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Blood Values in Wild and Captive Komodo Dragons (*Varanus komodoensis*)

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The Komodo dragon (*Varanus komodoensis*) is the largest living lizard and occupies a range smaller than that of any other large carnivore in the world. Samples from 33 free-ranging animals at five localities in Komodo National Park, Indonesia were evaluated to assess underlying health problems. To build a comparative database, samples from 44 Komodo dragons in both Indonesian and U.S. zoos were also analyzed. Tests performed included complete blood counts, clinical chemistry profiles, vitamin A, D_3, and E analyses, mineral levels, and screening for chlorinated pesticides or other toxins in wild specimens. Blood samples from wild dragons were positive for hemogregarines, whereas captive specimens were all negative. Total white blood cell counts were consistently higher in captive Komodo dragons than in wild specimens. Reference intervals were established for some chemistry analytes, and values obtained from different groups were compared. Vitamin A and E ranges were established. Vitamin D_3 levels were significantly different in Komodo dragons kept in captive, indoor exhibits versus those with daily ultraviolet-B exposure, whether captive or wild specimens. Corrective measures such as ultraviolet-permeable skylights, direct sunlight exposure, and self-ballasted mercury vapor ultraviolet lamps increased vitamin D_3 concentrations in four dragons to levels comparable with wild specimens. Toxicology results were negative except for background-level chlorinated pesticide residues. The results indicate no notable medical, nutritional, or toxic problems in the wild Komodo dragon population. Problems in captive specimens may relate to, and can be corrected by, husbandry measures such as regular ultraviolet-B exposure. Zoo Biol 19:495–509, 2000. © 2000 Wiley-Liss, Inc.

Key words: Komodo dragons; *Varanus komodoensis*; blood values; normal physiologic values

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INTRODUCTION

Komodo dragons (*Varanus komodoensis*) are the world's largest living lizards and range over several tiny islands in central Indonesia. These monitor lizards have the smallest range of any large carnivore in the world. Because of the harsh climate and difficult terrain, studies have been short term except for a 15-month ecology and behavior study [Auffenburg, 1981]. Various population estimates, including current studies, place the total individuals at between 3,000 and 4,000, most of which live in Komodo National Park [Ciofi, 1999]. Because of increasing ecotourism and national park status, Komodo National Park (KNP) is currently being effectively managed as a preserve for the species. On the adjacent island of Flores, the Komodo dragon population is in rapid decline because of prey depletion and human encroachment. Other potential threats include political instability, climate changes, and volcanic activity. Komodo dragons are currently classified as a CITES Appendix 1 species [Auffenburg, 1981] or as Vulnerable under the IUCN Red List [IUCN, 1996].

Baseline health assessments of wild reptiles have been reported for several species [Ghebremeskel et al., 1991; Calle et al., 1994; Raphael et al., 1994], but none for the Komodo dragon. Studies such as these are vital for detection of underlying health problems and provide comparative values for future health assessments. Particularly in the case of a species with such a limited range as the Komodo dragon, early detection of potentially severe health problems could prevent catastrophe.

Normal physiologic values should also be valuable in improving captive medical management. This is often accomplished in Komodo dragons through good husbandry practice such as correct temperature gradients, sufficient exhibit space, whole animal diets, and daily access to natural or artificial ultraviolet-B (UVB) light [Walsh, 1996; Ullrey and Bernard, 1999]. Overall management becomes even more important because the Komodo dragon breeds well currently in zoos, and thus the captive group acts as a vital reserve for the wild population [Walsh, 1996].

MATERIALS AND METHODS

Komodo Dragons

Samples were collected from 77 Komodo dragons, of which 44 were captive and 33 were wild. Wild Komodo dragons were sampled in four locations in KNP including the islands of Komodo, Rintja, and Gili Motang, as well as Wai Wuul preserve on the island of Flores. Age and gender of most dragons were not known, but most specimens were subadult (>1.0 m total length and usually >5.0 kg body weight) to mature in age. The smallest dragons weighed approximately 2.0 kg. Pathologic states except seasonal inanition during the dry season were not seen in wild specimens. Wild Komodo dragons were designated as the "wild" group for this study.

Of the 44 captive dragons, 28 specimens were located at the Yogjakarta Zoo, Java, Indonesia and were all housed for 1 to 2 hours daily in direct sunlight. The 16 U.S. zoo specimens representing seven U.S. zoos were divided into eight animals housed indoors with no daily ultraviolet exposure in any form, and eight specimens were housed outdoors or with some form of daily UVB exposure (sunlight, UV-permeable roof exhibit panels, or artificial UVB lighting). All 28 of the Indonesian zoo specimens and the eight U.S. zoo animals with daily UVB access were designated as a "captive outdoor" group. The eight U.S. Komodo dragons housed indoors

