

## Blood profile in captive adult male leopard geckos (*Eublepharis macularius*)

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**Abstract:** The aim of this study was to determine blood profile data in captive adult male leopard geckos. Animals were manually restrained with the head and neck extended. The right external jugular vein was punctured with a pre-heparinised needle and insulin syringe. The means and standard deviations for haemoglobin concentration, packed cell volume, total red blood cell count, total white blood cell count and counts for heterophils, basophils, eosinophils, monocytes, azurophils and lymphocytes for 20 healthy male leopard geckos were  $72.58 \pm 11.03$  g/l,  $25.40 \pm 3.68\%$ ,  $0.85 \pm 0.14 \cdot 10^{12}/l$ ,  $10.47 \pm 2.58 \cdot 10^9/l$ ,  $1.83 \pm 0.92 \cdot 10^9/l$ ,  $1.67 \pm 1.04 \cdot 10^9/l$ ,  $0.29 \pm 0.33 \cdot 10^9/l$ ,  $0.48 \pm 0.40 \cdot 10^9/l$ ,  $2.03 \pm 1.07 \cdot 10^9/l$  and  $4.17 \pm 2.12 \cdot 10^9/l$ , respectively. The means and standard deviations for total protein, albumin, globulins, glucose, uric acid, aspartate aminotransferase, creatine kinase, calcium, phosphorus and potassium for 20 healthy adult captive male leopard geckos were  $55.60 \pm 7.52$  g/l,  $16.45 \pm 2.37$  g/l,  $39.15 \pm 5.74$  g/l,  $6.18 \pm 1.35$  mmol/l,  $67.95 \pm 42.63$   $\mu$ mol/l,  $0.83 \pm 0.42$   $\mu$ kat/l,  $25.40 \pm 29.46$   $\mu$ kat/l,  $3.05 \pm 0.18$  mmol/l,  $1.4 \pm 0.23$  mmol/l, and  $5.78 \pm 0.58$  mmol/l, respectively. This is the first study to report blood haematology and biochemistry values for a group of captive adult male leopard geckos.

**Keywords:** reptiles; lizards; venepuncture; haematology; blood chemistry

The leopard gecko (*Eublepharis macularius*) is one of the most common species of captive lizards and the most traded species of the family *Eublepharidae*. Blood analysis in geckos has been a subject of interest for some authors (Sacchi et al. 2007; Mayer et al. 2011; Olayemi 2011; Salamat et al. 2013). Results of previous studies showed variations, which were due to animal selection and the methods used for venepuncture and the processing of blood samples (Redrobe and MacDonald 1999; Pejrilova et al. 2004; Hernandez-Divers 2006; Knotkova et al. 2005; Knotkova et al. 2008; Mayer

et al. 2011). Blood collection from the ventral tail vein is difficult and can potentially lead to caudal autotomy. Ventral abdominal vein venepuncture has been described by some authors (Redrobe and MacDonald 1999; Hernandez-Divers 2006). However, for this technique, the geckos have to be anaesthetised and placed in dorsal recumbency; the needle is then inserted through the skin in the ventral midline with a risk of lacerating the vessel and the formation of haematoma (Redrobe and MacDonald 1999; Hernandez-Divers 2006). Recently, a newly developed method for safe blood

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collection from leopard geckos has been published (Morici et al. 2016). The aim of this study was to establish reference data for blood parameters in a group of healthy adult male leopard geckos in captivity.

## MATERIAL AND METHODS

**Animals.** Forty-four (44) clinically healthy adult male leopard geckos (*Eublepharis macularius*) kept in captivity were included in this study. They originated from one large captive bred population in the Czech Republic and ranged in age from 15 months to 16 months. All geckos in this study were kept and used in accordance with directive 2010/63/EU and ethical approval was obtained. Geckos were housed in glass terraria (90 × 45 × 45 cm) with air temperature maintained at 30–31 °C, and air humidity at 40–50%. The substrate consisted of folded paper towel. Recycled paper box and paper towel rolls were offered to geckos in order to provide more surface area for climbing and as hiding spots. A heat pad (RH-6, ReptiTherm®, Zoo Med, USA) was placed under each terrarium as a heat source, while a UVA/UVB light bulb (Reptisun 5.0 UVB Mini Compact Fluorescent Bulb, Zoo Med, USA) was placed inside each terrarium as a light and UV light source. The geckos were maintained under a 12/12 light/dark regime. Feeding was performed three times a week with crickets or cockroaches.

