.05$) between male and female prairie dogs.

In this study, compared with juvenile animals, older prairie dogs tended to show lower values for albumin, ALP, ALT, BUN, and glucose and higher values for creatinine, sodium, total proteins, and globulins. Total bilirubin was previously reported to be lower in older prairie dogs, but this trend was not observed in animals tested in our current study.[50,51] However, our older prairie dogs tended to show a decrease in albumin concentration and increases in the total protein and globulin concentrations compared with juvenile animals, and similar trends have been reported for guinea pigs, rats, and degus (*Octodon degus*).[27,29,46]

Older mice and rats tend to show lower blood glucose concentrations.[14,29] In our current study, older prairie dogs had significantly lower blood glucose than juvenile animals, and this was also previously described in wild-caught prairie dogs tested using the same veterinary POC analyzer.[50,51] In addition to showing age-associated effects, blood glucose concentrations in prairie dogs vary considerably depending on the animals' origin, diet, fasting time, stress, and the testing methodology.[22,28,51]

When compared with juveniles, older prairie dogs in the current study tended to show lower BUN values and higher creatinine concentrations. Primarily diet and diminished glomerular filtration rate lead to increased BUN concentrations.[29,30,51]The same age-related effect on BUN has been described in older degus, and the difference was suggested to be due to higher protein metabolism in younger animals.[27] Similar to older prairie dogs, older guinea pigs tend to show