with no UVB exposure were classified as a "captive indoor" group for this study. Samples were collected in captive specimens that ranged in size similar to wild animals in the study (subadult to mature). Wild Komodo dragons opportunistically catch a wide variety of prey, which consists mainly of large mammals and also birds, rodents, other reptiles, and even insects [Auffenburg, 1981]. Diet for Indonesian zoo dragons consisted of 20% whole mice and 80% beef or chicken meat. U.S. specimens were fed a variety of whole animals (rats, mice, quail, fish, rabbits) or whole animals and no more than 20% muscle meat (horsemeat, beef) as part of the total diet. All eight U.S. Komodo dragons were subjected to daily UVB exposure later in the study, and four of these animals were opportunistically sampled for vitamin D_3 levels. Two animals moved into summer outdoor exhibits had samples collected after 1 and then 3 months of direct temperate sunlight exposure. Another dragon housed with no daily UV exposure had vitamin D_3 levels measured after 6 weeks under UVB-permeable exhibit panels (sample 1) followed by 2 months of direct sunlight (sample 2), and then two samples during 1 year after the animal was returned to indoor housing because of cold weather. Daily UVB exposure was provided during this time by a 300-W self-ballasted mercury vapor lamp (Active UV Heat, Metamorph Enterprises, Santa Barbara, CA) at a distance of 2.44 m for 8 to 10 hours daily. A fourth specimen and exhibit mate to the preceding Komodo dragon was transferred to outdoor housing to improve vitamin D_3 levels, sampled after 1 month of direct temperate sunlight, and then moved because of cold weather to indoor housing and provided with the same artificial lighting. Two samples were drawn during the next year from this animal.

Sample Collection and Processing

After the venepuncture site was scrubbed, blood was taken by a lateral approach to the ventral tail vein and collected into plain, lithium-heparin syringes, or sodium-heparin-flushed syringes. Blood was transferred to lithium-heparin, sodium-heparin, or no-additive (royal-blue top tubes for trace mineral analysis) tubes. These were placed in cooled, insulated containers until processed.

Whole blood was used to make blood smears, measuring blood hemoglobin concentrations, determination of blood hematocrit, and measuring total heterophil/eosinophil count. Plasma was harvested within 4 to 8 hours of blood collection, placed in cryogenic polypropylene vials, and immediately frozen in liquid nitrogen. Blood collected from captive specimens was processed within 1 hour. Frozen plasma samples were generally stored as 1- to 2-mL aliquots in cryogenic vials to be distributed to the various laboratories used during this study. This amount proved to be sufficient to run the various test panels (serum chemistries, trace minerals, vitamin D_3, etc.) without the need to subject samples to thaw and freeze cycle(s) that may have been detrimental to test results. After frozen samples were returned to the United States, they were shipped via overnight carrier to veterinary diagnostic laboratories (Veterinary Medical Diagnostic Laboratory [VMDL], University of Missouri, Columbia, MO; Animal Health Diagnostic Laboratory [AHDL], Michigan State University, East Lansing, MI) or a commercial reference laboratory (Antech, Phoenix, AZ).

Analytical methods

Hematocrit values were determined by centrifugation. Blood hemoglobin concentrations were determined by a spectrophotometric method. Total heterophil/eosi-

nophil counts were done using a manual counting technique previously described [Dein, 1984; Gross, 1984]. Calculations using the leukocyte differential count were used to establish total white blood cell (WBC) counts.

Blood films were fixed with methanol in the field and stored in protective containers until examination in the United States. Blood films were stained with Kleinebeger-Noble Giemsa, and 200 leukocytes were identified to establish the leukocyte differential count. Leukocytes were differentiated according to standard methods [Frye, 1991]. Fourteen heparinized plasma samples collected during the initial survey of wild Komodo dragons on the island of Komodo were shipped to a commercial reference laboratory (Antech, Phoenix, AZ) for measuring concentrations of albumin, calcium, chloride, creatinine, glucose, inorganic phosphorus, potassium, protein (total), sodium, and uric acid and activities of aspartate transaminase (AST), lactate dehydrogenase (LD), and creatine kinase (CK).

Clinical chemistry assays were performed at the VMDL for all Komodo dragons both wild and captive during the remainder of the study. Heparinized plasma samples were brought to room temperature before measuring concentrations of albumin, calcium, chloride, creatinine, glucose, inorganic phosphorus, potassium, protein (total), sodium, urea, and uric acid and activities of AST, LD, and CK. If fibrin was present, a clear sample was obtained by centrifugation and pipetting. All plasma clinical chemistry assays were performed on the Vitros 550 (Ortho-Clinical Diagnostics, Rochester, NY) either by reflectance photometry or ion-selective electrode. Values obtained by these methods were used to determine reference intervals.