The geckos were acclimatised in terraria for 14 months before the procedures were undertaken. Twenty-two geckos (group A) were subjected to blood collection for haematology analyses, and twenty-two geckos (group B) were used for blood collection for blood biochemistry analyses. The body condition of each gecko was evaluated in a standard clinical examination. The body weight of each of the geckos was measured on digital scales (Kern and Sohn GmbH, Balingen, Germany). The mean body weight of the 44 leopard geckos was  $37.6 \pm 5.9$  g (range 28.6–54.7 g). Geckos were fasted for 24 hours prior to blood collection. Access to water was not limited.

**Blood collection from the jugular vein.** The room air temperature was maintained at 26 °C. Geckos were manually restrained with the head and neck extended. Blood was collected using the method published recently by Morici et al. (2016) and Morici et al. (2018). Briefly, the left index of

the operator was placed on the head just behind the right eye while the left middle finger was positioned on the left side of the neck. The head of the gecko was slightly rotated to the left, exposing the area of the right jugular vein (Figure 1). The skin on the right side of the neck was disinfected with a diluted alcohol solution. The needle of the pre-heparinised (heparinum natricum, Heparin inj., 5000 IU/ml, Léčiva Praha, Czech Republic) insulin syringe (0.5 ml – 29G insulin syringe, BD medical, France) was gently inserted rostro-caudally into the right jugular vein (Figure 2). After the total volume of



Figure 1. Leopard gecko manually restrained for jugular vein venepuncture

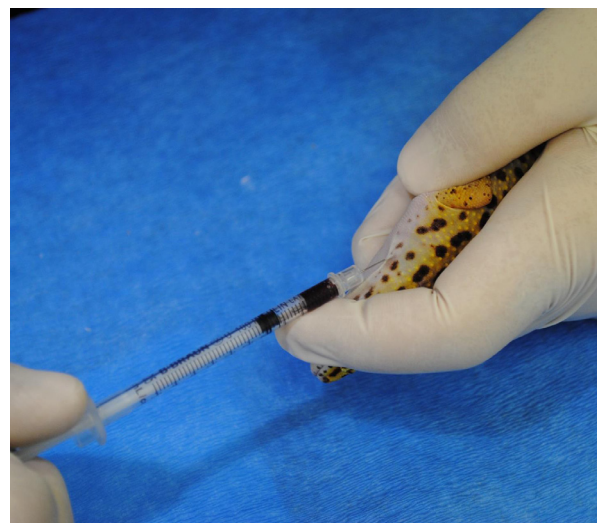


Figure 2. Jugular vein venepuncture in a leopard gecko (*Eublepharis macularius*)

0.15–0.20 ml of blood was collected the needle was gently withdrawn and a cotton swab was pushed against the neck to prevent bleeding and haematoma formation. The phlebotomy was conducted by the same person (M.M.) for all animals. Blood samples were immediately transferred to a laboratory in the same building for processing.

**Haematological analyses.** All analyses were conducted by the same person (Z.K.). Haematocrit (packed cell volume) measurements were performed using the micro-haematocrit method (Pejrilova et al. 2004). Haemoglobin concentration was determined spectrophotometrically using a standard cyanmethaemoglobin method with one modification: the samples were centrifuged following red blood cell lysis to remove the nuclear and cytoplasmic debris. The total red blood cell count and white blood cell count were determined manually using a haemocytometer with Natt and Herrick's stain. Air-dried blood smears were stained using the Pappenheim method (May-Grünwald + Giemsa-Romanowski stains, Pejrilova et al. 2004). The leukocyte differential counts were analysed using an Olympus BX 51TF light microscope and documented with an Olympus C 3030 digital camera. Two hundred cells were evaluated for the differential count. White blood cells were classified as heterophils, basophils, eosinophils, monocytes, azurophils or lymphocytes.

**Biochemical analyses.** The biochemical analyses were performed with the use of the Avian/Reptilian Profile Plus rotor on the VetScan VS 2<sup>®</sup> analyser (Abaxis, Inc., Union City, CA). All samples were run with exactly 100 µl of plasma. The following parameters were measured: total protein, albumin, globulins, glucose, bile acids, aspartate aminotransferase, creatine kinase, calcium, sodium, potassium, phosphorus and uric acid.

**Statistics.** The distribution of data was evaluated with the use of a standard Excel program test for

Windows 7 (Microsoft<sup>®</sup>, USA). For all data, the mean, median, standard deviation and minimum-maximum (min-max) were calculated.