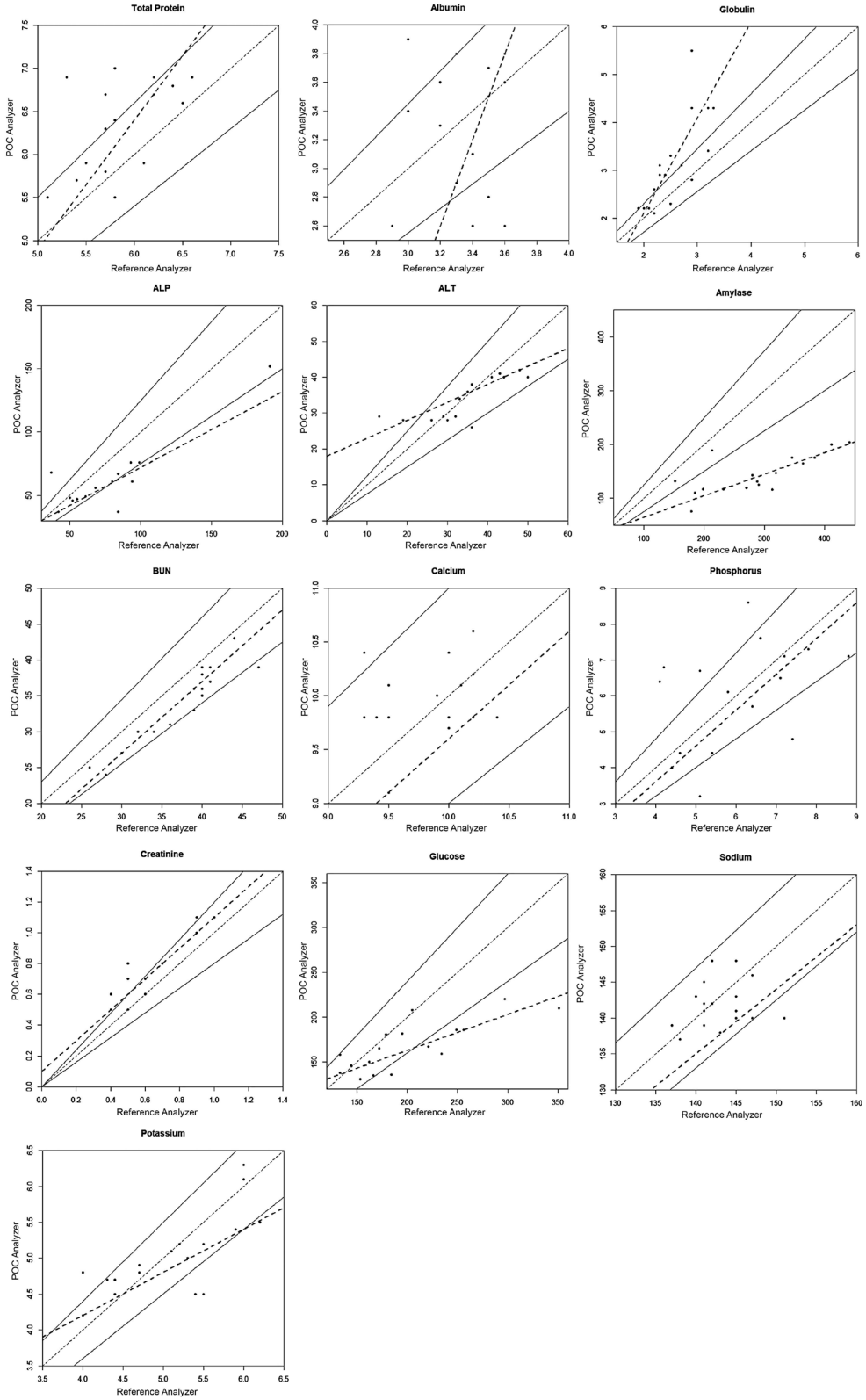

Figure 2. Differential plots of various plasma biochemical analyte concentrations measured by using veterinary POC and laboratory reference analyzers. The dotted line is the line of perfect agreement ($y = x$), the solid lines are the clinical allowable error limits around the line of perfect agreement, and the thick dotted line is the Passing–Bablock regression line (the bias). For acceptable clinical agreement, 95% of the data points must lie between within the solid lines.

higher creatinine values.[29] In addition, age-related increases in creatinine are commonly accompanied by increases in BUN concentration in guinea pigs, rats, and mice, and this azotemia is suggested to result from renal pathologies that likewise tend to increase with age in these species.[29] The prairie dogs in the current study were deemed healthy, without clinical evidence of renal dysfunction or systemic disease, and the age-related increase in the plasma creatinine concentrations was not

Table 4. Agreement statistics between the laboratory reference and veterinary POC biochemical analyzers for 17 prairie dogs in this study

Analyte	Constant bias	95% CI	Proportional bias	95% CI	95% LOA	TEa	WCAEL
Albumin (g/dL)	–7.0[a]	–∞ to –0.9	3.0[a]	1.2 to ∞	±1.0	15%	No
ALP (IU/L)[a]	12[a]	4 to 26	0.6[a]	0.4 to 0.8	±75	25%	No
ALT (IU/L)[a]	18[a]	8 to 26	0.5[a]	0.2 to 0.8	±170	25%	No
Amylase (IU/L)	25	–16 to 68	0.4[a]	0.2 to 0.5	±126	25%	No
Total bilirubin (mg/dL)[a]	NA	NA	NA	NA	±0.1	30%	No
BUN (mg/dL)	–3	–14 to 4	1.0	0.8 to 1.3	±4	15%	Yes
Calcium (mg/dL)	–0.4	–15.7 to 6.0	1.0	0.4 to 2.6	±0.9	10%	Yes
Phosphorus (mg/dL)	–0.4	–5.7 to 4.2	1.0	0.3 to 1.9	±3.0	20%	No
Creatinine (mg/dL)[a]	0.1	–0.5 to 0.3	1.0	0.7 to 2.0	±0.3	20%	No
Glucose (mg/dL)[a]	83[a]	23 to 122	0.4[a]	0.2 to 0.7	±83	20%	No
Na (mmol/L)[a]	9	–213 to 113	0.9	0.2 to 2.5	±8	5%	No
K (mmol/L)	1.8[a]	0.1 to 2.9	0.6	0.4 to 1.0	±0.9	10%	No
Total protein (g/dL)	–2.6	–19.7 to 1.8	1.5	0.8 to 4.5	±1.3	10%	No
Globulin (g/dL)[a]	–1.9[a]	–5.0 to –0.3	2.0[a]	1.3 to 3.3	±1.3	15%	No

LOA, limits of agreement; Max, maximum; Min, minimum; TEa, allowable total error; WCAEL, within clinical allowable error limits.
[a]Statistically significant bias (different from 0 for constant bias; different from 1 for proportional bias).

accompanied by an increased BUN. Future studies that compare blood biochemical results in the context of renal histopathology and urine specific gravity in prairie dogs might help to explain the observed age-related changes in plasma creatinine concentration.