Mineral and vitamin A, D, and E analyses were performed at the AHDL. Mineral analyses for calcium, molybdenum, phosphorus, boron, copper, zinc, barium, chromium, iron, sodium, magnesium, cobalt, and potassium were conducted by inductively coupled plasma emission spectroscopy as previously described [Stowe et al., 1985]. Vitamin A levels (retinol) in samples were measured by high-performance liquid chromatography (HPLC) using a fluorescent detection method. Vitamin E (α/γ-tocopherol) levels were determined on an HPLC using an ultraviolet detection method. Plasma selenium levels in Komodo dragons were assayed by a wet chemistry method using acid digestion and then fluorescent detection. Vitamin D_3, 25-hydroxy vitamin D_3 (25[OH]D_3), was measured using a commercial radioimmunoassay kit (DiaSorin, Stillwater, MN).

Selected toxicologic testing was performed on two Komodo island specimens and three Rintja island specimens by mass spectrometry and gas chromatography at the AHDL. Results were checked against AHDL's library of several thousand compounds. Komodo dragons at Wai Wuul Preserve, Flores, Gili Motang (KNP), and a second locality on Rintja (KNP) were more specifically tested for chlorinated pesticides (aldrin, α-BHC, beta-BHC, O, P′-DDD;P, P′-DDD; P, P′-DDE; P, P′-DDT, dieldrin, endrin, heptachlor, epoxide, lindane, γ-BHC, and nonachlor) by electron capture gas chromatography [Price et al., 1986].

Analysis of data

If measured analyte concentrations were reported in traditional units, they were converted to Système International (SI) units by recommended conversion factors and minimal increments [Lundberg et al., 1986].

Calculated ratios (albumin/globulin, calcium/phosphorus, sodium/potassium) were calculated using concentrations expressed in SI units. Calculated osmolarity

was obtained when analyte concentrations (in mmol/L) were available for its formula: calculated osmolarity = 1.86 (Na + K) + urea + glucose.

Outlier values application of an outlier test [Reed et al., 1971], excludes values when the difference between the two highest (or two lowest) values in the distribution exceeds one third of the range of all values. After removal of outlier values, data from 33 wild, 30 captive outdoor, and 8 captive indoor Komodo dragons were separated into respective groups. Data for each analyte or laboratory test result of each group were tested for normality using the Kolmogorov-Smirnov normality test and for equal variance across groups (Sigma-Stat Windows version 2.03, SPSS Inc., Chicago, IL), and *P* values of 0.05 were used for rejections. Test results less than or greater than an assay's sensitivity limits were not used in the normality or comparison testing.

If data passed tests for normality and equal variance, between-group comparisons were done with one-way analysis of variance with a *P* value of 0.05.

If there were statistically significant differences between groups, a Tukey test was performed to isolate the group or groups of data that were different ($P < 0.05$).

If data did not pass tests for normality and equal variance, between-group comparisons were done with Kruskal-Wallis one-way analysis of variance on ranks with a *P* value of 0.05. If there were statistically significant differences between groups, the Dunn's method was used to isolate the group or groups of data that were different ($P < 0.05$).

The above methods were used for the comparison of groups except for the blood hemoglobin concentration for which there were no values for the captive indoor group. The Mann-Whitney rank sum test was used to determine whether there was a statistically significant difference between groups if the data did not pass the normality testing.

If statistical tests did not find significant differences between data in the wild, captive outdoor and captive indoor groups, laboratory test results were combined before computing reference intervals.

If statistical tests indicated a statistically significant difference among groups, results of the Tukey test or Dunn's method were examined to identify the group that was different. If results of the Tukey test or Dunn's method indicated that the wild and captive outdoor groups were not statistically different, their data were combined for computation of reference intervals. Analysis of variance might indicate there was a difference between the three groups but did not indicate whether one or more differences existed. If there was a statistical difference, then the Tukey test and Dunn's method were used to attempt to isolate the different group (if only one group differed). If the Tukey and Dunn's failed to isolate one group as different, then Group 1 (example only) was different from Groups 2 and 3. Group 2 was considered different from Groups 1 and 3, and Group 3 was different from Groups 1 and 2. For the purposes of this study, no group was considered "normal." If there was statistical difference among groups, then data could not be combined for calculation of reference intervals or for reporting of observed ranges.

Outlier values that were identified as described above were removed from the combined data. Reference intervals were established by nonparametric analysis when at least 40 reference values remained in combined groups [Solberg, 1983]. The number of values to be included in the reference interval was determined by multiplying the number of reference values for an analyte or calculated result by 95% and then rounding down to the next lower integer.

To find reference limits, the highest and lowest remaining values were excluded.

For most analytes, a third value was excluded by removing either the remaining highest or lowest value, depending on which had the greatest interval between it and the remaining second highest or lowest. After the above exclusions, the remaining lowest and highest values were the reference limits of the reference intervals. The observed range for each analyte or calculated laboratory result represented all measured values except the values excluded by the outlier range test.

For some of the trace minerals, the plasma concentrations were below an assay's analytical sensitivity. For those analytes, the number of observed values below the limit was determined.

Hematologic data were analyzed as described for the clinical chemistry data already given. Because statistically significant differences between the three groups were common, particularly the total leukocyte counts, data from the three groups were not combined for reference interval determination.