## RESULTS

In two leopard geckos from group A the blood collection from both the right and the left jugular veins was unsuccessful. These two animals were excluded from the study. Blood was collected easily from the right jugular vein in 12 of the 20 leopard geckos. In seven animals, collection of blood from the right jugular vein was difficult, and the blood samples had to be collected from both the right and left jugular vein. In one animal, our attempts to collect blood from the right jugular vein were not successful and the blood sample had to be collected from the left jugular vein.

In group B, blood was collected from the right jugular vein easily in 17 leopard geckos. In two animals, we failed to collect the whole volume of blood necessary for analysis from the right jugular vein, and blood samples had to be collected from the right and left jugular veins. In one animal, our attempts to collect blood from the right jugular vein were not successful and the blood sample had to be collected from the left jugular vein only.

In five cases, only small haematomas were present immediately after the venepuncture, which persisted for between one and two days. The effect of the phlebotomy was monitored for a time span of six to twelve weeks and all animals were considered healthy without any complications.

Blood samples collected from two animals in group B had to be excluded from the study due to haemolysis. The total number of blood samples used for laboratory analyses was therefore 20 in group A and 20 in group B. The mean body weight of the 20 leopard geckos in group A and

Table 1. Haematology profiles of 20 male leopard geckos (*Eublepharis macularius*)

Parameter	Haemoglobin (g/l)	PCV (%)	RBCs (10 <sup>12</sup> /l)	WBCs (10 <sup>9</sup> /l)	Heterophils (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)	Azurophils (%)	Lymphocytes (%)
Mean	72.58	25.40	0.85	10.47	17.6	1.67	2.7	0.29	4.7	39
SD	11.03	3.68	0.14	2.58	8.1	1.04	2.7	0.33	4.1	14.7
Max	96.05	31.00	1.08	12.50	34	3.62	8	1.2	15	66
Min	54.94	18.00	0.48	6.00	4	0.14	0	0	6	15
Median	70.07	25.00	0.83	10.00	17.5	1.47	2	0.14	3	36.5

PCV = packed cell volume; RBCs = red blood cells; SD = standard deviation; WBCs = white blood cells

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Table 2. Blood chemistry profiles of 20 male leopard geckos (*Eublepharis macularius*)

Parameter	TP (g/l)	Albumin (g/l)	Globulin (g/l)	Glucose (mmol/l)	UA ( $\mu$ mol/l)	AST ( $\mu$ kat/l)	CK ( $\mu$ kat/l)	Ca (mmol/l)	P (mmol/l)	K+ (mmol/l)
Mean	55.60	16.45	39.15	6.18	67.95	0.83	25.40	3.05	1.40	5.78
SD	7.52	2.37	5.74	1.35	42.63	0.42	29.46	0.18	0.23	0.58
Max	67.00	23.00	47.00	10.10	160.00	1.80	117.30	3.44	1.87	7.00
Min	42.00	13.00	29.00	4.30	18.00	0.30	1.20	2.76	1.14	4.50
Median	56.50	15.50	40.00	6.20	54.00	0.70	15.75	3.00	1.30	5.90

AST = aspartate aminotransferase; Ca = calcium; CK = creatine kinase; K+ = potassium; Na+ = sodium; P = phosphorus; SD = standard deviation; TP = total protein; UA = uric acid

20 leopard geckos in group B was  $36.7 \pm 5.2$  g (range 28.6–49.7 g) and  $38.5 \pm 6.7$  g (range 29.2–54.7 g), respectively.

Haematology values and blood biochemistry profiles of male leopard geckos are presented in Tables 1 and 2. The most common leukocytes in the peripheral blood were lymphocytes; azurophils, heterophils and basophils were relatively common, monocytes were uncommon and eosinophils were the least common. The calcium to phosphorus ratio (Ca : P) was about 2 in all leopard geckos. The results of bile acids in all 20 male leopard geckos were the same with bile acids  $< 3.5 \mu\text{mol/l}$ . Therefore, bile acids was excluded from the blood biochemistry analysis.

## DISCUSSION

Leopard geckos are very commonly kept as a pet species and have become a lizard model for research purposes. Despite the fact that the leopard gecko is one of the three most common species of captive lizards in Europe (with the veiled chameleon *Chamaeleo clypeatus* and the inland bearded dragon *Pogona vitticeps*), data dealing with haematological and biochemical blood profiles of captive leopard gecko have not been reported. To the best of our knowledge, this is the first study to report haematology and blood biochemistry values for a group of captive adult male leopard geckos. The study incorporated a large population of male leopard geckos kept in a standardised environment, with monitoring of the animals' health and activity performed daily.