Compared with adults in this study, juvenile prairie dogs tended to show higher ALP and ALT concentrations—similar to the findings from wild-caught prairie dogs tested with the same veterinary POS analyzer.[50,51] Similar observations have been reported for guinea pigs, rats, mice, degus, and giant rats (*Cricetomys gambianus*).[14,27,29,37] Increased serum or plasma ALT activity is a relatively specific indicator of hepatocellular damage in relevant species, but as with all biochemical analytes, abnormal results must be interpreted in light of other laboratory data and clinical signs.[6] Because our animals showed no clinical or other biochemical indications of liver pathology, the observed changes in ALT may not be clinically relevant. In canines, there are 3 recognized isoenzymes of ALP, including bone, liver, and corticosteroid-induced forms.[42] Prairie dog are susceptible to developing hepatocellular carcinomas, and 2 confirmed cases of the neoplasm showed marked increases in ALP concentration when compared with healthy animals and other published RI in this species.[40,47] The combined data from the current study and the reports regarding hepatocellular carcinoma in prairie dogs suggest that this species has the bone and liver ALP isoenzymes. Future studies in prairie dogs can test directly for the presence of different ALP isoenzymes, as is done in other species.[42]

Significantly higher plasma albumin concentrations were present in female prairie dogs in the current study, and a similar effect has been reported in female rats, mice and Syrian hamsters (*Mesocricetus auratus*).[26,34,39] However, because the female prairie dogs in our study tended to be older than males, the observed effect might also have been age-related. The higher creatinine and lower ALT in females than the males in the current study might also reflect age-related changes. However, these changes were also observed in female rats regardless of age.[21] Future studies aimed at age-related blood biochemistry comparisons in black-tailed prairie dogs should evaluate evenly distributed sex and age groups.

Although we noted significant age- and sex-related effects in the plasma biochemistry, we did not generate separate RI for the prairie dogs in this study, because statistically significant difference may not necessarily be clinically relevant and because we had insufficient numbers of reference animals for partitioning.[21,26] Table 1 shows RI for the entire prairie dog population. Although RI for the entire population largely overlap age- and sex-specific RI for most analytes, providing separate data for each of the subgroups may still be useful; separate descriptive statistics are provided in Tables 2 and 3.

Limitations of the current study mainly relate to the sample size, which ideally needs to comprise at least 120 tested animals.[13,31] However, this optimal number is rarely available when working with uncommon species, and the number of prairie dogs included in the current study is much higher than previously used in similar studies in this species.[10] In addition, the precision and accuracy of the testing methodology can be evaluated through duplicate or triplet repeated testing of the same blood samples and by using different machines. In reality, this ideal validation process is often performed by the assay manufacturers or by reference laboratories and is not routinely performed in most RI studies.[25] The lack of data regarding the analytical performance of this veterinary POC analyzer in prairie dogs further complicates transference of these RI for other laboratories, regardless of whether the same analyzer or a different analyzer or methodology is used for sample analysis. As such, future studies in this species should include assessment of precision and the accuracy of this veterinary POC analyzer.

In conclusion, the plasma biochemical values generated by using veterinary POC and a laboratory reference analyzer showed poor agreement for the majority of the analytes, thus suggesting that the resulting data cannot be interpreted directly by comparison. Our current study reports RI for veterinary-POC–based plasma biochemical analytes in captive-raised black-tailed prairie dogs and relevant age- and sex-related differences. The established data can be useful in the interpretation of plasma biochemical analysis in black-tailed prairie dogs, thereby improving diagnosis and treatment of diseases this species.

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