Clinical chemistry data (excluding outlier values) from the VMDL and the commercial reference laboratory were compared for differences by either the *t*-test (if passed normality or variance testing as described above) or the Mann-Whitney rank sum test (if failed either normality or variance testing).

RESULTS

Results of the analysis of clinical chemistry data are reported in Table 1. References intervals for urea concentrations were not established because all measured plasma concentrations were at or below the analytical sensitivity of the assay (i.e., 1.0 μmol/L). Because reliable urea concentrations were not measured, urea/creatinine ratios were not calculated.

For 11 analytes, statistically significant differences were seen among wild specimens, captive dragons that had daily UVB exposure, and captive specimens housed only indoors (Table 2). Of the differences, only the vitamin D_3 levels were considered clinically important.

Serial vitamin D_3 results in Komodo dragons housed indoors with no UVB source changed dramatically when daily UVB exposure was given. Two specimens with values of 4 and 32 nmol/L rose to 252 and 271 nmol/L, respectively, in 1 month of temperate sunlight exposure. At the end of 3 months of direct sunlight, these two Komodo dragons showed values of 236 and 249 nmol/L vitamin D_3. A third Komodo dragon under UV-permeable roof panels (Polycast SUVT, Polycast Technology Corp., Stamford, CT) increased vitamin D_3 from 25 to 158 nmol/L in 6 weeks. At this point, the animal was subjected to 2 months of direct sunlight, and vitamin D_3 levels were measured at 196 nmol/L. After transfer to indoor holding because of cold weather, this Komodo dragon recorded values of 252 and 212 nmol/L vitamin D_3 during the next year. UVB exposure was provided only by a 300-W self-ballasted mercury vapor lamp (Active UV Heat, Metamorph Enterprises) set at 2.44 m distance for 8 to 10 hours daily. The fourth Komodo dragon opportunistically sampled during this study had an initial value of 14 nmol/L vitamin D_3 that rose to 200 nmol/L after 1 month of temperate sunlight exposure. This specimen was housed in conditions identical to those of the third animal in indoor holding at this point because of cold weather. Vitamin D_3 levels taken during the next year stayed at more than 250 nmol/L in this Komodo dragon under the same artificial UVB exposure conditions.

Hematologic results are tabulated in Table 3. Significant differences were seen

between hematocrit and total WBC. Wild Komodo dragons typically had lower hematocrits than captive animals kept indoors or outdoors. Median values for total leukocyte counts were lowest in wild dragons and significantly higher for captive outdoor dragons (2.3×10^9/L versus 6.0×10^9/L).

Measured or calculated clinical chemistry values determined by a commercial reference laboratory are reported in Table 4. There were statistically significant differences between 11 of the 18 clinical chemistry values of the commercial reference laboratory and the VMDL. Urea concentrations were not compared because of the analytical sensitivity of the assays.

Hemoparasites identified as hemogregarines were present in erythrocytes on stained blood films. These organisms were present in some of the wild dragons from every island studied (Komodo, Rintja, Flores, Gili Motang). No hemoparasites were seen in any captive specimens in U.S. or Indonesian zoos. None of the captive specimens had ever been treated previously for blood parasites.

Toxicology testing yielded negative results on five wild Komodo dragons from Rintja and Komodo islands. Chlorinated pesticide testing on dragon plasma from Flores, Rintja (second locality) and Gili Motang did reveal extremely low levels (parts per billion) of P, P′-DDE, β-BHC, α-BHC, and nonachlor.

DISCUSSION

Baseline physiologic values for wild populations are important to define current health status and as a reference point for future studies [Calle et al., 1994]. Age, sex, seasonality, or temperature were not used as differentiating factors, although studies have found differences in hematologic values based on these factors [Calle et al., 1994; Raphael et al., 1994; Anderson et al., 1996]. Precise age and gender could not be determined consistently, especially when surveying wild populations. Determining the sex of Komodo dragons by physical traits alone, as would be the situation in the field, has been inconsistent and requires specialized testing [Morris et al., 1996]. The numerous differences in results between a commercial reference laboratory and the VMDL negated effective comparison of the two groups of data. Use of one laboratory throughout the study would have resulted in a larger reference population.

Statistically different results for chemistry analytes and nutrition values were not clinically apparent except in vitamin D_3 levels. Concentrations of serum or plasma 25[OH]D_3 are considered useful in assessing the vitamin D status in humans and animals [Ullrey and Bernard, 1999]. This also appears to be the case with the Komodo dragon. Wild and captive animals with daily UVB exposure exhibited values greater than 100 nmol/L (except for one captive specimen) and typically had values in the 150 to 250 nmol/L range (~75% of all Komodo dragons with daily UVB exposure). Captive animals with no daily UVB exposure were always more than 100 nmol/L and often more than 50 nmol/L. Similar values have been found in indoor crocodile monitors (*Varanus salvadorii*) and water monitors (*Varanus salvator*) [D. Gillespie, unpublished data].