The present study differs from similar studies that have been focused on haematology and biochemistry in wild or laboratory-housed populations of

other gecko species (Sacchi et al. 2007; Mayer et al. 2011; Olayemi 2011; Salamat et al. 2013). The effects of age, sex, reproductive activity, altitude, season and feeding activity on lizard blood profiles have been well documented (Knotek et al. 2003; Pejrilova et al. 2004; Knotkova et al. 2005; Gonzalez-Morales et al. 2015, 2017; Guadarrama et al. 2019) and confirmed for different gecko species (Sacchi et al. 2007; Mayer et al. 2011; Olayemi 2011). Based on these findings, only captive adult males in good health condition were included in the present study. Similar to Mayer et al. (2011), the presented data have been generated from a single colony and we acknowledge that care has to be taken in interpreting the results as a reference range.

In comparison with the other methods for venepuncture in geckos that have been suggested by other authors (e.g. cardiocentesis, phlebotomy of the ventral abdominal vein (Redrobe and MacDonald 1999; Hernandez-Divers 2006)), the technique of blood collection from the jugular vein was not stressful or dangerous for any of the leopard geckos used in the present study. In only two of 44 animals was the method not successful. In 11 animals, the blood sample had to be collected from the right and/or left jugular vein. Blood collection using this method proved to be easy, reliable and repeatable, and the geckos did not show any adverse effects.

Our mean values for white blood cell counts were similar to the results of Mayer et al. (2011), but in the present study the range for the white blood cell counts was narrower. The most common leukocytes in the peripheral blood of 20 male leopard geckos were lymphocytes, azurophils and heterophils. In accordance with the study of Mayer et al. (2011), lymphocytes were the most common leukocyte seen on the blood smears. The classifica-



tion of leukocytes in reptiles is difficult since these cells show morphological variation among species (Sacchi et al. 2007). Olayemi (2011) identified only four types of leukocytes in the peripheral blood of house geckos (*Hemidactylus frenatus*): heterophils, lymphocytes, eosinophils and monophils, while Sacchi et al. (2007) and Mayer et al. (2011) identified five different types of leukocytes: heterophils, basophils, eosinophils, monocytes and lymphocytes. In the present study, six different types of leukocytes were identified in leopard geckos: heterophils, basophils, eosinophils, monocytes, lymphocytes and azurophils. In reptiles, azurophils differ from monocytes and lymphocytes by the presence of typical azurophilic granules in the cytoplasm (Knotkova et al. 2002; Pejrilova et al. 2004). The percentage of the most common leukocytes, lymphocytes, was similar to results for Moorish geckos (Sacchi et al. 2007), but the percentages of heterophils and eosinophils were lower and the percentages of basophils and monocytes were higher for the leopard gecko.

The use of the VetScan VS 2<sup>®</sup> automatic chemistry analyser with the Avian/Reptilian Profile Plus rotor for clinical practice and research with reptiles has been well documented (Knotkova et al. 2010; Mayer et al. 2011). Similar to results from previous studies (Knotkova et al. 2010; Mayer et al. 2011), bile acid concentrations in all blood samples from leopard geckos in the present study were reported as < 35 µmol/l. The lower end of the dynamic range of the bile acids on the rotor is < 35 µmol/l. These results were therefore excluded from the blood biochemistry analysis. In cases where the true bile acid concentration is required another analyser must be used. The ability to measure the concentration of bile acids in the peripheral blood proved interesting and feasible for veterinary practice with chelonians and medium-sized lizards (Knotek et al. 2007; Knotkova et al. 2008; Knotek et al. 2009). The concentration of bile acids can be determined with the use of different automatic analyser systems. However, the volume of blood sample that could be easily collected from small reptile species like the leopard gecko (e. g. 0.100–0.200 µl) is not large enough for the use of standard laboratory analysers. Further studies focused on feasible methods for determining bile acid concentrations in small reptile species are necessary.

Blood concentrations of total protein, albumin, globulins, cholesterol, uric acid and total calcium in female lizards are strongly influenced by their re-

productive activity (Knotek et al. 2003; Pejrilova et al. 2004; Knotkova et al. 2005; Mayer et al. 2011). In accordance with this knowledge and the expected differences in blood biochemistry profiles between males and females, the evaluation of blood profiles for female leopard geckos within different reproductive activity periods is needed. This research is currently ongoing.

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