One Komodo dragon with extremely low vitamin D_3 levels (14 nmol/L) exhibited clinical lameness and poor bone density on radiographs, with fractures in several long bones. This finding is similar to another study of low vitamin D_3 values and corresponding bone abnormalities in hatchling Komodo dragons that were corrected by providing daily UVB exposure [Allen et al., 1994]. Daily sunlight exposure was

TABLE 1. Clinical chemistry results for Komodo dragon plasma including reference intervals, observed ranges, and statistical data

Analyte or calculated result	Units	Reference intervals[a]	Number of reference values[b]	Observed range for measured values[c]	Number of measured values[c]	Mean value[c]	Mean value[c]	P[d]
Albumin	g/L	30–45	51	21–46	54	36	37	0.690[e]
Albumin/globulin ratio	—	0.7–1.2	51	0.6–1.4	54	1.0	0.9	0.521[e]
Aspartate transaminase	IU/L	—	—	7–39	53	16	14	<0.001[f]
Barium	µmol/L	—	—	≤0.2	5	—	—	—
Boron	µmol/L	—	—	≤20	48	—	—	—
Calcium/phosphorus ratio	—	1.6–4.4	48	1.3–5.3	51	2.8	2.6	0.267[f]
Calcium[g]	mmol/L	3.0–4.2	49	3.00–4.40	52	3.54	3.42	0.913[f]
Calcum[h]	mmol/L	—	—	2.94–4.30	48	3.62	3.60	0.013[e]
Chloride	mmol/L	98–129	51	91–130	54	116	117	0.198[f]
Chromium	µmol/L	—	—	<0.2	43	—	—	—
Creatine kinase	IU/L	130–2,403	50	54–3,428	53	656	439	0.145[f]
Cobalt	µmol/L	—	—	<0.3	5	—	—	—
Copper	µmol/L	10.2–20.8	45	10.0–24.4	48	15.2	15.0	0.883[e]
Creatinine	µmol/L	20–90	51	20–110	54	50	50	0.106[f]
Globulins	g/L	—	—	22–70	54	39	38	0.030[f]
Glucose	mmol/L	6.7–14.4	48	5.6–16.4	53	10.1	9.8	<0.001[e]
Iron	µmol/L	—	—	11–22[i]	22	15	14	0.194[e]
Lactate dehydrogenase	IU/L	108–2,139	50	100–3,107	53	904	781	0.078[f]
Magnesium	µmol/L	1.02–1.68	45	0.84–1.86	48	1.34	1.30	0.354[e]
Molybdenum	µmol/L	—	—	<0.4	5	—	—	—
Osmolarity, calculated	mmol/L	318–350	50	301–352	53	330	330	0.099[e]
Phosphorus[g]	mmol/L	0.75–2.80	51	0.40–3.40	54	1.75	1.70	0.447[e]
Phosphorus[h]	mmol/L	0.90–2.65	45	0.45–3.40	48	1.70	1.70	0.095[e]
Potassium[g]	mmol/L	3.2–5.4	51	2.8–6.5	54	4.4	4.3	0.579[e]
Potassium[h]	mmol/L	3.3–6.4	45	2.8–7.1	48	4.8	4.8	0.126[e]
Protein, total	g/L	51–99	51	43–113	54	75	75	0.095[f]

Continued

TABLE 1. (*Continued*)

Analyte or calculated result	Units	Reference intervals[a]	Number of reference values[b]	Observed range for measured values[c]	Number of measured values[c]	Mean value[c]	Mean value[c]	*P*[d]
Protein, total[j]	g/L	—	—	73–88	14	82	83	0.324[f]
Selenium	µmol/L	—	—	0.7–3.3	53	1.9	1.9	<0.001[f]
Sodium/potassium ratio	—	30–47	50	27–56	53	39	38	0.703[e]
Sodium[k]	mmol/L	158–174	50	154–176	53	167	167	0.096[e]
Sodium[h]	mmol/L	—	—	123–178	48	161	161	0.010[e]
Urea	mmol/L	—	—	≤1	54	—	—	—
Uric acid	µmol/L	—	—	50–940	54	260	260	<0.001[f]
Vitamin A	µmol/L	—	—	0.10–0.85	43	0.25	0.25	0.015[f]
Vitamin D[l]	nmol/L	—	—	4–324	54	164	172	0.008[f]
Vitamin E	µmol/L	2–15	50	2–23	53	6	6	0.239[f]
Zinc	µmol/L	—	—	31.8–163.7	48	62.7	54.6	0.002[f]

[a]Except for glucose concentraton, computed from combined values of wild, captive outdoor, and captive indoor groups if differences between groups were not statistically significant and there were at least 40 remaining reference values after removal of outlier values. Reference interval for glucose concentration computed from combine data from Wild and Captive Outdoor Groups.
[b]Reference values used for determination for reference limits; does not contain the outlier values.
[c]Does not include outlier values that were excluded by the Dixon range test.
[d]Differences among three groups considered significantly different if *P* values were <0.05.
[e]*P* value from parametric one-way analysis of variance.
[f]*P* value from Kruskal-Wallis one-way analysis of variance on ranks.
[g]Spectrophotometric assay method.
[h]Inductively coupled plasma emmission spectroscopy.
[i]There were 25 additional reference values for iron that were <9 µmol/L.
[j]Measured by refractometry on heparinized plasma.
[k]Ion-selective electrode method.
[l]There were nine additional reference values for vitamin D that were reported to be >250 nmol/L.

TABLE 2. Clinical chemistry data in wild, captive outdoor, and captive indoor groups of Komodo dragons; includes data when there was a statistically significance difference among groups

Analyte or calculated result	Units	Wild			Captive outdoor			Captive Indoor			P[a]
		Range	Median	No.	Range	Median	No.	Range	Median	No.	
AST	IU/L	10–39	19	21	7–30	13	27	7–14	10	5	<0.001[b,c]
Calcium[d]	mmol/L	2.94–4.06	3.50	28	3.40–4.20	3.80	10	3.06–4.12	3.80	8	0.013[e,f]
Globulins	g/L	22–46	40	21	26–70	36	28	25–59	42	5	0.030[b,h]
Glucose	mmol/L	6.2–16.4	11.2	20	6.7–13.0	9.7	28	5.6–8.3	6.9	5	<0.001[e,i]
Selenium	µmol/L	1.4–3.3	2.1	28	0.7–2.4	1.2	16	1.2–3.2	1.9	8	<0.001[b,i]
Sodium[d]	mmol/L	123–175	159	28	156–178	171	10	150–168	161	8	0.010[b,f]
Uric acid	µmol/L	170–680	310	21	70–740	170	28	50–940	90	5	<0.001[b,c]
Vitamin A	µmol/L	0.15–0.75	0.25	28	0.10–0.50	0.20	7	0.10–0.85	0.20	8	0.015[b,h]
Vitamin D	nmol/L	117–324	183	26	83–260	168	17	4–97	29	8	<0.008[e,i]
Zinc	µmol/L	31.8–74.4	50.9	28	43.0–139.4	95.4	10	41.2–163.7	49.1	8	0.002[b,i]

[a]Differences among three groups considered significantly different if P values <0.05.
[b]P value from Kruskal-Wallis one-way analysis of variance on ranks.
[c]Significant difference between wild and captive outdoor groups and between wild and captive indoor groups (Dunn's method, $P < 0.05$).
[d]Inductively coupled plasma spectroscopy.
[e]P value from parametric one-way analysis of variance.
[f]Significant difference between wild and captive outdoor groups (Tukey test, $P < 0.05$).
[g]Significant difference between captive outdoor and captive indoor groups (Tukey test, $P < 0.05$).
[h]Group or groups that differ from the others were not isolated (Dunn's method, $P < 0.05$).
[i]Significant difference between wild and captive indoor groups and between captive outdoor and captive indoor groups (Dunn's method, $P < 0.05$).

TABLE 3. Hematologic data in wild, captive outdoor, and captive indoor groups of Komodo dragons

		Wild			Captive outdoor			Captive Indoor			
	Units	Range	Median	No.	Range	Median	No.	Range	Median	No.	P[a]
Hematocrit		0.25–0.40	0.34	31	0.29–9.45	0.33	19	0.37–0.44	0.42	3	0.017[b,c]
Hemoglobin	g/L	95–159	117	16	97–125	110	16			0	0.132[d]
Erythrocyte width	µm	11.2–15.8	14.0	31	10.5–16.5	14.0	21	14.0–14.8	14.0	5	0.189[e]
Erythrocyte length	µm	21.0–27.9	23.0	31	21.5–28.0	24.5	21	24.0–24.8	24.5	5	0.003[e,f]
Leukocyte	$\times 10^9$/L	0.7–12.5	2.3	30	3.0–10.9	6.0	21	2.4–3.2		2	<0.001[e,f]
Heterophil	$\times 10^9$/L	0.1–4.6	0.8	30	0.7–5.0	2.7	21	1.7–2.1		2	<0.001[e,f]
Lymphocyte	$\times 10^9$/L	0.5–6.4	1.2	30	1.1–6.3	3.2	21	0.7–1.1		2	<0.001[e,g]
Prolymphocyte	$\times 10^9$/L	0.0–0.1	0.0	30	0.0–0.2	0.0	21	0.0–0.0		2	0.225[e]
Plasmacyte	$\times 10^9$/L	0.0–0.5	0.0	30	0.0–1.4	0.2	21	0.0–0.0		2	0.001[e,g]
Monocyte	$\times 10^9$/L	0.0–1.5	0.1	30	0.0–1.1	0.2	21	0.0–0.0		2	0.005[e,h]
Azurophil	$\times 10^9$/L	0.0–0.3	0.0	30	0.0–0.8	0.1	21	0.0–0.0		2	0.225[e]
Basophil	$\times 10^9$/L	0.0–0.6	0.0	30	0.0–0.1	0.0	21	0.0–0.0		2	0.008[e,g]
Eosinophil	$\times 10^9$/L	0.0–0.0	0.0	30	0.0–0.0	0.0	21	0.0–0.0		2	0.303[e]
Heterophil[i]	Fraction	0.08–0.58	0.38	31	0.14–0.65	0.40	22	0.33–0.69	0.53	6	0.008[b,c]
Lymphocyte[i]	Fraction	0.35–0.82	0.52	31	0.20–0.73	0.46	22	0.28–0.53	0.34	6	0.009[e,j]
Prolymphocyte[i]	Fraction	0.00–0.03	0.00	31	0.00–0.02	0.00	22	0.00–0.00	0.00	6	0.249[e]
Plasmacyte[i]	Fraction	0.00–0.08	0.01	31	0.00–0.16	0.02	22	0.00–0.11	0.03	6	0.045[e,g]
Monocyte[i]	Fraction	0.00–0.16	0.02	31	0.00–0.18	0.04	22	0.00–0.07	0.02	6	0.289[e]
Azurophil[i]	Fraction	0.00–0.07	0.00	31	0.00–0.15	0.02	22	0.00–0.07	0.02	6	0.007[e,g]
Basophil[i]	Fraction	0.00–0.13	0.01	31	0.00–0.03	0.00	22	0.00–0.05	0.00	6	<0.001[e,f]
Eosinophil[i]	Fraction	0.00–0.01	0.00	31	0.00–0.00	0.00	22	0.00–0.00	0.00	6	0.246[e]

[a]Differences among three groups considered significantly different if P values <0.05.
[b]P value from one-way analysis of variance tests.
[c]Significant difference between wild and captive outdoor groups and between wild and captive indoor groups (Tukey test, $P < 0.05$).
[d]P value from Mann-Whitney rank sum test.
[e]P value from Kruskal-Wallis one-way analysis of variance on ranks.
[f]Significant difference between wild and captive outdoor groups (Dunn's method, $P < 0.05$).
[g]Group or groups that differ from the others were not isolated (Dunn's method, $P < 0.05$).
[h]Significant difference between wild and captive indoor groups and between captive outdoor and captive indoor groups (Dunn's method, $P < 0.05$).
[i]From leukocyte differential count.
[j]Significant difference between wild and captive indoor groups (Dunn's method, $P < 0.05$).

TABLE 4. Clinical chemistry results for Komodo dragon plasma from a reference laboratory[a]

Analyte or calculated result	Units	Observed range for measured values	Number of measured values	Mean value	Median value
Albumin[b]	g/L	20–30	14	25	25
Albumin/globulin ratio[c]	—	0.3–0.6	14	0.4	0.4
Aspartate transaminase	IU/L	5–38	14	16	13
Calcium	mmol/L	3.10–4.22	14	3.70	3.70
Calcium/phosphorus ratio	—	1.2–3.2	14	2.4	2.5
Chloride[c]	mmol/L	89–111	13	104	106
Creatine kinase	IU/L	143–3,212	14	760	512
Creatinine	µmol/L	20–90	14	50	40
Globulins[c]	g/L	47–84	14	47	84
Glucose[c]	mmol/L	4.0–15.7	14	7.9	6.6
Lactate dehydrogenase[c]	IU/L	133–918	12	321	243
Osomolarity, calculated[b]	mmol/L	292–320	13	307	307
Phosphorus	mmol/L	1.15–2.60	14	1.65	1.55
Potassium	mmol/L	3.5–5.4	13	4.4	4.3
Protein total[c]	g/L	72–106	14	83	82
Sodium[b]	mmol/L	149–165	13	156	155
Sodium/potassium ratio[b]	—	28.5–44.3	13	36.3	35.7
Urea nitrogen	mmol/L	0.4–1.8	13	1.0	0.7
Uric acid[c]	µmol/L	170–1,620	12	640	510

[a]Antech Laboratory, Phoenix, AZ.
[b]Data statistically different (*t*-test, $P < 0.05$) from values measured or calculated by the Clinical Pathology Laboratory, Veterinary Medical Diagnostic Laboratory.
[c]Data statistically different (Mann-Whitney rank sum test, $P < 0.05$) from values measured or calculated by the Clinical Pathology Laboratory, Veterinary Medical Diagnostic Laboratory.

given to this Komodo dragon, and clinical lameness resolved in 1 to 2 months. Unfortunately, follow-up radiographs or vitamin D_3 values were not available. The calcium level of this specimen at the time of apparent clinical hypovitaminosis D, 4.17 mmol/L, is at the upper end of the reference interval (3.0–4.2 mmol/L). In fact, calcium levels did not appear to vary with vitamin D_3 values in this study. This is in contrast to green iguanas (*Iguana iguana*), which demonstrated variable blood calcium levels when hypovitaminosis D was experimentally induced in a study group [Ullrey and Bernard, 1999]. Possibilities include species-specific differences in vitamin D and calcium metabolism or diet. Whole animals used in captive Komodo dragon diets (usually not a feature of herbivorous iguana diets) should represent balanced food items at least with respect to calcium/phosphorus levels [Frye, 1991]. Dietary vitamin D_3 levels were not done for wild or captive specimens. However, apparently dietary sources did not provide enough vitamin D_3 for captive Komodo dragons to elevate 25[OH]D_3 blood levels comparable to those of wild specimens even with whole mammal, bird, or fish items usually offered.

Komodo dragons housed indoors (with no daily UVB exposure) quickly return to vitamin D_3 levels comparable to those of wild specimens through daily UVB exposure. Direct sunlight offers the most natural route of exposure, but UV-permeable acrylic roof panels appear to be an effective alternative. It took only 2 weeks longer (6 weeks total) for a Komodo dragon under these roof panels to reach levels comparable to those of wild specimens as compared to two dragons in direct temperate

sunlight (1 month total). Polycast SUVT (Polycast Technology Corp.) used in this study transmits 74% and 81% of the critical UVB wavelengths of 295 and 300 nm, respectively, the site of most vitamin D_3 biosynthesis [Ullrey and Bernard, 1999]. Acrolite OP-4 (Cyro Industries, Newark, NJ; 75.4%—295 nm, 79.9%—300 nm) or Solacryl SUVT (Polycast Technology Corp.; 85%—295 nm, 85%—300 nm) should provide similar or better results [Ullrey and Bernard, 1999; Ullrey, unpublished data].

Ultraviolet-emitting lamps may be used in husbandry situations where direct sunlight or UV-permeable roof panels cannot be used. In addition to the Komodo dragons documented in our study, several crocodile and water monitors with no previous UVB exposure for 2 to 3 years have shown plasma vitamin D_3 levels comparable to those of wild Komodo dragons (>150 nmol/L) after 2 to 3 months of exposure under a commercially available self-ballasted mercury vapor lamp (UV Active Heat, Metamorph Enterprises). Animal exposure distances of 1.0 to 2.44 m were irradiated by 160- or 300-W lamps. This represents a major advantage in providing adequate daily UVB exposure in large exhibits, whereas fluorescent UVB lamps are only effective at distances of 0.3 to 0.46 m [Ullrey and Bernard, 1999].

It appears that Komodo dragons absorb vitamin D_3 orally. Three Komodo dragons without any UVB source have maintained blood levels between 100 and 330 nmol/L vitamin D_3 during a 6- to 12-month period. Specimens were maintained on a whole animal diet as well as approximately 3,000 IU vitamin D_3/kg dietary dry matter [R. Burns, unpublished data; D. Gillespie, unpublished data]. This route of supplementation may result in hypo- or hypervitaminosis D_3. Sunlight by direct exposure or through UV-permeable acrylic roof panels avoids this problem because natural biosynthesis is thought to be self-regulating [Ullrey and Bernard, 1999]. Sunlight also provides UVA wavelengths, thought to be important in inducing many behaviors and other factors not understood at this time [Frye, 1991]. Indoor lamps should be used as supplemental UVB sources when changing husbandry situations dictate.

Hemoparasites seen were microgametocytes and macrogametocytes of the genus *Hepatozoon*, commonly referred to as hemogregarines [Frye, 1991; Wozniak et al., 1994]. These appear to be incidental findings, because hemogregarines appear to cause few problems in most reptiles [Telford, 1984; Wozniak, 1994]. Nevertheless, it is interesting to note that no hemogregarines were seen in wild Komodo dragons in another study, only in wild water monitors [Auffenburg, 1981]. No hemogregarines were seen in captive specimens, even those apparently captured from the wild. This may suggest that hemogregarine infections are self-limiting in captivity for the Komodo dragons.

Low levels of chlorinated pesticides found in Komodo dragons in several parts of their range were not surprising. Levels are considered extremely low and likely not of toxicologic significance. The Komodo dragon sits at the top of the food chain, and these substances are used in agriculture in much of the world. All areas surveyed except Gili Motang island either had active or past agriculture use (as in areas of KNP). No present or previous source could be found, except that the island is close to Flores and has strong currents running between the two islands.

CONCLUSIONS

1. The wild Komodo dragon has no notable nutritional, toxic, infectious, or parasitic problems at this time. Field studies in these areas must continue to monitor changing situations in the future.

2. Captive Komodo dragons can maintain vitamin D_3 levels comparable to wild specimens through daily UV exposure such as direct sunlight, UV-permeable roof panels, or artificial UVB-emitting lamps placed at appropriate distances. Dietary supplementation may represent a viable alternative but has inherent problems with over- or undersupplementation. Studies are needed to define proper dietary levels.

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